Interactions between fungi, forest management, and ecosystem services

Kerstin Varenius
Faculty of Forest Sciences
Department of Forest Mycology and Plant Pathology
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2017

Acta Universitatis agriculturae Sueciae
2017:87

Cover: Ecosystem services provided by fungi in forests. Illustration: Cajsa Lithell

ISSN 1652-6880
ISBN (print version) 978-91-7760-064-0
ISBN (electronic version) 978-91-7760-065-7

Print: Repro, Uppsala 2017

Interactions between fungi, forest management, and ecosystem services

Abstract

Fungi provide a wide range of ecosystem services (ES) in forests but have for long been difficult to study. Knowledge of their role in soil processes has therefore been lacking and not accounted for in forest management. With the recent development in methodology, we have begun to learn more about fungi and their role in ES provisioning.

Interactions between fungi, forest management, and ES were investigated in boreal forests in Sweden. Soil fungal communities were characterised using high-throughput sequencing. Long-term impacts of tree harvesting versus retaining trees on the composition of ectomycorrhizal fungal (EMF) communities were investigated. Furthermore, as part of the Swedish Forest Soil Inventory and National Forest Inventory, and using structural equation modelling, the interplay between soil fungal community composition and tree growth was investigated. Finally, ES provided by fungi in forests were reviewed and potential consequences of Swedish forest management on selected ES were discussed.

Temporary retention of trees for ten years did not seem to compensate for harvesting induced changes in EMF communities, which seemingly lasted for several decades. Permanent retention trees enabled local maintenance of some EMF. These results indicate that harvesting induced soil chemistry changes are more important than tree continuity in deciding post-harvest composition of EMF communities. Therefore, if aiming to conserve EMF, it is recommended to retain intact forest patches. Soil fungal community composition seemed to correlate with tree growth. Increased knowledge on fungal functional traits may therefore improve predictions of boreal forest productivity.

Intensive management may alter fungal communities leading to undesired consequences for ES provided by fungi. A proposed way to ensure resilience of managed forests is therefore to consider potential impacts of forestry on fungal communities and to simultaneously aim for multiple ES.

Keywords: soil fungi, forest management, ecosystem services, high-throughput sequencing, seed trees, retention trees, clear-cutting, structural equation modelling

Author's address: Kerstin Varenius, SLU, Department of Forest Mycology and Plant Pathology, P.O. Box 7026, 750 07 Uppsala, Sweden. *E-mail:* kerstin.varenius@slu.se

Interaktioner mellan svampar, skogsbruk och ekosystemtjänster

Sammanfattning

Svampar bidrar med många olika ekosystemtjänster (ET) i skog men har länge varit svåra att studera. Kunskap om svampars roll i markprocesser har därför saknats och inte kunnat beaktas inom skogsbruk. Med den senaste tidens metodutveckling har vi börjat lära oss mer om svampar och deras roll i generering av ekosystemtjänster.

Interaktioner mellan svampar, skogsbruk och ET undersöktes i boreala skogar i Sverige. Svampsamhällen identifierades med hjälp av högupplöst sekvensering. Långsiktiga konsekvenser av avverkning respektive kvarlämnade träd för ektomykorrhizasvampars (EMS) samhällssammansättning undersöktes. Dessutom, som en del i Markinventeringen och Riksskogstaxeringen, och med hjälp av strukturell ekvationsmodellering analyserades samspelet mellan samhällssammansättning av marksvampar och trädtillväxt. Slutligen beskrevs svamprelaterade ET i skog och potentiella konsekvenser av det svenska skogsbruket på utvalda ET diskuterades.

Att tillfälligt lämna fröträd i tio år efter avverkning verkade inte kunna kompensera för avverkningsinducerade förändringar i EMS-samhällen, vilka verkade bestå i flera decennier. Permanenta hänsynsträd möjliggjorde lokal överlevnad av EMS. Dessa resultat tyder på att avverkningsinducerade förändringar i markkemi har större betydelse än trädkontinuitet för sammansättningen av EMS-samhällen efter avverkning. Om man avser att förvalta EMS rekommenderas därför avsättning av intakta skogspartier. Samhällssammansättningen av marksvampar verkade korrelera med trädtillväxt. Ökande kunskap i svampars funktionella egenskaper kan därför förbättra bedömningar av skogens framtida produktivitet.

Intensivt skogsbruk orsakar förändringar i svampsamhällen som i sin tur kan leda till oönskade konsekvenser för de ET som svamparna bidrar med. Ett föreslaget sätt att trygga brukade skogars resiliens är därför att beakta möjliga konsekvenser av skogsbruk för svampsamhället och att sträva mot flera ET samtidigt.

Nyckelord: marksvampar, skogsbruk, ekosystemtjänster, högupplöst sekvensering, fröträd, hänsynsträd, kalavverkning, strukturell ekvationsmodellering

Författarens adress: Kerstin Varenius, SLU, Institutionen för skoglig mykologi och växtpatologi, Box 7026, 750 07 Uppsala. *E-post:* kerstin.varenius@slu.se

Dedication

To all the women before me who wanted to write a PhD thesis but never got the chance.

