School of Doctoral Studies in Biological Sciences

University of South Bohemia in České Budějovice

Faculty of Science

Fungi at land use gradient in temperate region of central Nepal

Ph.D. Thesis

Sujan Balami M.Sc.

Supervisor: Mgr. Martina Vašutová, Ph.D.

Department of Botany, Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic

České Budějovice 2023

The thesis should be cited as:

Balami, S, 2023, Fungi at land use gradient in temperate region of central Nepal. Ph.D. Thesis Series, No. 3. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, 185 pp.

Annotation

The aim of this thesis is to summarize worldwide studies regarding land use change impact on the soil fungal communities. Second, to evaluate the effect of land use change, particularly abandoned agricultural land, on soil and fine roots fungal communities in the temperate region of central Nepal. Lastly, to contribute to the knowledge on the Nepal mycobiota by describing new species from the study area.

Reviewed studies showed a negative response of fungal quantitative parameters after land use change from less-intensive site management to intensive site management and mostly significant shifts in the fungal community composition. Due to dependence on the local context, current knowledge is insufficient to generalize the effect of single land use change on fungi.

In central Nepal, the community composition of soil fungi on permanent plots in abandoned agricultural land differed significantly from that of agricultural land and seminatural forests. The fungal succession is not expected to be straightforward because the fungal community in long-term regenerated forests differed from other land use type by having a higher proportion of ericoid mycorrhizal fungi, abundant ectomycorrhizal fungi, and different soil properties.

Focusing on tree species level, land use had a significant effect on arbuscular and total fungi associated with the roots of economically important trees accompanying succession, *Alnus nepalensis* and *Schima*

wallichi. This effect was more pronounced than tree species identity. Low ectomycorrhizal fungal morphotype diversity was found in fine roots of *A. nepalensis*. Although the effect of land use on the ectomycorrhizal fungal community was not significant, sequences of *Cortinarius* spp. were significantly more abundant in forests than in the other land use types.

Three ectomycorrhizal fungi *Alnicola alni-nepalensis*, *Cortinarius nepalensis*, and *Lactarius alni-nepalensis* were described as new species associated with *Alnus nepalensis*.

Declaration

I hereby declare that I am the author of this dissertation and that I have used only those sources and literature detailed in the list of references.

Kathmandu, 04. 01. 2023 Sujan Balami

Signature

Financial support

The work in this thesis was supported partly by the Ministry of Education, Youth, and Sports of Czech Republic within the CzeCOS program (grant no. LM2018123) and partly by using resources of the Department of Botany, Faculty of Science, University of South Bohemia.

Acknowledgements

I would like to express my sincere thanks to everyone who contributed to this thesis, namely:

1. My supervisor Martina Vašutová for constantly helping me in every possible way to accomplish this work.
2. Pavel Cudlín and Global Change Research Institute, Czech Academy of Sciences for their scientific and financial support.
3. Department of Plant Resources (DPR), Ministry of Forests and Environment (MoFE), Government of Nepal (GoN), for giving permission to carry out this work in the study area.
4. Rural Reconstruction Nepal for providing logistics and transportation support.
5. Plant Quarantine and Pesticide Management Centre, GoN, for providing a phytosanitary certificate to transport soil and root samples. National Herbarium and Plant Laboratories, DPR, MoFE, GoN, for giving permission to transport herbarium specimens to the Czech Republic.
6. All the co-authors –Douglas Godbold, Jiri Košnar, Petr Kotas, Giri Tripathi, Chiranjeewee Khadka, Ratna Karki and Vijay Chaudhary.
7. Jan Willem Jongepier for English language correction of introduction and summary.
8. All the colleagues of Department of Botany, especially Aleš Jirsa, Hedvika Synková, Pavlína Matysková, and Jan Fiala for their valuable support.
9. Last but not least, to my beloved family.

List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

I. **Balami S**, Vašutová M, Godbold D, Kotas P, Cudlín P (2020) Soil fungal communities across land use types. IFOREST 13:548. [IF= 1.8 (2020)]
SB collected the data, performed formal analysis, wrote the original draft, reviewed, and edited the manuscript. His contribution was 75%.

II. **Balami S**, Vašutová M, Košnar J, Karki R, Khadka C, Tripathi G, Cudlín P (2021) Soil fungal communities in abandoned agricultural land has not yet moved towards the seminatural forest. For Ecol Manag 491:119181. [IF= 3.5 (2021)]
SB collected the data, performed formal analysis, wrote the original draft, reviewed and edited the manuscript. His contribution was 65%.

III. **Balami S**, Vašutová M, Chaudhary V, Cudlín P (2022) Land use effect on the root-associated fungi of *Alnus nepalensis* and *Schima wallichii*. (Manuscript)
SB collected the data, performed formal analysis, wrote the original draft, reviewed and edited the manuscript. His contribution was 70%.

IV. **Balami S**, Vašutová M (2022) Three new ectomycorrhizal fungi from central Nepal: *Alnicola alni-nepalensis*, *Cortinarius nepalensis*, and *Lactarius alni-nepalensis.* (Manuscript)
SB collected the data, wrote the original draft, reviewed and edited the manuscript. His contribution was 80%.

Co-authors' agreement

Martina Vašutová, the supervisor of the Ph.D. thesis and co-author of all the papers and manuscripts presented fully acknowledges the major contribution of Sujan Balami in all the presented papers and manuscripts.

……

Mgr. Martina Vašutová, Ph.D.

Pavel Cudlín, the co-author of papers I, II and manuscript III fully acknowledges the major contribution of Sujan Balami in those presented papers and manuscript.

……

doc. RNDr. Pavel Cudlín, CSc.

Table of contents

General Introduction

At present, several global change scenarios, such as land use change, are so widespread that they affect almost all biological systems in a substantial part of the world. Land use change is ranked as one of the major drivers of decline in nature and biodiversity (IPBES 2019). It disrupts various provisioning, supporting and regulatory ecosystem services (Hasan et al. 2020). Generally, the consequences of land use change are detrimental when natural areas are converted to intensive land use forms (e.g. agricultural land, pasture, urban areas). By contrast, abandonment of intensive land use types (e.g. abandonment of agricultural land) and restoration of seminatural vegetation have less detrimental to positive impacts (Bell et al. 2020; Mantero et al. 2020). Abandonment of agricultural land is becoming a common process in many parts of the world, which provides the opportunity to restore degraded ecosystems (Castillo et al. 2021; Zhu et al. 2021). However, not only vegetation succession but also belowground microbial communities are vital for successful restoration (Farrel et al. 2020).

Fungi are the dominant eukaryotes in the soil and are one of the most diverse groups of organisms with distinct ecological roles in the terrestrial ecosystem (Hawksworth and Lücking 2017; Money 2016; Blackwell 2011). They are involved in various ecosystem processes, either directly through symbiosis and pathogenesis or indirectly through decomposition and nutrient cycling (Dighton 2018). Meanwhile, these soil fungi are in constant interaction with their environment such as climate, vegetation, edaphic and topographic factors, and biotic factors/interactions. Generally, rural land use change represents changes in vegetation, soil properties, and anthropogenic activities on land with a particular type of usage as defined by e.g. Brinkmann et al. (2019) and Zhang et al. (2018). However, due to the hyperdiversity of fungi and the large number of undescribed species as well as their undefined functional role, our understanding of the effect of land use change on the soil fungal community is insufficient to interpret consequences of these processes. This is particularly so in underexplored regions like Nepal, where the knowledge gap is huge.

In this context, the first contribution of this thesis is to fill the knowledge gap concerning general information about the impact of land use change on soil fungal communities by reviewing worldwide studies (**Chapter I**). After, this thesis will provide insight in the change in diversity and community composition of soil and root-associated fungi in response to abandonment of agricultural land in the temperate region of central Nepal (**Chapters II** and **III**). Finally, this thesis will contribute to the knowledge of fungal diversity on abandoned land by describing three new species of ectomycorrhizal fungi (**Chapter IV**).

1. Land use change and abandoned agricultural land

The term 'land use change' means a change in human employment of land for a certain use or function (Briassoulis 2020). Globally, approximately 32% of the land area has changed from 1960 to 2019 with overall a net loss of forest and a net expansion of agricultural land (Winkler et al. 2021). Changes in various land use categories (primary, secondary and plantation forest, grassland, woodland/rangeland, pasture, agricultural land, urban areas) have contributed to the overall land use change. Winkler et al. (2021) also reported that the pattern of land use change varies on temporal and spatial scales. There is a decrease in forest cover in Southeast Asia and West Africa due to the expansion of perennial croplands (Austin et al. 2019; Ordway et al. 2017), while there is an increase in forest cover in Europe as well as other developed countries such as the United States, Japan, China and in mountainous areas due to abandonment of agricultural land (Chaudhary et al. 2020; Kolecka et al. 2017; Li and Li 2017; Kaplan et al. 2012).

FAO (2016) defines 'agricultural land abandonment' as the cessation of agricultural activities on agricultural land and the complete withdrawal of agricultural management for at least four years. Approximately 385–472 million km^2 of farmland (8–10% of the world's cultivated land) have undergone abandonment (Winkler et al. 2021). Three main drivers. i.e. ecological drivers (environmental features of the land such as elevation, geological substrate, slope and erosion), socioeconomic drivers (new

economic opportunities, migration, rural depopulation) and mismanagement (overexploitation and desertification) are considered the responsible factors in agricultural land abandonment (Li and Li 2017).

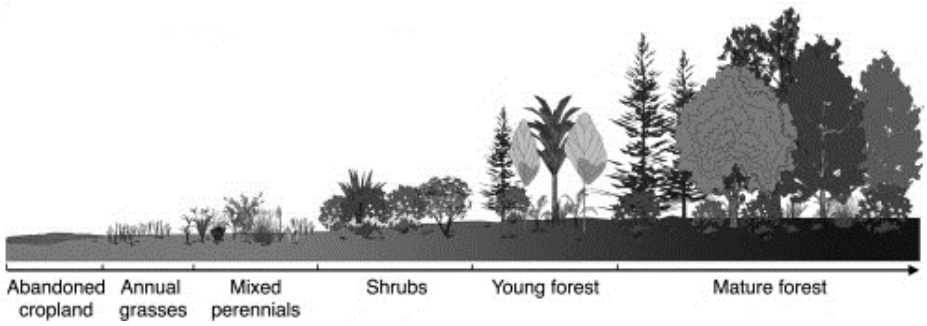

Fig. 1 Natural recovery of native forests, in which degraded land undergoes several stages, from annual grasses through mixed perennials and shrubs eventually to mature forest (Yang et al. 2020a).

Generally, abandonment of agricultural land can cause undesirable environmental, socioeconomic, and landscape impacts, such as biodiversity loss, landscape homogenisation, increased fire risk, soil erosion, and soil degradation, as well as an increase in the area of agricultural intensification (Benayas et al. 2007; Vega-García and Chuvieco 2006). However, abandonment of agricultural land also reduces the high pressure on biodiversity and natural resources (Fayet et al. 2022; Bell et al. 2020). It can provide space for the expansion of natural vegetation adapted to local conditions, which can lead to the development of secondary forests (Kolecka et al. 2015). Such restorations have the potential to increase carbon sequestration, soil restoration, improve nutrient cycling, and increase biodiversity (Fig. 1) (Liu et al. 2020; Yang et al. 2019). However, the succession of abandoned land vegetation often follows less predictable pathways (Guariguata and Ostertage 2001). Several abiotic and biotic factors, including the proportion of prior land use intensity, determine the speed of forest regeneration. Abiotic factors (fire, drought, soil nutrients, soil pH, slope, surface stoniness, canopy openness), land use history and time since abandonment are the major

factors explaining the course of succession on abandoned agricultural land. In addition, biotic factors such as seed dispersal limitations of plants, seed predation and competition also determine the successional process (Hooper 2008; Benjamin et al. 2005).

2. Soil fungal community

Fungi are a group of heterotrophic eukaryotic organisms forming a separate domain in the 'Tree of Life'. Hawksworth and Lücking (2017) estimated that 2–3.8 million species of fungi could exist on Earth, but only 3–8% are known to date. Although limited by the existence of many cryptic species, the recent advances in molecular methods have increased the estimated numbers further to 12 million species (Heitman et al. 2020). A recent classification system has grouped fungi into seven different phyla: Basidiomycota, Ascomycota, Entorrhizomycota, Mucoromycota, Zoopagomycota, Blastocladiomycota and Chytridiomycota (James et al. 2020). Due to their ability to adapt to various environmental conditions (e.g. pH and temperature), fungi are found in almost all habitats such as soil, water and phylloplane. Although it is evident that an important part of fungi are also associated with aboveground vegetation (endophytes, leaf pathogens, etc.) (Vašutová et al. 2021), most of the fungal species named and described so far are likely to occur in the soil environment at some stage in their life cycle, and are known as soil fungi (Tedersoo et al. 2014).

Soil fungi constitute an important component of belowground microbial diversity mainly comprising of saprotrophic fungi, soil-borne pathogenic fungi, mycorrhizal fungi, and spores of fungi growing aboveground (Limtong and Nasanit 2017; Tedersoo et al. 2014). All these diverse groups of fungi play a vital role in ecosystems, including decomposition, nutrient cycling, pathogenesis and plant nutrition (Moore et al. 2011).

Saprotrophic fungi are the largest group of soil fungi often dominating in the litter layer of the soil (Odriozola et al. 2021). Because saprophytic fungi release a wide range of ligninocellulolytic enzymes, they can hydrolyse a variety of organic residues (both easily degrading and recalcitrant

substrates) present in the soil. Additionally, hyphal aggregation of saprophytic fungi (e.g. mycelial cords) form highly dynamic channels for nutrient distribution (Cairney 2007; Boddy 2000). Thus, saprophytic fungi play an important role in the decomposition and transformation of organic residues, facilitating the nutrient cycling process, particularly of carbon (C), nitrogen (N) and phosphorus (P), along with other elemental cycles (Moore et al. 2011).

Several taxa within Ascomycota and Basidiomycota are among the most common soil-borne pathogens of plants which can survive in soil as saprophytes on plant residues or in the form of spores or resting structures such as sclerotia (Heitman et al. 2020; Heitman 2011). These fungi can significantly impair plant growth and development or even destroy large populations of plants (Hansen and Stone 2005; Dobson 1994). Pathogenic fungi play a substantial role in maintaining plant diversity and mediating plant succession in forest ecosystems (Bagchi et al. 2014). Negative plant-soil feedback facilitates plant species co-existence and positively affect diversity-productivity relationships (Mommer et al. 2018; Maron et al. 2011).

Symbiotrophs – mycorrhizal fungi – form mutualistic relationships with more than 90% of vascular plant species (Brundrett and Tedersoo 2018), and at least 50,000 fungal species of Ascomycota, Basidiomycota and Mucoromycota are estimated to form mycorrhizas (van der Heijden 2015). The mycorrhizal fungal association is one of the most relevant and significant interactions of plants in the terrestrial ecosystem (Tedersoo et al. 2020a; Klironomos et al. 2011; Smith and Read 2010). One of the primary functions of mycorrhizal fungi is to increase plant access to soil nutrients in exchange for C from photosynthesis (Smith and Read 2010). Extraradical hyphae of mycorrhizal fungi provide an efficient mechanism for distributing plant-derived C throughout the soil matrix (Simard and Durall 2004). Mycorrhizal fungi also play an important role in the stabilization of soil organic matter and the decomposition of residues (Shah et al. 2016; Bearden and Petersen 2000). Coevolution of plants and

fungi has generated four main types of mycorrhizal fungi, namely arbuscular mycorrhizal (AM), ectomycorrhizal (ECM), orchid mycorrhizal (ORM) and ericoid mycorrhizal (ERM) fungi (Smith and Read 2010) (Fig. 2). The richness of plants forming these mycorrhizal associations is unevenly distributed. Some plants may also form more than one type of mycorrhiza, for example dual mycorrhizas in *Alnus* species (Teste et al. 2020).

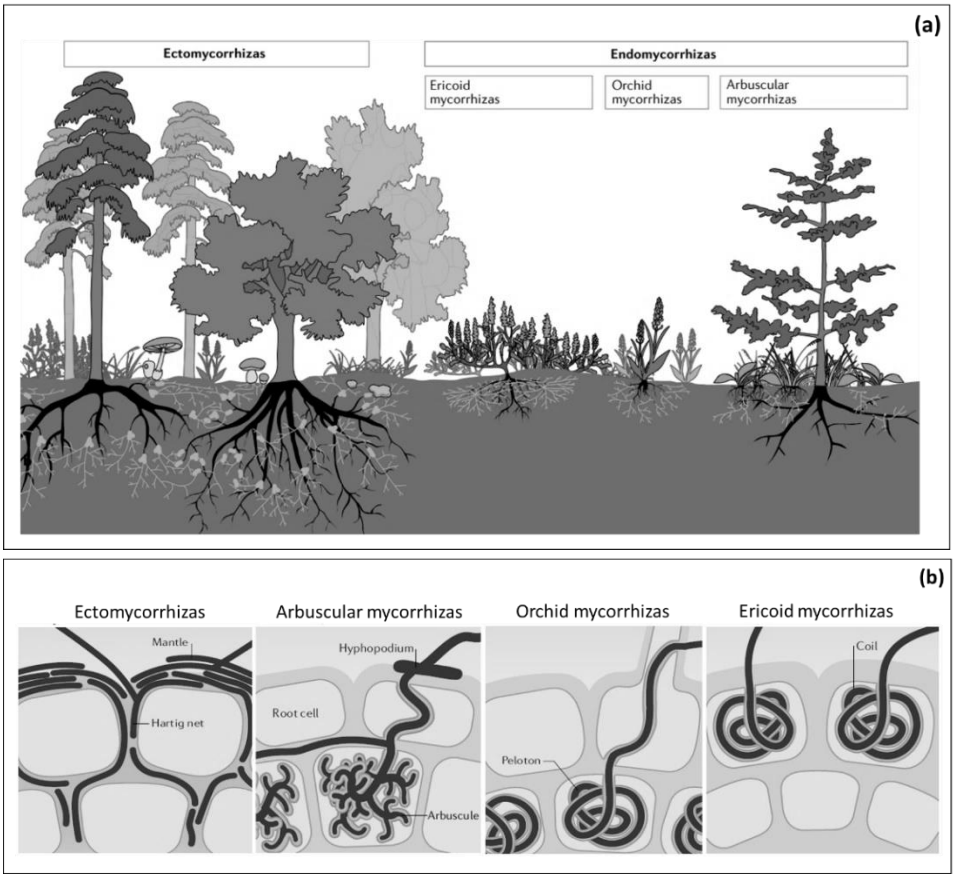

Fig. 2 (a) Major mycorrhizal types. (b) Main cellular features of ectomycorrhizas (ECM), arbuscular mycorrhizas (AM), orchid mycorrhizas (ORM), and ericoid mycorrhizas (ERM) (Genre et al. 2020).

Arbuscular mycorrhizal fungi are the most ancient and widespread mycorrhizal fungi, colonizing approximately 71% species of angiosperms

(Brundrett and Tedersoo 2018). To date, the MaarjAM database lists 300 phylogroups of AM fungi (Öpik et al. 2010). However, the actual diversity of AM fungi is expected to be much higher because AM fungal species show remarkable intraspecific variation (Mathieu et al. 2018). They are considered to be less host-specific and are mostly/predominantly formed by Glomeromycotinian species (e.g. *Glomus*, *Acaulospora*, *Ambispora*, *Archeospora*, *Diversispora*, *Gigaspora*, *Scutellospora*, *Paraglomus*) and by a few Mucoromycotinian species (e.g. *Endogone* and *Planticonsortium*) of the phylum Mucoromycota. AM fungi provide their plant symbionts with substantial proportions of their P and N requirements (Smith and Read 2010). The AM association also improves resistance to a variety of stresses (drought, salinity, herbivory, temperature, metals and diseases) in plants (Zou et al. 2021; Middleton 2015; Hameed et al. 2014; Evelin 2009).

Ectomycorrhizal fungi are the most species-rich mycorrhizal fungi but colonize only 2.2% of angiosperms and gymnosperms, mainly woody perennials (Brundrett and Tedersoo 2018). These fungi have evolved many times from saprophytic fungi and their diversity is estimated at 20,000–25,000 species of Basidiomycota and Ascomycota (Tedersoo et al. 2010; Brundrett 2009; Rinaldi et al. 2008). ECM fungi are reported to be more or less host plant specific and are reported to be common in temperate and boreal ecosystems and large forested areas of tropical and subtropical regions (Kumar and Atri 2018). Most of these ECM fungi belong taxonomically to the Basidiomycota, i.e. Agaricomycetes (e.g. *Amanita*, *Laccaria*, *Cortinarius*, *Inocybe*, *Boletus*, *Leccinum*, *Paxillus*, *Suillus*, *Lactarius*, *Russula* and *Thelephora*), and some to the Ascomycota, i.e. Pezizomycetes (*Helvella*, *Hydnotrya*, *Wilcoxina*), Eurotiomycetes (*Elaphomyces*) and Dothideomycetes (*Cenococcum*) (Tedersoo et al. 2010). Regarding their ecological role, ECM fungi can decompose N-rich polymers, extract phosphate from various organic sources, and then supply these nutrients to their plant symbionts along with other micronutrients (Makarov 2019; Kafle et al. 2019). ECM fungi also increase stress resistance in plants by colonizing the fine roots (Chu et al. 2021).

Ericoid mycorrhizal fungi are a group of mycorrhizal fungi colonizing plants of Ericaceae, Epacridaceae and Empetraceae. The diversity of ERM fungi includes approximately 150 species of Ascomycota (primarily) and some Basidiomycota (Brundrett and Tedersoo 2018). Despite their low diversity, ERM fungi are known to have a nearly global distribution and are often abundant in habitats with harsh edaphic conditions (soils with low pH and temperatures, and low soil moisture, which limit the uptake of soil nutrients in plants and slow down the decomposition of organic matter) (Kohout 2017). ERM fungi show some ecological flexibility that has both mutualistic and saprotrophic capabilities and is less dependent on plant-derived C (Grelet et al. 2016). The ERM fungi are known to produce a broad range of hydrolytic and oxidative enzymes which can mobilize nutrients from organic complexes in the soil (Read et al. 2004; Cairney and Meharg 2003; Näsholm et al. 1998). However, our knowledge of ERM fungi is very limited compared to that of AM and ECM fungi.

Orchid mycorrhizal fungi specifically colonize plants of the Orchidaceae family, where the plant partners are either partially or fully mycoheterotrophic (Smith and Read 2010). The most common ORM fungi are members of the Basidiomycota traditionally grouped under the name rhizoctonias included in the families Serendipitaceae, Ceratobasidiaceae and Tulasnellaceae (Li et al. 2021). Since orchid seedlings (protocorms) lack chlorophyll, they rely on C and nutrients obtained from ORM fungi (Favre-Godal et al. 2020; Schiebold et al. 2017). The majority of adult orchids retain their ORM fungi and have been reported to gain up to 20% of organic molecules (C and N) from ORM fungi (Schweiger et al. 2018; Smith and Read 2010).

3. Factors affecting soil fungal community

As part of soil microbial communities, the soil fungal community is strongly influenced by abiotic and biotic factors of an ecosystem (deterministic process) (Yang et al. 2020b; Jamiołkowska et al. 2018; Wang et al. 2015; Broeckling et al. 2008). Soil physicochemical properties, climatic factors, anthropogenic activities, etc. are important abiotic factors

regulating soil fungal communities (Bui et al. 2020; Yang et al. 2017a; Kabir 2005). Along with the co-occurring microbiota (Crowther et al. 2012), plant factors (diversity, density, and community composition) are crucial biotic drivers of the soil fungal community (Francioli et al. 2021; Chen et al. 2017; Gao et al. 2015). In addition, particular fungal characteristics such as life history strategy and dispersal mechanism (stochastic process) co-determine the composition of a fungal community (Cline and Zak 2014).

Various soil factors such as soil pH, and C, N and P concentrations are known to affect the soil fungal community (Xiao et al. 2020; van Geel et al. 2020). Soil pH is one of the most important soil factors affecting fungi either directly by affecting their growth and metabolism or indirectly by affecting other abiotic factors such as C and nutrient availability (Zhang et al. 2016; Rousk et al. 2009; Lauber et al. 2008). Generally, soil fungi are found to have a relatively wide range of pH tolerance (Wardle and Lindahl 2014), but only a small number of taxa prefer a very low or very high pH level. Some taxa prefer a lower pH, others prefer a higher pH level. For example, Wang et al. (2015) found soil pH to be a significant factor affecting the diversity and richness of soil fungi in *Quercus-Rhododendron-Abies* forests. Similarly, Liu et al. (2018) found soil pH to have an effect on the fungal community composition along elevational gradients of *Betula-Abies-Rhododendron* forests. A study from North American temperate mixed hardwood forests showed that with a rise in soil pH by 1.7 units in the acidic range, AM and ECM community composition changed significantly (Carrino-Kyker et al. 2016). Thus, soil pH affects both diversity and community composition of soil fungal communities.

Soil C is another driving factor of fungal diversity and community composition (Yang et al. 2017b; Liu et al. 2015). Because fungi depend on exogenous C for growth, both the quality and the quantity of C determine the soil fungal community. The effect of soil C on fungi can be different depending on functional group. In the case of mycorrhizal fungi, which

mainly depend on their host for a C source, the composition of fungi varies with host plant physiology and growth. In the case of saprophytic fungi, the quality of the litter plays a significant role. Easily available substrates (e.g. plant root exudates, parts of leaf litter) and recalcitrant substrates facilitate different species of fungi depending upon their ligninocellolytic capability (Li et al. 2017; Broeckling et al. 2008). Liu et al. (2015) found that soil C content affects the fungal community in a maize-soybean-wheat field, where the abundance of Mucoromycota and Ascomycota increased, and Basidiomycota decreased with increasing soil C content. The relative abundance of lignin was also found to be significantly correlated with ECM and saprophytic fungal abundance in different North American secondary forests composed of *Betula*, *Quercus*, *Acer* and *Fagus* (Pec et al. 2021).

Similar to C, the soil fungal community is also affected by the addition of N (Xiao et al. 2020). Long-term N fertilization has been found to decrease soil fungal diversity and community composition in temperate croplands of China (Zhou et al. 2016), in subtropical Australia (Paungfoo-Lonhienne et al. 2015), and in *Pinus taeda* plantation forests of North America (Weber et al. 2013). In general, increasing N deposition has been found to decrease the abundance of lignolytic and ECM fungi (Lilleskov et al. 2019; van der Linde et al. 2018). For example, a increase in N deposition results in a decrease in abundance of lignolytic fungi in northern hardwood forests dominated by *Acer* sp. in Michigan, USA (Entwistle et al. 2018). In both temperate and tropical forests, AM fungi respond to N inputs with a significant decrease in root colonization, spore density and external hyphal length (Sheldrake et al. 2018; Treseder 2004a). Morrison et al. (2016) found a decrease in the species richness and diversity of ECM fungi and an increase in richness and diversity of saprophytic fungi with N enrichment in mixed temperate hardwood forests dominated by *Quercus* spp. in Massachusetts, USA. The composition of ECM fungi was found to be determined by N fertilization in temperate deciduous oak forests in Indiana, USA (Avis et al. 2008). Thus, increasing N deposition or fertilization usually have negative effects on soil fungi parameters.

Along with C and N, also soil P plays an important role in structuring the soil fungal community (He et al. 2016). Sometimes the amount of P in the soil was found to be more important than N in determining fungal community composition, for example in an alpine meadow (He et al. 2016). The concentration of available P in agricultural land (andosols) was known to affect the diversity and composition of the soil fungal community significantly (Bao et al. 2013). Yan et al. (2022) found a reduction in β-diversity of soil fungi in temperate grasslands due to enrichment of available P in the soil. However, some studies also reported no significant effect of P addition in soil fungal community, for example in peatland in China (Cao et al. 2022). Regarding mycorrhizal fungi, P fertilization was found to influence the composition of AM fungi in subtropical forests dominated by *Castanopsis*, *Schima*, and *Lithocarpus* species (Maitra et al. 2021). During vegetation restoration, the richness of AM fungi was reported to be negatively related to the available P in subtropical montane forests (Liang et al. 2015). The richness of Glomerales was found to decrease significantly with P addition in tropical lower montane forests of South America (Camenzind et al. 2014). In the case of ECM fungi, P deficiency was reported to promote ECM diversity in a *Quercus* and *Pinus* stand in southwest China (Khalid et al. 2021).

In addition to pH and soil nutrients, other soil physical properties, for example soil texture, also soil aggregations are responsible for shaping fungal community composition. For example, soil texture was found to be significantly correlated with fungal diversity and community composition in different land use types (agricultural land, grassland, marshland and woodland) because soil texture heterogeneity provides a diversity in niche space (Seaton et al. 2020).

Vegetation properties – plant species richness, diversity and composition are the major biotic factors significantly influencing fungal biomass, diversity and community composition (Yang et al. 2017b; Chen et al. 2017). A global meta-analysis has shown that an increase in plant species richness significantly increases the soil fungal biomass (Chen et al. 2019).

Increases in plant species richness have been reported to enhance saprophytic and ECM fungal biomass due to an increase in root biomass and amount of root exudates (Awad et al. 2019; Eisenhauer et al. 2017). The *plant diversity hypothesis* (Waldrop et al. 2006) proposes that greater plant diversity increases the range of organic substrates entering the soil, which create more niche space and facilitate high diversity. For example, increased plant diversity has been found to increase fungal diversity in grasslands of the Tibetan Plateau (Wang et al. 2022; Yang et al. 2017b), in subtropical forests of China (Li et al. 2020), and in forests of the northern Baltic region (Tedersoo et al. 2020b). Furthermore, the plant family identity also affects the relative abundance of total fungi at the phylum level (Zhong et al. 2020). Several studies have found a significant correlation between plant species composition and total fungal species composition, such as in temperate grasslands of China (Chen et al. 2017) and in a tropical forest of Costa Rica (Kivlin et al. 2016). Thus, plants are an important factor in determining the soil fungal community.

Another important factor affecting soil fungal communities is disturbance (both natural and anthropogenic). Individual fungal species respond differently to the intensity of disturbance depending on their life history strategies. Competitive fungal species are favoured at low levels of disturbance (K-selected strategies), whereas good colonizers (r-selected) are favoured by high levels of disturbance (Carroll and Wicklow 1992). Depending on disturbance type, the effect of disturbance on fungi can be direct (e.g. fragmentation of hyphae, reduced propagules/inoculum) or indirect by affecting physical and chemical properties of the soil (Jones et al. 2003). Regarding agricultural disturbances, the intensity of tillage strongly alters the soil fungal community. For example, the relative abundance of saprotrophs was found to increase with long-term tillage practice in a tomato-cotton field. (Schmidt et al. 2019). In the case of disturbance in forests, Shi et al. (2019) found a decline in the abundance of saprophytic fungi and an increase in facultative pathogenic fungi with disturbance of the forest (mainly composed of Fagaceae and Lauraceae), which was related to increased availability of P, soil pH, and reduced

competition. It is also evident that AM fungi are sensitive to a wide range of anthropogenic disturbances (Jansa et al. 2006; Treseder et al. 2004b). For example, Brundrett and Ashwath (2013) found a higher AM fungal diversity in undisturbed sites compared to former mining sites. Sharmah and Jha (2014) reported a high AM spore density in undisturbed forest compared to plantation forest. Rosendahl and Matzen (2008) found differences in AM abundance and composition between arable land (with different crops) and fallow land. However, some studies also showed an increase (Guadarrama et al. 2014) or no change in AM diversity (Lekberg et al. 2012) as a result of soil disturbance. In the case of ECM fungi, the effect of disturbances such as wildfires, clear-cuts, stump removal and windthrow on ECM fungal diversity and composition have been reported in several studies (Vašutová et al. 2018; Barker et al. 2013; Menkis et al. 2010). Jones et al. (2003) suggested that clear-cut logging modifies ECM composition which is related to the modification of soil biology and chemistry after clear-cutting. Hartmann et al. (2014) reported a negative effect of disturbance (logging and subsequent soil compaction) on ECM fungi such as *Russula*, *Inocybe*, *Clavulina*, *Hygrophorus*, *Elaphomyces*, *Hyphodontia*, *Boletus*, *Cortinarius* and *Tarzetta*. Vašutová et al. (2018) found a significant effect of management regimes in spruce forest affected by windthrow, where the percentage of ECM fungi decreased with increasing disturbance and management intensity. Thus, depending on the type and scale of the disturbance and life strategies of fungi, disturbances mostly decrease the soil fungal diversity and change species composition.