Contents

Popular science summary

Fungi take part in many complex processes resulting in products and aspects of nature that humans consider valuable. However, since many fungi live belowground, where they are hard to identify, and rarely form visible mushrooms, they have for a long time not received attention for their important contribution. Recently, DNA-based techniques for identifying fungal species underground have been developed, which make it possible to learn more about fungi and what they do.

Humans manage forests, often with the main goal of producing timber. In Sweden, the majority of timber is harvested through clear-cutting, which means that almost all trees in a forest are removed. Since many fungi are dependent on living trees, the abundance of many fungal species can change after clear-cutting. However, it is uncertain for how long these changes persist as the new forest ages and if it has any long-term consequences for the processes fungi take part in.

We investigated effects of clear-cutting on fungi living as collaborators of trees in young- and up to 60-year-old forests and evaluated whether leaving trees at harvest influenced the abundance of these fungi. Moreover, we identified soil fungi in over hundred forests in Sweden and included data on their abundances in a statistical model, with the aim to investigate whether the composition of fungi in soil matters for tree growth. Finally, we described the variety of benefits provided by fungi in forests, which are valued by humans, and how these may be affected by current forestry practices in Sweden.

The effects of tree harvesting on the composition of fungal species were still detectable several decades after the harvest and could not be compensated for by temporary retention of trees for teen years after harvest. Trees left permanently standing on the clear-cut seemed to act as a lifeboat for some species in a restricted area around the trees. Furthermore, our model indicated a relationship between the composition of soil fungal species and tree growth, although causes and effects remain uncertain. From our results it is clear that fungi are of huge importance for a wide variety of forest products and experiences that humans value. Therefore, it is important to account for fungi in forest management.

Populärvetenskaplig sammanfattning

Svampar deltar i många komplexa processer som ger upphov till produkter och aspekter av naturen som vi människor värdesätter. Eftersom många svampar lever i jorden där de är svåra att identifiera och sällan bildar synliga fruktkroppar har de dock under lång tid inte fått uppmärksamhet för sina viktiga bidrag. Nyligen utvecklade DNA-baserade metoder för att identifiera svamparter i jord gör det möjligt att lära sig mer om svampar och vad de gör.

Människor brukar skogen, ofta med målet att producera timmer. I Sverige avverkas majoriteten av den brukade skogen genom kalavverkning, vilket innebär att de flesta av träden skördas. Eftersom många svampar är beroende av levande träd, förändras förekomsterna av många svamparter vid kalavverkning. Det är dock osäkert hur länge dessa förändringar finns kvar när den nya skogen växer upp och om det har några långtgående konsekvenser för de processer som svamparna deltar i.

Vi undersökte effekterna av kalavverkning på svampar som lever i samarbete med träd i unga och upp till 60-åriga skogar och utvärderade om förekomsten av dessa svampar påverkas av att lämna kvar träd vid avverkning. Dessutom identifierade vi marksvampar från över hundra skogar i norra Sverige och inkluderade deras förekomster i en statistisk modell för att undersöka om sammansättningen av svamparter i jorden påverkar trädens tillväxt. Slutligen beskrev vi olika nyttor som svampar bidrar med i skogen, vilka människor värdesätter, och hur dessa kan påverkas av dagens skogsbruk i Sverige.

Effekter av avverkning på sammansättningen av svamparter var fortfarande mätbara flera decennier efter avverkningen och kunde inte kompenseras för genom att tillfälligt lämna träd i tio år efter avverkning. Träd som lämnats permanent på kalhygget verkade fungera som livbåt för en del arter inom ett avgränsat område runt träden. Vår modell tydde även på ett samband mellan sammansättningen av svamparter i jorden och trädens tillväxt, även om orsak och verkan är oklar. Våra resultat visar att svampar har stor betydelse för många av skogens produkter och upplevelser som människor uppskattar. Därför är det viktigt att svampar beaktas inom skogsbruket.

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Varenius K.*, Kårén O., Lindahl B. D., and Dahlberg A. 2016. Long-term effects of tree harvesting on ectomycorrhizal fungal communities in boreal Scots pine forests. *Forest Ecology and Management* 380, 41-49.

II Varenius K.*, Lindahl B. D., and Dahlberg. A. 2017. Retention of seed trees fails to lifeboat ectomycorrhizal fungal diversity in harvested Scots pine forests. *FEMS Microbiology Ecology* 93 (in press).

III Varenius K., Kyaschenko J., Stendahl J., Lindahl B. D., and Dahlberg A.* 2017. Tree growth relates to soil fungal community composition in Swedish boreal forests (manuscript).

IV Varenius K., Dahlberg A.*, Lindahl B. D., Finlay R., and Rist L. 2017. Jeopardizing forest resilience: the overlooked role of fungi (manuscript).

Papers I-II are reproduced with the permission of the publishers.
* Corresponding author.

The contribution of Kerstin Varenius to the papers included in this thesis was as follows:

I Participated in the sampling and laboratory work and analysed the data. Wrote the manuscript with supervision and was responsible for correspondence with the journal.

II Designed and planned for the study with supervision. Inspected aerial photographs of study objects and collected samples with assistance. Analysed the data, wrote the manuscript and was responsible for correspondence with the journal.

III Designed the study with supervision. Performed the lab work for the samples collected in 2014, developed the R script designed by J. S. Lefcheck, analysed the data, and wrote the manuscript.

IV Idea of paper developed together with supervisors. Designed the survey and analysed the answers. Wrote the manuscript with supervision.

Abbreviations

CA	Correspondence analysis
CCA	Canonical correspondence analysis
DCA	Detrended correspondence analysis
EMF	Ectomycorrhizal fungi
ES	Ecosystem services
ITS	Internal transcribed spacer
NFI	National forest inventory
NMDS	Non-metric multidimensional scaling
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
SEM	Structural equation modelling
SFSI	Swedish forest soil inventory
SH	Species hypothesis

1 Introduction

1.1 Resilience

Disturbances may cause changes in composition of biological communities, which can impact ecosystem functions and processes. Ecosystems differ in resilience – the capacity to undergo disturbance and maintain functions and controls (Gunderson and Holling 2001). By considering resilience we can acknowledge that nature is not stable but dynamic and subject to both natural and anthropogenic disturbances.

There has been much discussion aimed at identifying the major drivers of resilience. According to the *insurance hypothesis,* high species richness serves as an insurance of resilience, in that if some species are lost, others can perform the same functions (Mori, Lertzman and Gustafsson 2017). The *mass ratio hypothesis* instead acknowledges that most ecosystem functions are carried out by a few dominant taxa constituting the majority of the biomass (Mori, Lertzman and Gustafsson 2017). Thus, as long as these key species remain, loss of other species may not threaten ecosystem resilience.

1.2 Fungi

Fungi are eukaryotic organisms with cell walls primarily made up of glucans and chitin and may reproduce both somatically and sexually, using spores (Cole 1996; Bowman and Free 2006; Petersen 2013). The two major phylogenetic groups of fungi are *Ascomycota* and *Basidiomycota*, which are distinguished by the way they produce spores (Petersen 2013). Since many fungi live underground, they have for a long time been inaccessible for studies and therefore poorly understood (Douhan et al. 2011; Heilmann-Clausen et al. 2014). However, with recent methodological advances we have begun to learn more about the roles of fungi in ecosystems (Lindahl et al. 2013).

Fungal life forms vary from short-lived unicellular yeasts to mycelial networks covering hundreds of square meters and living for hundreds of years (Ferguson et al. 2003; McLaughlin et al. 2009). The main fungal life strategies are to decompose non-living organic matter (saprotrophs), to form mutualistic associations with autotrophic organisms (mycorrhiza and lichens), parasitize other organisms, or to live inside living plant tissues (endophytes) (Boddy, Franklin and van West 2008; Smith and Read 2008; Heilmann-Clausen et al. 2014).

Fungi represent a diverse kingdom, equal in species richness to animals and exceeding plants (Blackwell 2011; Tedersoo et al. 2014). Fungal diversity seems to be highest in soils and a few grams of soil can harbour hundreds of fungal species (van der Heijden Bardgett, and van Straalen 2008; Peay, Kennedy and Talbot 2016). Because of the diversity of fungi and since fungal communities with considerable differences in composition of species have been observed in similar habitats, fungal communities have for long been assumed to be functionally redundant – one community performing a function equally well as another under the same environmental conditions (Allison and Martiny 2008; Peay, Kennedy, and Talbot 2016).

1.3 Fungi provide ecosystem services

Fungi take part in key ecosystem processes resulting in many benefits for humans. However, knowledge about fungi has long been lagging behind that of other organism groups in forests.

1.3.1 Ecosystem services

The concept of ecosystem services (ES) is a utilitarian metaphor of nature as the benefits that humans obtain from it (MEA 2005; Norgaard 2010). The concept was introduced in the 1970s by conservation biologists and ecological economists aiming to increase interest in biodiversity conservation among the general public (Gómez-Baggethun et al. 2010). Since then, it has increased in popularity and after the Millennium Ecosystem Assessment (MEA 2005) it has increasingly also been used as a framework for scientific assessments of ecosystem change (Norgaard 2010). ES are usually categorised as supporting services forming the basis for other services, regulating services that maintain desired properties of the environment, provisioning services like food, material or energy, and cultural services (MEA 2005).

It is debated whether ES is a useful metaphor for interdisciplinary communication of values humans attribute to nature or whether it narrows our perception of nature (Gómez-Baggethun et al. 2010; Norgaard 2010; Kallis, Gómez-Baggethun, and Zografos 2013). One critique of the ES concept is that aspects of nature only receive attention when they can be attributed a monetary value (Kallis, Gómez-Baggethun, and Zografos 2013). However, ES frameworks do not necessarily imply monetary valuation and different valuation frameworks may be applied depending on circumstances and goals (Kallis, Gómez-Baggethun, and Zografos 2013).