4. Mechanisms of fungal community succession

Due to continuous changes in vegetation, soil nutrient level, interaction between existing species, disturbance factors, etc. in particular habitats, the soil fungal community hardly remains in a steady state over time and thereby undergoes succession (Dix and Webster 1995). Similarly to the succession of other biotic communities, fungal succession can conveniently be considered as a three-stage process in which fungal propagules first arrive at the site of succession, then establish, interact,

compete, disperse, and are later replaced by other fungal species (Frankland 1992).

The arrival of fungal spores or mycelia in a particular substratum is usually a random event. However, distance from source, topography, season, life cycle and dispersal mechanisms may affect the spread of fungal spores. After the arrival of fungal propagules, the speed with which the species is established in the substratum depends on selection pressure. Generally, r-selected species with fast germination and rapid growth are successful. The r-type behaviour enables r-selected species to capture new resources ahead of competitors and to compete efficiently for ephemeral energy supplies (Pugh and Boddy 1988). Subsequently, as time elapses and habitat conditions change, pioneer fungal species are replaced by other seral fungal species. Three different models (the facilitation, tolerance, and inhibition models) could be valid for fungal succession, particularly succession in decaying substrate (Frankland 1992). The facilitation model considers that the earlier species pave way for later species and facilitate replacement, whereas the tolerance model assumes that the late colonists replace early species because they can grow better at lower levels of resources. The inhibition model considers that the early species at the site modify the microenvironment, so that it becomes less suitable for subsequent colonization by both early and late species. However, unlike plant communities, fungal communities hardly reach an endpoint (climax community) during succession. However, unlike plant communities, fungal communities hardly reach an endpoint (climax community) during succession. Reaching a climax community seems to be the most probable for the succession of ECM fungi because they are the most linked to vegetation (Li et al. 2020).

5. Land use change and soil fungal community

Typically, a change in rural land use is a function of changes in vegetation, soil properties and anthropogenic activities in particular land use types. As discussed in Section 3, these factors affect the soil fungal community invariably. Several studies have shown that land use change has various

impacts on both quantitative and qualitative parameters of the soil fungal community (Pereira et al. 2022; Zhu et al. 2021; Baral et al. 2021; Brinkmann et al. 2019). The occurrence of equivocal results is related to the variation in land use types considered in particular studies, the intensity of land use and the climatic, topographic and edaphic conditions. Additionally, taxonomic and functional groups of fungi respond differently to land use change.

In general, change in land use types have been found to affect soil fungal species richness and diversity significantly (Yang et al. 2017a; Mueller et al. 2016). Several studies have reported that an increase in land use intensity (e.g. conversion of natural forest and grassland to plantation forest, pasture or cropland) leads to a decrease in fungal species richness and diversity (Hui et al. 2018; Fracetto et al. 2013), but not always (Cerqueira et al. 2018). Regarding community composition, conversion of primary forest to various intensive land use types, such as perennial cropland (Brinkmann et al. 2019), pasture (Mueller et al. 2016) or plantation forest (Nakayama et al. 2019), has been found to alter soil fungal community composition significantly. During such land use change, the relative abundance of different taxonomic and functional groups of fungi also changes (Meng et al. 2020; Brinkmann et al. 2019; Hannula et al. 2017). In the case of mycorrhizal fungi, changes in land use also significantly affect different AM and ECM fungal parameters. Land use change has been found to significantly alter AM spore density, glomalin-related soil protein, root colonization, diversity, and species composition (Xu et al. 2017; Trejo et al. 2016; Pereira et al. 2014; González-Cortés et al. 2012; Bedini et al. 2007; Rillig et al. 2003). Conversion of tropical primary forest to plantation forest (Zhang et al. 2021) and to agricultural land (Pereira et al. 2014) have been found to lower AM fungal diversity significantly. Likewise, changes in AM species composition have been reported during conversion of tropical dry forest to pasture (Gavito et al. 2008), subtropical primary forest to plantation forest (Sharmah and Jha 2014), and grassland to farmland in arid and semi-arid steppes (Xiang et al. 2014). Compared to AM fungi, the response of ECM fungi to change in

land use has been studied less. A few reports, for example Correia et al. (2021), found a significant difference in the ECM fungal community between long-established and recent beech forests. Klavina et al. (2022) found significant differences in the richness of ECM between Norway spruce forests developed on former agricultural land and former forest land. Overall, differences in soil fungal parameters are found to be associated with either one or more factors such as land use intensity, soil factors (soil pH, moisture, ion exchange capacity, organic matter, total C, N and P concentrations, and C/N ratio) and plant factors (diversity, composition, biomass, litter composition, etc.) (Sui et al. 2019; Yang et al. 2017a).

6. Land use change and abandoned agricultural land in Nepal

Nepal is a mountainous country located on the southern slope of the central Himalayan region which is bounded by the cold and arid Tibetan plateau to the north and the hot and humid Indian plains to the south (Dhital 2015). Stearn (1960) proposed a broad categorization of Nepal into three phytogeographic regions: –western Nepal, eastern Nepal and central Nepal (Fig. 3). Along the elevational gradient, Nepal is classified into five major physiographic zones: –Terai, Siwalik, Middle Mountains, High Mountains and High Himal representing six different bioclimatic zones (Table 1).

Like many other regions, also several parts of Nepal have undergone substantial land use change in recent decades due to environmental and socioeconomic changes (Thapa et al. 2021; Maharjan et al. 2020; Uddin et al. 2015). This is reflected in the changes in land cover as listed in Table 2 (FRTC 2022). The data show that there is a gain of 1.7% in forest cover and a loss of 2.1% in agricultural land. A comparison of land use changes between different physiographic zones of Nepal has shown that the extent of change in forest and agricultural land use is greater in the Terai, Siwalik and Middle Mountains regions (FRTC 2022). Particularly in the Middle Mountains, the forest area has increased by approximately 249 thousand hectares and the area of agricultural land has decreased by ~233 thousand hectares (FRTC 2022). Besides the government policy in forest

conservation, one of the factors for the increased forest cover in Nepal is related to the abandonment of agricultural land and subsequent expansion of vegetation (Kc and Race 2019).

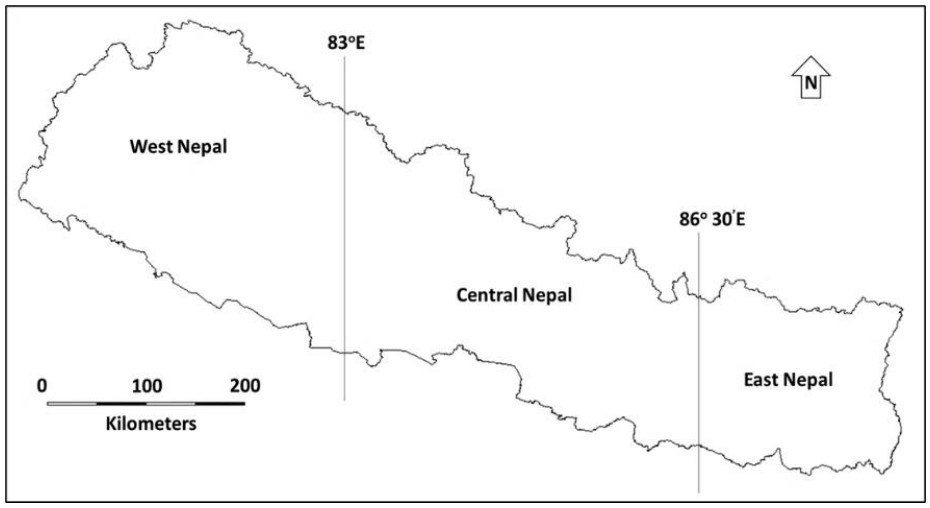

Fig. 3 Phytogeographic regions of Nepal. (Source: Sujan Balami)

Table 1: Physiographic and bioclimatic zones of Nepal (MFSC 2014).

SN	Physiographic zone	Elevation	Bioclimatic zone
1	Terai	Below 500 m	Tropical
2	Siwalik	500-1000 m	
3	Middle Mountains	1000-2000 m	Subtropical
		2000-3000 m	Temperate
4	High Mountains	3000-4000 m	Subalpine
		4000-5000 m	Alpine
5	High Himal	Above 5000 m	Nival

Since most of the agricultural land in mountainous areas of Nepal are spatially scattered small and narrow terraces, the rural inhabitants gradually reduce farming intensity leading to an underutilization and abandonment of agricultural land (Subedi et al. 2021; Dahal et al. 2020; Ojha et al. 2017; Poudel et al. 2014). Ojha et al. (2022) reported that several biophysical and socioeconomic factors are driving factors of the

abandonment of agricultural land (Fig. 4). After abandonment, the areas are exposed to different forms of geomorphic damage, for example landslides, debris flows, formation of gullies and sinkholes, etc. (Chaudhary et al. 2020).

Table 2: Land cover types and percent cover in Nepal (FRTC 2022)

SN	Land cover type	Percent cover		Percent change
		2000	2019	
1	Waterbody	0.44	0.48	0.04
2	Glacier	3.14	3.14	0
3	Snow	3.9	6.29	2.39
4	Forest	39.99	41.69	1.7
5	Riverbed	1.15	1.11	-0.04
6	Built-up	0.17	0.53	0.36
7	Cropland	26.31	24.21	-2.1
8	Bare soil	0	0.03	0.03
9	Bare rock	7.38	5.64	-1.74
10	Grassland	13.95	13.27	-0.68
11	Other forested land	3.57	3.62	0.05

There are very few studies of vegetation succession on abandoned agricultural land in Nepal, but Ojha et al. (2022) pointed out that such abandoned areas are gradually colonized by vegetation after abandonment. Chaudhary et al. (2019) reported that in the subtropical mountainous region of western Nepal, native tree species such as *Pinus roxburghii*, *Schima wallichii*, *Quercus semicarpifolia*, *Shorea robusta*, *Castanopsis* sp. and *Engelhardtia* sp. spread spontaneously on abandoned agricultural land with the highest cover of shrub adapted to those areas. Likewise, Jaquet et al. (2015) found that invasive plants such as *Ageratina adenophora* gradually colonized abandoned terraces of paddy fields in western Nepal after abandonment. Also based on my observation, abandoned agricultural land in the lower temperate and upper subtropical parts of central Nepal is spontaneously colonized by vegetation after abandonment. After abandonment, such areas are first colonized by grasses

with sparse shrubs such as *Berberis* spp., *Pyracantha cernulata* and *Rubus ellipticus*, and saplings of tree species like *Alnus nepalensis* and *Schima wallichii*. Later, the coverage of shrubs and tree species such as A. *nepalensis*, *S. wallichii*, *Symplocos* spp., *Daphniphyllum himalayense* and *Rhododendron arboreum* increases gradually. Because *A. nepalensis* and *S. wallichii* usually accompany the succession of abandoned agricultural land in the mountainous areas of central Nepal and are the components of some parts of mature forests, their successful colonization and establishment can be crucial for the spontaneous succession of such abandoned fields.

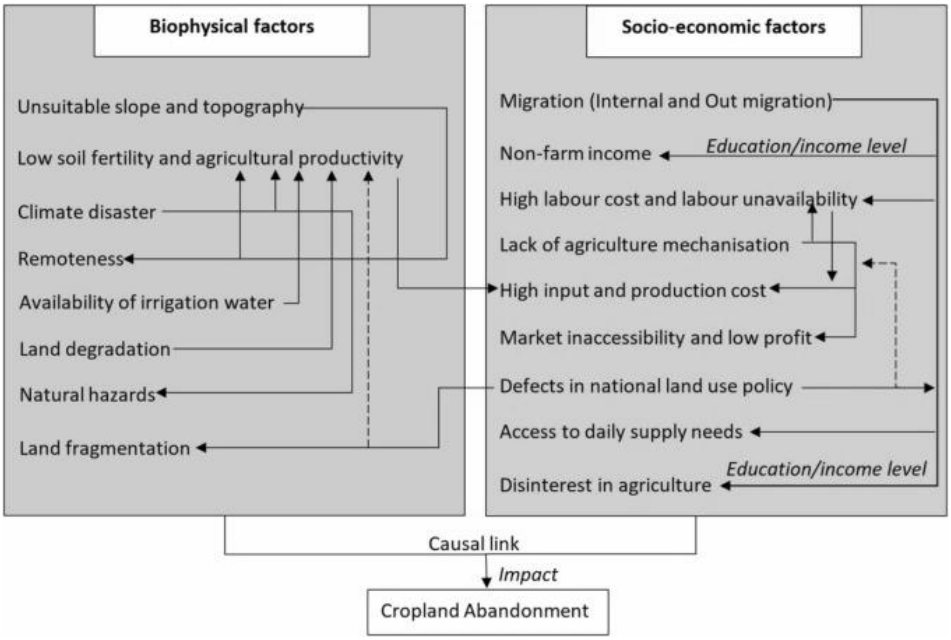

Fig. 4 Loop diagram showing the casual links between biophysical and socioeconomic factors for the abandonment of agricultural land in Nepal. The solid and dashed lines show the direct and indirect linkages between the factors, respectively (Ojha et al. 2022).

Alnus nepalensis D. Don (Betulaceae), commonly known as Nepalese alder (Fig. 5a), is a large deciduous tree growing up to 30 m high and is found in the subtropical to lower temperate regions of Nepal. It is

distributed at elevations of 500–3000 m and grows best on loamy soils and on sand and gravel. *A. nepalensis* is an ecologically and economically important native tree in Nepal. It is a nitrogen-fixing tree vital in controlling landslides and erosion in hilly areas of Nepal, and is thus used in land reclamation. It is also used as low-quality timber and to make charcoal and firewood.

Fig. 5 (a) *Alnus nepalensis* D. Don. (b) *Schima wallichii* (DC.) Korth. (Source: Sujan Balami)

Schima wallichii (DC.) Korth. (Theaceae) is an evergreen, medium to large tree growing up to 25 m in height (Fig. 5b). It is a mostly subtropical tree but can be found in the lower montane forests of Nepal. This tree can grow

in a wide range of habitats and on many types of soil but is most common in disturbed and secondary forests. *S. wallichii* is also an economically and ecologically important tree in Nepal. It is useful for reforestation and water conservation in catchment areas, and for erosion control. It is also used as top-quality firewood, medium-quality timber, and in agroforestry.

7. Fungi of Nepal

Fungal diversity in Nepal is less explored as compared to flora and fauna, and most of the exploration is done for macrofungal species only (Adhikari 2015). Just like vegetation, the mycobiota in Nepal represents a connecting link between the mycobiotas of the Sino-Japanese, Western and Central Asian, Northeast American, partially North African, and mainly Indian subcontinent (reviewed by Adhikari 2016). The eastern areas possess some of the typical mycobiota of Southeast Asia, while the remainder has elements similar to those found in the north-western Himalayas.

The latest data on the fungal diversity of Nepal shows that 2,467 species of fungi are found in Nepal. If the plant/fungi ratio (i.e. 1:6) (Hawksworth and Lücking 2017) is considered, then the currently known plant diversity (6,653 species) (Kunwar et al. 2010) would estimate fungal diversity in Nepal at approximately 38 thousand species. This means that only ~6% of fungi are currently known in Nepal. Regarding macrofungi, a review study by Devkota and Aryal (2020) reported that 1,291 species belonging to 375 genera and 108 families are found in Nepal. However, Christensen et al. (2008) estimated that about 3000 macrofungal species could be present in Nepal based on comparison with Europe and North America. Devkota and Aryal (2020) also reported that 131 species of macrofungi are endemic to Nepal. However, studies on the Nepalese mycobiota are very few due to several limitations such as the low number of mycologists, the lack of mycological trainings, literature, funding, etc. Furthermore, most of this limited number of studies are concentrated in central Nepal only (Adhikari 2015). Currently, Nepal's National Herbarium (KATH) houses only 3500 specimens of macrofungi, which also reveals a poor description of the fungal diversity in Nepal. Due to these circumstances, taxonomic,

ecological and ethnomycological studies in Nepal are inadequate. Many macrofungal species in Nepal are undescribed. Moreover, due to misidentification, the incidence of mushroom poisoning is high in rural areas of Nepal.

Focusing on mycorrhizal fungi, which are studied in this work in more detail, 50 species of both ECM genus *Russula* and *Amanita* and 22 species of *Lactarius* have been recorded from central Nepal. Interestingly, only nine species of the most species-rich genus *Cortinarius* have been recorded in Nepal, six of them from central Nepal (Adhikari 2015). Also other ECM fungi such as six species of *Suillus* and 11 species of *Boletus* have been recorded in central Nepal. Compared to ECM fruitbodies, AM fungi are explored less in central Nepal. Only a few studies, e.g. Baral et al. (2021) and Vaidya et al. (2007), have confirmed the occurrence of AM genera such as *Ambispora*, *Acaulospora*, *Pacispora*, *Gigaspora*, *Claroideoglomerus*, *Glomus*, *Paraglomus*, *Scutellospora* and *Piriformospora*.

8. Thesis framework and aims

The presented study includes four different chapters. **Chapter I** provides a review of global results of studies on the impact of land use change on soil fungi. **Chapters II** and **III** are based on an analysis of soil and root-associated fungi, respectively, on abandoned agricultural land in temperate region of central Nepal. Together, five different types of land use were selected, namely agricultural land (AG), short- and long-term abandoned agricultural land (SA, LA) and short- and long-term regenerated forests (SR, LR). **Chapter IV** is solely taxonomic, describing three new ectomycorrhizal fungal species found in permanent plots.

The aim of **Chapter I** is to summarise the present knowledge of the impact of land use change on soil fungi, to highlight the limitations of various methodological approaches, and to describe current knowledge gaps. The objectives of **Chapter II** are to evaluate change in soil fungal communities including AM fungi in response to the spontaneous vegetation succession

of abandoned agricultural land, and to find out if there is any indication of a delay or constraint in the succession of soil fungal communities. **Chapter III** aims to reveal more detailed relationships at the plant-fungus species level and to evaluate the variation in root-associated fungal communities of two selected tree species, *Alnus nepalensis* and *Schima wallichii*, accompanying succession in response to abandonment of agricultural land. **Chapter IV** aims to contribute to the knowledge of the diversity of fungal species on abandoned land by describing three new ectomycorrhizal fungal species associated with *Alnus nepalensis* based on morphological characteristics and phylogenetic analysis.

Chapter I

Soil fungal communities across land use types

Sujan Balami, Martina Vašutová, Douglas Godbold, Petr Kotas,

Pavel Cudlín

Manuscript published as: Balami S, Vašutová M, Godbold D, Kotas P, Cudlín P (2020) Soil fungal communities across land use types. IFOREST 13:548–558.

Abstract

Land use change is one of the major causes of biodiversity loss, mostly due to habitat change and fragmentation. Belowground fungal diversity is very important in terrestrial ecosystems, however, the effect of land use change on soil fungal community is poorly understood. In this review, a total of 190 studies worldwide were analyzed. To monitor the effect of land use change, different fungal parameters such as richness, diversity, community composition, root colonization by arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi, spore density, ergosterol, and phospholipid fatty acid (PLFA) content and AM fungal glomalin related soil protein (GRSP) were studied. In general, results from analyzed studies often showed a negative response of fungal quantitative parameters after land use change from less-intensive site management to intensive site management. Land use change mostly showed significant shifts in fungal community composition. Considering land use change types, only 18 out of 91 land use change types were included in more than 10 studies, conversion of primary and secondary forest to various, more intensive land use was most often represented. All these 18 types of land use change influenced fungal community composition, however, the effects on quantitative parameters were mostly inconsistent. Current knowledge is not sufficient to conclude general land use impacts on soil fungi as the reviewed studies are fragmented and limited by the local context of land use change. Unification of the methodology, detailed descriptions of environmental factors, more reference sequences in public databases, and especially data on ecology and quantitative parameters of key fungal species would significantly improve the understanding of this issue.

Keywords: Soil Fungi, Land Use Change, Fungal Diversity, Species Composition, Mycorrhizal Fungi

1. Introduction

Soil fungi represent one of the most diverse assemblages of organisms on the Earth. They inhabit a wide range of ecological niches and play an essential role in ecological and biogeochemical processes (Dix & Webster 2012). Taxonomically, they are grouped mainly within the nine phyla – Opisthosporidia, Chytridiomycota, Neocallimastigomycota, Blastocladiomycota, Zoopagomycota, Mucoromycota, Glomeromycota, Basidiomycota and Ascomycota (Naranjo-Ortiz & Gabaldón 2019) and categorized into four ecological guilds –endophytes, mycorrhizal fungi, saprotrophs, and pathogens (Zanne et al. 2020). Endophytic fungi inhabit living plants and do not cause disease. Mycorrhizal fungi are symbionts that assist plants in nutrient and water uptake from soil and coping with stress conditions (Smith & Read 2008). Most terrestrial plants live in symbiosis with arbuscular mycorrhizal (AM) fungi, phylogenetically uniform groups within Glomeromycota and characterized by a low host specificity. Ectomycorrhizal (ECM) fungi are highly host specific symbionts of only 4.5% of terrestrial plants (mainly trees, often dominated in boreal and temperate forests) and evolved several times within Basidiomycota and Ascomycota (Brundrett 2009). For this reason, there is no simple marker that would distinguish them from other trophic groups without identification to the genus. In contrast, pathotrophs cause diseases to plants and other biota (Agrios 2005). The saprotrophs serve as primary degraders of organic matter in the soil (Setala & McLean 2004). Thus, being a component of soil microbiota, soil fungi are sensitive to any kind of alteration in the soil environment and any associated changes in the aboveground environment, such as human-mediated land use change.

The term “land use” can be defined as human employment of land cover type (biophysical state of the earth’s surface and immediate subsurface, e.g., forest, grassland, cropland, etc.) for certain purpose or function (Briassoulis 2019). It is often difficult to define the types of land use because it varies with land cover and the anthropogenic activities on it. In a broader sense, the changes in land use types from one state (e.g., primary forest, pasture) to other states (e.g., secondary forest, secondary grassland,

cropland, etc.) can be defined as land use change. A particular land use type can be either intensive or extensive based on the degree of anthropogenic activities.

In general, land use change is a function of changes in vegetation structure, soil properties, and anthropogenic activities which are known to influence the soil fungal communities (Sepp et al. 2018). Change in aboveground plant diversity, coverage, and community composition can influence soil fungi by regulating microclimatic conditions, nutrient addition to soil or exudation of allelochemicals, presence/absence of host plants for mycorrhizal fungi, etc. (Sene et al. 2012, Voriskova et al. 2016, Bainard et al. 2017). Moreover, soil factors (soil porosity, soil pH, carbon, nitrogen, phosphorus content, etc.) can significantly shape soil fungal communities (Oliveira et al. 2016, Wang et al. 2017). Likewise, anthropogenic activities like tillage, logging/mowing, fertilization, etc. have pronounced effects on soil fungal communities (Tomao et al. 2020).

Although many studies have shown the land use change impact on fungal parameters (Oehl et al. 2003, 2010, Peerawat et al. 2018), no review studies were published so far. For soil carbon, two meta-analyses were carried out on the effect of land use change on a global scale (Guo & Gifford 2002) and for tropical areas only (Don et al. 2011). These two studies illustrate that the greatest loss in soil carbon occurs after the conversion of primary sites to cropland but pointed out the occurrence of local constraints that limit the formulation of a broad conclusion. Accordingly, in the case of soil fungi also, published studies differ in several parameters (geographic region, habitat, methods, and target fungal group) such that it can be very difficult to reach a clear conclusion. As land use change is so widespread in a global context, we aimed (i) to summarize the present knowledge on land use change impact on soil fungi, (ii) highlight the limits of the various methodological approaches, and (iii) describe the current knowledge gaps to encourage further research on this topic.

2. Current studies

A total of 190 published original research articles worldwide were identified (April 2020) using keywords “Land use AND Fungi”, “Land use AND Microorganisms”, “Restoration AND Fungi”, “Restoration AND Microorganisms” “Secondary succession AND Fungi”, “Secondary succession AND Microorganisms”, “Succession AND Fungi”, “Succession AND Microorganisms” through Web of Science®, Google Scholar® and from the references of the selected papers (Tab. S1 in Supplementary material). These studies were selected based on the following criteria: (i) at least two land use types were compared (Tab. 1); (ii) at least one soil fungal parameter was assessed (Tab. 2). Studies dealing with only one land use type with different management (forest clearing, logging, or thinning, fertilization, crop rotation, tillage system, etc.) and chronosequence in single land use were not included. We focused this study on rural land-use change, thus publications dealing with urban and mining areas were excluded. In such land use types severe disruption of spontaneous soil development, heavy metals, and other chemicals can seriously affect the soil fungal community. For each publication, the land use type, soil fungal parameters, the fungal group (ECM/AM/total fungi) under study, the identification method used, and an overview of results were summarized. Also, the place of study, publication date, soil types, and climate information were tabulated (Tab. S1, Tab. S2, Tab. S3, Tab. S4 in Supplementary material). Most of the studies were carried out after 2008 (Fig. 1).

An increase in the number of studies in the last decade appears to be driven by an increasing concern of the effects of land use change, as well as by the use of next-generation sequencing methods (NGS) of environmental samples that has facilitated soil fungal research. A higher number of studies were carried out in Asia (73 studies) followed by Europe (49), South America (32), North America (17), Africa (12), Oceania (7). European and South American studies were distributed throughout the continent, whereas the Asian studies were mainly concentrated in the temperate region of China.

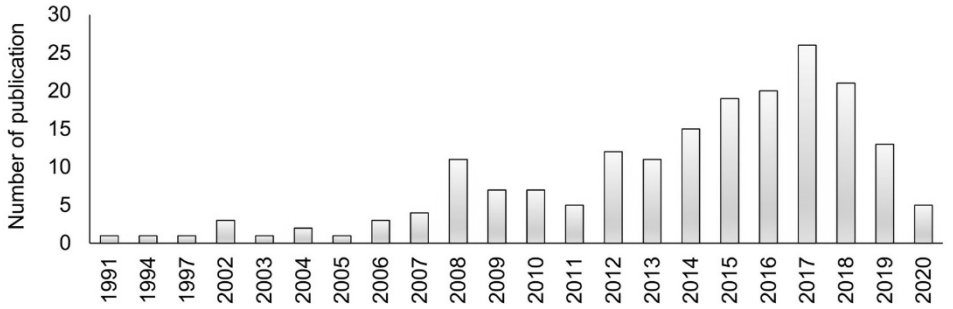

Fig. 1 - Number of publications based on years of appearance.

A higher proportion of studies compared natural and semi-natural habitats (primary/secondary forest, grassland, shrubland, and woodland/rangeland) with croplands, which represent a typical example of extensive land use and intensive land use types, respectively. In addition, extensive land use types resulting from agricultural land abandonment, abandonment of pasture, plantation in disturbed areas, or natural successional changes, are also commonly compared (33 studies). Tab. 1 shows the number of studies analyzed for various types of land use change. The majority of studies (27) compared secondary forest with cropland, 26 studies croplands with secondary grassland, 24 studies primary with secondary forests, and 23 studies primary forest with cropland.

In general, all fungal groups were assessed in studies after the introduction of the NGS methods (ca. 2008). Before this date, most studies were focused on AM fungi and considerably fewer studies dealt with ECM fungi (9) or saprotrophic fungi (micromycetes) assessed by cultivation methods (6 – Fig. 2). The low numbers of studies on ECM fungi could be due to the absence of ECM trees for comparative evaluation across the land use types. In the earlier studies identification of the fungi was based mostly on morphology.

Tab. 1 - Land use type change considered in the reviewed publications (The figure in each cell indicate the number of publications that considered corresponding land use change). If one paper dealt with several land use types, e.g., change of forest to pasture and then to cropland, score one was added to each land use change. Abbreviations of land use types: (PF) Primary forest; (SF) Secondary forest; (PL) Plantation forest; (PS) Primary shrubland; (SS) Secondary shrubland; (PG) Primary grassland; (SG) Secondary grassland; (WL/RL) Woodland or Rangeland; (PA) Pasture; (AL) Abandoned land; (AF) Agroforestry; (PC) Perennial cropland; (CR) Cropland; (Others) Bareland/Eroded land. Increase in land use intensity follows the order of: PF<SF<PL, PG<SG<CL, SG<PA<PC<CR, SF<AL<CR, etc.

Land use types	PF	SF	PL	PS	SS	PG	SG	WL/RL	PA	AL	AF	PC	CR
SF	24												
PL	17	15											
PS	0	1	0										
SS	5	6	2	1									
PG	0	2	2	1	1								
SG	11	20	6	1	7	5							
WL/RL	0	1	0	0	1	1	4						
PA	13	11	8	1	1	1	10	1					
AL	4	8	7	0	2	2	8	1	2				
AF	2	4	1	0	0	0	1	0	2	1			
PC	16	10	5	0	0	0	5	0	4	3	1		
CR	23	27	15	1	6	13	26	5	18	19	3	10	
Others	2	2	2	0	0	0	0	0	0	0	1	0	4

Tab. 2 - Effect of land use change (intensification) on different fungal parameters. The figure in each cell indicates the total number of papers dealing with fungal parameter. Publication list is given in Tab. S1 (Supplementary material).