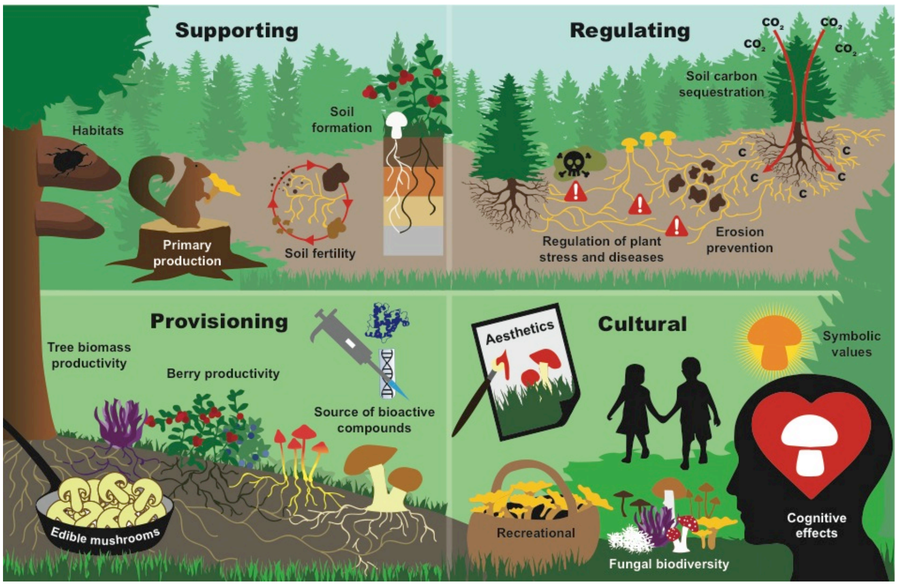

Figure 1. Ecosystem services (ES) provided by fungi in forests. Illustration: Cajsa Lithell

1.3.2 Ecosystem services provided by fungi in forests

Fungi provide a wide range of ES in forests (Fig. 1). They form a basis of existence for other organisms by providing habitats and a source of food (Heilmann-Clausen et al. 2014). Fungi also have the capacity to decompose complex organic compounds and take part in mineral weathering, which is why they are important players in processes regulating soil formation, soil fertility, and carbon sequestration (Finlay et al. 2010; Clemmensen et al. 2013; Shah et al. 2015). Some fungi cause plant diseases while others decrease the effects of plant diseases and abiotic stresses (Smith and Read 2008; Fisher et al. 2012).

Fungi may influence plant productivity and thereby services related to plant biomass production (van der Heijden Bardgett, and van Straalen 2008). Compounds derived from fungi are also used within the medical and biotechnology sector (Østergaard and Olsen 2010; De Silva et al. 2013). Edible mushrooms serve as an important food source worldwide (Boa 2004) and mushroom picking is an appreciated recreational activity in many countries (Boa 2004; Peintner et al. 2013). Fungi are also are used in rituals and serve as a source of inspiration for art (Nicholson 2009; Nai and Meyer 2016). Irrevocable loss of species is a source of concern to most people, which motivates conservation of fungal biodiversity for its own sake (Heilmann-Clausen et al. 2014).

A Swedish Scots pine forest. Photo: Kerstin Varenius

1.4 Fungi in boreal forest soils

Boreal forests cover the major part of the northern hemisphere, are exposed to a cold climate with short growing seasons, and have nutrient limited and acidic soils (Bonan and Shugart 1989; Lindahl and Clemmensen 2017). Even though these harsh conditions from a global perspective select for a limited subset of fungal species, the relative diversity of fungi is high compared to plants and bacteria (Tedersoo et al. 2014; Lindahl and Clemmensen 2017).

The composition of fungal communities in boreal forest soils seems to be influenced soil acidity, availability of water and nutrients, and dominating tree species (Prescott and Grayston 2013; Lindahl and Clemmensen 2017). Among mycorrhizal fungi, host generalism seems to be the rule but there are examples of host specificity (Peay et al. 2016). The type of litter produced depending on tree species may also select for a subset of saprotrophic fungi (Prescott and Grayston 2013). Acidic and dry soils seem to be dominated by stress tolerant ascomycetes (Sterkenburg et al. 2015). Decreasing soil acidity and increasing resource availability are proposed to favour basidiomycetes with high decomposing capacities but less stress tolerance (Sterkenburg et al. 2015; Kyaschenko et al. 2017a). As soil fertility increase more and earthworms increasingly mix the soil, fungal colonisation may decrease, which would favour bacteria (Butenshoen et al. 2007).

A replanted clear-cut in Northern Sweden. Photo: Kerstin Varenius

1.4 Nordic forest management affects fungi

The human influence on Nordic forests goes back thousands of years but has increased greatly since industrialisation (Framstad et al. 2013). In the 1950s large-scale mechanised clear-cutting was introduced, which today is the major harvesting method (Framstad et al. 2013; Skogsstyrelsen 2016). A minor part of the forests are naturally regenerated by temporary retention of scattered seed trees for ten years after harvest (Karlsson and Örlander 2004). Historically, Nordic forests have experienced wildfires of relatively low intensity resulting in major tree survival (Kuuluvainen and Aakala 2011). Therefore, clear-cutting represents a type of disturbance that is new to the area.