Fungal parameter	No difference	Decrease	Increase	Varying	Change in community
1. Species richness and diversity					
a) species / OTUs richness	10	28	12	29	
b) diversity	14	23	9	20	
2. Community structure					
a) Higher taxa/guilds abundance	4	5		37	
b) Species/OTUs composition	4				89
3. Root colonization	3	14	4	8	
4. Spore density	4	17	10	11	
5. Fungal biomass					
a) Phospholipid fatty acid content	3	17	4	17	
b) Soil ergosterol content		3			
c) ITS copy number/ Gene copy number	1	8	1	1	
5. Glomalin related soil protein	1	7		4	
6. Others					
a)% hyphal length	1	3		1	
b) Colony forming unit	1	1	1	2	

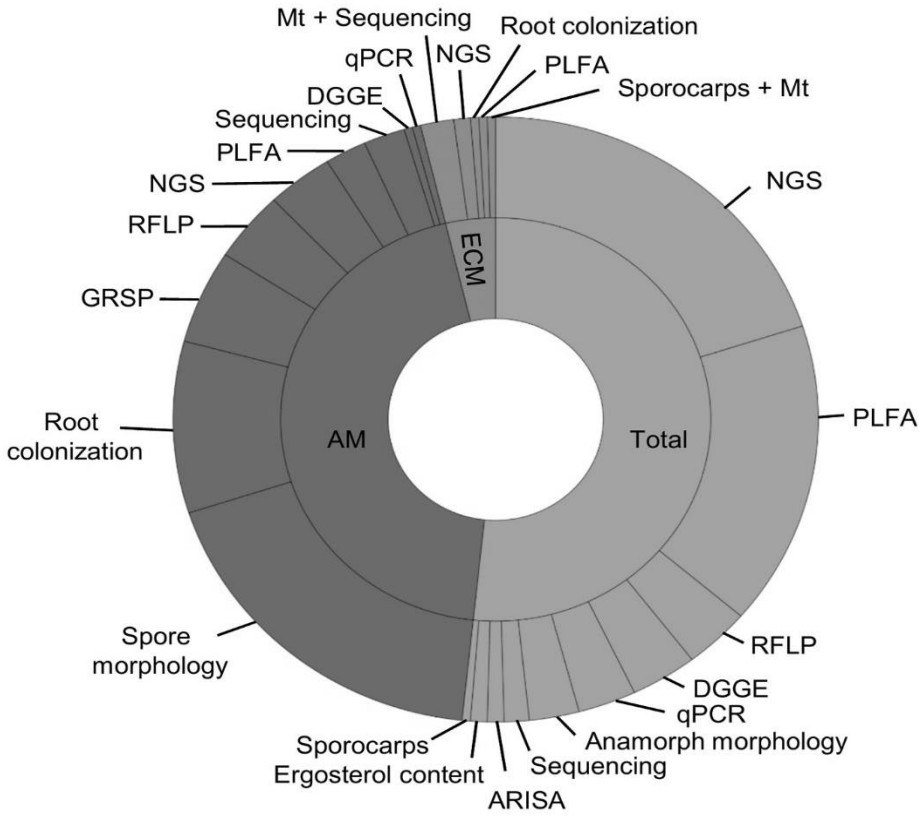

Fig. 2 - Proportion of published papers dealing with monitoring the impact of land use change according to the fungal group studied (AM: arbuscular mycorrhizal fungi; ECM: ectomycorrhizal fungi; and total fungi) and the different methods used to measure fungal parameters. If particular paper deals with more than one group of fungi (total, AM, or ECM fungi), each group is scored with 1. (PLFA): phospholipid fatty acid analysis; (RFLP): restriction fragment length polymorphism; (DGGE): denaturation gradient gel electrophoresis; (Mt): morphotyping; (ARISA): automated ribosomal intergenic spacer analysis; (GRSP): glomalin related soil protein); (qPCR): quantitative PCR; (Sequencing): Sanger sequencing; (NGS): next generation sequencing.

3. Land use change impact on soil fungi

Following a land use change, different fungal parameters showed a negative response to land use intensification (e.g., primary forest <

secondary forest < plantation forest < cropland). However, positive response or varying/no-difference in observed fungal parameters were also found. The clear result was found in the case of fungal community composition. Eighty-nine out of 93 studies focusing on changes in community composition showed a significant change in fungal community composition (Tab. 2). The majority of the studies also showed a decrease in quantitative fungal parameters following land use intensification. In the following subsections, some representative results have been described briefly.

3.1.Species richness and diversity

Studies on the effects of land use change on soil fungal richness and diversity (measured using different indices) used direct analysis of soils, or used soils incubated in a greenhouse with trap plants, such as *Sorghum* sp., *Plantago* sp., *Allium* sp. (Oehl et al. 2003). In early studies, identification of species to quantify species richness and diversity was based on morphological characteristics, such as spore morphology of AM fungi (Bedini et al. 2007), ECM morphotypes, sporulation of anamorphs (saprotrophs – McLean & Huhta 2002), and sporocarp morphology (Tedersoo et al. 2006). Along with these methods, molecular fingerprint methods, such as denaturing gradient gel electrophoresis (DGGE – Dong et al. 2008), restriction fragment length polymorphisms (RFLPs – Kasel et al. 2008), and automated ribosomal intergenic spacer analysis (ARISA – Okubo & Sugiyama 2009) were also used. Later, next-generation sequencing methods (454 pyrosequencing, Illumina sequencing, and Ion Torrent platform) have been introduced (Taylor et al. 2017). For DNA based analysis, the SSU (Moora et al. 2014), LSU (Voriskova et al. 2016) or ITS region (Wakelin et al. 2016) were usually amplified using appropriate primers. The estimates were then expressed in terms of actual species number or operational taxonomic unit (OTUs).

Generally, soil fungal richness decreased with the conversion of primary forest/ grassland/shrubland to the more intensive land use sites, like secondary forest, plantation forest, perennial cropland, cropland

(monoculture or crop rotation system – Oehl et al. 2003, Sene et al. 2012). For instance, during the conversion of forested areas to cropland, or to perennial cropland and fallow land (Tchabi et al. 2008), a reduction in the AM fungal richness was found. Likewise, when analyzing ECM fungal richness in roots of poplar plants in different land use (former cropland, former grassland, and former spruce stand), lower ECM fungal richness was found in former cropland sites compared to that found in the former spruce forest or former grassland (Gherghel et al. 2014). Although a decrease in species/OTUs richness values were common after increasing land use intensity, some contrasting results were also observed. The richness value either increases with increasing land use intensity (10 studies) or there is no clear direction of change (29 studies – Tab. 2). For example, higher AM fungal species richness was found in the secondary forest compared to the primary forest in the Amazon (Stürmer &Siqueira 2011). Higher observed OTUs richness was found in Jungle Rubber than in the rain forest in Sumatra, Indonesia (Brinkmann et al. 2019). While comparing perennial cropland (vineyard) to the adjacent sclerophyllous forest in the Mediterranean, there was no significant difference in total fungal species richness (Castaneda et al. 2015).

Similar to the total species richness estimates, diversity estimates based on the Shannon index of AM fungi (Honnay et al. 2017), ECM fungi and total fungi were found to decrease significantly with land use intensification (Gherghel et al. 2014). The comparison of perennial cropland sites with the secondary forest and fallow land showed a lower value of the Shannon diversity index of AM fungi for cropland (Snoeck et al. 2010). Likewise, higher OTUs diversity of AM fungi was observed by comparing grassland sites with agricultural sites (Manoharan et al. 2017). Lumini et al. (2010) found that the sites with low inputs (pasture and covered vineyard) showed a higher AM fungal diversity than those subjected to human inputs (managed meadow and tilled vineyard). But there were some equivocal results also. For instance, the conversion to intensive land use sites (primary forest to secondary forest) did not necessarily lead to the decrease in species richness, and/or a change in the diversity index of soil fungi

(Kerfahi et al. 2016). Comparable diversity was reported while comparing the soybean field with plantation forests in temperate China (Sui et al. 2019). A higher Shannon diversity index of AM fungi was found in cropland sites compared to Atlantic forest sites (Pereira et al. 2014). Conversion of the pristine forest into other land uses (in tropical humid Amazon) did not reduce AM fungal diversity (Stürmer & Siqueira 2011). Similarly, McGuire et al. (2015) did not found significant differences in total fungal diversity between primary forest, regenerating forest, and oil palm plantation.

The maintenance of species richness may be due to the increase in plant diversity, density/coverage, as well as abiotic factors among land uses that promote diversity even in intensive land use systems. A pattern of increased AM fungal richness in cropland, agroforestry, and secondary forest sites compared to mature tropical forest sites has been explained by an increasing number of plant species that harbor different AM fungi growing in these land use types (Stürmer & Siqueira 2011). It has been observed that some mature tropical forests with climax plant communities are associated with the low richness of AM fungi (Siqueira et al. 1998). However, unaltered richness and diversity estimates do not mean that species composition has not changed. Using species richness as a parameter, replacement of species can result in a similar level of diversity and is indistinguishable from no impacts of land use change (Ying et al. 2013).

3.2.Community structure

Modification of soil fungal species/OTU composition (estimated by the same methods mentioned in the previous chapter) after land use change is quite common and most obvious while comparing forest sites with cropland sites. As expected, various native forest types showed different fungal species composition compared to secondary forest (Mueller et al. 2016), plantation forest (Kasel et al. 2008, Moora et al. 2014, Brinkmann et al. 2019), pasture (Wang et al. 2014), grassland (Yurkov et al. 2012), and perennial cropland (Li et al. 2013). After the conversion of rain forest

sites to rubber plantation, Kerfahi et al. (2016) observed changes from a Basidiomycota dominated to an Ascomycota dominated community. However, a study from China's Loess Plateau showed increasing abundance of Ascomycota and decreasing abundance of Basidiomycota while comparing cropland to plantation (Yang et al. 2017). During the conversion of primary forest to oil palm monoculture (in lowland evergreen rain forest of peninsular Malaysia dominated by Dipterocarpaceae), representatives of Archaeorhizomycetales, Hypocreales, and Saccharomycetales were found more abundant, whereas Cantharellales and Mortierellales dominated the primary forest sites. For ECM genera *Amanita* and *Lactarius* were more abundant in the regenerating forest, whereas *Craterellus* and *Russula* were more abundant in the primary forest (McGuire et al. 2015). Replacement of grassland with willow and poplar plantation leads to the enrichment of putative pathogenic and ECM fungi associated with trees (Xue et al. 2016). Significant differences in fungal communities were found in different land use types (bog, rough grazing site, and forest plantation sites) when investigating ericoid mycorrhizal diversity in two different ericaceous plant species, *Vaccinium macrocarpon* and *Calluna vulgaris* (Hazard et al. 2014).

Comparing AM composition of forests, croplands, and grasslands in the southern part of Tibetan Plateau, Glomeraceae were less abundant in forests, Gigasporacae in grasslands, and Acaulosporaceae in croplands than in other land use types (Xu et al. 2017). In agricultural systems, the occurrence of *Acaulospora denticulata*, *Glomus ambisporum*, and *Claroideoglomus etunicatum* was significantly affected by cropping and non-cropping systems in Taita Hills, Kenya (Jefwa et al. 2012). A study in Terceira Island (Azores, Atlantic Ocean) showed that seminatural pastures were dominated by species of *Acaulospora* and *Scutellospo*ra, particularly *S. calospora* and *A. cf. myriocarpa*. Whereas, for intensively farmed pastures, species with glomoid spores, and members of the two genera *Claroideoglomus* and *Paraglomus* were found most frequent and abundant (Melo et al. 2014).

3.3.Root colonization by AM and ECM fungi

Mycorrhizas on roots have also been quantified to evaluate the land use change impacts on AM and ECM communities. In ECM fungi, the percentage of root colonization (proportion of mycorrhizal and non-mycorrhizal root tips) and for AM fungi the percentage of arbuscules, vesicles, and hyphae were quantified. For both types of mycorrhizas, the extent of colonization showed variation with land use change and could be explained by alteration of soil properties after land use change (Muthukumar et al. 2003).

The percentage of colonization by ECM fungi of *Salix* sp. was found to be higher in the natural forest compared to short rotation coppice (Hrynkiewicz et al. 2012). The difference was explained by soil water content, an initial inoculum of ECM fungi, host genotype, and soil pH. In a study of edaphic effects on symbiosis during soil development, mycorrhizal colonization shifted from AM fungi to ECM fungi (the plants had both types of mycorrhizal association) in response to decreasing phosphorus content in the soil. Such a shift in mycorrhizal association enables the acquisition of organic phosphorus via extracellular phosphatase activity (Albornoz et al. 2016).

The effect of increasing intensity of land use on AM fungal colonization is less clear than that for ECM fungi, being this possibly due to lower host specificity of AM fungi (Guadarrama et al. 2008). In the investigation of Islas et al. (2016), no relationship between the degree of land use intensification (pristine soil to agricultural soil) and root colonization of AM fungi was observed. On the other hand, the conversion of natural forested areas to grassland/pasture increased AM fungal colonization (Palta et al. 2016). Analysis of AM colonization in experimental plants established in degraded forest and in planted forest sites, showed lower root colonization in planted forest sites than in degraded forest (Sene et al. 2012). This difference was explained by the increased canopy of the planted forest reducing light and temperature at the forest floor, and thus plant density and hence lower root colonization.

Such increases or decreases in root colonization by ECM and AM fungi after land use intensification can be explained also with soil nutrient availability and perturbation in soil (Palta et al. 2016). Often the increase in soil nutrient availability has a negative impact on mycorrhizal colonization (Treseder 2004). Perturbation of the soil (e.g., soil compaction) reduces the porosity of soil, thereby reducing passages for organic nutrients and decreasing fungal mycelium ramification (Drew et al. 2003). On the other hand, the tillage in agricultural land breaks the extraradical mycelium into smaller units, and thus it increases the infectivity of fungal mycelium (Boddington & Dodd 2000).

3.4.Spore density

AM fungi produce thick-walled spores as their propagules, and morphological variations in such spores were traditionally employed for genus identification (Schenck & Perez 1990). Quantifying spore number per volume of soil by direct microscopic counting (Daniels & Skipper 1982) and expressing this count as a spore density/abundance, was used to observe land use effects.

Undisturbed areas, like natural grasslands and rangelands, were reported to harbour higher spore density in comparison to fallow land, pasture, plantation sites, and cropland (Jefwa et al. 2012, Li et al. 2013). The effect of land use intensification on spore density was observed also at the species level (Oehl et al. 2010). For instance, in a comparison of natural sites with pasture, the spore density of *Glomus* sp. was higher in natural sites while *Acaulospora* sp. was higher in pasture sites (Oliveira et al. 2016). Alternatively, spore density was also expressed as infective propagule numbers (the number of spores that can infect the test host plant, determined by the Most Probable Number technique). The total number of infective propagules was found to be significantly higher in pasture compared to traditionally managed cropland (Sousa et al. 2013). However, other studies reported comparable spore density of AM fungi in forested sites and fallow sites (Tchabi et al. 2008). They reported that apart from the overall land use impacts, soil organic carbon and pH levels affect the

sporulation of AM fungi. While comparing short fallow land with the perennial cropland and secondary forest, significantly higher spore density was observed in short fallow and found to be related with C:N ratio in soil (Snoeck et al. 2010). Also, the impact of vegetation has been highlighted. It is known that grass land species have a more abundant fine root system than trees in a mature forest, and this correlates positively with AM fungal colonization and sporulation (Muleta et al. 2008).

3.5.Fungal biomass

Quantification of fungal biomass using lipid components of fungi, namely soil ergosterol content and phospholipid fatty acids (PLFA), including neutral fatty acids (NLFA), have also been employed to monitor land use impacts on soil fungi. The unsaturated fatty acid 18:2ω-6,9c was used as a marker of fungal biomass in the community profile (Olsson 1999). For AM fungi, 16:1ω-5c (Sousa et al. 2013), and for ECM and saprotrophic fungi, 18:2ω-6,9c and 18:1ω-9c (Balser et al. 2005) were also applied. Additionally, estimates of ITS copy number (a proxy estimate of fungal biomass) from quantitative PCR were also used to show land use change effects on fungi (Grantina et al. 2011).

In a comparison of farmland and forest sites, soil ergosterol contents were found to follow the order of the intensity of land use, i.e., intensively cropland < pasture < forest soils (Turgay & Nonaka 2002). The plantation in mangrove forest sites with coconut, rubber, etc. was also found to reduce the soil ergosterol content by up to 90% (Dinesh & Chaudhuri 2013).

The decreasing value of fungal PLFA (18:2ω-6,9c) content with increasing land use intensity was reported by Hamer et al. (2008). Similarly, higher fungal PLFA content was found in forest sites compared to perennial cropland (Dong et al. 2008). The content of PLFAs of AM fungi was lower in forest soils compared to abandoned and heathland soils, whereas the PLFAs of saprophytic and ECM fungi were more abundant in ancient forest soils (Olsson 1999). Such trends shown by AM and ECM fungi plus saprophytic fungi have been explained by soil nutrient conditions and differences in host plants. Higher amounts and structural complexity of

carbon sources in forests form a bigger niche availability for ECM and saprophytic fungi, whereas higher concentrations of N and P in the former arable site support the AM fungi. However, some contrasting results have also been observed. With some exceptions, higher fungal biomass measured with the PLFA (18:2ω-6,9c) was observed in perennial agricultural soil compared to coastal grassland (Drenovsky et al. 2010). Similarly, PLFA content (18:2ω-6,9c) was found to be increased in the transition from forest to pasture to an abandoned field, due to the availability of nutrients as grass litter decomposed faster compared to the forest (Potthast et al. 2012). However, a study of the conversion of native grassland to pasture showed no significant difference in fungal PLFAs (Wong et al. 2015). It was suggested that no significant differences in soil properties (e.g., available P, soil pH, and vegetation cover) maintained an equal amount of fungal PLFAs in native grassland and pasture. Wang et al. (2017) also found no differences in fungal PLFA content with the conversion of natural forest to rubber plantations, but the AM fungi PLFA marker (16:1ω-5c) was found to be higher in the natural forest than in the rubber plantation. It has been explained that soil acidification and higher phosphorus content reduced AM fungal biomass in rubber plantation sites.

Using a qPCR approach, no difference in the log number of copies of gene/gm of soil was found in abandoned land compared with plantation forest, but its values were significantly lower than mixed forest (Li et al. 2013). Similarly, no significant change in the log number of copies of gene/gm of soil between secondary grassland, pine plantation, and cropland was found (Ying et al. 2013). A study by Grantina et al. (2011), showed the order of log number of copies/gm of soil as former forest > agriculture > pasture > agricultural land.

3.6.Glomalin related soil protein (GRSP) of AM fungi

Glomalin is a proteinaceous substance produced by AM fungi which is released to the soil during hyphal turnover and after the death of the fungus (Driver et al. 2005). Different fractions of this protein, namely immunoreactive soil protein (IRSP) and easily extractable immunoreactive

soil protein (EE-IRSP) were quantified in soil to assess land use change impacts (Rillig 2004).

In the soil of grasslands, total glomalin related soil protein (GRSP) content was higher compared to a plantation site and was even lower in a maize monoculture (Bedini et al. 2007). Similarly, native mixed deciduous forest (*Acer*, *Alnus*, *Juglans*, *Carya*, etc.) contained a higher concentration of GRSP compared to agricultural and afforested sites (Sousa et al. 2013). Total GRSP was lower in cropland and fallow land when compared to forest soils covered by the AM-forming timber species *Combretodendron macrocarpum*, *Piptadeniastrum africanum* and *Disternonanthus benthamianus* (Fokom et al. 2012). Likewise, glomalin concentration was found lower in cropland than the native forest of temperate Himalaya (Nautiyal et al. 2019). Here, it has been explained that an increase in the root network of trees in the forest leads to an increase in AMF colonization which contributed to higher glomalin concentration. Prolonged arable land use of former grass land soils reduced the content of GRSP further (Preger et al. 2007). However, some studies showed no changes in GRSP content after land use change. For example, when poplar plantation was compared with cropland (maize field) no significant change in GRSP content was found (Wang et al. 2015).

4. Summary of the individual land use change impacts on soil fungal parameters

Generally, conclusions on the impact of land use change on soil fungi are limited by the number of replications. However, 18 types of land use change were considered in ≥10 replicated studies (Tab. 1). Thus, to extract generalized information needed for stakeholders, a summary of these replicated studies was made. Fig. 3 shows the overall impacts of these 18 types of land use change on qualitative (species composition) and quantitative fungal parameters (richness, diversity, root colonization, spore density of AM fungi, and fungal biomass – Tab. S5 and Tab. S6 in Supplementary material). If we set ≥50% of the results as a boundary of increase/decrease or change, all 18 land use types showed changes in the

community composition of all fungal groups (Tab. S5, Tab. S6). When comparing primary forest to secondary forest, secondary grassland, pasture, and cropland, no fungal quantitative parameters were changed. AM fungal quantitative parameters decreased when the primary forest was changed to plantation forest, perennial cropland, or cropland. Surprisingly, total fungal quantitative parameters increased when the primary forest was changed for plantation forests. Brinkmann et al. (2019) explained this pattern based on the intermediate disturbance hypothesis, which proposed that the highest diversities will occur in ecological systems with moderate disturbances.

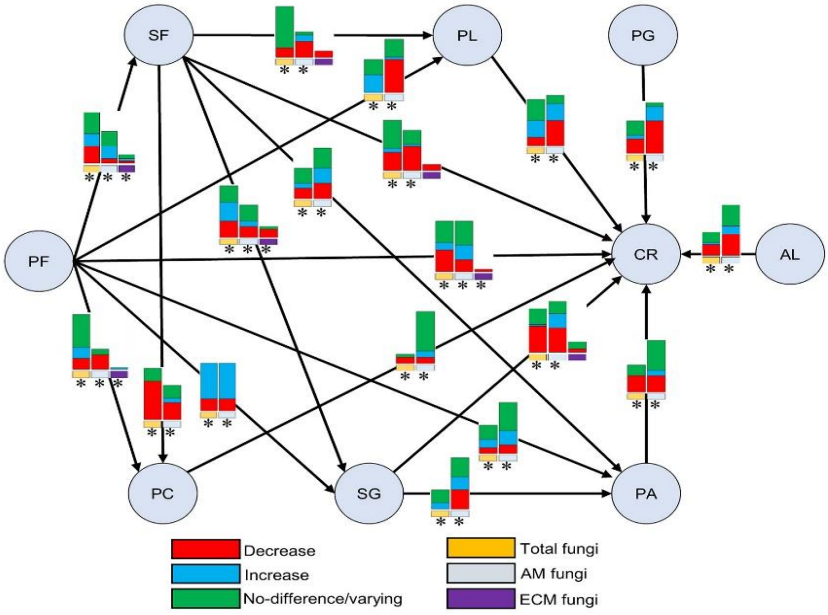

Fig. 3 - Summary of the individual land use change impacts on quantitative parameters and species composition of total, AM, and ECM fungi. (Bar graph represents the number of studies showing various impacts positive/negative/varying or no impacts on quantitative parameters and asterisk "*" sign represents the change in species composition >50% of the studies). (PF): Primary forest; (SF): Secondary forest; (PL): Plantation forest; (PG): Primary grassland; (SG): Secondary grassland; (PA): Pasture; (AL): Abandoned land; (PC): Perennial cropland; (CR): Cropland.

There are no clear changes in fungal parameters when the secondary forest is compared with secondary grassland and pasture. But converting of secondary forest to plantation forest, or perennial cropland or cropland, showed decreases in AM fungal parameters. There are also negative impacts on total fungal parameters following changes of secondary forest to perennial cropland. Only AM fungal parameters decreased when the plantation forest is changed to cropland.

These results demonstrate that it is very difficult to evaluate the response of soil fungal communities based on quantitative parameters. The fact that AM fungi provide a clearer response is probably due to their similar life strategy and low specificity. In contrast, total fungi contain many groups with so many different life strategies that their responses overlap, and the response is very much context-dependent.

5. Limitations of methodological approaches

The major limitation in evaluating the effect of the land use change on soil fungal communities is a poor consensus in land use type categorization. For instance, some studies defined land use types through the climatic zone (Thomson et al. 2015), some by plant cover type (Sousa et al. 2013), and some considered parent bedrock composition in single land use types (Oehl et al. 2003). In addition, some studies poorly described the vegetation, soil types, and climate of their experimental plots (Makiola et al. 2019). So, the effect of land use change cannot be correctly interpreted, because these factors could surpass land use change impact.

The other difficulty in the interpretation of land use change effects is a sampling design. As the experimental plots are distributed at a landscape level, homogeneous replicate plots selection is often difficult. However, there is also the limitation of selecting all studied land use types at one locality. Consequently, plot replicates in a distant location could influence soil fungi due to changes in environmental conditions. For instance, in fragmented agricultural fields, the management of soil fertility may be different, which affects soil fungi. Similarly, during the abandonment of the agricultural field, less productive fields are abandoned earlier, such that

it can be presumed that the soil conditions are already different compared to previous land use. Therefore, as much information as possible should be gathered about land use history by interviewing local people.

Discrepancies in sampling is also another important factor. Some studies sampled soil along a transect with as small as 1 m^2 plot size (Bainard et al. 2017), whereas others sampled 1 ha plots with replicates of soil cores (Pereira et al. 2014). The preparation of composite soil samples from replicates of soil cores was a common method among studies. A few studies also considered fractions of different soil horizons (Rilling 2003). The preparation of composite soil samples may overlay the vertical differences, i.e. the land use change effect might be only in the topsoil layer, and deeper soil layer remains unaffected. In the case of predictor variables, many studies considered land use types as a typical predictor and soil factors as additional predictors. However, a comprehensive description of vegetation (as a next predictor) was missing in most studies (e.g. Zhang et al. 2017, Brinkmann et al. 2019).

In early studies, delimitation of species (evaluation of species richness and diversity) based on morphological characteristics such as AM fungal spore morphology (Bedini et al. 2007), ECM morphotypes, (McLean & Huhta 2002), and sporocarps morphology (Tedersoo et al. 2006) that relied on expert knowledge and could differ between studies. Molecular fingerprinting methods like denaturing gradient gel electrophoresis (DGGE) can overlook less abundant species. Introduction of NGS methods (454 pyrosequencing, Ion Torrent platform, Illumina sequencing) (Taylor et al. 2017) has solved the aforementioned issue. However, it has the limitation that appropriate primers for all fungal groups during PCR amplification must be used (Tedersoo et al. 2015) and several other technical issues (Zinger et al. 2019). The species-level identification could be also limited due to missing reference sequences in public databases. Moreover, knowledge of the ecological requirements of many taxa remains unknown (Nguyen et al. 2016) that arises difficulties in the interpretation of obtained data (Peay 2014). During root colonization assays, obtaining the identity of fine roots from particular plants could be difficult in a field-

based investigation. Although the use of trap plants solves this problem, trap plants may not reflect the exact field conditions. The age of plants and the proportion of fine roots could also affect root colonization by AM and ECM fungi. Also, studying spore density in soil could be limited in the sporulation capacity of a particular taxon. Sporulation of AM fungi could differ between AM species and could be influenced by differences in seasonal dynamics (Oehl et al. 2009). Assessment of fungal and AM biomass was also employed in several land use change studies. PLFA method has its advantages of being rapid and inexpensive, sensitive, and reproducible but the interpretation of PLFAs as indicative of different groups of organisms is not straightforward. The specificity of different fatty acid markers (e.g. 16:1ω-5c for AM fungi, 18:2ω-6,9c for ECM fungi and 18:1ω-9c for saprophytic fungi) has been questioned (Frostegård et al. 2011). In the case of AM fungi, GRSP is reputed to be a protein of AM fungi, but its presence in AM fungi only is unclear (Whiffen et al. 2007). Humic substances present in the soil could also interfere with the measurement of GRSP using colorimetric assays (Moragues-Saitua et al. 2019). Consequently, origin of the soil will influence the results. For instance, GRSP represents a good indicator of land use change effect in herb dominated ecosystems, being more concentrated in permanent than temporal systems. Its content in forests depends probably on mycorrhizal types of dominant trees (AM/ECM). As the amount of GRSP content in soil depends upon hyphal growth and spore production, individual species (that remains unaffected by land use change) may also have roles in determining the differences in concentrations of GRSP across sites (Rillig 2001). To obtain more robust and reliable data usage of several methods, i.e. NGS method accompanied by any morphological method (sporocarps, morphotypes, spores, root colonization) and biomass measurement (PLFA content, GRSP content), is highly recommended.

6. Conclusion and future perspectives

We found 190 relevant studies dealing with land use impact on soil fungi. The studies came mostly from Asia and Europe and were based mainly on next-generation sequencing, PFLA analysis, or spore morphology of AM

fungi. Around 80% of these studies were published in the last decade. Land use type classification in these studies was mainly dependent on the local context. The most studied land use change was a conversion from primary forest to cropland/secondary forests, cropland to plantation forest/secondary grassland/pasture. From 91 types of land use change, only 18 were included in more than 10 studies. Most of the results showed that species richness and diversity were negatively impacted by land use change, but the results are not unequivocal. This means land use change does not necessarily lead to the changes in the soil fungal diversity. Generally, land use change has greater impacts on community composition. Root colonization of AM and ECM decreases in intensive land use sites. For AM fungi, several studies showed decreases in spore density with increasing land use intensity. Soil ergosterol, PFLA, and glomalin contents were found to follow the order of the intensity of land use, however, also equivocal results were published.

Our study suggests that current knowledge is not sufficient to reach conclusions on the effect of single land use change on soil fungi. This is due to the low number of replicates, extreme variability of local conditions worldwide, different methodological approaches and their limitations, the high species richness of fungi, and different responses of various fungal guilds, dependent on plant cover and soil conditions. A proper description of land use history and environmental factors and a sufficient number of replicates is required for good comparisons. During soil sampling, it should be taken into account that larger plots are limited by sufficient replication and smaller plots by the representativeness of samples. Thus, widely accepted soil sampling strategies can be devised to have at least some uniformity of plot size, soil core size, and the number of replications, etc. Controlled long-term experiments can be one possible option on how to disentangle loss of diversity due to disturbance and management from natural changes caused by a change of associated host plants or soil properties (Heinemeyer et al. 2004). As NGS methods provide a new level of information in soil fungal research, it should be a basic method to assess community composition. However, it is often unclear whether DNA

sequences originate from hyphae, spores, or relic DNA (Carini et al. 2017). Thus, non-molecular methods, such as biomass estimation, provide an important cross-check and additional information. This is especially useful in studies of AM fungi because, due to redundancy caused by a high diversity and many life strategies, quantitative parameters of total fungi often do not change.

Although different parts of the world are covered to date, studies in tropical Asia, temperate areas of the Hindukush region, East Asia, Eurasia, and Africa are very few. Forest conversion to agricultural lands is increasing in tropics, Asia, and Africa (FAO 2016). Because these areas are also an important habitat for biodiversity protection (Yesson et al. 2007), more studies are needed to fill this gap. Apart from methodological issues, the main problem of the reviewed studies is in the interpretation of data obtained. In the case of quantitative parameters, there are no generally accepted average values for a particular land use type. Due to the above-mentioned variability, such values can be obtained only in a local context, with sufficient replicates.

A promising tool for species data interpretation is a trait approach that has been widely used in vegetation science (Violle et al. 2007). However, data on fungal ecology are limited to main guilds assessment only (Nguyen et al. 2016) without any details about biology and niche requirements of selected species. In a European context, some information could be assessed based on red lists, indicator species lists, or monographies, but in other parts of the world, these sources are mostly not yet available.

Moreover, many fungal species are undescribed for science, and if so, have no reference sequence in public databases (e.g., Dothideomycetes, Leotiomycetes). Data on the properties of higher taxa are very rough and often contrasting life strategies are present within one genus. Therefore, we strongly recommend building the database of key species characteristics to move our knowledge from description and comparison to functionality. It is crucial to obtain more data on the ecology of key species (Peay 2014) to develop ideas on the functional significance of

changes in species composition. This review reveals that soil fungal response to land use change needs further evaluation. Soil fungal community dynamics can be reflected as an indicator of land use change. This understanding will help in combating land change impacts on other biotic communities and assist land-management strategies.

Most of the results showed that species richness and diversity were negatively impacted by land use change, but the results are not unequivocal. This means land use change does not necessarily lead to the changes in the soil fungal diversity. Generally, land use change has greater impacts on community composition. Root colonization of AM and ECM decreases in intensive land use sites. For AM fungi, several studies showed decreases in spore density with increasing land use intensity. Soil ergosterol, PFLA, and glomalin contents were found to follow the order of the intensity of land use, however, also equivocal results were published.

Our study shows current knowledge is not sufficient to reach conclusions on the effect of single land use change on soil fungi. This is due to the low number of replicates, extreme variability of local conditions worldwide, different methodological approaches and their limitations, the high species richness of fungi, and different responses of various fungal guilds, dependent on plant cover and soil conditions.

A proper description of land use history and environmental factors and a sufficient number of replicates is required for good comparisons. During soil sampling, it should be taken in account that larger plots are limited by sufficient replication and smaller plots by the representativeness of samples. Thus, widely accepted soil sampling strategies can be devised to have at least some uniformity of plot size, soil core size, and the number of replications, etc. Controlled long-term experiments can be one possible option on how to disentangle loss of diversity due to disturbance and management from natural changes caused by a change of associated host plants or soil properties (Heinemeyer et al. 2004).

As NGS methods provide a new level of information in soil fungal research, it should be a basic method to assess community composition.

However, it is often unclear whether DNA sequences originate from hyphae, spores, or relic DNA (Carini et al. 2017). Thus, non-molecular methods, such as biomass estimation, provide an important cross-check and additional information. This is especially useful in studies of AM fungi because due to redundancy caused by a high diversity and many life strategies, quantitative parameters of total fungi often do not change.

Although different parts of the world are covered to date, studies in tropical Asia, temperate areas of the Hindukush region, East Asia, Eurasia, and Africa are very few. Forest conversion to agricultural lands is increasing in tropics, Asia, and Africa (FAO 2016). Because these areas are also an important habitat for biodiversity protection (Yesson et al. 2007), more studies are needed to fill this gap.

Apart from methodological issues, the main problem of the reviewed studies is in the interpretation of data obtained. In the case of quantitative parameters, there are no generally accepted average values for a particular land use type. Due to the above-mentioned variability, such values can be obtained only in a local context, with sufficient replicates.

A promising tool for species data interpretation is a trait approach that has been widely used in vegetation science (Violle et al. 2007). However, data on fungal ecology are limited to main guilds assessment only (Nguyen et al. 2016) without any details about biology and niche requirements of selected species. In a European context, some information could be assessed based on red lists, indicator species lists, or monographies, but in other parts of the world, these sources are mostly not yet available.

Moreover, many fungal species are undescribed for science, and if so, have no reference sequence in public databases (e.g., Dothideomycetes, Leotiomycetes). Data on the properties of higher taxa are very rough and often contrasting life strategies are present within one genus. Therefore, we strongly recommend building the database of key species characteristics to move our knowledge from description and comparison to functionality. It is crucial to obtain more data on the ecology of key

species (Peay 2014) to develop ideas on the functional significance of changes in species composition.

This review reveals that soil fungal response to land use change needs further evaluation. Soil fungal community dynamics can be reflected as an indicator of land use change. This understanding will help in combating land change impacts on other biotic communities and assist land-management strategies.

List of abbreviations

AM –Arbuscular Mycorrhiza, ARISA –Automated Ribosomal Intergenic Spacer Analysis, DGGE –Denaturation Gradient Gel Electrophoresis, ECM –Ectomycorrhiza, GRSP –Glomalin Related Soil Protein, ITS –Internal Transcribe Sequence, LSU –Large Subunit, NGS –Next Generation Sequencing, OTU –Operational Taxonomic Unit, PLFA –Phospholipid Fatty Acid Analysis, RFLPs –Random Amplified Fragment Length Polymorphism, SSU –Small Subunit

Acknowledgement: This work was supported by the Ministry of Education, Youth and Sports of CR within the CzeCOS program, grant number LM2018123. We thank all the authors who contributed to the original research article.

Supplementary materials

Tab. S1 - List of publications reviewed: land use types and sites.

Tab. S2 - List of publications reviewed: fungal guilds, fungal parameters and methods used.

Tab. S3 - List of publications reviewed: parameters analyzed.

Tab. S4 - List of publications reviewed: geographic and environmental variables.

Tab. S5 - Impact of particular land use change on soil fungi.

Tab. S6 - Summary of Tab. S5.

Link: https://iforest.sisef.org/pdf/?id=ifor3231-013&sm=1&ext=pdf

References

Agrios GN (2005). Plant pathology (5th edn). Academic Press, London, UK, pp. 358-611.

Albornoz FE, Lambers H, Turner BL, Teste FP, Laliberté E (2016). Shifts in symbiotic associations in plants capable of forming multiple root symbioses across a long-term soil chronosequence. Ecology and Evolution 6: 2368-2377. -doi: 10.1002/ece3.2000

Bainard LD, Chagnon PL, Cade-Menun BJ, Lamb EG, LaForge K, Schellenberg M, Hamel C (2017). Plant communities and soil properties mediate agricultural land use impacts on arbuscular mycorrhizal fungi in the Mixed Prairie ecoregion of the North American Great Plains. Agriculture, Ecosystems and Environment 249: 187-195. -doi: 10.1016/j.agee.2017.08.010

Balser TC, Treseder KK, Ekenler M (2005). Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biology and Biochemistry 37: 601-604. -doi: 10.1016/j.soilbio.2004.08.019

Bedini S, Avio L, Argese E, Giovannetti M (2007). Effects of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein. Agriculture, Ecosystems and Environment 120: 463-466. -doi: 10.1016/j.agee.2006.09.010

Boddington CL, Dodd JC (2000). The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. I. Field studies in an Indonesian ultisol. Plant and Soil 218: 137-144. -doi: 10.1023/A:1014966801446

Briassoulis H (2019). Analysis of land use change: theoretical and modeling approaches. Web Book of Regional Science 3, Regional Research Institute, West Virginia University, Morgantown, WV, USA, pp 11-12. Brinkmann N, Schneider D, Sahner J, Ballauff J, Edy N, Barus H, Irawan B, Budi SW, Qaim M, Daniel J, Polle A (2019). Intensive tropical land use massively shifts soil fungal communities. Scientific Reports 9 (1): 16732. -doi: 10.1038/s41598-019-39829-4

Brundrett MC (2009). Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil 320: 37-77. -doi: 10.1007/s11104-008-9877-9

Carini P, Marsden PJ, Leff JW, Morgan EE, Strickland MS, Fierer N (2017). Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nature Microbiology 2 (3): 37. -doi: 10.1038/nmicrobiol.2016.242

Castaneda LE, Godoy K, Manzano M, Marquet PA, Barbosa O (2015). Comparison of soil microbial communities inhabiting vineyards and native sclerophyllous forests in central Chile. Ecology and Evolution 5: 3857-3868. -doi: 10.1002/ece3.1652

Daniels BA, Skipper HD (1982). Methods for the recovery and quantitative estimation of propagules from soil. In: “Methods and Principles of Mycological Research”

(Schenck NC). The American Phytopathological Society, St. Paul, MN, USA, pp. 29-35.

Dinesh R, Chaudhuri SG (2013). Soil biochemical/microbial indices as ecological indicators of land use change in mangrove forests. Ecological Indicators 32: 253-258. -doi: 10.1016/j.ecol ind.2013.03.035

Dix NJ, Webster J (2012). Fungal ecology (1st edn). Springer Science and Business Media, London, UK, pp. 1-5. [online] URL: http://books. google.com/books?id=3XXwCAAAQBAJ

Don A, Schumacher J, Freibauer A (2011). Impact of tropical land-use change on soil organic carbon stocks-a meta-analysis. Global Change Biology 17: 1658-1670. -doi: 10.1111/j.1365-2486.2010.02336.x

Dong X, Huai-Ying Y, De-Yong GE, Huang CY (2008). Soil microbial community structure in diverse land use systems: a comparative study using Biolog, DGGE, and PLFA analyses. Pedosphere 18: 653-663. -doi: 10.1016/S1002-0160(08)60060-0

Drenovsky RE, Steenwerth KL, Jackson LE, Scow KM (2010). Land use and climatic factors structure regional patterns in soil microbial communities. Global Ecology and Biogeography 19: 27-39. -doi: 10.1111/j.1466-8238.2009.00486.x

Drew EA, Murray RS, Smith SE, Jakobsen I (2003). Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. Plant and Soil 251: 105-114. -doi: 10.1023/A:1022932414788

Driver JD, Holben WE, Rillig MC (2005). Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 37: 101-106. -doi: 10.1016/j.soilbio.2004.06.011

Fokom R, Adamou S, Teugwa MC, Boyogueno AB, Nana WL, Ngonkeu MEL, Zollo PA (2012). Glomalin related soil protein, carbon, nitrogen and soil aggregate stability as affected by land use variation in the humid forest zone of south Cameroon. Soil Tillage Research 120: 69-75. -doi: 10.1016/j.still.2011.11.004

FAO (2016). State of the world's forests 2016 -Forests and agriculture: land use challenges and opportunities. FAO Report, Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 10-23.

Frostegård A, Tunlid A, Bååth E (2011). Use and misuse of PLFA measurements in soils. Soil Biology and Biochemistry 43: 1621-1625. -doi: 10.1016/j.soilbio.2010.11.021

Gherghel F, Behringer D, Haubrich S, Schlau M, Fey-Wagner C, Rexer KH, Kost G (2014). Former and host genotype influence the mycorrhizal colonization of poplar roots. Forests 5: 2980-2995. -doi: 10.3390/f5122980

Grantina L, Seile E, Kenigsvalde K, Kasparinskis R, Tabors G, Nikolajeva V, Muiznieks I (2011). The influence of the land use on abundance and diversity of soil fungi: comparison of conventional and molecular methods of analysis. Environmental and Experimental Biology 9: 9-21.

Guadarrama P, Castillo-Argüero S, Ramos-Zapata JA, Camargo-Ricalde SL, Alvarez-Sánchez J (2008). Propagules of arbuscular mycorrhizal fungi in a secondary dry

forest of Oaxaca, Mexico. Revista de Biologia Tropical 56: 269-277. [online] URL: http://www.scielo.sa.cr/pdf/rbt/v56n1/art19v56n1.pdf

Guo LB, Gifford RM (2002). Soil carbon stocks and land use change: a meta-analysis. Global Change Biology 8: 345-360. -doi: 10.1046/j.1354-1013.2002.00486.x

Hamer U, Makeschin F, Stadler J, Klotz S (2008). Soil organic matter and microbial community structure in set-aside and intensively managed cropland soils in NE-Saxony, Germany. Applied Soil Ecology 40: 465-475. -doi: 10.1016/j.apsoil. 2008.07.001

Hazard C, Gosling P, Mitchell DT, Doohan FM, Bending GD (2014). Diversity of fungi associated with hair roots of ericaceous plants is affected by land use. FEMS Microbiology Ecology 87: 586-600. -doi: 10.1111/1574-6941.12247

Heinemeyer A, Ridgway KP, Edwards EJ, Benham DG, Young JPW, Fitter AH (2004). Impact of soil warming and shading on colonization and community structure of arbuscular mycorrhizal fungi in roots of a native grassland community. Global Change Biology 10: 52-64. -doi:10.1111/j.1365-2486.2003.00713.x

Honnay O, Helsen K, Van Geel M (2017). Plant community reassembly on restored semi-natural grasslands lags behind the assembly of thearbuscular mycorrhizal fungal communities. Biological Conservation 212: 196-208. -doi: 10.1016/j.biocon.2017.06.017

Hrynkiewicz K, Toljander YK, Baum C, Fransson PM, Taylor AF, Weih M (2012). Correspondence of ectomycorrhizal diversity and colonisation of willows (Salix spp.) grown in short rotation coppice on cropland sites and adjacent natural stands. Mycorrhiza 22: 603-613. -doi: 10.1007/s00572-012-0437-z

Islas AT, Guijarro KH, Eyherabide M, Rozas HS, Echeverría HE, Covacevich F (2016). Can soil properties and agricultural land use affect arbuscular mycorrhizal fungal communities indigenous from the Argentinean Pampas soils? Applied Soil Ecology 101: 47-56. -doi: 10.1016/j.apsoil.2016.01.005

Jefwa JM, Okoth S, Wachira P, Karanja N, Kahindi J, Njuguini S, Huising J (2012). Impact of land use types and farming practices on occurrence of arbuscular mycorrhizal fungi (AM fungi) Taita-Taveta district in Kenya. Agriculture, Ecosystems and Environment 157: 32-39. -doi: 10.1016/j.agee.2012.04.009

Kasel S, Bennett LT, Tibbits J (2008). Land use influences soil fungal community composition across central Victoria, south-eastern Australia. Soil Biology and Biochemistry 40: 1724-1732. - doi: 10.1016/j.soilbio.2008.02.011

Kerfahi D, Tripathi BM, Dong K, Go R, Adams JM (2016). Rainforest conversion to rubber plantation may not result in lower soil diversity of bacteria, fungi, and nematodes. Microbial Ecology 72: 359-371. -doi: 10.1007/s00248-016-0790-0

Li JJ, Zheng YM, Yan JX, Li HJ, He JZ (2013). Succession of plant and soil microbial communities with restoration of abandoned land in the Loess Plateau, China. Journal of Soils and Sediments 13: 760-769. -doi: 10.1007/s11368-013-0652-z

Lumini E, Orgiazzi A, Borriello R, Bonfante P, Bianciotto V (2010). Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. Environmental Microbiology 12: 2165-2179. -doi: 10.1111/j.1462-2920.2009.02099x

Makiola A, Dickie IA, Holdaway RJ, Wood JR, Orwin KH, Glare TR (2019). Land use is a determinant of plant pathogen alpha - but not beta - diversity. Molecular Ecology 16: 3786-3798. -doi:10.1111/mec.15177

Manoharan L, Rosenstock NP, Williams A, Hedlund K (2017). Agricultural management practices influence AM fungi diversity and community composition with cascading effects on plant productivity. Applied Soil Ecology 115: 53-59. -doi: 10.1016/j.apsoil.2017.03.012

McGuire KL, D'Angelo H, Brearley FQ, Gedallovich SM, Babar N, Yang N, Mansor P (2015). Responses of soil fungi to logging and oil palm agriculture in Southeast Asian tropical forests. Microbial Ecology 69: 733-747. -doi: 10.1007/s00248-014-0468-4

McLean M, Huhta V (2002). Microfungal community structure in anthropogenic birch stands in central Finland. Biology and Fertility of Soils 35: 1-12. -doi: 10.1007/s00374-001-0431-7

Melo CD, Walker C, Rodríguez-Echeverría S, Borges PA, Freitas H (2014). Species composition of arbuscular mycorrhizal fungi differ in seminatural and intensively managed pastures in an isolated oceanic island (Terceira, Azores). Symbiosis 64: 73-85. -doi: 10.1007/s13199-014-0303-1

Moora M, Davison J, Opik M, Metsis M, Saks U, Jairus T, Zobel M (2014). Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. FEMS Microbiology Ecology 90: 609-621. -doi: 10.1111/1574-6941.12420

Moragues-Saitua L, Merino-Martín L, Stokes A, Staunton S (2019). Towards meaningful quantification of glomalin-related soil protein (GRSP) taking account of interference in the Coomassie Blue (Bradford) assay. European Journal of Soil Science 70: 727-735. -doi: 10.1111/ejss.12698

Mueller RC, Rodrigues JL, Nüsslein K, Bohannan BJ (2016). Land use change in the Amazon rain forest favours generalist fungi. Functional Ecology 30: 1845-1853. -doi: 10.1111/1365-2435.12651

Muleta D, Assefa F, Nemomissa S, Granhall U (2008). Distribution of arbuscular mycorrhizal fungi spores in soils of smallholder agroforestry and monocultural coffee systems in southwestern Ethiopia. Biology and Fertility of Soils 44: 653-659. -doi: 10.1007/s00374-007-0261-3

Muthukumar T, Sha L, Yang X, Cao M, Tang J, Zheng Z (2003). Distribution of roots and arbuscular mycorrhizal associations in tropical forest types of Xishuangbanna, southwest China. Applied Soil Ecology 22: 241-253. -doi: 10.1016/S0929-1393(02)00156-7

Nautiyal P, Rajput R, Pandey D, Arunachalam K, Arunachalam A (2019). Role of glomalin in soil carbon storage and its variation across land uses in temperate Himalayan regime. Biocatalysis and Agricultural Biotechnology 21: 101311. -doi: 10.1016/j.bcab.2019.101311

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke JS, Kennedy PG (2016). FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20: 241-248. -doi: 10.1016/j.funeco.2015.06.006

Naranjo-Ortiz MA, Gabaldón T (2019). Fungal evolution: diversity, taxonomy and phylogeny of the Fungi. Biological Reviews 94: 2101-2137. -doi: 10.1111/brvv94.6

Oehl F, Sieverding E, Ineichen K, Mäder P, Boller T, Wiemken A (2003). Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Applied and Environmental Microbiology 69: 2816-2824. -doi:10.1128/AEM.69.5.2816-2824.2003

Oehl F, Sieverding E, Ineichen K, Maeder P, Wiemken A, Boller T (2009). Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. Agriculture, Ecosystems and Environment 134: 257-268. -doi: 10.1016/j.agee.2009.07.008

Oehl F, Laczko E, Bogenrieder A, Stahr K, Bösch R, Van Der Heijden M, Sieverding E (2010). Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biology and Biochemistry 42: 724-738. -doi: 10.1016/j.soilbio.2010.01.006

Okubo A, Sugiyama SI (2009). Comparison of molecular fingerprinting methods for analysis of soil microbial community structure. Ecological Research 24 (6): 1399-1405. -doi: 10.1007/s11284-009-0602-9

Oliveira SP, Cndido MJD, Weber OB, Xavier FAS, Escobar MEO, Oliveira TS (2016). Conversion of forest into irrigated pasture I. Changes in the chemical and biological properties of the soil. Catena 137: 508-516. -doi: 10.1016/j.catena.2015.10.017

Olsson PA (1999). Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology 29: 303-310. - doi: 10.1111/j.1574-69411999.tb00621.x

Palta S, Lermi AG, Beki R (2016). The effect of different land uses on arbuscular mycorrhizal fungi in the northwestern Black Sea Region. Environmental Monitoring and Assessment 188 (6): 1379. - doi: 10.1007/s10661-016-5350-z

Peay KG (2014). Back to the future: natural history and the way forward in modern fungal ecology. Fungal Ecology 12: 4-9. - doi: 10.1016/j.funeco.2014.06.001

Peerawat M, Blaud A, Trap J, Chevallier T, Alonso P, Gay F, Suvannang N (2018). Rubber plantation ageing controls soil biodiversity after land conversion from cassava. Agriculture, Ecosystems and Environment 257: 92-102. -doi: 10.1016/j.agee.2018.01.034

Pereira CMR, Da Silva DKA, De Almeida Ferreira AC, Goto BT, Maia LC (2014). Diversity of arbuscular mycorrhizal fungi in Atlantic forest areas under different

land uses. Agriculture, Ecosystems and Environment 185: 245-252. -doi: 10.1016/j.agee.2014.01.005

Potthast K, Hamer U, Makeschin F (2012). Land use change in a tropical mountain rainforest region of southern Ecuador affects soil microorganisms and nutrient cycling. Biogeochemistry 111: 151-167. -doi: 10.1007/s10533-011-9626-7

Preger AC, Rillig MC, Johns AR, Du Preez CC, Lobe I, Amelung W (2007). Losses of glomalin related soil protein under prolonged arable cropping: a chronosequence study in sandy soils of the South African Highveld. Soil Biology and Biochemistry 39: 445-453. -doi: 10.1016/j.soilbio.2006.08.014

Rillig MC, Wright SF, Nicholas KA, Schmidt WF, Torn MS (2001). Unusually large contribution of arbuscular mycorrhizal fungi tosoil organic matter pools in tropical forest soils. Plant and Soil 233: 167-177. -doi: 10.1023/A:1010364221169

Rillig MC, Ramsey PW, Morris S, Paul EA (2003). Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to land use change. Plant and Soil 253 (2): 293-299. -doi: 10.1023/A:1024807820579

Rillig MC (2004). Arbuscular mycorrhizae, glomalin, and soil aggregation. Canadian Journal of Soil Science 84 (4): 355-363. -doi: 10.4141/S04-003

Schenck NC, Perez Y (1990). Manual for the identification of VA mycorrhizal fungi (3rd edn). Synergistic Publications, Gainesville, FL, USA, pp.286.

Sene G, Samba-Mbaye R, Thiao M, Khasa D, Kane A, Manga A, Sylla SN (2012). The abundance and diversity of legume-nodulating rhizobia and arbuscular mycorrhizal fungal communities in soil samples from deforested and man-made forest systems in a semiarid Sahel region in Senegal. European Journal of Soil Biology 52: 30-40. -doi: 10.1016/j.ejsobi.2012.05.005

Sepp SK, Jairus T, Vasar M, Zobel M, Opik M (2018). Effects of on arbuscular mycorrhizal fungal communities in Estonia. Mycorrhiza 28: 259-268. -doi: 10.1007/s00572-018-0822-3

Setala H, McLean MA (2004). Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia 139: 98-107. -doi: 10.1007/s00442-003-1478-y

Siqueira JO, Carneiro MAC, Curi N, Da Silva Rosado SC, Davide AC (1998). Mycorrhizal colonization and mycotrophic growth of native woody species as related to successional groups in Southeastern Brazil. Forest Ecology and Management 107 (1-3): 241-252. -doi: 10.1016/S0378-1127(97)00336-8

Smith SE, Read DJ (2008). Mycorrhizal symbiosis (3rd edn). Elsevier - Academic Press, London, UK, pp. 1-9. Snoeck D, Abolo D, Jagoret P (2010). Temporal changes in VAM fungi in the cocoa agroforestry systems of central Cameroon. Agroforestry Systems 78: 323-328. -doi: 10.1007/s10457-009-9254-6

Sousa CDS, Menezes RSC, Sampaio EVDSB, Lima FDS, Oehl F, Maia LC (2013). Arbuscular mycorrhizal fungi within agroforestry and traditional land use systems

in semi-arid Northeast Brazil. Acta Scientiarum Agronomy 35: 307-314. -doi:10.4025/actasciagron.v35i3.16213

Stürmer SL, Siqueira JO (2011). Species richness and spore abundance of arbuscular mycorrhizal fungi across distinct land uses in Western Brazilian Amazon. Mycorrhiza 21 (4): 255-267. -doi: 10.1007/s00572-010-0330-6

Sui X, Zhang R, Frey B, Yang L, Li MH, Ni H (2019). Land use change effects on diversity of soil bacterial, Acidobacterial and fungal communities in wetlands of the Sanjiang Plain, northeastern China. Scientific Reports 9: 1-14. -doi: 10.1038/s41598-018-37186-2

Taylor JD, Helgason T, Opik M (2017). Molecular community ecology of arbuscular mycorrhizal fungi. In: "The Fungal Community: its Organization and Role in the Ecosystem" (Dighton J, White JF eds). CRC Press, Boca Raton, FL, USA, pp. 3-23. -doi: 10.1201/9781315119496-2

Tchabi A, Coyne D, Hountondji F, Lawouin L, Wiemken A, Oehl F (2008). Arbuscular mycorrhizal fungal communities in sub-Saharan Savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone. Mycorrhiza 18: 181-195. -doi: 10.1007/s00572-008-0171-8

Tedersoo L, Suvi T, Larsson E, Kõjalg U (2006). Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. Mycological Research 110: 734-748. -doi: 10.1016/j.mycres.2006.04.007

Tedersoo L, Anslan S, Bahram M, Põlme S, Riit T, Liiv I (2015). Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. MycoKeys 10: 1-43. -doi: 10.3897/mycokeys.10.4852

Thomson BC, Tisserant E, Plassart P, Uroz S, Griffiths RI, Hannula SE, Martin F (2015). Soil conditions and intensification effects on soil microbial communities across a range of European field sites. Soil Biology and Biochemistry 88: 403-413. -doi: 10.1016/j.soilbio.2015.06.012

Tomao A, Bonet JA, Castaño C, De-Miguel S (2020). How does forest management affect fungal diversity and community composition? Current knowledge and future perspectives for the conservation of forest fungi. Forest Ecology and Management 457: 117678. -doi: 10.1016/j.foreco.2019.117678

Treseder KK (2004). A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. New Phytologist 164: 347-355. -doi: 10.1111/j.1469-8137.2004.01159.x

Turgay OC, Nonaka M (2002). Effects of land use and management practices on soil ergosterol content in andosols. Soil Science and Plant Nutrition 48: 693-699. - doi: 10.1080/00380768.2002.10409259

Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E (2007). Let the concept of trait be functional! Oikos 116: 882-892. -doi:10.1111/j.0030-1299.2007.15559.x

Voriskova A, Janoušková M, Slavíková R, Pánková H, Daniel O, Vazačová K, Münzbergová Z (2016). Effect of past agricultural use on the infectivity and composition of a community of arbuscular mycorrhizal fungi. Agriculture, Ecosystems and Environment 221: 28-39. -doi: 10.1016/j.agee.2016.01.012

Wakelin SA, Gerard E, Van Koten C, Banabas M, O'Callaghan M, Nelson PN (2016). Soil physicochemical properties impact more strongly on bacteria and fungi than conversion of grassland to oil palm. Pedobiologia 59: 83-91. -doi: 10.1016/j.pedobi.2016.03.001

Wang Q, Bao Y, Liu X, Du G (2014). Spatio-temporal dynamics of arbuscular mycorrhizal fungi associated with glomalin-related soil protein and soil enzymes in different managed semiarid steppes. Mycorrhiza 24: 525-538. -doi: 10.1007/s00572-014-0572-9

Wang Q, Wang W, He X, Zhang W, Song K, Han S (2015). Role and variation of the amount and composition of glomalin in soil properties in farmland and adjacent plantations with reference to a primary forest in North-Eastern China. PloS One 10: e0139623. -doi: 10.1371/journal.pone.0139623

Wang J, Ren C, Cheng H, Zou Y, Bughio MA, Li Q (2017). Conversion of rainforest into agroforestry and monoculture plantation in China: consequences for soil phosphorus forms and microbial community. Science of the Total Environment 595: 769-778. -doi: 10.1016/j.scitotenv.2017.04.012

Whiffen LK, Midgley DJ, McGee PA (2007). Polyphenolic compounds interfere with quantification of protein in soil extracts using the Bradford method. Soil Biology and Biochemistry 39:691-694. -doi: 10.1016/j.soilbio.2006.08.012

Wong MR, Morgan JW, Wong NK, Cavagnaro TR (2015). The incorporation of fungal to bacterial ratios and plant ecosystem effect traits into a state-and-transition model of land use change in semi-arid grasslands. Agriculture, Ecosystems and Environment 201: 11-19. -doi: 10.1016/j.agee.2014.10.012

Xu M, Li X, Cai X, Li X, Christie P, Zhang J (2017). Land use alters arbuscular mycorrhizal fungal communities and their potential role in carbon sequestration on the Tibetan Plateau. Scientific Reports 7 (1): 151. -doi: 10.1038/s41598-017-03248-0

Xue C, Penton CR, Zhang B, Zhao M, Rothstein DE, Mladenoff DJ, Tiedje JM (2016). Soil fungal and bacterial responses to conversion of open land to short-rotation woody biomass crops. GCB Bioenergy 8: 723-736. -doi: 10.1111/gcbb.12

Yang Y, Dou Y, Huang Y, An S (2017). Links between soil fungal diversity and plant and soil properties on the Loess Plateau. Frontiers in Microbiology 8: 34. -doi: 10.3389/fmicb.2017.02198

Yesson C, Brewer PW, Sutton T, Caithness N, Pahwa JS, Burgess M, Culham A (2007). How global is the global biodiversity information facility? PLoS One 2 (11): e1124. -doi: 10.1371/journal.pone.0001124

Ying JY, Zhang LM, Wei WX, He JZ (2013). Effects of land utilization patterns on soil microbial communities in an acid red soil based on DNA and PLFA analyses. Journal of Soils and Sediments 13: 1223-1231. -doi: 10.1007/s11368-013-0704-4

Yurkov AM, Kemler M, Begerow D (2012). Assessment of yeast diversity in soils under different management regimes. Fungal Ecology 5: 24-35. -doi: 10.1016/j.funeco.2011.07.004

Zhang C, Liu G, Song Z, Qu D, Fang L, Deng L (2017). Natural succession on abandoned cropland effectively decreases the soil erodibility and improves the fungal diversity. Ecological applications 27: 2142-2154. -doi: 10.1002/eap.15

Zinger L, Bonin A, Alsos IG, Bálint M, Bik H, Boyer F, Chariton AA, Creer S, Cossac E, Deagle BE, Barba MD, Dickie IA, Dumbrell AJ, Ficentola GF, Fierer N, Fumagalli L, Gilbert MTP, Jarman S, Jumpponen A, Kauserud H, Orlando L, Pansu J, Pawlowski J, Tedersoo L, Thomsen PF, Willerslev E, Tabrlet P (2019). DNA metabarcoding Need for robust experimental designs to draw sound ecological conclusions. Molecular Ecology 28: 1857-1862. -doi: 10.1111/mec.15060

Zanne AE, Abarenkov K, Afkhami ME, Aguilar Trigueros CA, Bates S, Bhatnagar JM, Busby PE, Christian N, Cornwell WK, Crowther TW, Flores Moreno H, Floudas D, Gazis R, Hibbett D, Kenndey P, Lindner DL, Maynard DS, Milo AM, Nilsson RH, Powell J, Schildhauer M, Schilling J, Treseder KK (2020). Fungal functional ecology: bringing a trait-based approach to plant-associated fungi. Biological Reviews 95: 409-433. -doi: 10.1111/brv.12570

Chapter II

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

Sujan Balami, Martina Vašutová, Jiri Košnar, Ratna Karki,

Chiranjewee Khadka, Giri Tripathi, Pavel Cudlín

Manuscript published as: Balami S, Vašutová M, Košnar J, Karki R, Khadka C, Tripathi G, Cudlín P (2021) Soil fungal communities in abandoned agricultural land has not yet moved towards the seminatural forest. For Ecol Manag 491:119181.

Forest Ecology and Management 491 (2021) 119181

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

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

Sujan Balami [a,*], Martina Vašutová [a,b], Jiří Košnar [a], Ratna Karki [c], Chiranjewee Khadka [b], Giri Tripathi [d], Pavel Cudlín [b]

[a] Department of Botany, Faculty of Science, University of South Bohemia, Na Zlaté stoce 1, České Budějovice 370 05, Czech Republic
[b] Department of Carbon Storage in the Landscape, Czech Academy of Sciences, Lipová 1789/9, České Budějovice 370 05, Czech Republic
[c] Faculty of Agriculture, University of South Bohemia, Studentská 1668, České Budějovice 370 05, Czech Republic
[d] Department of Biotechnology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

ARTICLE INFO

Keywords:
Land use
Abandoned agricultural land
Soil fungi
Mycorrhizal fungi
Species composition
Illumina sequencing

ABSTRACT

The increasing trend in the abandonment of agricultural land provides a unique opportunity for the restoration of forests. However, not only vegetation but also belowground microbial communities are vital for the development of stable forest stands. To assess whether soil fungi can affect the succession of abandoned agricultural lands, we analyzed soil fungal communities, including total and arbuscular mycorrhizal (AM) fungi in agricultural lands (AG), short- and long-term abandoned agricultural lands (SA and LA), and short- and long-term regenerating seminatural forests (SR and LR). Vegetation analysis showed that the abandoned lands were gradually converting to seminatural forests. However, significantly lower soil pH values and higher C and N concentrations were found in the LR forests than in the other land use types. Although there were no significant differences observed in the total and AM fungal diversity among land use types, the species compositions of seminatural forests (LR and SR), abandoned lands (SA and LA), and agricultural land (AG) were clearly differentiated in the ordination analysis. Significant differences were observed between AG and other land use types and between LR and abandoned land in the case of total fungal communities. The ECM fungi associated with *Alnus* occurred on the LA plots, whereas the LR plots were characterized by different ECM fungi (*Russula, Lactifluus*) and a high abundance of ericoid fungi along with hygrocyboid and clavarioid taxa. These results indicate that fungal succession is not as straightforward as vegetation changes. AM fungi will probably not constrain the abandoned land succession towards seminatural forests, but total fungal communities could be hindered by soil properties and absence of host trees.