Nordic boreal forests are dominated by Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*), which both associate with ectomycorrhizal fungi (EMF) (Framstad et al. 2013; Smith and Read 2008). EMF depend on their host trees and are therefore negatively affected by tree harvest (Jones, Durall, and Cairney 2003). After clear-cutting, some EMF species may have survived on roots of retained living trees, or as a dormant spore bank in the soil, but most species have to re-establish by spore dispersal from surrounding forests (Rosenvald and Lõmus 2008; Peay et al. 2012; Glassman et al. 2015). Clear-cutting therefore causes a shift in the composition of EMF communities, which seems to persist for several decades (Twieg et al. 2007; Wallander et al. 2010; Kyaschenko et al. 2017b).

Management of Nordic forests is considered to be the main cause of fungal species endangerment and red listing (Rassi et al. 2010; ArtDatabanken 2015; Henriksen and Hilmo 2015). In Swedish forestry, the practice of permanently retaining some trees at harvest was introduced in the 1990s, with the aim to mitigate negative effects on biodiversity (Fedrowitz et al. 2014). Trees retained through the clear-cut phase seem to enable survival of some EMF within about 10 m from the tree (Cline, Ammirati and Edmonds 2005; Luoma et al. 2006; Jones et al. 2008). However, some fungi appear to require conditions that can only be found in natural and in principle unmanaged forests (Dahlberg, Genney, and Heilmann-Clausen 2010) and may therefore not cope with harvesting even though some trees are retained.

1.5 Do soil fungi affect tree growth?

Mycorrhizal fungi supply their hosts with nutrients and water and may thereby directly stimulate tree growth (Smith and Read 2008; van der Heijden, Bardgett, and van Straalen 2008). However, some mycorrhizal fungal species are thought to be more parasitic than mutualistic (Smith and Read 2008; Näsholm et al. 2013; Baskaran et al. 2016), why the outcome of mycorrhizal associations for trees may vary.

Tree growth may also be benefited indirectly by decomposition of complex organic matter resulting in release of organically bound nutrients, which is performed by saprotrophic and to some extent mycorrhizal fungi (Boddy, Franklin and van West 2008; Lindahl and Tunlid 2015). However, both saprotrophic and mycorrhizal fungal species differ in nutrient mobilising capacity (Floudas et al. 2012; Kohler et al. 2015). Hence, the composition of soil fungi may influence the rate of tree growth (van der Heijden, Bardgett, and van Straalen 2008).

Inoculations of tree seedlings with single or few EMF strains have resulted in negative, positive or neutral effects on seedling performance depending on fungal species (Jones, Durall and Cairney 2003; Menkis et al. 2007). Furthermore, Kranabetter, Durall, and MacKenzie (2009) observed a shift in EMF community composition along a stand productivity gradient. These results could indicate that composition of soil fungi influence tree growth rate. However, Kranabetter (2004) observed that seedlings originating from a disturbed road grew as well or better as seedlings taken from a forest, when planted in clear-cuts. Jones (2017) interpreted this observation as an indication that soil fungal communities could be functionally redundant with respect to their influence on tree growth.

1.6 Methods to study soil fungi

Studies of soil fungi were for long restricted to cultures or collections of fruitbodies (Douhan et al. 2011). However, some soil fungi cannot be cultured and produce no, small, or hidden fruitbodies, which explains why these methods do not fully cover the belowground species distribution (Horton and Bruns 2001; Douhan et al. 2011). Methods of identifying species using DNA have therefore revolutionised fungal research.

1.5.1 Characterising fungal communities using DNA

An early method for fungal identification by DNA was restriction fragment length polymorphism (RFLP) that used restriction enzymes to identify fungi (Douhan et al. 2011). Since then, the resolution of species detected per sample has increased greatly, and current technologies are still developing (Lindahl et al. 2013).

A common current practice to characterise fungal communities is through universal extraction of DNA followed by fungal-specific amplification by polymerase chain reaction (PCR) and high-throughput sequencing of species-specific DNA barcodes (Clemmensen et al. 2016). A commonly used fungal barcode is the internal transcribed spacer (ITS) region of the ribosome encoding genes (Clemmensen et al. 2016). This region is highly variable among fungal species but flanked by conserved regions making it suitable for PCR primers (Ihrmark et al. 2012).

The rapid development of sequencing platforms is illustrated in this thesis, as the previously prevailing technology, 454®-pyrosequencing, had just been announced to be phasing out at the start of the work. The platform used for paper I, IonTorrent®, was later shown to be more sensitive to variation in length of the amplified fragments (amplicons) than the 454 method (Clemmensen et al. 2016). The Pacific Biosciences® technology, which was used for papers II-III, seems to have less length bias than other platforms (Clemmensen et al. 2016).

Even though high-throughput sequencing provides high-resolution description of fungal communities, the analysed samples represent a tiny fraction of the fragmented mosaic of fungal mycelia in forest soils (Douhan et al. 2011). Furthermore, since fungal communities, like most biological communities, are composed of a few dominating species and many rare ones (Horton and Bruns 2001), random sampling of soil infers low probability of capturing rare species at sufficient frequencies for reliable statistics.