1. Introduction

Abandonment of agricultural land is a major land use change in different parts of the world with non-intensive and less-productive farming systems (MacDonald et al., 2000; Li and Li, 2017; Ustaoglu and Collier, 2018). In addition to conversion to other human-activity-based land use forms, these abandoned areas provide an opportunity to restore forests, which often have positive consequences for biodiversity and other ecosystem services (Kuemmerle et al., 2011; Navarro and Pereira, 2012; Niwaxu and Shackleton, 2019; Liu et al., 2020). However, the trajectories of such forest successions are not straightforward and depend upon several ecological (e.g. soil pH, soil biota, canopy openness, slope), historical (time since abandonment, duration of cultivation), and spatial factors (distance from adjacent forest, landscape structure, etc.) (Gardescu and Marks, 2004; Benjamin et al., 2005; Kardol et al., 2006; Cramer et al., 2008; Piché and Kelting, 2015).

Together with other soil microbes, soil fungi are crucial for plant community succession as they play an important role in the nutrient cycling of an ecosystem and interact directly with plants via mutualisms (mycorrhizal fungi) or pathogenesis (van Der Heijden et al., 2008; Johnson et al., 2016; Tedersoo et al., 2020). In long-term abandoned fields, the fungal community change with gradual decreases in pathogens and yeasts and increasing mycorrhizal fungi and slow-growing saprotrophic species, which is expected to affect carbon flow through the soil food web, thus affecting plant succession (Hannula et al., 2017; Bosso et al., 2017). The presence or absence of certain species of arbuscular mycorrhizal (AM) fungi has been found to alter plant communities by affecting soil fertility and nutrient acquisition (Vogelsang

* Corresponding author.
E-mail address: balamisujan@gmail.com (S. Balami).

https://doi.org/10.1016/j.foreco.2021.119181
Received 19 January 2021; Received in revised form 12 March 2021; Accepted 17 March 2021
Available online 8 April 2021

et al., 2006; Neuenkamp et al., 2019). Zobel and Öpik (2014) proposed that during secondary succession, changes in a plant community might be primarily constrained by the slower dispersal of symbiotic fungal partners than by the dispersal of plants. Likewise, pathogenic soil fungi can affect plant survival and fitness by causing root diseases in certain plants, thus regulating plant communities (Holah et al., 1997; Holah and Alexander, 1999). On the other hand, soil fungal communities are regulated by abiotic soil properties and plant diversity (Lauber et al., 2008; Yang et al., 2017). Soil pH and soil carbon (both types and amount of input) are found to directly affect the fungal community composition of soils (Goldmann et al., 2015; Liu et al., 2015; Ren et al., 2018). Plants can shape a fungal community structure by providing nutrient resources and hosts (Hanif et al., 2019). Studies have also shown that invasion by alien invasive plants (e.g. *Ageratina adenophora, Centaurea maculosa, Chromolaena odorata*) can alter AM fungal communities (Mummey and Rillig, 2006; Niu et al., 2007) or cause accumulation of pathogenic fungi (Mangla et al., 2008) that favor invasive plants and could hinder the succession of vegetation (Flory and Clay, 2010). This evidence implies that soil fungi are important players involved in vegetation succession.

Few previous studies on the responses of soil fungal communities during the secondary succession of abandoned agricultural land have presented equivocal results. Generally, the fungal parameters (richness, diversity, AM fungal spore density, and glomalin-related soil protein content) of soils in abandoned agricultural land are comparable to those in forests and higher than those in agricultural lands (Li et al., 2007; Snoeck et al. 2010), but of soil fungal diversity in abandoned agricultural land may be either lower or higher than in forests (Grantina et al., 2011; Li et al., 2013; Zhang et al., 2018). The most obvious results are seen in studies of fungal species composition, in which abandoned agricultural land has clearly differed from forest and agricultural land (Ciccolini et al., 2015; Hannula et al., 2017; Zhang et al., 2018). However, due to variations in local contexts and land use classifications, it is difficult to apply these results worldwide.

Abandonment of agricultural land is also common in the mid-hills of Nepal due to environmental and socioeconomic changes, e.g. unpredicted weather conditions, changes in income sources replacing agriculture, outmigration to city areas and foreign employment that has led to deficiencies in workers (Ojha et al., 2017; Chapagain et al., 2018). Typically, agricultural practices in the study area are traditional and low-intensive. After the cessation of agricultural practices, the abandoned areas are gradually covered by vegetation. The conversion of such vegetation into seminatural forests would be beneficial for local inhabitants (due to the timber, fodder, firewood, edible fungi, and medicinal plants the seminatural forests would provide) and would maintain biodiversity and ecosystem functions. To determine whether the succession of abandoned agricultural land leads spontaneously to seminatural forests or if there are some indications of barriers, it is necessary to take into account several aspects of this process. In this regard, we aimed to evaluate how soil fungal communities, including those of AM fungi, change in response to the spontaneous succession of abandoned agricultural land, if there is any indication of a delay or constraint in the succession of soil fungal communities. Seminatural forests were included as reference. Consequently, we analyzed fungal communities in terms of species diversity (*i*), the proportion of functional groups, and species composition (*ii*). Furthermore, we were interested in which environmental factors affect fungal species composition during this process (*iii*) and what the effect of an invasive subshrub (*A. adenophora*) that is abundant in abandoned areas may be on soil fungal communities (*iv*). We hypothesized that abandoned agricultural land will have a similar level of total and AM fungal diversity as those in seminatural forests but higher levels than those of agricultural lands (*i*); that the fungal community composition of abandoned agricultural lands will be more similar to those of seminatural forests than to those of agricultural lands (*ii*); that fungal species composition will be influenced by both vegetation and abiotic soil factors (*iii*); and that fungal species composition will be mainly affected by increasing coverage of the invasive subshrub *A. adenophora* (*iv*).

2. Materials and methods

2.1. Study area, vegetation, and soil sampling

The study was conducted in the Dolakha district, Bagmati Province, Nepal. This area has a temperate monsoon climate with warm and humid summers followed by dry and cold winters. The mean annual temperature is ~ 14 °C (a minimum of 7.5 °C occurs in January and a maximum of 19.5 °C occurs in July). The annual precipitation is 2091 mm (80% of precipitation occurs from June to August). The growing season lasts from early April to late October. Five different land use types –agricultural land (AG), short-term abandoned land (SA), long-term abandoned land (LA), short-term regenerated forest (SR), and long-term regenerated forest (LR) as a reference– were identified by extensive field visits (May to August 2017) (Table 1). Within each land use type, 5 circular plot replicates (500 m^2) were selected (Fig. 1, Table S1), which were distributed in the mid-hill areas (latitude: 27.62 to 27.77 N, longitude: 85.83 to 86.17 E; 1800 to 2300 m a.s.l).

For the analysis of vegetation, each circular plot was divided into four subplots. The cover of three vegetation layers (tree, shrub and herb) and cover of individual plant species were estimated for each subplot separately, and average values were calculated for each plot. Eight soil cores (25 cm × 3.2 cm) were taken from each plot by systematic random sampling (along 8 different directions, 4 m and 8 m away from the centre in a regular alternate fashion) after removing the litter layer in 2018. Soil samples were first sieved through a 2 mm sterile mesh and homogenized to make a composite sample and then dried in silica gel (3 × 15 g wet soil per sample). Approximately 200 g of each composite soil sample was used for the determination of the physicochemical properties (soil pH, total carbon (C), and nitrogen (N) concentration). The total C and N concentrations (%) were analyzed by the dry combustion method using an elemental analyzer (Elementar Analysensysteme GmbH. Modell vario MAX CN).

2.2. Molecular methods

Genomic DNA was extracted from 3 × 0.2 g of each soil sample (triplicate) using a Nucleospin Power Soil Kit (Machery-Nagel, Inc., Düren, Germany) according to the manufacturer's instructions. Triplicate DNA samples were then pooled into a single sample. The amplification of the template DNA was also performed in triplicate. The ITS2 region of total fungi was amplified using the barcoded primers gITS7 and ITS4 (Ihrmark et al., 2012). Each 25 μl of PCR reaction mixture contained 1 μl of template DNA, 0.75 μl of a polymerase mix (4 U:96 U of Pfu DNA polymerase; Fermentas, Waltham, MA, USA) and MyTaq Polymerase (Bioline, USA), 5 μl of 5x polymerase buffer including PCR nucleotide mix, 0.75 μl of bovine serum albumin (BSA) (20 mg/ml), 1 μl of each primer (5 pmol/μl) and 15.5 μl of sterile water. The PCR

Table 1
Land use types and their land use history/ characteristics.

Land use types	Characteristics/land use history
Agricultural land (AG)	Active agricultural land, traditional or not fully intensive agricultural system
Short-term abandoned land (SA)	Abandoned for 4 to 6 years, dominated by herbs and sparse shrubs, no pasture pressure
Long-term abandoned land (LA)	Abandoned for 15 to 20 years, colonized with young trees and shrubs, no pasture pressure
Short-term regenerated forest (SR)	Regenerated 30 to 40 years after the disturbance like pasture, cutting and logging, and litter removal, etc.
Long-term regenerated forest (LR)	Near natural with no known history of forest clearing but affected by disturbance like pasture and litter removal etc.

The history of agricultural land abandonment and forests were ascertained by interviewing local inhabitants with ethics approval.

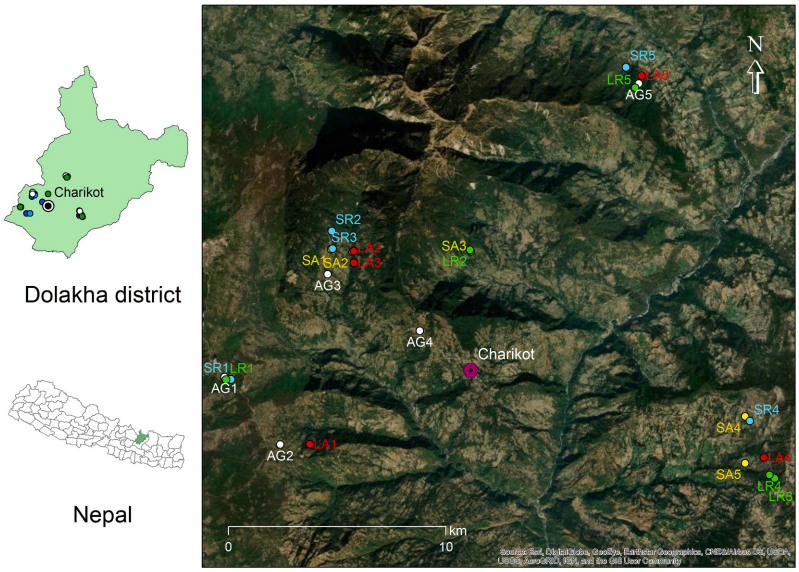

Fig. 1. Map of study area showing 25 permanent plots in 5 land use types. (AG –agricultural land, SA –short-term abandoned land, LA –long-term abandoned land, SR –short-term regenerated forest, and LR –long-term regenerated forest).

conditions were set at a denaturation temperature of 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min, and final elongation at 72 °C for 7 min.

As the commonly used ITS primers tend to underestimate the abundance of AM fungi, we used specific AM primers for the SSU region to focus on this group. The SSU rDNA fragment was amplified by semi-nested PCR. In the first PCR, each 10 µl of PCR reaction mixture contained 0.5 µl of template DNA, 0.1 µl (2 U/µl) of polymerase (Phusion High-Fidelity DNA polymerase, New England Biolabs), 2 µl of 5x HF buffer, 0.2 µl of dNTP (10 mM each dNTP), 0.8 µl of each primer NS31 (Simon et al., 1992) and AML2 (Lee et al., 2008) and 5.6 µl of sterile water. The PCR conditions were set at 94 °C for 3 min, then 35 cycles were run at 94 °C for 30 s, 65 °C for 30 s, 72 °C for 1 min, and the final elongation was run at 72 °C for 10 min. The reaction mixture (10 µl) for the second PCR contained 0.5 µl of template DNA, 0.1 µl (2 U/µl) of the polymerase (Phusion High-Fidelity DNA polymerase, New England Biolabs), 2 µl of 5x HF buffer, 0.2 µl of dNTP (10 mM of each dNTP), 0.8 µl of each primer (WANDA (Dumbrell et al., 2011) and AML2) and 5.6 µl of sterile water. The PCR conditions were set at 94 °C for 3 min, then 10 cycles were run at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, and the final elongation was run at 72 °C for 10 min. The amplicons were pooled and purified using a MinElute PCR purification kit (Qiagen) according to the manufacturer's instructions. The concentration of PCR products was measured using the Qubit® 2.0 Fluorometer (Thermo Scientific). Sequencing of the amplicons was performed on an Illumina MiSeq (250 bp paired-end) at the Institute of Microbiology, CAS, Prague, Czech Republic. Sequence data were deposited in MG-RAST (https://www.mg-rast.org) under accession number mgm4889418.3 & mgm4889419.3.

2.3. Bioinformatics analysis

The amplicon sequencing data were processed using the pipeline SEED v.2.1 (Větrovský et al., 2018). Briefly, pair-end reads were merged using fastq-join (Aronesty, 2013). The ITS2 region was extracted using ITSx v. 1.0.11 (Bengtsson-Palme et al., 2013) before processing. Chimeric sequences were removed using VSEARCH v. 2.4.3 (Rognes et al., 2016), and the sequences at the 97% similarity level were clustered using UPARSE, which was implemented in USEARCH v.8.1.1861 (Edgar 2013). Afterward, the most abundant sequences per OTU were obtained using MAFFT v.7.222 (Katoh et al., 2009) and subjected to identification against the UNITE+INSD dataset (Abarenkov et al., 2020) using BLASTn (at the species level, sequence similarity ≥ 97%; at the genus level, sequence similarity ≥ 80%). Because our study was conducted in a poorly studied area, we expected many undescribed fungal species/genera without reference sequences in public databases. Therefore, unidentified OTUs represented by more than 10 sequences were blasted against the GenBank database (accessed in February 2020) to evaluate their similarity to the fungal sequences. For further analyses, the singletons, non-fungal sequences, and sequences that did not match similarity and coverage with any fungal sequence ≥ 70% were removed. The OTU table was constructed from a random resampling of 10,350 sequences per sample, resulting in a total of 6612 OTUs. The fungal trophic modes (symbiotroph, saprotrophs, and pathotrophs) were identified by FUNGuild (Nguyen et al., 2016). For species composition analysis, only the OTUs (380) with a abundance ≥ 0.5% per sample were used (Table S2).

For the AM fungi, due to the length of the amplified SSU region, only the sequences starting from the forward primer were used. The chimeric sequences were removed by the USEARCH v.8.1.1861 UCHIME algorithm (Edgar et al., 2011), and the sequences with an expected error rate higher than 0.005 were removed. After resampling (5000 sequences per sample), the dataset was subjected to a BLAST+ v.2.5.0 search against the MaarjAM database (accessed in February 2020) using the SSU pipeline (Vasar et al., 2017). The following criteria were required for a match: sequence similarity ≥ 97%; alignment length not differing from the length of the shorter query and subject sequences by greater than 5%; and a BLAST e-value < 1e-50. The sequences with similarity to the closest available AM sequence exceeding 90% but below 97% were considered putative novel AMF taxa and were clustered using USEARCH

with a 97% similarity threshold. Only the clusters containing more than 100 sequences were retained. Twenty sequences of each cluster were selected at random and aligned with the type sequences of the MaarjAM database (accessed November 2018) using MAFFT with the default settings and trimmed according to the length of Illumina sequences. The NJ tree was constructed using TOPALi v.2.5 (Milne et al., 2009) with default settings and 500 bootstrap replicates. Only the clusters that formed well-supported (bootstrap values ≥ 75) monophyletic clades (3 novel virtual taxa) were accepted. The virtual taxa (VT) table was constructed from a random resampling of 900 sequences per sample (151 VTs) (Table S3). One SR plot was removed from the nalysis of AM fungi because of an insufficient number of AM sequences (231). For the species composition analysis, only VTs (115) with a higher abundance than 0.5% per sample were used (Table S3).

2.4. Statistical analysis

Detrended correspondence analysis (DCA) was used to evaluate the vegetation composition in different land use types and land use type effect was tested using the global permutation test of canonical correspondence analysis (CCA). Variation in abiotic soil properties among land use types was tested by one-way ANOVA test followed by TukeyHSD post-hoc test. For fungal sequence data, the rarefaction curve of each sample was prepared using the package *mobr* in the R statistical program (R Core Team 2019). Diversity estimates (Shannon index, InvSimpson index, observed OTU and VT richness, and Chao1 richness) were calculated using the *vegan* and *Otu_table* packages. Differences in the diversity indices and the abundance of selected taxa and functional groups among land use types were analyzed by one-way ANOVA (or Kruskal Wallis test) and post-hoc test Tukey HSD (or Wilcoxon signed-rank test). Only OTUs/VTs with an abundance of $\geq 0.5\%$ were considered for community analysis by DCA. The data were first normalized using log-transformation. The effects of land use type and abiotic soil properties were tested by the global permutation test of CCA. The effects of vegetation composition on the species composition of total and AM fungi were tested by the co-correspondence analysis (CoCA) (ter Braak and Schafferes, 2004). The dissimilarities in the total fungal OTU and VT compositions between the land use types were tested by the analysis of similarities (ANOSIM) using the package *vegan* in the R statistical program. For variance partitioning, significant CoCA case scores were taken as vegetation factors as a first group and abiotic soil factors as a second group. To observe the effect of *A. adenophora* coverage on the total fungal and AM occurrences, a generalized-linear model of CCA was used. All multivariate analyses were performed with the Canoco v.5.00 software (ter Braak and Šmilauer, 2012).

3. Results

3.1. Vegetation composition differed among land use types

Ordination analysis showed that the vegetation composition differed significantly across five different land use types (Fig. 2). Land use type explained 29% of the variation in the vegetation composition (pseudo-F = 2.1, P < 0.01). Briefly, AG plots were cropped with maize, potato, millet, and invaded by some weed species e.g. *Persicaria runciniata, Ageratum conyzoides, Galinsoga quadriradiata, Oxalis corniculata, Polygonum* sp., etc. Subsequently after cessation of agricultural practices the abandoned lands (SA) were covered by grass species e.g. *Imperata cylindrica*, *Cyperus* sp.; forbs e.g. *Drymaria cordata, Centella* sp., *Taraxacum* sp., etc. and sparse shrubs e.g. *Rubus ellipticus, Berberis aristata*. Later, in LA, the vegetation was gradually replaced by the ruderal tree *Alnus nepalensis* along with a few *Schima wallichii* and *Pinus wallichiana* and a dense cover of the shrubs *Rubus ellipticus, Berberis aristata,* and *Pyricantha cernulata.* The invasive alien subshrub *Ageratina adenophora* also established simultaneously in LA (Fig. 2B). Two of the LA plots (LA4 and LA5) were separated from other LA plots in ordination diagram due to higher coverage of *Schima wallichii, Lyonia ovalifolia, Viburnum erubuscence*, *Myrsine semiserrata, Rubus rugosus,* and some herbs (Fig. 2). SR and LR plots were mainly composed of ericoid trees e.g. *Daphniphyllum himalayense, Symplocos ramosissima, Lyonia ovalifolia, Rhododendron arboreaum,* etc. and similar shrubs like in LA. ECM trees occurred rarely, mainly in the LR plots (*Castanopsis hystrix, Quercus semicarpifolia*), or in their vicinity (*Quercus* spp., *Engelhardia spicata)*.

3.2. Soil properties among different land use types

The ANOVA test showed significant differences in soil nitrogen (P < 0.01), carbon (P < 0.01) concentrations (%) and pH (P < 0.01) among the land use types. However, this difference was solely from the estimates of LR. Soil pH value was significantly lower in LR than in the abandoneds (SA and LA) and agricultural (AG) land use types. On the other hand, the mean values of N% and C% were significantly higher in LR than in the other land use types (Table 2). While comparing SR with LA, there were no significant differences in any of the measured abiotic

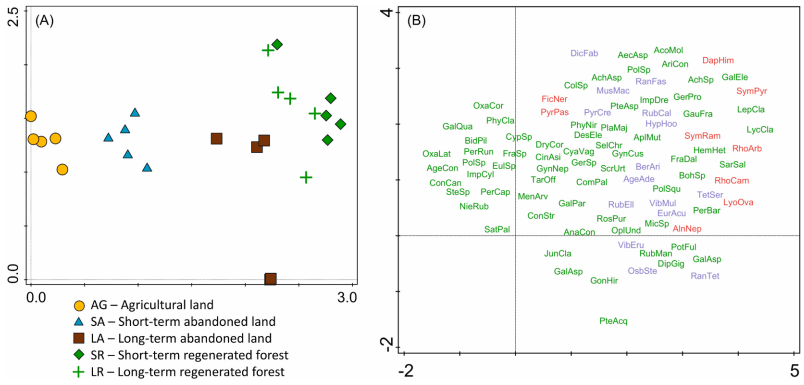

Fig. 2. DCA of vegetation (A) plots and (B) plants (species abbreviations with green coloured text are herbs, blue – shrubs, and red – trees; only 80 species with the highest abundance value are shown in the diagram and details of species abbreviations are in Table S1). AG –agricultural land, SA –short-term abandoned land, LA –long-term abandoned land, SR –short-term regenerated forest, and LR –long-term regenerated forest. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Soil pH, N%, and C% (Mean ± SD) in AG, SA, LA, SR, and LR land use types. To compare different land use types one way ANOVA and post hoc (TukeyHSD) test were used. Values sharing the different letters in parenthesis are significantly different (N = 25, P < 0.05). AG –agricultural land, SA –short-term abandoned land, LA –long-term abandoned land, SR –short-term regenerated forest, and LR –long-term regenerated forest.

Land use type	pH	Nitrogen (N %)	Carbon (C%)
AG	5.54 ± 0.23 (a)	0.24 ± 0.07 (a)	3.98 ± 1.71 (a)
SA	5.25 ± 0.29 (a)	0.24 ± 0.11 (a)	4.45 ± 1.8 (a)
LA	5.08 ± 0.23 (ab)	0.21 ± 0.06 (a)	3.99 ± 0.42 (a)
SR	4.73 ± 0.31 (bc)	0.39 ± 0.25 (a)	7.25 ± 2.53 (a)
LR	4.52 ± 0.25 (c)	0.71 ± 0.17 (b)	10.39 ± 2.17 (b)

soil properties.

3.3. Land use barely influences total fungal and AM diversity estimates

Rarefaction analysis of the total fungal OTUs assigned at 97% similarity indicated undersampling of actual richness whereas AM fungal VTs indicated sufficient sampling (Fig. S1). Altogether, 6612 OTUs were identified within the resampled total fungal dataset and 151 VTs within AM dataset. The diversity analysis showed significantly higher α-diversity (InvSimpson) in the LA plots than in the AG plots (P < 0.05) and higher estimated richness (Chao1) (P < 0.05) in the SR plots than in the SA plots. No significant differences were found in the AM fungal communities among the land use types. The total fungi showed a higher Shannon index, Invsimpson index, observed richness, and Chao1 values than the AM fungi (Table 3).

3.4. Land use determined the proportion of different taxa and functional groups

Among the identified OTUs, the majority were classified into Ascomycota (54.1 ± 4.8%), followed by Basidiomycota (18.3 ± 4.7%) and Mucoromycota (10.9 ± 3.3%). Basidiomycota were significantly more abundant in the LR plots than in the AG plots (Fig. S2). The other fungal groups did not differ significantly between the different land use types. At the order level, differences in the relative abundance were observed (Fig. 3A). Sordariales, Hypocreales, and Filobasidiales were significantly more abundant in AG than in other land use types (P < 0.01, P < 0.05, P < 0.05), Pleosporales was significantly more abundant in AG than in SA, SR, and LR (P < 0.05), and Mortierellales were significantly more abundant in AG than in SA (P < 0.05). On the other hand, Agaricales was significantly less abundant in AG than in SA, LA, and LR (P < 0.05). In the case of AM fungi, members of Glomeraceae were dominant in the SA, LA, SR, and LR plots but were significantly less abundant in the AG plots (P < 0.001, Fig. 3B). Claroideoglomeraceae was significantly more abundant in AG than in the other types of plots (P < 0.05). Acaulosporaceae was significantly more abundant in AG, SR, and LR than in SA and LA (AG-LA, P < 0.01; AG-SA, P < 0.05; SR-SA, P < 0.05; SR-LA, P < 0.01; LR-LA, P < 0.05), Gigasporaceae in AG than in SA (P < 0.05) and Paraglomeraceae in AG than in LR (P < 0.05). The relative abundance of different functional groups of total soil fungi varied with land use type (Table 4, Fig. 4A). Pathotrophs were significantly more abundant in AG than in SA, SR, and LR. Symbiotrophs (mainly ECM and ericoid fungi) were significantly more abundant in LA, SR, and LR than in AG and SA. Regarding mycorrhizal sequences, significant differences were only found in the case of ECM (Fig. 4B). Their sequences were significantly less abundant in the AG plots than in the LA (P < 0.01), SR (P < 0.05), and LR plots (P < 0.05). The most abundant ECM fungi in LA were *Tomentella, Alnicola,* and *Inocybe,* in SR *Alnicola, Tomentella, Elaphomyces* and *Inocybe* whereas *Russula* and *Lactifluus* dominated in LR (Table S2).

Table 3
OTU richness and diversity indices in different land use types (Mean ± SD). Values sharing the different letters in parenthesis are significantly different (N = 25, P < 0.05). AG –agricultural land, SA –short-term abandoned land, LA –long-term abandoned land, SR –short-term regenerated forest, and LR –long-term regenerated forest.

	Land use type	Shannon	InvSimpson	Observed richness	Chao1 richness
Total fungi	AG	5.15 ± 0.1 (a)	21.21 ± 3.04 (a)	790 ± 48.65 (a)	1051.77 ± 131.19 (ab)
	SA	5.07 ± 0.25 (a)	24.87 ± 3.46 (ab)	729.6 ± 64.94 (a)	975.49 ± 59.43 (a)
	LA	5.08 ± 0.11 (a)	25.78 ± 1.92 (b)	764.2 ± 94.43 (a)	1037.9 ± 145.78 (ab)
	SR	5.16 ± 0.26 (a)	24.25 ± 7.62 (ab)	882.4 ± 99.48 (a)	1265.83 ± 122.37 (b)
	LR	4.95 ± 0.46 (a)	21.07 ± 11.2 (ab)	834 ± 124.18 (a)	1185.46 ± 146.05 (ab)
AM fungi	AG	2.84 ± 0.34 (a)	12.08 ± 4.97 (a)	38.2 ± 8.67 (a)	44.40 ± 16.20 (a)
	SA	2.59 ± 0.21 (a)	8.70 ± 2.24 (a)	35.4 ± 5.36 (a)	47.45 ± 12.35 (a)
	LA	2.61 ± 0.34 (a)	8.27 ± 3.046 (a)	36.6 ± 10.41 (a)	40.05 ± 11.39 (a)
	SR	2.93 ± 0.35 (a)	13.26 ± 4.75 (a)	40.75 ± 14.93 (a)	44.13 ± 15.66 (a)
	LR	2.67 ± 0.32 (a)	8.79 ± 3.11 (a)	34.4 ± 13.95 (a)	36.75 ± 16.91 (a)

3.5. Shift in the species composition of the total fungal and AM communities

Ordination analysis showed that both the total and AM fungal community composition differed significantly among five different land use types (Fig. 5). Land use type explained 23% of the variation in the species composition of the total fungi (pseudo-F = 1.5, P < 0.01) and 25% of the variation in the species composition of the AM fungi (pseudo-F = 1.6, P < 0.01). Specifically, the forest plots (LR and SR), abandoned plots (SA and LA), and agricultural plots (AG) were clearly separated in the ordination space. This pattern is not in agreement with vegetation (Fig. 2). The results from the ANOSIM test showed that the total fungal and AM communities of AG differed significantly from those of all other land use types (Table 5). For the total fungi, the LR plots also significantly differed from the abandoned plots (Table 5). For the AM fungi, the dissimilarities in community composition between the abandoned plots and forested plots were not significant (Table 5).

3.6. Soil and vegetation factors determine the variation in the total fungal and AM community composition

CoCA analysis showed a significant effect of vegetation composition on total fungal species composition (trace value = 1.85, P < 0.01) and AM species composition (trace value = 0.98, P < 0.05). Partitioning of the variance between vegetation and soil factors together showed that both factors were equally important, with a higher overlap in the case of AM fungi (Fig. 6). Comparison of the sub-factors of soil (pH, N%, and C %) showed that the soil C% affected both the total fungal and AM species composition (P < 0.01). The total fungal community was further influenced by pH (P < 0.05), whereas AM fungi were influenced by N% (P < 0.05).

3.7. Influence of increasing Ageratina adenophora coverage on total fungal species composition

It was found that increasing coverage of *A. adenophora* did not influence the overall species composition of either total fungi or AM fungi. However, 22 fungal OTUs and 4 AM VTs were significantly correlated with coverage of *A. adenophora*, e.g. OTU0109, OTU0131, *Pseudeurotium* sp. (OTU0209), *Fibulochlamys* sp. (OTU0379), *Glomus* spp. (VT072, VT234), *Archaeospora* sp. (VT005) and *Acaulospora* sp. (VT026).

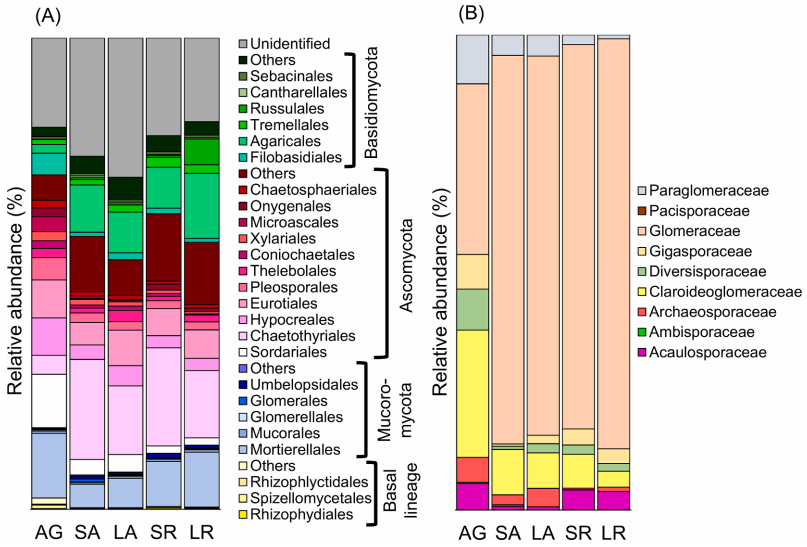

Fig. 3. The relative abundance (%) of (A) total fungal taxa (at order level) and (B) AM taxa (at the family level) in 5 different land use types. AG –agricultural land, SA –short-term abandoned land, LA –long-term abandoned land, SR –short-term regenerated forest, and LR –long-term regenerated forest.