1.5.2 Defining and naming fungal species post-sequencing

Species concepts are useful to define groups of organisms and understand how they evolved. However, genetic boundaries between species are not always clear. Also, since many fungal species still lack scientific names there is a need for a standardised way to refer to a certain species (Kõljalg et al. 2013). Therefore, the species hypothesis (SH) concept, in which a SH is defined based on DNA sequence dissimilarity to the closest neighbour (single linkage), is practical when studying fungal communities (Lindahl et al. 2013; Kõljalg et al. 2013). After sequencing, the resulting sequences are therefore clustered into SHs using a bioinformatics pipeline (Lindahl et al. 2013). Because of the high variability in length and sequence of the ITS region, such clustering is usually performed based on pairwise alignments, such as the algorithm applied in the SCATA bioinformatics pipeline (Lindahl et al. 2013; Clemmensen et al. 2016).

When SHs are defined, they are assigned names based on comparison with previously sequenced expert-curated fungal SHs in the UNITE database (Kõljalg et al. 2013). SHs lacking scientific names can be assigned standardised accession numbers, which enables future tracing (Kõljalg et al. 2013).

1.5.3 Analysis of fungal community data

A common method to explore the large data sets generated by high-throughput sequencing is by some kind of visual data projection, known as an ordination (Ramette 2007; Pality and Shankar 2016). Ordinations that do not include pre-defined relationships between variables are called unconstrained or indirect ordinations and are useful for data exploration (Pality and Shankar 2016). When testing hypotheses, ordination algorithms with pre-defined relationships between variables are useful, and such ordinations are called constrained or direct (Pality and Shankar 2016).

Correspondence analysis (CA) is an unconstrained ordination method, in which arbitrary scores are initially assigned to the SHs (Ramette 2007). Based on these scores and SH abundances, weighted average scores of samples are computed. These sample scores are then used to compute new weighted average SH scores. The correspondence between SH and sample scores is then maximised by standardising the scores in an iterative process until there is no change in scores. The final scores are used for visual projection. CA sometimes results in artificial horseshoe patterns in the representation of the data. This so-called "arch" effect can be compensated for by a mathematical procedure called detrending, which is why such an ordination is referred to as detrended CA (DCA).

Another unconstrained ordination method is non-metric multidimensional scaling (NMDS), which depends on a matrix of pairwise dissimilarities in SH composition of samples (Ramette 2007). The samples are placed randomly in an ordination space where after a new dissimilarity matrix is computed and compared with the initial matrix. This procedure is then iteratively repeated in order to achieve an optimal fit. The final ordination is used to visually map samples onto a space with a predefined number of dimensions. Ordination axes of NMDS can be rescaled, rotated, or inverted with preserved orientation of samples but require more computational power than DCA.

Canonical CA (CCA) is a constrained form of CA, in which the axes are linear combinations of environmental variables (ter Braak and Verdonschot 1995; Ramette 2007). The statistical validity of relationships can be evaluated using a permutation test, which iteratively estimates a test statistic in random subsets of the data (Pality and Shankar 2016).

In order to achieve representative ordinations, the data may benefit from a transformation. Community data usually have asymmetric representation of SHs, explaining why transformations accounting for such asymmetry can be useful (Legendre and Legendre 2012; Pality and Shankar 2016). Environmental variables can also benefit from transformations in order to fulfil the assumptions underlying the analysis algorithms, such as normality or scale (Ramette 2007).

1.5.4 Analysis of complex ecological interactions

Biological communities often interact with multiple environmental factors in complex ways, which cannot always be analysed using ordinations. Structural equation modelling (SEM) provides an opportunity to model and estimate predefined multiple causal relationships simultaneously (Grace, Scheiner and Schoolmaster 2015; Lefcheck 2016). Such relationships are usually presented in path diagrams with arrows between variables denoting assumed causal directions (Grace, Scheiner and Schoolmaster 2015; Lefcheck 2016). Variables can appear both as predictors and responses (Lefcheck 2016).

There are several different SEM algorithms and software. For the work of paper III, piecewise SEM was chosen. Piecewise SEM translates predefined causal relationships into a set of linear regressions, which are evaluated individually and then combined in a common test statistic (Lefcheck 2016). This procedure makes piecewise SEM relatively straightforward to use. One disadvantage with the method is that it is not possible to test bidirectional interactions (Lefcheck 2016).

Objectives

This thesis concerns interactions between fungi, ecosystem services (ES), and forest management. The aim was to gain insights into forest ES provided by fungi and how forest management affects fungal communities as well as the ES they provide.

The specific objectives were to

I Investigate whether regeneration of Scots pine forests by temporary retention of seed trees enable survival of ectomycorrhizal fungi (EMF) resulting in an EMF community composition different from clear-cuts replanted with nursery-grown seedlings, and whether such differences persist for 50 years (Paper I and II).

II Evaluate the importance of permanent retention trees for the survival of EMF following harvesting (Paper II).

III Analyse whether the composition of soil fungal communities correlates with tree growth in boreal forests (Paper III).