Table 4
Relative abundance (%) (Mean ± SD) of a total fungal functional group. Values sharing the same letters in the parenthesis are not significantly different at $P < 0.05$. AG –agricultural land, SA –short-term abandoned land, LA –long-term abandoned land, SR –short-term regenerated forest, and LR –long-term regenerated forest.

Functional group	Land use type				
	AG	SA	LA	SR	LR
Pathotroph	5.7 ± 1.4 (a)	2.7 ± 2.4 (b)	3.0 ± 1.2 (ab)	1.8 ± 0.9 (b)	1.2 ± 0.4 (b)
Saprotroph	26.2 ± 3.0 (a)	20.4 ± 2.9 (ab)	18.0 ± 5.1(b)	18.6 ± 3.6 (ab)	19.9 ± 5.9 (ab)
Symbiotroph	0.9 ± 0.2 (a)	4.6 ± 5.6 (a)	8.7 ± 3.6 (b)	6.6 ± 5.0 (b)	8.3 ± 8.6 (b)
Uncertain	37.0 ± 5.2	33.2 ± 8.1	28.3 ± 3.1	39.3 ± 2.6	41.9 ± 9.1
Unidentified	29.9 ± 3.9	38.9 ± 9.9	41.8 ± 8.9	33.4 ± 6.7	28.5 ± 4.6

4. Discussion

4.1. No change in diversity estimates of total and AM fungi among land use types

Similar levels of total and AM fungal diversity were found across all studied land use types, and changes in diversity are thus not expected to affect abandoned land succession (Table 3). These results are contradictory to the expected result of lower diversity estimates in AG land because of disturbances related to agricultural activities. A study from subtropical China by Zhang et al. (2018) showed an increase in diversity estimates in the early stages following abandonment of agricultural land and then a decrease in later stages and in forests, but our data did not show such a pattern. Most likely, changes in land use types could limit some taxa but favor others, thus balancing the overall land use change effect. In our study, AG land represented a traditional or not fully intensive agricultural system (the AG study site underwent agricultural practices involving the use of livestock manure, intercropping, and crop rotation, and only small quantities of artificial fertilizers), which might not be as detrimental to some fungal taxa as intensive systems. Studies have shown significant increases in fungal diversity when using crop rotation and intercropping practices (Jiang et al., 2016; Li and Wu, 2018). One reason for the lack of observed difference in the total fungal diversity could be the hyper-diversity of fungi, which was not possible to capture sufficiently by our approach (Fig. S1A). The lack of change in AM diversity between the AG and other land use types could be related to maize cultivation and frequent tillage (draws AM fungi from lower soil layers to upper layers) in AG, which could have increased AM richness in AG. In the case of SA, the dominant grasses and forbs are commonly AM-associated, which could have led to the comparable AM fungal richness with LA, SR, and LR.

4.2. Fungal community composition changes

Due to limitations in the FUNGuild database and uncertain trophic behavior (functional groups), a relatively high proportion of taxa were unassigned to functional groups (Fig. 3A, Table 4). This emphasizes the necessity of classical taxonomic studies in impoverished areas of the world, i.e. formal descriptions of new species, observations of their ecology (Peay, 2014), and provision of reference sequences to public databases, as suggested by Truong et al. (2017). At the functional group level, significantly lower abundances of pathotrophs were found in SA, SR, and LR than in AG due to the cessation of agricultural activities. The observed decrease in the abundance of pathotrophs after abandonment is in agreement with the results of Hannula et al. (2017). Reduced disturbance in SA, SR, and LR compared to AG probably leads to a decrease in the abundance of pathotrophs because many pathogens are r-selected species that rapidly respond to agricultural disturbances (Almeida et al., 2001). There were comparable abundances of saprotrophs and symbiotrophs between abandoned lands (LA and SA) and seminatural forests (SR and LR).

The ECM fungi were comparably abundant in LA and LR, but the

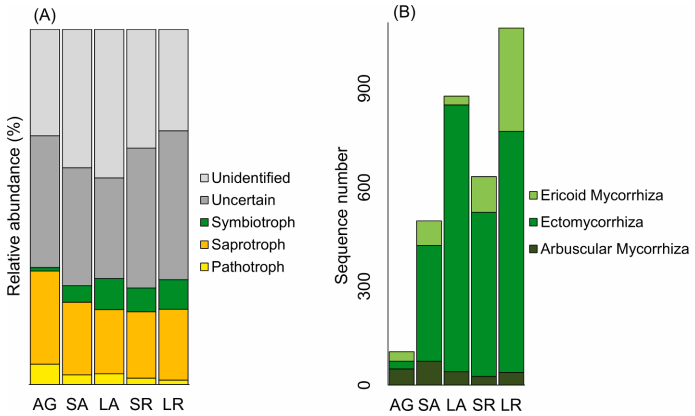

Fig. 4. (A) Mean relative abundance of trophic groups (pathotrophs, saprotrophs, symbiotrophs) along with taxa of uncertain tropic behavior and unidentified taxa (B) mean number of sequences assigned to mycorrhizal fungi in five land use types (AG –agricultural land, SA –short-term abandoned land, LA –long-term abandoned land, SR –short-term regenerated forest and LR –long-term regenerated forest).

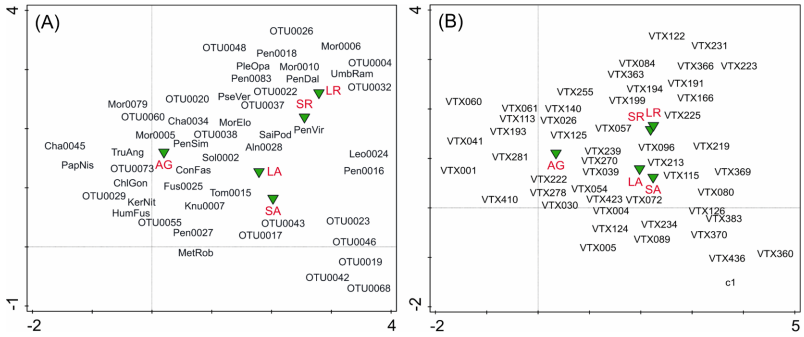

Fig. 5. Community composition of (A) total fungi (percentage variation explained by DCA1 = 12.6% and DCA2 = 8.8%). (B) AM fungi (percentage variation explained by DCA1 = 16.6% and DCA2 = 8.6%). AG –agricultural land, SA –short-term abandoned land, LA –long-term abandoned land, SR –short-term regenerated forest and LR –long-term regenerated forest. Only 50 most dominant OTUs/VTs are shown. OTUs identified to species level are abbreviated as ConFas –*Coniochaeta fasciculata,* ChlGon –*Chloridium gonytrichii*, HumFus –*Humicola fuscoatra*, KerNit –*Kernia nitida,* MetRob –*Metarhizium robertsii*, MorElo –*Mortierella elongata*, PapNis –*Papulaspora nishigaharana*, PenVir –*Penicillium virgatum*, PenSim –*Penicillium simplicissimum*, PseVer –*Pseudogymnoascus verrucosus*, PenDal –*Penicillium daleae*, PleOpa –*Pleotrichocladium opacum,* SaiPod –*Saitozyma podzolica*, TruAng –*Truncatella angustata,* UmbRam –*Umbelopsis ramanniana*. Species identified to genus level are abbreviated with the first 3 alphabets of a name followed by OTU ID e.g. Cha0045 –*Chaetomium* 0045. Unidentified OTUs are presented as name OTU plus its ID e. g. OTU0046. In AM fungal ordination diagram, C1 – C3 are novel VTs found in this study (Table S3).

species compositions were different. This result was caused by different host trees – *Alnus nepalensis* in LA (*Tomentella, Alnicola, Inocybe*) and probably by *Quercus, Castanopsis* and *Engelhardia*, which sparsely occurred in the LR plots or their vicinity. Some previous studies on ECM fruitbody succession in abandoned agricultural land showed a somewhat similar pattern; macrofungi such as *Inocybe* and *Thelephora* were found as early colonizers and, subsequently, species of *Russula* appeared (Visser et al., 1995; Twieg et al., 2007; Kalucka, 2009). The reason that SR comprised a lower number of ECM sequences than did LR was probably due to the low abundance of ECM trees in SR due to their removal as timber in the past. The occurrence of late-successional ECM species is probably limited by the absence of host trees rather than by a lack of fungal propagules because seminatural forests occur in the neighborhood of abandoned land areas.

Ericoid fungi (mainly *Oidiodendron chlamydosporicum*) comprised an important portion of fungal communities in LR forests, where they were most likely associated with *Rhododendron, Daphniphyllum*, and *Symplocos*. Although SR and LR forests are difficult to distinguish in the field without knowledge of their history, soil properties seem to be good indicators, as has already been determined in different forest types (Boeraeve et al., 2018). The relationship among higher soil C content, stand age (Liang et al., 2018) and the higher proportion of ericoid plants and associated ericoid fungi (Talbot et al., 2008; Leopold, 2016) needs further investigation.

In agreement with our hypothesis, we found more similar species compositions between abandoned agricultural land and seminatural forest than between abandoned agricultural land and agricultural land (Fig. 4). The AG significantly differed from the other land use types for total fungi and AM fungi, but LR was significantly different from SA and LA only for total fungi. The reason may be a long history of habitat development and niche differentiation in LR, which was quite pronounced in the hyper-diverse total fungal community compared to AM

Table 5
Dissimilarities in total and AM fungal species composition along land use types determined by analysis of similarities (ANOSIM). AG –agricultural land, SA –short-term abandoned land, LA –long-term abandoned land, SR –short-term regenerated forest, and LR –long-term regenerated forest.

	Land use type	AG	SA	LA	SR
Total fungi	SA	**0.49**			
	LA	**0.57**	−0.02		
	SR	**0.63**	0.23	0.25	
	LR	**0.79**	**0.44**	**0.47**	−0.31
AM fungi	SA	**0.64**			
	LA	**0.7**	−0.13		
	SR	**0.95**	0.35	0.28	
	LR	**0.78**	0.33	0.21	0.18

R-value close to + 1 means that there is a dissimilarity between the group and R-value near 0 indicates no significant dissimilarity between the groups. Values in bold indicate significant dissimilarities ($P < 0.05$).

fungi. The higher abundance of some fungal taxa (Sordariales, Hypocreales, Pleosporales) in AG than in other land use types (Fig. 3A) could be attributed to the presence of easily accessible carbon sources from plant debris, livestock manure, and regular disturbances (twice a year); the presence of carbon gives an advantage to ruderal species and facilitates their dispersal. Seminatural forest communities differ from LA communities by higher abundances of hygrocyboid and clavarioid taxa. In addition, as mentioned above, LR is characterized by the presence of the members of the ECM genera *Russula* and *Lactifluus,* whereas SR possessed more *Elaphomyces*, *Inocybe, Alnicola* and *Tomentella* sequences. This indicates that a succession of fungal communities could be affected by the absence of host trees or certain soil properties because some hygrocyboid and clavarioid taxa are known to have narrow ecological niches (Jordal et al., 2016).

Based on AM fungal community analysis, AM fungi would not hinder succession because there were no significant differences between abandoned lands and seminatural forests. A remarkable observation was the differences in the abundance of Acaulosporaceae; these species were more abundant in AG. The Acaulosporaceae family is expected to be tolerant to mechanical disturbances and sensitive to other disturbances (van der Heyde et al., 2017). However, any generalization to a family level must be interpreted with caution. Out of the 12 species found, only three *Acaulospora* sp. (VT026, VT028, and VT030) occurred in all AG plots, whereas in forest plots, various *Acaulospora* sp. were dominant. The low abundance of Glomeraceae in AG was surprising because these species are considered ideal r-selected species (van der Heyde et al., 2017). Nevertheless, as reported by Avio et al. (2013) and Sharmah and Jha (2014), different strategies have been observed within this family.

4.3. Significant effect of vegetation and abiotic soil factors on fungal species composition

Both vegetation and soil factors were found to have a significant effect on the species composition of total and AM fungi. Interestingly, the patterns found in the ordination diagrams of vegetation (Fig. 2A) and fungi (Fig. S3) differed. The vegetation change was more straightforward than fungi. This could be due to differences in the specificity of the interaction between plant and fungal species, implying that the plant species present are not equally important to the fungal communities. For example, the absence of ECM host tree species has a more pronounced effect than the absence of non-mycorrhizal herb species. Also, the dispersal possibilities and competition abilities of fungal species can greatly vary, so the establishment of hyper-diverse fungal communities may require much more time than that of plants.

The higher importance of soil factors for total fungi than for AM could be explained by the more diverse ecological niches (litter, excrement, manure, soil, animals, etc.) of total fungi than of AM fungi. It was interesting to observe the higher importance of vegetation factors than soil factors in the case of AM fungi; this difference could be related to some sort of specificity to plants (Eom et al., 2000; Wang et al., 2019; Šmilauer et al., 2020). All soil parameters (C%, N%, and pH) are generally known to influence fungal species composition. The effect of nitrogen on AM species composition was probably caused by the close involvement of AM fungal species in nutrient uptake processes due to their symbiotic lifestyle. Nitrogen addition in soil has been found to decrease carbon allocation in roots (Zheng et al., 2018). Thus, nitrogen availability could lead to competition for carbon among fungal species, which could change AM species composition. Regarding the effect of soil pH on AM species composition, AM fungi differ in their pH preference, with some AM species being generalists (*Glomus intraradices*) while some are limited to a narrow pH range (An et al., 2008). Compared to these symbiotic lifestyles, the very diverse total fungal community has many combined strategies and adaptations, so this effect may not be as pronounced. However, such results are possibly context-dependent, as Moora et al. (2014) reported a more important effect of pH than of C% on AM fungal communities. In contrast, Qin et al. (2017) found that nitrogen had a larger effect on the AM communities of Moso bamboo forests than did pH. Similarly, Mueller et al. (2016) and Li et al. (2018) found no significant effects of pH on total fungal communities along the gradients of primary forest, secondary forest, pasture or agricultural land, planted forests, planted shrubs, and natural grasslands.

4.4. Effect of invasive plant species cover on fungal species composition

Contrary to previous observations based on the culture method (Balami et al., 2017) that revealed that *Ageratina adenophora* invasion caused changes in 29% of fungal species composition, we did not detect any significant effect of *A, adenophora* on fungal species composition in this study. The reason for this result could be that we did not sample the rhizospheric soil of *A. adenophora* in the current study. Nevertheless, some of the fungal OTUs and VTs were positively correlated with increasing coverage of *A. adenophora*. These fungal OTUs were mainly unidentified, so it is difficult to make any inferences from these observations. It is known that the effects of alien plants can be either direct or mediated by allelochemicals (Cantor et al., 2011). For instance, Mangla et al. (2008) reported the accumulation of pathogenic fungi (*Fusarium semitectum*) in rhizospheric soils of the invasive plant *Chromolaena odorata.* In general, we do not expect that *A. adenophora* hinders the succession of abandoned agricultural land towards seminatural forests

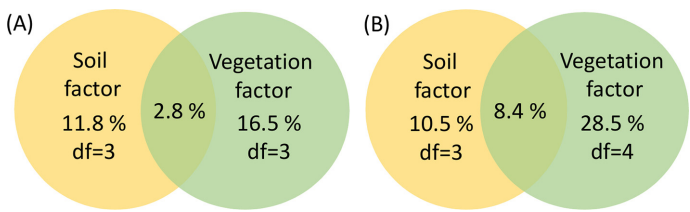

Fig. 6. Variance partitioning between vegetation factor and abiotic soil factors (A) total fungal species composition (B) AM fungal species composition.

via belowground interactions; its aboveground competitive abilities could be a much greater threat to native communities (Kumar et al., 2020).

5. Conclusion

Land use change was found to have a significant effect on the species composition of both the total fungal and AM fungal communities. Due to the differences in abiotic soil properties and vegetation properties (e.g. absence of host trees) between abandoned agricultural land and semi-natural forests, the succession of soil fungal communities are not expected to be straightforward. The LA plots were characterized by high coverage of invasive *Ageratina adenophora*, *Alnus nepalensis,* and the associated ECM fungi *Tomentella, Alnicola,* and *Inocybe*, whereas both seminatural forests were characterized by high abundances of hygrocyboid and clavarioid fungi and different ECM fungi species. LR was further characterized by a high abundance of ericoid trees and ericoid fungi. Although the presence of the invasive subshrub *A. adenophora* was not found to affect the overall fungal community composition, some unidentified OTUs/VTs were associated with the presence of the subshrub. In general, based on our results, we do not expect that fungi would hinder the succession of abandoned agricultural land. The conversion of abandoned agricultural land to that of seminatural forested land could be facilitated by the planting of local trees.

CRediT authorship contribution statement

Sujan Balami: Writing - original draft, Data curation, Software, Formal analysis. **Martina Vašutová:** Supervision, Methodology, Writing - review & editing. **Jiří Košnar:** Methodology, Formal analysis, Software, Writing - review & editing. **Ratna Karki:** Writing - review & editing, Funding acquisition. **Chiranjewee Khadka:** Conceptualization, Writing - review & editing. **Giri Tripathi:** Conceptualization, Writing - review & editing. **Pavel Cudlín:** Conceptualization, Writing - review & editing, Resources, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Ministry of Education, Youth and Sports of CR within the CzeCOS program, grant number LM2018123. We thank Padam Dahal and Saran Thami for field assistance, Gyanu Thapa Magar and Dipesh Pathak for vegetation sampling, and Jan Purkyt for the map of the study area.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2021.119181.

References

Abarenkov, K., Zirk, A., Piirmann, T., 2020. Full UNITE+INSD dataset for Fungi. Version 04.02.2020. UNITE Community 2020. https://doi.org/10.15156/BIO/786372.

Almeida, Á.M.R., Saraiva, O.F., Farias, J.R.B., Gaudêncio, C.A., Torres, E., 2001. Survival of pathogens on soybean debris under no-tillage and conventional tillage systems. Pesq. Agropec. Bras. 36, 1231–1238. https://doi.org/10.1590/S0100-204X2001001000003.

An, G.H., Miyakawa, S., Kawahara, A., Osaki, M., Ezawa, T., 2008. Community structure of arbuscular mycorrhizal fungi associated with pioneer grass species Miscanthus sinensis in acid sulfate soils: habitat segregation along pH gradients. Soil Sci. Plant Nutr. 54, 517–528. https://doi.org/10.1111/j.1747-0765.2008.00267.x.

Aronesty, E., 2013. Comparison of Sequencing Utility Programs. Open Bioinform. J. 7, 1–8. https://doi.org/10.2174/1875036201307010001.

Avio, L., Castaldini, M., Fabiani, A., Bedini, S., Sbrana, C., Turrini, A., Giovannetti, M., 2013. Impact of nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a Mediterranean agroecosystem. Soil Biol. Biochem. 67, 285–294. https://doi.org/10.1016/j.soilbio.2013.09.005.

Balami, S., Thapa, L.B., Jha, S.K., 2017. Effect of invasive *Ageratina adenophora* on species richness and composition of saprotrophic and pathogenic soil fungi. Biotr. 24, 212–219. https://doi.org/10.11598/btb.2017.24.3.702.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., Wit, P., Sanchez-Gracia, M., Ebersberger, I., Sousa, F.D., Amend, A., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, K.M., Vik, U., Veldre, V., Nilsson, R.H., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. 4, 914–919. https://doi.org/10.1111/2041-210X.12073.

Benjamin, K., Domon, G., Bouchard, A., 2005. Vegetation composition and succession of abandoned farmland: effects of ecological, historical and spatial factors. Landsc. Ecol. 20, 627–647. https://doi.org/10.1007/s10980-005-0068-2.

Boeraeve, M., Honnay, O., Mullens, N., Vandekerkhove, K., De Keersmaeker, L., Thomaes, A., Jacquemyn, H., 2018. The impact of spatial isolation and local habitat conditions on colonization of recent forest stands by ectomycorrhizal fungi. Forest Ecol. Manag. 429, 84–92. https://doi.org/10.1016/j.foreco.2018.06.043.

Bosso, L., Lacatena, F., Varlese, R., Nocerino, S., Cristinzio, G., Russo, D., 2017. Plant pathogens but not antagonists change in soil fungal communities across a land abandonment gradient in a Mediterranean landscape. Acta Oecol. 78, 1–6. https://doi.org/10.1016/j.actao.2016.11.002.

Cantor, A., Hale, A., Aaron, J., Traw, M.B., Kalisz, S., 2011. Low allelochemical concentrations detected in garlic mustard-invaded forest soils inhibit fungal growth and AMF spore germination. Biol. Invasions 13, 3015–3025. https://doi.org/10.1007/s10530-011-9986-x.

Chapagain, P.S., Rai, M.K., Paudel, B., 2018. Land-use Land Cover Change and its Pathways in Sidin VDC, Panchthar District. Nepal. Geogr. J. Nepal 11, 77–94. https://doi.org/10.3126/gjn.v11i0.19550.

Ciccolini, V., Bonari, E., Pellegrino, E., 2015. Land-use intensity and soil properties shape the composition of fungal communities in Mediterranean peaty soils drained for agricultural purposes. Biol. Fertil. Soils 51, 719–731. https://doi.org/10.1007/s00374-015-1013-4.

Cramer, V.A., Hobbs, R.J., Standish, R.J., 2008. What's new about old fields? Land abandonment and ecosystem assembly. Trends Ecol. Evol. 23, 104–112. https://doi.org/10.1016/j.tree.2007.10.005.

Dumbrell, A.J., Ashton, P.D., Aziz, N., Feng, G., Nelson, M., Dytham, C., Fitter, A.H., Helgason, T., 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytol. 190, 794–804. https://doi.org/10.1111/j.1469-8137.2010.03636.x.

Edgar, R.C., 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996. https://doi.org/10.1038/nmeth.2604.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

Eom, A.H., Hartnett, D.C., Wilson, G.W., 2000. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. Oecologia 122, 435–444. https://doi.org/10.1007/s004420050050.

Flory, S.L., Clay, K., 2010. Non-native grass invasion suppresses forest succession. Oecologia 164, 1029–1038. https://doi.org/10.1007/s00442-010-1697-y.

Gardescu, S., Marks, P.L., 2004. Colonization of old fields by trees vs. shrubs: seed dispersal and seedling establishment. J. Torrey Bot. Soc. 131, 53–68. https://doi.org/10.2307/4126928.

Goldmann, K., Schöning, I., Buscot, F., Wubet, T., 2015. Forest management type influences diversity and community composition of soil fungi across temperate forest ecosystems. Front. Microbiol. 6, 1300. https://doi.org/10.3389/fmicb.2015.01300.

Grantina, L., Seile, E., Kenigsvalde, K., Kasparinskis, R., Tabors, G., Nikolajeva, V., Jungerius, P., Muiznieks, I., 2011. The influence of the land use on abundance and diversity of soil fungi: comparison of conventional and molecular methods of analysis. Env. Exp. Biol. 9, 9–21.

Hanif, M., Guo, Z., Moniruzzaman, M., He, D., Yu, Q., Rao, X., Liu, S., Tan, X., Shen, W., 2019. Plant Taxonomic Diversity Better Explains Soil Fungal and Bacterial Diversity than Functional Diversity in Restored Forest Ecosystems. Plants 8, 479. https://doi.org/10.3390/plants8110479.

Hannula, S.E., Morriën, E., de Hollander, M., Van Der Putten, W.H., van Veen, J.A., De Boer, W., 2017. Shifts in rhizosphere fungal community during secondary succession following abandonment from agriculture. ISME J. 11, 2294–2304. https://doi.org/10.1038/ismej.2017.90.

Holah, J.C., Alexander, H.M., 1999. Soil pathogenic fungi have the potential to affect the co-existence of two tallgrass prairie species. J. Ecol. 87, 598–608. https://doi.org/10.1046/j.1365-2745.1999.00383.x.

Holah, J.C., Wilson, M.V., Hansen, E.M., 1997. Impacts of a native root-rotting pathogen on successional development of old-growth Douglas fir forests. Oecologia 111, 429–433. https://doi.org/10.1007/s004420050255.

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x.

Jiang, Y., Liang, Y., Li, C., Wang, F., Sui, Y., Suvannang, N., Zhou, J., Sun, B., 2016. Crop rotations alter bacterial and fungal diversity in paddy soils across East Asia. Soil Biol. Biochem. 95, 250–261. https://doi.org/10.1016/j.soilbio.2016.01.007.
Johnson, N.C., Gehring, C., Jansa, J., 2016. Mycorrhizal mediation of soil: fertility, structure, and carbon storage. Elsevier, Amsterdam.
Jordal, J.B., Evju, M., Gaarder, G., 2016. Habitat specificity of selected grassland fungi in Norway. Agarica 37, 5–32.
Kalucka, I., 2009. Macrofungi in the secondary succession on the abandoned farmland near the Białowieża old-growth forest. Monogr. Bot. 99, 1–156. https://doi.org/10.5586/mb.2009.001.
Kardol, P., Martijn Bezemer, T., van Der Putten, W.H., 2006. Temporal variation in plant–soil feedback controls succession. Ecol. Lett. 9, 1080–1088. https://doi.org/10.1111/j.1461-0248.2006.00953.x.
Katoh, K., Asimenos, G., Toh, H., 2009. Multiple alignment of DNA sequences with MAFFT. Methods Mol. Biol. 537, 39–64. https://doi.org/10.1007/978-1-59745-251-9_3.
Kuemmerle, T., Olofsson, P., Chaskovskyy, O., Baumann, M., Ostapowicz, K., Woodcock, C.E., Houghton, R.A., Hostert, P., Keeton, W.S., Radeloff, V.C., 2011. Post-Soviet farmland abandonment, forest recovery, and carbon sequestration in western Ukraine. Glob. Chang. Biol. 17, 1335–1349. https://doi.org/10.1111/j.1365-2486.2010.02333.x.
Kumar, M., Verma, A.K., Garkoti, S.C., 2020. Lantana camara and *Ageratina adenophora* invasion alter the understory species composition and diversity of chir pine forest in central Himalaya. India. Acta Oecol. 109, 103642 https://doi.org/10.1016/j.actao.2020.103642.
Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol. Biochem. 40, 2407–2415. https://doi.org/10.1016/j.soilbio.2008.05.021.
Lee, J., Lee, S., Young, J.P.W., 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol. Ecol. 65, 339–349. https://doi.org/10.1111/j.1574-6941.2008.00531.x.
Leopold, D.R., 2016. Ericoid fungal diversity: challenges and opportunities for mycorrhizal research. Fungal Ecol. 24, 114–123. https://doi.org/10.1016/j.funeco.2016.07.004.
Li, J.J., Zheng, Y.M., Yan, J.X., Li, H.J., He, J.Z., 2013. Succession of plant and soil microbial communities with restoration of abandoned land in the Loess Plateau. China. J. Soil Sediment. 13, 760–769. https://doi.org/10.1007/s11368-013-0652-z.
Li, L.F., Li, T., Zhao, Z.W., 2007. Differences of arbuscular mycorrhizal fungal diversity and community between a cultivated land, an old field, and a never-cultivated field in a hot and arid ecosystem of southwest China. Mycorrhiza 17, 655–665. https://doi.org/10.1007/s00572-007-0143-4.
Li, S., Li, X., 2017. Global understanding of farmland abandonment: A review and prospects. J. Geogr. Sci. 27, 1123–1150. https://doi.org/10.1007/s11442-017-1426-0.
Li, S., Wu, F., 2018. Diversity and co-occurrence patterns of soil bacterial and fungal communities in seven intercropping systems. Front. Microbiol. 9, 1521. https://doi.org/10.3389/fmicb.2018.01521.
Liang, X., Liu, S., Wang, H., Wang, J., 2018. Variation of carbon and nitrogen stoichiometry along a chronosequence of natural temperate forest in northeastern China. J. Plant Ecol. 11, 339–350. https://doi.org/10.1093/jpe/rtx008.
Liu, J., Sui, Y., Yu, Z., Shi, Y., Chu, H., Jin, J., Liu, X., Wang, G., 2015. Soil carbon content drives the biogeographical distribution of fungal communities in the black soil zone of northeast China. Soil Biol. Biochem. 83, 29–39. https://doi.org/10.1016/j.soilbio.2015.01.009.
Liu, Y., Zhu, G., Hai, X., Li, J., Shangguan, Z., Peng, C., Deng, L., 2020. Long-term forest succession improves plant diversity and soil quality but not significantly increase soil microbial diversity: Evidence from the Loess Plateau. Ecol. Eng. 142, 105631 https://doi.org/10.1016/j.ecoleng.2019.105631.
MacDonald, D., Crabtree, J.R., Wiesinger, G., Dax, T., Stamou, N., Fleury, P., Lazpita, G., Gibon, A., 2000. Agricultural abandonment in mountain areas of Europe: environmental consequences and policy response. J. Environ. Manage. 59, 47–69. https://doi.org/10.1006/jema.1999.0335.
Mangla, S., Inderjit, Callaway, R.M., 2008. Exotic invasive plant accumulates native soil pathogens which inhibit native plants. J. Ecol. 96, 58–67. https://doi.org/10.1111/j.1365-2745.2007.01312.x.
Milne, I., Lindner, D., Bayer, M., Husmeier, D., McGuire, G., Marshall, D.F., Wright, F., 2009. TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. Bioinformatics 25, 126–127. https://doi.org/10.1093/bioinformatics/btn575.
Moora, M., Davison, J., Öpik, M., Metsis, M., Saks, U., Jairus, T., Vasar, M., Zobel, M., 2014. Anthropogenic land-use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. FEMS Microbiol. Ecol. 90, 609–621. https://doi.org/10.1111/1574-6941.12420.
Mueller, R.C., Rodrigues, J.L.M., Nüsslein, K., Bohannan, B.J.M., 2016. Land-use change in the Amazon rain forest favours generalist fungi. Funct Ecol. 30, 1845–1853. https://doi.org/10.1111/1365-2435.12651.
Mummey, D.L., Rillig, M.C., 2006. The invasive plant species Centaurea maculosa alters arbuscular mycorrhizal fungal communities in the field. Plant Soil 288, 81–90. https://doi.org/10.1007/s11104-006-9091-6.
Navarro, L.M., Pereira, H.M., 2012. Rewilding abandoned landscapes in Europe. Ecosystems 15, 900–912. https://doi.org/10.1007/s10021-012-9558-7.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006.
Neuenkamp, L., Zobel, M., Lind, E., Gerz, M., Moora, M., 2019. Arbuscular mycorrhizal fungal community composition determines the competitive response of two grassland forbs. PloS one 14, e0219527. https://doi.org/10.1371/journal.pone.0219527.
Niu, H.B., Liu, W.X., Wan, B.L., 2007. An invasive aster (*Ageratina adenophora*) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. Plant Soil 294, 73–85. https://doi.org/10.1007/s11104-007-9230-8.
Niwaxu, A., Shackleton, C.M., 2019. The availability of non-timber forest products under forest succession on abandoned fields along the Wild Coast. South Africa. Forests 10, 1093. https://doi.org/10.3390/f10121093.
Ojha, H.R., Shrestha, K.K., Subedi, Y.R., Shah, R., Nuberg, I., Heyojoo, B., Cedamon, E., Rigg, J., Tamang, S., Paudel, K.P., Malla, Y., McManus, P., 2017. Agricultural land underutilisation in the hills of Nepal: Investigating socio-environmental pathways of change. J. Rural. Stud. 53, 156–172. https://doi.org/10.1016/j.jrurstud.2017.05.012.
Peay, K.G., 2014. Back to the future: Natural history and the way forward in modern fungal ecology. Fungal Ecol. 12, 4–9. https://doi.org/10.1016/j.funeco.2014.06.001.
Piché, N., Kelting, D.L., 2015. Recovery of soil productivity with forest succession on abandoned agricultural land. Restor. Ecol. 23, 645–654. https://doi.org/10.1111/rec.12241.
Qin, H., Chen, J., Wu, Q., Niu, L., Li, Y., Liang, C., Xu, Q., 2017. Intensive management decreases soil aggregation and changes the abundance and community compositions of arbuscular mycorrhizal fungi in Moso bamboo (*Phyllostachys pubescens*) forests. Forest Ecol. Manag. 400, 246–255. https://doi.org/10.1016/j.foreco.2017.06.003.
R Core Team, 2019. R: A language and environment for statistical computing. R Found Stat Comput Vienna, Austria URL http://wwwR-project.org/.
Ren, C., Wang, T., Xu, Y., Deng, J., Zhao, F., Yang, G., Han, X., Feng, Y., Ren, G., 2018. Differential soil microbial community responses to the linkage of soil organic carbon fractions with respiration across land-use changes. For. Ecol. Manag. 409, 170–178. https://doi.org/10.1016/j.foreco.2017.11.011.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahe, F., 2016. VSEARCH: A versatile open source tool for metagenomics. PeerJ. 4, e2584 https://doi.org/10.7717/peerj.2584.
Sharmah, D., Jha, D.K., 2014. Diversity of Arbuscular Mycorrhizal Fungi in Disturbed and Undisturbed Forests of Karbi Anglong Hills of Assam. India. Agric. Res. 3, 229–238. https://doi.org/10.1007/s40003-014-0110-1.
Simon, L., Lalonde, M., Bruns, T.D., 1992. Specific amplification of 18S fungal ribosomal genes from vesicular- arbuscular endomycorrhizal fungi colonizing roots. Appl. Environ. Microbiol. 58, 291–295.
Šmilauer, P., Šmilauerová, M., Kotilínek, M., Košnar, J., 2020. Foraging speed and precision of arbuscular mycorrhizal fungi under field conditions: An experimental approach. Mol. Ecol. 29, 1574–1587. https://doi.org/10.1111/mec.15425.
Snoeck, D., Abolo, D., Jagoret, P., 2010. Temporal changes in VAM fungi in the cocoa agroforestry systems of central Cameroon. Agrofor. Syst. 78, 323–328. https://doi.org/10.1007/s10457-009-9254-6.
Talbot, J.M., Allison, S.D., Treseder, K.K., 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Funct. Ecol. 22, 955–963. https://doi.org/10.1111/j.1365-2435.2008.01402.x.
Tedersoo, L., Bahram, M., Zobel, M., 2020. How mycorrhizal associations drive plant population and community biology. Science 367, 6480. https://doi.org/10.1126/science.aba1223.
ter Braak, C.J.F., Schaffers, A.P., 2004. Co-correspondence analysis: a new ordination method to relate two community compositions. Ecology 85, 834–846. https://doi.org/10.1890/03-0021.
ter Braak, C.J.F., Šmilauer, P., 2012. CANOCO reference manual and user's guide: software for ordination (version 5.0). Microcomputer Power, Ithaca.
Truong, C., Mujic, A.B., Healy, R., Kuhar, F., Furci, G., Torres, D., Niskanen, T., Sandoval-Leiva, P.A., Fernandez, N., Escobar, J.M., Moretto, A., Palfner, G., Pfister, D., Nouhra, E., Swenie, R., Sanchez-Garcia, M., Matheny, P.B., Smith, M.E., 2017. How to know the fungi: combining field inventories and DNA-barcoding to document fungal diversity. New. Phytol. 214, 913–919. https://doi.org/10.1111/nph.14509.
Twieg, B.D., Durall, D.M., Simard, S.W., 2007. Ectomycorrhizal fungal succession in mixed temperate forests. New Phytol. 176, 437–447. https://doi.org/10.1111/j.1469-8137.2007.02173.x.
Ustaoglu, E., Collier, M.J., 2018. Farmland abandonment in Europe: an overview of drivers, consequences, and assessment of the sustainability implications. Environ. Rev. 26, 396–416. https://doi.org/10.1139/er-2018-0001.
van der Heijden, M.G., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11, 296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x.
van der Heyde, M., Ohsowski, B., Abbott, L.K., Hart, M., 2017. Arbuscular mycorrhizal fungus responses to disturbance are context-dependent. Mycorrhiza 27, 431–440. https://doi.org/10.1007/s00572-016-0759-3.
Vasar, M., Andreson, R., Davison, J., Jairus, T., Moora, M., Remm, M., Young, J.P.W., Zobel, M., Öpik, M., 2017. Increased sequencing depth does not increase captured diversity of arbuscular mycorrhizal fungi. Mycorrhiza 27, 761–773. https://doi.org/10.1007/s00572-017-0791-y.
Větrovský, T., Baldrian, P., Morais, D., 2018. SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. Bioinformatics 34, 2292–2294. https://doi.org/10.1093/bioinformatics/bty071.
Visser, S., 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytol. 129, 389–401. https://doi.org/10.1111/j.1469-8137.1995.tb04309.x.
Vogelsang, K.M., Reynolds, H.L., Bever, J.D., 2006. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. New Phytol. 172, 554–562. https://doi.org/10.1111/j.1469-8137.2006.01854.x.

Wang, Z., Jiang, Y., Deane, D.C., He, F., Shu, W., Liu, Y., 2019. Effects of host phylogeny, habitat and spatial proximity on host specificity and diversity of pathogenic and mycorrhizal fungi in a subtropical forest. New Phytol. 223, 462–474. https://doi.org/10.1111/nph.15786.

Yang, Y., Dou, Y., Huang, Y., An, S., 2017. Links between soil fungal diversity and plant and soil properties on the Loess Plateau. Front. Microbiol. 8, 2198. https://doi.org/10.3389/fmicb.2017.02198.

Zhang, K., Cheng, X., Shu, X., Liu, Y., Zhang, Q., 2018. Linking soil bacterial and fungal communities to vegetation succession following agricultural abandonment. Plant Soil 431, 19–36. https://doi.org/10.1007/s11104-018-3743-1.

Zheng, Z., Ma, P., Li, J., Ren, L., Bai, W., Tian, Q., Sun, W., Zhang, W.H., 2018. Arbuscular mycorrhizal fungal communities associated with two dominant species differ in their responses to long-term nitrogen addition in temperate grasslands. Funct. Ecol. 32, 1575–1588. https://doi.org/10.1111/1365-2435.13081.

Zobel, M., Öpik, M., 2014. Plant and arbuscular mycorrhizal fungal (AMF) communities–which drives which? J. Veg. Sci. 25, 1133–1140. https://doi.org/10.1111/jvs.12191.

Chapter III

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

Sujan Balami, Martina Vašutová, Vijay Chaudhary, Pavel Cudlín

Manuscript submitted as: Balami S, Vašutová M, Chaudhary V, Cudlín P (2021) Land use effect on root-associated fungi of *Alnus nepalensis* and *Schima wallichii*. Mycorrhiza

Abstract

Abandoned agricultural land can be a potential site for natural forest restoration. *Alnus nepalensis* and *Schima wallichii* are economically important native trees that occurred in short-term (SA), long-term (LA) abandoned land and seminatural regenerated forest (RF) could facilitate the succession. We studied the diversity and species composition of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi including total fungi associated with their fine roots, to determine how land use change affects their key symbionts important for nutrient uptake and resilience. Additionally, ECM morphotypes were examined. The results did not show any differences in the diversity of AM and total fungi in either tree species among land use types. Similarly, no difference in ECM diversity was found in *A. nepalensis*. However, land use has a significant effect on species composition, which is more pronounced than tree species identity. SA was the most different in terms of AM species composition, and RF in total fungal composition. The AM taxon *Acaulosporaceae* showed a lower relative abundance in SA than in LA and RF. The relative abundance of *Archeosporaceae* followed a different trend between trees (in *A. nepalensis* LA > SA and in *S. wallichii* SA > LA). Nine ECM morphotypes and 31 ECM operational taxonomic units (OTUs) were found in the root tips of *A. nepalensis*. *Cortinarius* sp. was significantly more abundant in RF than in the other land use types, whereas *Alnicola*, *Tomentella* and *Russula* preferred young stages. Differences between root-associated fungal communities in abandoned agricultural land and seminatural forests do not seem to hinder the proper nutrition and regeneration of the studied trees.

Keywords: Arbuscular fungi, Ectomycorrhiza, *Alnus nepalensis*, *Schima wallichii*, Short-term and long-term abandoned soil

Pages 75-116 (unpublished part)

Chapter IV

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

Sujan Balami, Martina Vašutová

Manuscript

Abstract

Three ectomycorrhizal species, *Alnicola alni-nepalensis*, *Cortinarius nepalensis* and *Lactarius alni-nepalensis*, from the middle mountain region of central Nepal are described as new to science. All three species have small basidiomata and grow under the canopy of *Alnus nepalensis* on abandoned agricultural land and in regenerated forests. A description of macroscopic and microscopic characters together with ITS and RPB2 sequences and a comparison with similar taxa are provided.

Keywords- *Alnus nepalensis*, Hymenogastraceae, Cortinariaceae, Russulaceae, ITS and RPB2 rDNA sequences

Pages 119-160 (unpublished part)

Summary

In a time of increasing concern over the impacts of global land use change on soil microbial communities (Liu et al. 2022; Romdhane et al. 2022; Stephanou et al. 2021), this thesis expands our understanding of how changes in land use affect soil fungal communities. Since soil fungi play a key role in e.g. biogeochemical cycling, plant nutrition and terrestrial productivity (Vassileva et al. 2022; Powell and Rillig 2018), the response of soil fungi to any land use change is of great ecological significance. This thesis presents a first critical review of soil fungal response to land use change based on results from studies worldwide (**Chapter I**). Two of the chapters in this thesis, **Chapters II** and **III**, are among the few studies (e.g. Zhang et al. 2018; Hannula et al. 2017) on soil and root-associated fungal response to abandonment of agricultural land. At the same time, this is the first use of environmental DNA sequencing of soil and root tip fungal communities in Nepal, as well as the first analysis of the *Schima wallichii* root fungal community. The species described in **Chapter IV** are the first ectomycorrhizal species for science to be associated with *Alnus nepalensis*.

The review results in **Chapter I** concerning the global distribution of relevant studies shows that an evaluation of the fungal response to land use change is missing in many important parts of the world. The study has also identified poor consensus on land use categorization in global studies, most of which depend on the local context. Accordingly, an implementation of standard methods to categorize land use types appears to be less feasible because the local context varies widely. To avoid inconsistencies in the results concerning land use category, enough explanatory variables should be documented during data collection and then used to make a constrained analysis. Additionally, discrepancies in soil and root sampling strategies are found in the studies. In general, the summary of global results shows that a change in land use results in a change in fungal species composition and that increasing intensification of land use reduces quantitative soil fungal parameters. However, equivocal results are also evident in cases where land use intensification does not necessarily reduce quantitative fungal parameters. This could be related to several factors such as a small number of replicates per land use type, variation in local conditions

(climate, topography, vegetation, soil, etc.), limitations of various methods used to assess fungal parameters, varying reactions of fungal guilds, and exclusion of a high proportion of unidentified taxa from analysis. Therefore, most of the findings demonstrate that changes in the fungal community composition caused by land use change are more pronounced than changes in quantitative parameters, which is difficult to evaluate without detailed knowledge of the context.

Considering the dependence of soil fungal response on the land use context, in **Chapter II,** the effect of abandonment of agricultural land on the soil fungal community in mountainous areas of central Nepal was investigated. Two different stages of agricultural land abandonment (short- and long-term abandoned land) were evaluated with respect to agricultural land and seminatural forests (short- and long-term regenerated forests). A significant variation in AM and total fungal community composition among land use types was revealed. Differences were related to the variation in abiotic soil properties and vegetation. The vegetation factor was more important than the abiotic soil factors in determining the variation in the soil fungal community composition. The abandoned land differed more from the agricultural land than seminatural forests in terms of AM and total fungal composition. Compared to seminatural forests, abandoned land showed some differences in total fungal composition, which is notable in the case of ECM and ericoid fungi. Both long-term abandoned land and seminatural forests had abundant ECM fungal sequences, but differed in species composition, and abandoned land also had fewer ericoid fungal sequences than seminatural forests. In general, the results of **Chapter II** indicate that quantitative fungal parameters do not change with variation in land use types, but there is a significant difference in species composition, which is consistent with the review results of **Chapter I**. Together, the data on vegetation and fungi suggested that it is unlikely that fungi will hinder the succession of aboveground vegetation on abandoned agricultural land. It seems difficult for the soil fungal community to reach the stage of long-term regenerated forests due to differences in vegetation (dominance of ericoid trees) and acidic soil

with a high concentration of nitrogen and carbon. Nevertheless, the fungal succession can be improved if long-term abandoned fields are planted with native ericoid tree species such as *Symplocos* spp., *Daphniphyllum himalayanse* and *Rhododendron arboreum*. Furthermore, although we did not find any influence of the invasive plant *Ageratina adenophora* on the soil fungal community, the removal of *Ageratina* from abandoned land may be crucial to provide space for native ericoid trees.

To disentangle the complex processes in plant and fungal communities, **Chapter III** focuses on an analysis of root-associated fungi of two tree species accompanying succession, *Alnus nepalensis* and *Schima wallichii*. Interestingly, the effect of land use was more pronounced than that of tree identity in both AM and total fungal composition. The AM fungal composition of short-term abandoned land differed most strongly from that of long-term abandoned and seminatural forests, while the total fungal composition differed most strongly in the case of seminatural forests. In the case of *A. nepalensis*, also the ECM community showed a low diversity, just like that of other *Alnus* species (Tedersoo et al. 2009). Although ECM diversity and community composition did not differ between land use types in *A. nepalensis*, some taxa prefer young stages (e.g. *Tomentella*), while others seminatural forests (e.g. *Cortinarius*, *Alnicola*). Consequently, the findings of **Chapter III** infer that previous land use history influences the root-associated fungal community in the studied tree species. The total fungal community is more affected than the AM fungal community. However, the differences in both AM and total fungal communities are difficult to interpret due to a lack of knowledge on the ecology of the respective taxa. For the same reason, it is difficult to draw inferences about the adaptability of *A. nepalensis* and *S. wallichii* to changes in land use. However, it is assumed that differences in abundance of some ECM taxa in *A. nepalensis* between land use types provide the tree with a physiological adaptability to changing environmental conditions.

The large number of unidentified taxa in soil and root-associated fungal analysis in **Chapters II** and **III** emphasizes the importance of basic

taxonomic studies as presented in **Chapter IV**. Many species new to science can be associated with not well-studied tree species as in the case of *Alnus nepalensis*. **Chapter IV** also emphasizes the need for improvements in molecular methods for species delimitation, as well as difficulties in the phylogenetic resolution of *Cortinarius* species. As *A. nepalensis* plays a significant ecological and economic role in different parts of southeast Asia, from southwest China to part of the western Himalayan region (India and Nepal), knowledge about these ectomycorrhizal fungal would be important if we decided to cultivate or plant *A. nepalensis*, along with cooccurring ECM and ericoid trees, to support succession in abandoned areas.

In summary, it would be interesting to follow the permanent plots of this study in future research to see the actual trajectory of vegetation, soil properties and fungal succession. Also studies of root-associated fungal communities of other, mainly ericoid and ectomycorrhizal trees species can be performed to evaluate the effect of land use change. Since abandoned areas are in most cases colonized by invasive plants, it would be necessary to evaluate abandoned areas of subtropical and tropical regions of Nepal where the more noxious invasive plants *Chromolaena odorata*, *Lantana camara* and *Mikania micrantha* are distributed (Shrestha 2016). A more detailed study of the species associated with long-term regenerated forests (*Hygrocybe*, clavarioid fungi) would help to assess their ecological requirements and possible indication value. Furthermore, the role of ericoid shrubs and associated fungi in the vegetation and fungal community succession, which has so far been studied mainly in boreal forests, is another interesting aspect to study.

Overall, this study showed the impacts of land use change on soil and root-associated fungal communities based on global results and abandoned agricultural land use change in the temperate region of central Nepal. However, a more detailed understanding of the ecological consequences is limited by the poor description of fungal species (Hawksworth and Lücking 2017) and their functional roles. Therefore, this study emphasizes

the need to describe many new fungal species and to study their ecology. The classical taxonomy and Sanger sequencing are highly recommended to strengthen the fungal species data. The ecology of newly described species can be assessed by a combination of long-term observation, manipulative experiments, and the use of environmental sequencing data from other projects. Because a large portion of taxa are excluded from functional guild analysis due to their undefined functional role in public databases like FUNGuild (Nguyen et al. 2016), it is necessary to describe functional guilds of as many taxa as possible in such databases. Studies should consider many underexplored areas of tropical, subtropical, and temperate regions of the world, such as Asia Pacific, Southasia, Africa, etc. including biodiversity hotspots and different floristic zones. Thus, data on fungal taxonomy and traits will be crucial to understand the complex response of the fungal community to anthropogenic activities and its ecological consequences.

General bibliography

Adhikari MK (2015) Mushroom of Nepal. *Lalitpur, Nepal*: *Shree Press*, pp 236.

Adhikari MK (2016) Revised checklist to the mycotaxa proposed from Nepal. J Pl Reso 38:1–11.

Austin KG, Schwantes A, Gu Y, Kasibhatla PS (2019) What causes deforestation in Indonesia? Environ Res Lett 14:024007.

Avis PG, Mueller GM, Lussenhop J (2008) Ectomycorrhizal fungal communities in two North American oak forests respond to nitrogen addition. New Phytol 179:472–483.

Awad A, Majcherczyk A, Schall P, Schröter K, Schöning I, Schrumpf M, Pena R (2019) Ectomycorrhizal and saprotrophic soil fungal biomass are driven by different factors and vary among broadleaf and coniferous temperate forests. Soil Biol Biochem 131:9–18.

Bagchi R, Gallery RE, Gripenberg S, Gurr SJ, Narayan L, Addis CE, Lewis OT (2014) Pathogens and insect herbivores drive rainforest plant diversity and composition. Nature 506:85–88.

Bao Z, Matsushita Y, Morimoto S, Hoshino YT, Suzuki C, Nagaoka K, Tsushima S (2013) Decrease in fungal biodiversity along an available phosphorous gradient in arable Andosol soils in Japan. Can J Microbiol 59:368–373.

Baral K, Giri A, Shah PK, Kemmelmeier K, Stürmer SL, Gyawali S, Raut JK (2021) Diversity of arbuscular mycorrhizal fungi (Glomeromycota) in adjacent areas of different land use in Nepal. GSC Biol Pharm Sci 15:141–150.

Barker JS, Simard SW, Jones MD, Durall DM (2013) Ectomycorrhizal fungal community assembly on regenerating Douglas-fir after wildfire and clearcut harvesting. Oecologia 172:1179–1189.

Bearden BN, Petersen L (2000) Influence of arbuscular mycorrhizal fungi on soil structure and aggregate stability of a vertisol. Plant Soil 218:173–183.

Bedini S, Avio L, Argese E, Giovannetti M (2007) Effects of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein. Agric Ecosyst Environ 120:463–466.

Bell SM, Barriocanal C, Terrer C, Rosell-Melé A (2020) Management opportunities for soil carbon sequestration following agricultural land abandonment. Environ Sci Policy 108:104–111.

Benayas JR, Martins A, Nicolau JM, Schulz JJ (2007) Abandonment of agricultural land: an overview of drivers and consequences. CAB Rev: Perspect Agric Vet Sci Nutr Nat Resour 2:1–14.

Benjamin K, Domon G, Bouchard A (2005) Vegetation composition and succession of abandoned farmland: effects of ecological, historical and spatial factors. Landsc Ecol 20:627–647.

Blackwell M (2011) The Fungi: 1, 2, 3… 51 million species? Am J Bot 98:426–438.

Boddy L (2000) Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiol Ecol 31:185–194.

Briassoulis H (2020) Analysis of land use change: theoretical and modelling approaches. *Morgantown, West Virginia, USA*: *WVU Research Repository 2020*, 15–27.

Brinkmann N, Schneider D, Sahner J, Ballauff J, Edy N, Barus H, Polle A (2019) Intensive tropical land use massively shifts soil fungal communities. Sci Rep 9:1–11.

Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2008) Root exudates regulate soil fungal community composition and diversity. Appl Environ Microbiol 74:738–744.

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77.

Brundrett MC, Ashwath N (2013) Glomeromycotan mycorrhizal fungi from tropical Australia III Measuring diversity in natural and disturbed habitats. Plant Soil 370:419–433.

Brundrett MC, Tedersoo L (2018) Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol 220:1108–1115.

Bui A, Orr D, Lepori-Bui M, Konicek K, Young HS, Moeller HV (2020) Soil fungal community composition and functional similarity shift across distinct climatic conditions. FEMS Microbiol Ecol 96:fiaa193.

Cairney JW, Bastias BA (2007) Influences of fire on forest soil fungal communities. Can J For Res 37:207–215.

Cairney JW, Meharg AA (2003) Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. Eur J Soil Sci 54:735–740.

Camenzind T, Hempel S, Homeier J, Horn S, Velescu A, Wilcke W, Rillig MC (2014) Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. Glob Chang Biol 20:3646–3659.

Cao C, Huang J, Ge L, Li T, Bu ZJ, Wang S, Wang M (2022) Does shift in vegetation abundance after nitrogen and phosphorus additions play a key role in regulating fungal community structure in a northern Peatland? Front Microbiol 13:920382.

Carrino-Kyker SR, Kluber LA, Petersen SM, Coyle KP, Hewins CR, DeForest JL, Burke DJ (2016) Mycorrhizal fungal communities respond to experimental elevation of soil pH and P availability in temperate hardwood forests. FEMS Microbiol Ecol 92:fiw024.

Carroll GC, Wicklow DT (1992) The Fungal Community: Its organization and role in the ecosystem. *Boca Raton, Florida, USA*: *CRC Press*, 403–425.

Castillo CP, Jacobs-Crisioni C, Diogo V, Lavalle C (2021) Modelling agricultural land abandonment in a fine spatial resolution multi-level land-use model: An application for the EU. Environ Model Softw 136:104946.

Cerqueira AES, Silva TH, Nunes ACS, Nunes DD, Lobato LC, Veloso TGR, Silva CC (2018) Amazon basin pasture soils reveal susceptibility to phytopathogens and lower fungal community dissimilarity than forest. Appl Soil Ecol 131:1–11.

Chaudhary S, Wang Y, Dixit AM, Khanal NR, Xu P, Fu B, Li M (2019) Spatiotemporal degradation of abandoned farmland and associated eco-environmental risks in the high mountains of the Nepalese Himalayas. Land 9:1–19.

Chaudhary S, Wang Y, Dixit AM, Khanal NR, Xu P, Fu B, Yan K, Liu Q, Lu Y, Li M (2020) A synopsis of farmland abandonment and its driving factors in Nepal. Land 9:84.

Chen C, Chen HY, Chen X, Huang Z (2019) Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. Nat Commun 10:1–10.

Chen YL, Xu TL, Veresoglou SD, Hu HW, Hao ZP, Hu YJ, Chen BD (2017) Plant diversity represents the prevalent determinant of soil fungal community structure across temperate grasslands in northern China. Soil Biol Biochem 110:12–21.

Christensen M, Bhattarai S, Devkota S, Larsen HO (2008) Collection and use of wild edible fungi in Nepal. Econ Bot 62:12–23.

Chu H, Wang, Zhang H, Li Y, Wang Z, Dai C, Tang DM (2021) Inoculation with ectomycorrhizal fungi and dark septate endophytes contributes to the resistance of *Pinus* spp. to pine wilt disease. Front Microbiol 12:687304.

Cline LC, Zak DR (2014) Dispersal limitation structures fungal community assembly in a long-term glacial chronosequence. Environ Microbiol 16:1538–1548.

Correia M, Espelta JM, Morillo JA, Pino J, Rodríguez-Echeverría S (2021) Land-use history alters the diversity, community composition and interaction networks of ectomycorrhizal fungi in beech forests. J Ecol 109:2856–2870.

Crowther TW, Boddy L, Jones TH (2012) Functional and ecological consequences of saprotrophic fungus–grazer interactions. ISME J 6:1992–2001.

Dahal GR, Pandit BH, Shah R (2020) Abandoned agricultural land and its reutilisation by adoption of agroforestry: A case study from Kaski and Parbat districts of Nepal. J Forest Livelihood 19:1–16.

Devkota S, Aryal HP (2020) Wild mushrooms of Nepal. *In*: Siwakoti M, Jha PK, Rajbhandary S, Rai SK, *eds.* Plant diversity in Nepal. *Kathmandu, Nepal*: *Botanical Society of Nepal*, 41–54.

Dhital MR (2015) Physiography of Nepal. *In*: Dhital MR, *eds*. Geology of the Nepal Himalaya. *Cham, Switzerland*: *Springer*, 23–33.

Dighton J (2018) Fungi in ecosystem processes. 2nd edn. *Boca Raton, Florida, USA*: *CRC Press*, pp 434.

Dix NJ, Webster J (1995) Fungal ecology. 1st edn. *Dordrecht, Netherland*: *Springer*, pp 549.

Dobson A, Crawley M (1994) Pathogens and the structure of plant communities. Trends Ecol Evol 9:393–398.

Eisenhauer N, Lanoue A, Strecker T, Scheu S, Steinauer K, Thakur MP, Mommer L (2017) Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. Sci Rep 7:1–8.

Entwistle EM, Zak DR, Argiroff WA (2018) Anthropogenic N deposition increases soil C storage by reducing the relative abundance of lignolytic fungi. Ecol Monogr 88: 225–244.

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104:1263–1280.

FAO 2016 State of the World's Forests (2016) Forests and agriculture: land-use challenges and opportunities. *Rome, Italy*: *FAO*, pp 107.

Farrell HL, Léger A, Breed MF, Gornish ES (2020) Restoration, soil organisms, and soil processes: emerging approaches. Restor Ecol 28:S307–S310.

Favre-Godal Q, Gourguillon L, Lordel-Madeleine S, Gindro K, Choisy P (2020) Orchids and their mycorrhizal fungi: an insufficiently explored relationship. Mycorrhiza 30:5–22.

Fayet CM, Reilly KH, van Ham C, Verburg PH (2022) What is the future of abandoned agricultural lands? A systematic review of alternative trajectories in Europe. Land Use Policy 112:105833.

Fracetto GG, Azevedo LC, Fracetto FJ, Andreote FD, Lambais MR, Pfenning LH (2013) Impact of Amazon land use on the community of soil fungi. Sci Agric 70:59–67.

Francioli D, van Rijssel SQ, van Ruijven J, Termorshuizen AJ, Cotton TE, Dumbrell AJ, Mommer L (2021) Plant functional group drives the community structure of saprophytic fungi in a grassland biodiversity experiment. Plant Soil 461:91–105.

Frankland JC (1992) Mechanisms in fungal succession. *In*: Caroll GC, Wicklow DT, *eds.* The Fungal Community: its organization and role in the ecosystem. *New York, USA*: *Marcel Dekker*, 383–340.

FRTC (2022) National Land Cover Monitoring System of Nepal. *Kathmandu, Nepal*: *Forest Research and Training Centre (FRTC)*, 15–22.

Gao C, Zhang YU, Shi NN, Zheng Y, Chen L, Wubet T, Guo LD (2015) Community assembly of ectomycorrhizal fungi along a subtropical secondary forest succession. New Phytol 205:771–785.

Gavito ME, Pérez-Castillo D, González-Monterrubio CF, Vieyra-Hernández T, Martínez-Trujillo M (2008) High compatibility between arbuscular mycorrhizal fungal

communities and seedlings of different land use types in a tropical dry ecosystem. Mycorrhiza 19:47–60.

Genre A, Lanfranco L, Perotto S, Bonfante P (2020) Unique and common traits in mycorrhizal symbioses. Nat Rev Microbiol 18:649–660.

González-Cortés JC, Vega-Fraga M, Varela-Fregoso L, Martínez-Trujillo M, Carreón-Abud Y, Gavito ME (2012) Arbuscular mycorrhizal fungal (AMF) communities and land use change: the conversion of temperate forests to avocado plantations and maize fields in central Mexico. Fungal Ecol 5:16–23.

Grelet G, Martino E, Dickie IA, Tajuddin R, Artz R (2016) Ecology of ericoid mycorrhizal fungi: what insight have we gained with molecular tools and what's missing. *In*: Martin F, *eds*. Molecular Mycorrhizal Symbiosis. *New York, USA*: *John Wiley and Sons*, 405–419.

Guadarrama P, Castillo S, Ramos-Zapata JA, Hernández-Cuevas LV, Camargo-Ricalde SL (2014) Arbuscular mycorrhizal fungal communities in changing environments: The effects of seasonality and anthropogenic disturbance in a seasonal dry forest. Pedobiologia 57:87–95.

Guariguata MR, Ostertag R (2001) Neotropical secondary forest succession: changes in structural and functional characteristics. For Ecol Manag 148:185–206.

Hameed A, Wu QS, Abd-Allah EF, Hashem A, Kumar A, Lone HA, Ahmad P (2014) Role of AM fungi in alleviating drought stress in plants. *In*: Mohamad M, *eds.* Use of microbes for the alleviation of soil stresses. *New York, USA*: *Springer*, 55–75.

Hannula SE, Morriën E, de Hollander M, van der Putten WH, van Veen JA, De Boer W (2017) Shifts in rhizosphere fungal community during secondary succession following abandonment from agriculture. ISME J11:2294–2304.

Hansen EM, Stone JK (2005) Impacts of Plant Pathogenic Fungi on Plant Communities. *In*: Digiton J, White JF, Oudemans P, *eds.* The Fungal Community: Its organization and role in the ecosystem. *Boca Raton, Florida, USA, CRC Press*, 461–474.

Hartmann M, Niklaus PA, Zimmermann S, Schmutz S, Kremer J, Abarenkov K, Frey B (2014) Resistance and resilience of the forest soil microbiome to logging-associated compaction. ISME J 8:226–244.

Hasan SS, Zhen L, Miah MG, Ahamed T, Samie A (2020) Impact of land use change on ecosystem services: A review. Environ Dev 34:100527.

Hawksworth DL, Lücking R (2017) Fungal diversity revisited: 22 to 38 million species. Microbioal Spectr 5:5–4.

He D, Xiang X, He JS, Wang C, Cao G, Adams J, Chu H (2016) Composition of the soil fungal community is more sensitive to phosphorus than nitrogen addition in the alpine meadow on the Qinghai-Tibetan Plateau. Biol Fertil Soils 52:1059–1072.

Heitman J (2011) Microbial pathogens in the fungal kingdom. Fungal Biol Rev 25:48–60.

Heitman J, Howlett BJ, Crous PW, Stukenbrock EH, James TY, Gow NA (2020) The fungal kingdom. *New Jersey*, *USA*: *John Wiley and Sons*, pp 1136.