IV Describe the contribution of fungi to ES provision within forests and discuss potential consequences of forest management for these ES (Paper IV).

2 Project descriptions

2.1 EMF in seed tree regenerated stands vs. replanted clear-cuts after 50 years (Paper I)

With this study the hypotheses were tested (1) that effects of forest harvesting on the community composition and species richness of EMF last for at least 50 years and, (2) that seed tree regeneration has smaller effects than clear-cutting, due to differing levels of biological legacies of EMF during the early tree regeneration phase.

EMF communities were compared in nine Scots pine stands, of which three were regenerated around 1960 by replanting clear-cuts, three were naturally regenerated around the same time using seed trees and three were old natural stands (aged 157–174 years). All stands were located in west central Sweden in the Siljansfors Experimental Forest (N 60°54', E14°23').

Twenty-five soil cores were collected in each stand, from which DNA was extracted and ITS amplicons were sequenced using the IonTorrent platform. Sequences were clustered into SHs, which were compared with database records and assigned names. For each SH and stand, the frequency of presence (%) out of the total 25 samples was calculated. Frequencies of all SHs were then used in NMDS to compare composition of EMF communities between stands. The effects of regeneration method (seed trees vs. replanted clear-cuts), and whether the stands were old natural or regenerated, on the composition of EMF communities were statistically evaluated by permutation tests.

EMF communities in the studied stands were also investigated eighteen years earlier using RFLP and sporocarp collections (Kårén 1997). This data was re-evaluated and compared with the current high-throughput data, in order to assess the robustness of EMF community analysis using different methods.

2.2 EMF in seed tree regenerated stands vs. replanted clear-cuts during 60 years (Paper II)

This study aimed to test the hypotheses (1) that EMF communities are less impacted by regeneration using seed trees than by clear-cutting and replanting, (2) that EMF communities in stands regenerated using seed trees return to a composition similar to communities in old natural stands faster than communities in stands that were replanted after clear-cutting and (3) that local EMF communities close to permanent retention trees mirror communities of old natural stands but differ from those in surrounding harvested areas.

The study was replicated in three regions in Central and Northern Sweden located at increasing latitude, hence differing in climate (Fig. 2). In each region thirteen Scots pine dominated stands were selected, of which five were regenerated using seed trees, five were replanted clear-cuts and three were old natural stands with 100-200 year old trees. The regenerated stands were harvested between 1960 and 2000 and evenly distributed across stand ages. Regeneration method was confirmed using historical aerial photographs.

Ten soil samples were collected from each of the thirty-nine stands. Additional samples were collected around permanent retention trees in ten of the younger stands. DNA was extracted and ITS amplicons were amplified and sequenced using the PacBio RS II® platform. The resulting sequences were clustered into SHs and assigned names. Effects of regeneration method, stand age within regenerated stands, regenerated vs. old natural stands and permanent retention tree vs. harvested areas, on the composition of EMF communities (stand mean relative abundances of SHs) were tested using DCA ordinations and CCA permutation tests. The EMF community variation was then further explored using Sørensen index, *post hoc* generalised mixed models and indicator species analysis.

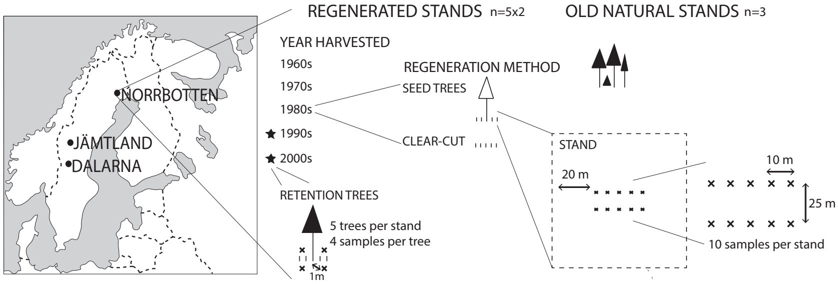

Figure 2. Study design, location of stands and sampling strategy. The study was replicated in three regions and in total 39 stands, of which nine were old natural stands and 30 were stands that had been regenerated between 1960 and 2000, either by seed-tree regeneration or by replanting after clear-cutting.

2.3 Evaluation of interplay between soil fungi and tree growth using national inventory data (Paper III)

This study aimed to investigate the hypothesis that community composition of soil fungi is a central driver of tree growth, while also considering other potentially influencing factors (Fig. 3).

Soil samples were collected in 131 conifer-dominated stands in Northern Sweden during 2014 and 2015, as part of the Swedish Forest Soil Inventory (SFSI) and the National Forest Inventory (NFI). Stand metadata and soil characteristics were collected simultaneously. Soil fungal communities in the samples were identified through extraction of DNA followed by ITS amplicon sequencing using the PacBio RS II platform. The obtained sequences were clustered into SHs and assigned names.