Hooper ER (2008) Factors affecting the species richness and composition of Neotropical secondary succession: a case study of abandoned agricultural land in Panama. *In*: Myster RW, *eds*. Post-agricultural succession in the neotropics. *New York, USA*: *Springer*, 141–164.

Hui N, Liu X, Jumpponen A, Setälä H, Kotze DJ, Biktasheva L, Romantschuk M (2018) Over twenty years farmland reforestation decreases fungal diversity of soils but stimulates the return of ectomycorrhizal fungal communities. Plant Soil 427:231–244.

IPBES (2019) Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. *Bonn, Germany*: *IPBES secretariat*, pp 55.

James TY, Stajich JE, Hittinger CT, Rokas A (2020) Toward a fully resolved fungal tree of life. Annu Rev Microbiol 74:291–313.

Jamiołkowska A, Księżniak A, Gałązka A, Hetman B, Kopacki M, Skwaryło-Bednarz B (2018) Impact of abiotic factors on development of the community of arbuscular mycorrhizal fungi in the soil: a Review. Int Agrophys 32:133.

Jansa J, Wiemken A, Frossard E (2006) The effects of agricultural practices on arbuscular mycorrhizal fungi. *In*: Fossard E, Blum WEH, Warkentin BP, *eds.* Function of Soils for Human Societies and the Environment. *London, UK*: *Geological Society of London*, 89–115.

Jaquet S, Schwilch G, Hartung-Hofmann F, Adhikari A, Sudmeier-Rieux K, Shrestha G, Kohler T (2015) Does outmigration lead to land degradation? Labour shortage and land management in a western Nepal watershed. Appl Geogr 62:157–170.

Jones MD, Durall DM, Cairney JW (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytol 157:399–422.

Kabir Z (2005) Tillage or no-tillage: impact on mycorrhizae. Can J Plant Sci 85:23–29.

Kafle A, Cope KR, Raths R, Yakha JK, Subramanian S, Bücking H, Garcia K (2019) Harnessing soil microbes to improve plant phosphate efficiency in cropping systems. Agronomy 9:127.

Kaplan JO, Krumhardt KM, Zimmermann NE (2012) The effects of land use and climate change on the carbon cycle of Europe over the past 500 years. Glob Chang Biol 18:902–914.

Kc B, Race D (2019) Outmigration and land-use change: A case study from the middle hills of Nepal. Land 9:2.

Khalid M, Du B, Tan H, Liu X, Su L, Ali M, Hui N (2021) Phosphorus elevation erodes ectomycorrhizal community diversity and induces divergence of saprophytic community composition between vegetation types. Sci Total Environ 793:148502.

Kivlin SN, Hawkes CV (2016) Tree species, spatial heterogeneity, and seasonality drive soil fungal abundance, richness, and composition in Neotropical rainforests. Environ Microbiol 18:4662–4673.

Klavina D, Tedersoo L, Agan A, Adamson K, Bitenieks K, Gaitnieks T, Drenkhan R (2022) Soil fungal communities in young Norway spruce-dominant stands: footprints of former land use and selective thinning. Eur J For Res 141:503–516.

Klironomos J, Zobel M, Tibbett M, Stock WD, Rillig MC, Parrent JL, Bever JD (2011) Forces that structure plant communities: quantifying the importance of the mycorrhizal symbiosis. New Phytol 189:366–370.

Kohout P (2017) Biogeography of ericoid mycorrhiza. *In*: Tedersoo L, *eds*. Biogeography of mycorrhizal symbiosis. *Cham*, *Switzerland*: *Springer*, 179–193.

Kolecka N, Kozak J, Kaim D, Dobosz M, Ginzler C, Psomas A (2015) Mapping secondary forest succession on abandoned agricultural land with LiDAR point clouds and terrestrial photography. Remote Sens 7:8300–8322.

Kolecka N, Kozak J, Kaim D, Dobosz M, Ostafin K, Ostapowicz K, Price B (2017) Understanding farmland abandonment in the Polish Carpathians. App Geogr 88:62–72.

Kumar J, Atri NS (2018) Studies on ectomycorrhiza: an appraisal. Bot Rev 84:108–155.

Kunwar RM, Shrestha K, Dhungana SK, Shrestha PR, Shrestha KK (2010) Floral biodiversity of Nepal: an update. J Nat Hist Mues 25:295–311.

Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol Biochem 40:2407–2415.

Lekberg Y, Schnoor T, Kjøller R, Gibbons SM, Hansen LH, Al-Soud WA, Rosendahl S (2012) 454-sequencing reveals stochastic local reassembly and high disturbance tolerance within arbuscular mycorrhizal fungal communities. J Ecol 100:151–160.

Li S, Huang X, Shen J, Xu F, Su J (2020) Effects of plant diversity and soil properties on soil fungal community structure with secondary succession in the *Pinus yunnanensis* forest. Geoderma 379:114646.

Li S, Li X (2017) Global understanding of farmland abandonment: A review and prospects. J Geogr Sci 27:1123–1150.

Li T, Yang W, Wu S, Selosse MA, Gao J (2021) Progress and prospects of mycorrhizal fungal diversity in orchids. Front Plant Sci 12:646325.

Li Y, Li Y, Chang SX, Liang X, Qin H, Chen J, Xu Q (2017) Linking soil fungal community structure and function to soil organic carbon chemical composition in intensively managed subtropical bamboo forests. Soil Biol Biochem 107:19–31.

Liang Y, He X, Chen C, Feng S, Liu L, Chen X, Su Y (2015) Influence of plant communities and soil properties during natural vegetation restoration on arbuscular mycorrhizal fungal communities in a karst region. Ecol Eng 82:57–65.

Lilleskov EA, Kuyper TW, Bidartondo MI, Hobbie EA (2019) Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: a review. Environ Pollut 246:148–162.

Limtong S, Nasanit R (2017) Phylloplane yeasts in tropical climates. *In*: Buzzni P, Lachance M, Yurkov A, *eds.* Yeasts in natural ecosystems: Diversity. *Cham, Switzerland*: *Springer*, 199–223.

Liu D, Liu G, Chen L, Wang J, Zhang L (2018) Soil pH determines fungal diversity along an elevation gradient in Southwestern China. Sci China Life Sci 61:718–726.

Liu G, Bai Z, Cui G, He W, Kongling Z, Ji G, Li D (2022) Effects of Land Use on the Soil Microbial Community in the Songnen Grassland of Northeast China. Front Microbiol 13:865184.

Liu J, Sui Y, Yu Z, Shi Y, Chu H, Jin J, Liu X, Wang G (2015) Soil carbon content drives the biogeographical distribution of fungal communities in the black soil zone of northeast China. Soil Biol Biochem 83:29–39.

Liu M, Han G, Zhang Q (2020) Effects of agricultural abandonment on soil aggregation, soil organic carbon storage and stabilization: Results from observation in a small karst catchment, Southwest China. Agric Ecosyst Environ 288:106719.

Maharjan A, Kochhar I, Chitale VS, Hussain A, Gioli G (2020) Understanding rural outmigration and agricultural land use change in the Gandaki Basin, Nepal. Appl Geogr 124:102278.

Maitra P, Zheng Y, Wang YL, Mandal D, Lü PP, Gao C, Guo LD (2021) Phosphorus fertilization rather than nitrogen fertilization, growing season and plant successional stage structures arbuscular mycorrhizal fungal community in a subtropical forest. Biol Fertil Soils 57:685–697.

Makarov MI (2019) The role of mycorrhiza in transformation of nitrogen compounds in soil and nitrogen nutrition of plants: a review. Eurasian Soil Sci 52:193–205.

Mantero G, Morresi D, Marzano R, Motta R, Mladenoff DJ, Garbarino M (2020) The influence of land abandonment on forest disturbance regimes: a global review. Landsc Ecol 35:2723–2744.

Maron JL, Marler M, Klironomos JN, Cleveland CC (2011) Soil fungal pathogens and the relationship between plant diversity and productivity. Ecol Lett 14:36–41.

Mathieu S, Cusant L, Roux C, Corradi N (2018) Arbuscular mycorrhizal fungi: intraspecific diversity and pangenomes. New Phytol 220:1129–1134.

Meng M, Chen HY, Lin J, Liu X, Guo X, Yuan Y, Zhang J (2020) Long term forest conversion affected soil nanoscale pores in subtropical China. Catena 185:104289.

Menkis A, Uotila A, Arhipova N, Vasaitis R (2010) Effects of stump and slash removal on growth and mycorrhization of *Picea abies* seedlings outplanted on a forest clear-cut. Mycorrhiza 20:505–509.

MFSC (2014) Nepal National Biodiversity Strategy and Action Plan 2014-2020. *Kathmandu, Nepal*: *Ministry of Forest and Soil Conservation (MFSC)*, 5–6.

Middleton EL, Richardson S, Koziol L, Palmer CE, Yermakov Z, Henning JA, Bever JD (2015) Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. Ecosphere 6:1–16.

Mommer L, Cotton TA, Raaijmakers JM, Termorshuizen AJ, van Ruijven J, Hendriks M, Dumbrell AJ (2018) Lost in diversity: the interactions between soil-borne fungi, biodiversity and plant productivity. New Phytol 218:542–553.

Money NP (2016) Fungal diversity. *In*: Watkinson SC, Boddy L, Money NP, *eds.* The fungi. *Amsterdam, Netherland*: *Academic Press*, 1–36.

Moore D, Robson GD, Trinci APJ (2011) 21st Century Guidebook to Fungi. *Cambridge, UK*: *Cambridge University Press*, pp 610.

Morrison EW, Frey SD, Sadowsky JJ, van Diepen LT, Thomas WK, Pringle A (2016) Chronic nitrogen additions fundamentally restructure the soil fungal community in a temperate forest. Fungal Ecol 23:48–57.

Mueller RC, Rodrigues JL, Nüsslein K, Bohannan BJ (2016) Land use change in the Amazon rain forest favours generalist fungi. Funct Ecol 30:1845–1853.

Nakayama M, Imamura S, Taniguchi T, Tateno R (2019) Does conversion from natural forest to plantation affect fungal and bacterial biodiversity, community structure, and co-occurrence networks in the organic horizon and mineral soil? For Ecol Manag 446:238–250.

Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M, Högberg P (1998) Boreal Forest plants take up organic nitrogen. Nature 392:914–916

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Kennedy PG (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol 20:241–248.

Odriozola I, Navrátilová D, Tláskalová P, Klinerová T, Červenková Z, Kohout P, Baldrian P (2021) Predictors of soil fungal biomass and community composition in temperate mountainous forests in Central Europe. Soil Biol Biochem 161:108366.

Ojha HR, Shrestha KK, Subedi YR, Shah R, Nuberg I, Heyojoo B, Cedamon E (2017) Agricultural land underutilisation in the hills of Nepal: Investigating socio-environmental pathways of change. J Rural Stud 53:156–172.

Ojha RB, Atreya K, Kristiansen P, Devkota D, Wilson B (2022) A systematic review and gap analysis of drivers, impacts, and restoration options for abandoned croplands in Nepal. Land Use Policy 120:106237.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M (2010) The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188:223–241.

Ordway EM, Asner GP, Lambin EF (2017) Deforestation risk due to commodity crop expansion in sub-Saharan Africa. Environ Res Lett 12:044015.

Paungfoo-Lonhienne C, Yeoh YK, Kasinadhuni NRP, Lonhienne TG, Robinson N, Hugenholtz P, Schmidt S (2015) Nitrogen fertilizer dose alters fungal communities in sugarcane soil and rhizosphere. Sci Rep 5:1–6.

Pec GJ, van Diepen LT, Knorr M, Grandy AS, Melillo JM, DeAngelis KM, Frey SD (2021) Fungal community response to long-term soil warming with potential implications for soil carbon dynamics. Ecosphere 12:e03460.

Pereira CMR, da Silva DKA, de Almeida Ferreira AC, Goto BT, Maia LC (2014) Diversity of arbuscular mycorrhizal fungi in Atlantic Forest areas under different land uses. Agric Ecosyst Environ 185:245–252.

Pereira CMR, López-García Á, Maia LC, Frøslev TG, Kjøller R, Rosendahl S (2022) Arbuscular mycorrhizal fungal communities of pristine rainforests and adjacent sugarcane fields recruit from different species pools. Soil Biol Biochem 167:108585.

Põlme S, Bahram M, Yamanaka T, Nara K, Dai YC, Grebenc T, Tedersoo L (2013) Biogeography of ectomycorrhizal fungi associated with alders (*Alnus* spp.) in relation to biotic and abiotic variables at the global scale. New Phytol 198:1239–1249.

Poudel KP, Tamang S, Shrestha KK (2014) Transforming land and livelihood: Analysis of agricultural land abandonment in the Mid Hills of Nepal. J For Livelihood 12:11–19.

Powell JR, Rillig MC (2018) Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. New Phytol 220:1059–1075.

Pugh GJF, Boddy L (1988) A view of disturbance and life strategies in fungi. Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences 94:3–11.

Read DJ, Leake JR, Perez-Moreno J (2004) Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Canad J Bot 82:1243–1263.

Rillig MC, Ramsey PW, Morris S, Paul EA (2003) Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to land-use change. Plant Soil 253:293–299.

Rinaldi AC, Comandini O, Kuyper TW (2008) Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Divers 33:1–45.

Romdhane S, Spor A, Banerjee S, Breuil MC, Bru D, Chabbi A, Philippot L (2022) Land-use intensification differentially affects bacterial, fungal and protist communities and decreases microbiome network complexity. Environ Microbiome 17:1–15.

Rosendahl S, Matzen HB (2008) Genetic structure of arbuscular mycorrhizal populations in fallow and cultivated soils. New Phytol 179:1154–1161.

Rousk J, Brookes PC, Bååth E (2009) Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl Environ Microbiol 75:1589–1596.

Schiebold JMI, Bidartondo MI, Karasch P, Gravendeel B, Gebauer G (2017) You are what you get from your fungi: nitrogen stable isotope patterns in *Epipactis* species. Ann Bot 119:1085–1095.

Schmidt R, Mitchell J, Scow K (2019) Cover cropping and no-till increase diversity and symbiotroph: saprotroph ratios of soil fungal communities. Soil Biol Biochem 129:99–109.

Schweiger JMI, Bidartondo MI, Gebauer G (2018) Stable isotope signatures of underground seedlings reveal the organic matter gained by adult orchids from mycorrhizal fungi. Funct Ecol 32:870–881.

Seaton FM, George PB, Lebron I, Jones DL, Creer S, Robinson DA (2020) Soil textural heterogeneity impacts bacterial but not fungal diversity. Soil Biol Biochem 144:107766.

Shah F, Nicolás C, Bentzer J, Ellström M, Smits M, Rineau F, Tunlid A (2016) Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. New Phytol 209:1705–1719.

Sharmah D, Jha DK (2014) Diversity of arbuscular mycorrhizal fungi in undisturbed forest, slash-and-burn field, and monoculture forest of Indo-Burma megadiverse region. Rev Bras Bot 37:339–351.

Sheldrake M, Rosenstock NP, Mangan S, Revillini D, Sayer EJ, Olsson PA, Wright SJ (2018) Responses of arbuscular mycorrhizal fungi to long-term inorganic and organic nutrient addition in a lowland tropical forest. ISME J 12:2433–2445.

Shi L, Dossa GG, Paudel E, Zang H, Xu J, Harrison RD (2019) Changes in fungal communities across a forest disturbance gradient. Appl Environ Microbiol 85:e00080–19.

Shrestha BB (2016) Invasive alien plant species in Nepal. *In*: Jha PK, Siwakoti M, Rajbhandary S, *eds.* Frontiers of Botany. *Kirtipur*, *Kathmandu*, *Nepal*: *Central Department of Botany*, *Tribhuvan University*, 269–284.

Simard SW, Durall DM (2004) Mycorrhizal networks: a review of their extent, function, and importance. Canad J Bot 82:1140–1165.

Smith SE, Read DJ (2010) Mycorrhizal symbiosis. *Amsterdam*, *Netherland*: *Academic Press*, pp 787.

Stearn WT (1960) *Allium* and *Milula* in the central and eastern Himalaya. Bull Br Mus Nat Bot 2:159–191.

Stephanou C, Omirou M, Philippot L, Zissimos AM, Christoforou IC, Trajanoski S, Ioannides IM (2021) Land use in urban areas impacts the composition of soil bacterial communities involved in nitrogen cycling A case study from Lefkosia (Nicosia) Cyprus. Sci Rep 11:1–12.

Subedi YR, Kristiansen P, Cacho O, Ojha RB (2021) Agricultural land abandonment in the Hill agro-ecological region of Nepal: Analysis of extent, drivers and impact of change. Environ Manage 67:1100–1118.

Sui X, Zhang R, Frey B, Yang L, Li MH, Ni H (2019) Land use change effects on diversity of soil bacterial, acidobacterial and fungal communities in wetlands of the Sanjiang Plain, northeastern China. Sci Rep 9:1–14.

Tedersoo L, Anslan S, Bahram M, Drenkhan R, Pritsch K, Buegger F, Abarenkov K (2020b) Regional-scale in-depth analysis of soil fungal diversity reveals strong pH and plant species effects in Northern Europe. Front Microbiol 11:1953.

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Abarenkov K (2014) Global diversity and geography of soil fungi. Science 346:1256688.

Tedersoo L, Bahram M, Zobel M (2020a) How mycorrhizal associations drive plant population and community biology. Science 367:eaba1223.

Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263.

Tedersoo L, Suvi T, Jairus T, Ostonen I, Põlme S (2009) Revisiting ectomycorrhizal fungi of the genus *Alnus*: differential host specificity, diversity and determinants of the fungal community. New Phytol 182:727–735.

Teste FP, Jones MD, Dickie IA (2020) Dual-mycorrhizal plants: their ecology and relevance. New Phytol 225:1835–1851.

Thapa P (2021) Impacts of land-use and land-cover change on land degradation: A case study in Dolakha District, Nepal. *In*: Singh R, *eds*. Re-envisioning Remote Sensing Applications. *Boca Raton, Florida, USA*: *CRC Press*, 209–215.

Trejo D, Barois I, Sangabriel-Conde W (2016) Disturbance and land use effect on functional diversity of the arbuscular mycorrhizal fungi. Agrofor Syst 90:265–279.

Treseder KK (2004a) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytol 164:347–355.

Treseder KK, Mack MC, Cross A (2004b) Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. Ecol Appl 14:1826–1838.

Uddin K, Shrestha HL, Murthy MSR, Bajracharya B, Shrestha B, Gilani H, Dangol B (2015) Development of 2010 national land cover database for the Nepal. J Environ Manage 148:82–90.

Vaidya GS, Shrestha K, Khadge BR, Johnson NC, Wallander H (2007) Study of biodiversity of arbuscular mycorrhizal fungi in addition with different organic matter in different seasons of Kavre district (Central Nepal). Sci World 5:75–80.

Vályi K, Rillig MC, Hempel S (2015) Land-use intensity and host plant identity interactively shape communities of arbuscular mycorrhizal fungi in roots of grassland plants. New Phytol 205:1577–1586.

van der Heijden MA, Martin FM, Selosse MA, Sanders IR (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytol 205:1406–1423.

van der Linde S, Suz LM, Orme CDL, Cox F, Andreae H, Asi E, Bidartondo MI (2018) Environment and host as large-scale controls of ectomycorrhizal fungi. Nature 558:243–248.

van Geel M, Jacquemyn H, Peeters G, van Acker K, Honnay O, Ceulemans T (2020) Diversity and community structure of ericoid mycorrhizal fungi in European bogs and heathlands across a gradient of nitrogen deposition. New Phytol 228:1640–1651.

Vassileva M, Mendes GDO, Deriu MA, Benedetto GD, Flor-Peregrin E, Mocali S, Vassilev N (2022) Fungi, P-solubilization, and plant nutrition. Microorganisms 10:1716.

Vašutová M, Edwards-Jonášová M, Veselá P, Effenberková L, Fleischer P, Cudlín P (2018) Management regime is the most important factor influencing ectomycorrhizal species community in Norway spruce forests after windthrow. Mycorrhiza 28:221–233.

Vašutová M, Jiroušek M, Hájek M (2021) High fungal substrate specificity limits the utility of environmental DNA to detect fungal diversity in bogs. Ecol Indc 121:107009.

Vega-García C, Chuvieco E (2006) Applying local measures of spatial heterogeneity to Landsat-TM images for predicting wildfire occurrence in Mediterranean landscapes. Landsc Ecol 21:595–605.

Waldrop MP, Zak DR, Blackwood CB, Curtis CD, Tilman D (2006) Resource availability controls fungal diversity across a plant diversity gradient. Ecol Lett 9:1127–1135.

Wang C, Ma L, Zuo X, Ye X, Wang R, Huang Z, Cornelissen JH (2022) Plant diversity has stronger linkage with soil fungal diversity than with bacterial diversity across grasslands of northern China. Glob Ecol Biogeogr 31:886–900.

Wang JT, Zheng YM, Hu HW, Zhang LM, Li J, He JZ (2015) Soil pH determines the alpha diversity but not beta diversity of soil fungal community along altitude in a typical Tibetan Forest ecosystem. J Soils Sediments 15:1224–1232.

Wardle DA, Lindahl BD (2014) Disentangling global soil fungal diversity. Science 346:1052–1053.

Weber CF, Vilgalys R, Kuske CR (2013) Changes in fungal community composition in response to elevated atmospheric CO_2 and nitrogen fertilization varies with soil horizon. Front Microbiol 4:78.

Winkler K, Fuchs R, Rounsevell M, Herold M (2021) Global land use changes are four times greater than previously estimated. Nat Commun 12:2501.

Xiang D, Verbruggen E, Hu Y, Veresoglou SD, Rillig MC, Zhou W, Chen B (2014) Land use influences arbuscular mycorrhizal fungal communities in the farming–pastoral ecotone of northern China. New Phytol 204:968–978.

Xiao Y, Li C, Yang Y, Peng Y, Yang Y, Zhou G (2020) Soil fungal community composition, not assembly process, was altered by nitrogen addition and precipitation changes at an alpine steppe. Front Microbiol 11:579072.

Xu M, Li X, Cai X, Li X, Christie P, Zhang J (2017) Land use alters arbuscular mycorrhizal fungal communities and their potential role in carbon sequestration on the Tibetan Plateau. Sci Rep 7:1–11.

Yan Y, Sun X, Sun F, Zhao Y, Sun W, Guo J, Zhang T (2022) Sensitivity of soil fungal and bacterial community compositions to nitrogen and phosphorus additions in a temperate meadow. Plant Soil 471:477–490.

Yang T, Adams JM, Shi Y, He JS, Jing X, Chen L, Chu H (2017b) Soil fungal diversity in natural grasslands of the Tibetan Plateau: associations with plant diversity and productivity. New Phytol 215:756–765.

Yang Y, Cheng H, Dou Y, An S (2020b) Plant and soil traits driving soil fungal community due to tree plantation on the Loess Plateau. Sci Total Environ 708:134560.

Yang Y, Dou Y, Huang Y, An S (2017a) Links between soil fungal diversity and plant and soil properties on the Loess Plateau. Front Microbiol 8:2198.

Yang Y, Hobbie SE, Hernandez RR, Fargione J, Grodsky SM, Tilman D, Chen WQ (2020a) Restoring abandoned farmland to mitigate climate change on a full earth. One Earth 3:176–186.

Yang Y, Tilman D, Furey G, Lehman C (2019) Soil carbon sequestration accelerated by restoration of grassland biodiversity. Nat Commun 10:1–7.

Zhang J, Quan C, Ma L, Chu G, Liu Z, Tang X (2021) Plant community and soil properties drive arbuscular mycorrhizal fungal diversity: A case study in tropical forests. Soil Ecol Lett 3:52–62.

Zhang K, Cheng X, Shu X, Liu Y, Zhang Q (2018) Linking soil bacterial and fungal communities to vegetation succession following agricultural abandonment. Plant Soil 431:19–36.

Zhang T, Wang NF, Liu HY, Zhang YQ, Yu LY (2016) Soil pH is a key determinant of soil fungal community composition in the Ny-Ålesund Region, Svalbard (High Arctic). Front Microbiol 7:227.

Zhao A, Liu L, Chen B, Fu W, Xie W, Xu T, Fu S (2020) Soil fungal community is more sensitive to nitrogen deposition than increased rainfall in a mixed deciduous forest of China. Soil Ecol Lett 2:20–32.

Zhong Z, Zhang X, Wang X, Fu S, Wu S, Lu X, Yang G (2020) Soil bacteria and fungi respond differently to plant diversity and plant family composition during the secondary succession of abandoned farmland on the Loess Plateau, China. Plant Soil 448:183–200.

Zhou J, Jiang X, Zhou B, Zhao B, Ma M, Guan D, Qin J (2016) Thirty-four years of nitrogen fertilization decreases fungal diversity and alters fungal community composition in black soil in northeast China. Soil Biol Biochem 95:135–143.

Zhu X, Yang W, Sun L, Song F, Li X (2021) Anthropogenic land use changes diversity and structure of arbuscular mycorrhizal fungal communities at 100-m scale in northeast China. Arc Agron Soil Sci 67:778–792.

Zou YN, Wu QS, Kuča K (2021) Unravelling the role of arbuscular mycorrhizal fungi in mitigating the oxidative burst of plants under drought stress. Plant Biol 23:50–57.

Appendix

Curriculum vitae

Sujan Balami

Ph.D. Student, Department of Botany, Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic.

Email: balamisujan@gmail.com

Age/ Nationality: 33/ Nepalese

Permanent address: Balaju-16, Kathmandu, Nepal

Education

2017 (October)–present: Ph.D. Student - University of South Bohemia

2015: Master of Science in Botany - Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal

2013: Bachelor of Science in Botany - Amrit Campus, Institute of Science and Technology, Tribhuvan University, Lainchour, Kathmandu, Nepal

2008: Intermediate in Science (Biology) - Amrit Campus, Institute of Science and Technology, Tribhuvan University, Lainchour, Kathmandu, Nepal

Publications and manuscripts

Chaudhary S, Magar GT, Shrestha S, **Balami S** (2022) Peoples' Perception on Invasive Alien Plant Species of Ramdhuni Municipality, Sunsari District, Eastern Nepal. Amrit Research Journal 3:1–9.

Balami S, Vašutová M, Košnar J, Karki R, Khadka C, Tripathi G, Cudlín P (2021) Soil fungal communities in abandoned agricultural land has not yet moved towards the seminatural forest. Forest Ecology and Management 491:119181.

Balami S, Vašutová M, Godbold D, Kotas P, Cudlín P (2020) Soil fungal communities across land use types. iForest-Biogeosciences and Forestry 13:548.

Balami S, Thapa LB, Jha SK (2019) Effects of invasive *Ageratina adenophora* on mycelial growth of some important soil fungi. Songklanakarin Journal of Science & Technology 41:465–470

Bhatta SP, Sharma KP, **Balami S** (2018) Variation in carbon storage among tree species in the planted forest of Kathmandu, Central Nepal. Current Science 115:274.

Balami S, Thapa LB, Jha SK (2017) Effect of invasive *Ageratina adenophora* on species richness and composition of saprotrophic and pathogenic soil fungi. BIOTROPIA-The Southeast Asian Journal of Tropical Biology 24:212–219.

Balami S, Thapa LB (2017) Herbivory damage in native *Alnus nepalensis* and invasive *Ageratina adenophora*. Botanica Orientalis: Journal of Plant Science 11:7–11.

Balami S, Thapa LB, Jha SK (2017) Sterilization effect of *Ageratina adenophora* invaded soil on root growth of native *Schima wallichii*. Physiological Ecology and Environmental Science 8:25–30.

Balami S, Vašutová M, Chaudhary V, Cudlín P. Assemblage of root-associated fungi in *Alnus nepalensis* and *Schima wallichii* on abandoned agricultural land. Mycorrhiza (***Submitted***).

Balami S, Vašutová M. Three new ectomycorrhizal fungi from central Nepal: *Alnicola alni-nepalensis, Cortinarius nepalensis*, and *Lactarius alni-nepalensis*. Phytotaxa (***Submission stage***).

Conferences and symposiums

Poster Presentation at the Festival of Ecology 14–18 December 2020, Organizer: British Ecological Society.

Papers and poster presentation at the "International Conference on Biodiversity, Climate Change Assessment and Impacts on Livelihood" 2017. Organizer: Central Department of Botany.

Paper presentation at "The 7th National Conference on Science and Technology" 2016. Organizer: Nepal Academy of Science and Technology.

Participation at "National Symposium on Pesticides Pollution", 2014. Organizer: Kathmandu University and University Grant Commission, Nepal.

Participation at the 2nd International Symposium on Biotechnology, 2010. Organizer: White House Education Network.

Interaction Program on Promotion of Scientific Concept 2017, Organizer: GoN, MoST, B.P Koirala Memorial Planetarium, Observatory, and Science Museum Development Board.

Research, teaching experiences and internship

2022 (September)–present: Teaching assistant at Department of Botany, Amrit Campus, Tribhuvan University, Lainchour, Kathmandu, Nepal

2019 (May): Internship at Institute of Forest Ecology, University of Natural Resources and Life Sciences, Vienna Gregor-Mendel-Straße, Vienna, Austria

2019 (February)–2019 (March): Technician in Restoration ecology group, Department of Botany, Faculty of Science, University of South Bohemia. Project: "Restoring biodiversity of disturbed peatlands as a basis for restoration of their future ecosystem functions and services".

2018 (February)–2018 (March): CzechGlobe – Global Change Research Institute, Czech Academy of Sciences, Czech Republic.

2017 (August)–2017 (November): Project leader in project "Domestication of wild edible mushrooms of the Dolakha District", Central Nepal, NGO Rural Reconstruction Nepal (RRN).

2015 (November)–2017 (September): Teaching assistant at Department of Botany, Amrit Campus, Tribhuvan University, Lainchour, Kathmandu, Nepal

Professional activities and awards

2022 (November)–present: Promotional editor: Nepal Journal of Biotechnology ISSN 2091-1130

2022 (July): Reviewer in *Scientific reports*.

2017 (October)–2022 (August): Scholarship as PhD student at Faculty of Science, University of South Bohemia, Czech Republic

2017: Vice president at Nature Conservation and Sustainable Development Society (NCDS), Maharajgung, Kathmandu, Nepal

2017: Gold Medal 'Vidhyabhusan Kha' from Ministry of Education, Science and Technology, Government of Nepal

2017 (March): Editor on weekly Newsletter “Headlines Himalaya” from Environmental Graduates of Himalayas (EGH), Resource Himalaya Foundation.

2016: Professor B.D. Pandey Memorial Award from the Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal

2013: Member in Botanical Student Society (BoSS) of Central Department of Botany.

2012–2013: Meritorious scholarship in M.Sc. Botany Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

2011: Gold Medal and Certificate of Merit from Amrit Campus, Tribhuvan University, Lainchour, Kathmandu Nepal

2011: Prabha Parajuli cash award, Department of Botany, Amrit Campus, Tribhuvan University, Lainchour, Kathmandu Nepal

e-mail: balamisujan@gmail.com

Fungi at land use gradient in temperate region of central Nepal
Ph.D. Thesis Series, 2023, No. 3.

Printed in the Czech Republic by Typodesign
Edition of 6 copies

University of South Bohemia in České Budějovice
Faculty of Science
Branišovská 1760
CZ-37005 České Budějovice, Czech Republic

Phone: +420 387 776 201
www.prf.jcu.cz, e-mail: sekret-fpr@prf.jcu.cz