Relative abundances of fungal SHs were calculated per stand, where after the soil fungal community composition of all stands were compared using NMDS. The NMDS scores were used to represent the fungal community in piecewise SEM models, which evaluated possible alternative interactions between soil fungi, tree growth, soil pH, tree species, precipitation and temperature. *Post hoc* logistic regressions were performed based on presence/absence data, in order to investigate trends in frequencies of individual SHs, whereas correlations between relative abundances of classes or functional guilds and tree growth were evaluated by linear regression.

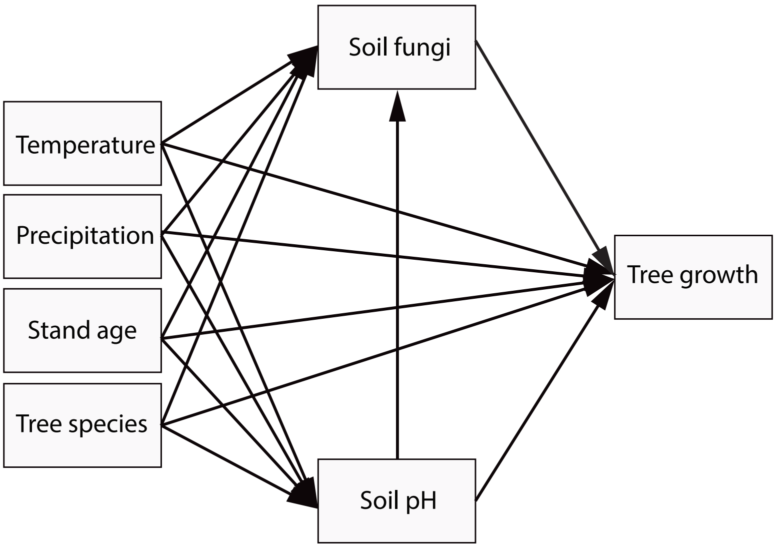

Figure 3. Hypothesised interactions between soil fungi, tree growth, and other influencing factors.

2.4 Fungi, ecosystem services and resilience of forests (Paper IV)

Knowledge of the role of fungi in ecosystem processes has until recently been scarce, but with recent methodological advances we have begun to learn more. This paper reviews the important contribution of fungi in forests using an ES framework. Furthermore, known and potential impacts of the current Swedish forest management on several ES provided by fungi are discussed. Since most biological studies are limited to a few years and a restricted area, selected experts on Fungal Ecology were asked to predict impacts on the discussed ES in a 100-year perspective, based on their collective knowledge. Finally, the outcomes of less or more intensive forest management for individual ES as well as overall forest resilience are discussed.

3 Results

3.1 Seed trees cannot compensate for long-term harvesting impacts

Several decades after harvest, EMF communities in regenerated stands still differed from those in old natural stands (Paper I and II). However, with increasing age of regenerated stands EMF communities became increasingly similar to old natural stand communities (Fig. 4, Paper II). No difference was observed in overall EMF community composition between stands regenerated using seed trees or by replanting clear-cuts (Paper I and II).

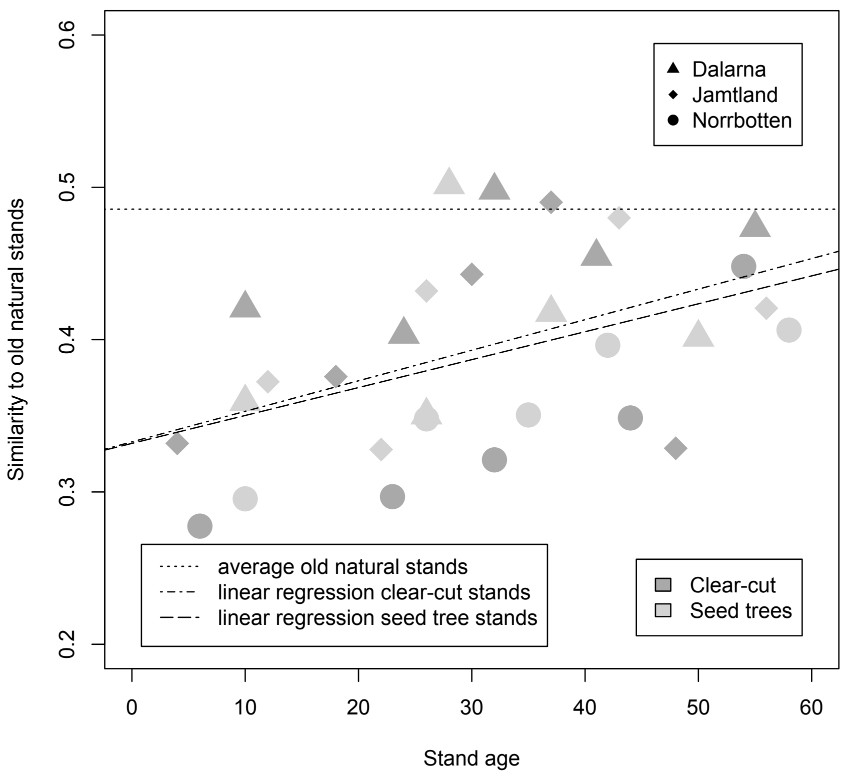

Figure 4. Similarity between ectomycorrhizal fungal (EMF) community compositions of stands regenerated either by replanting after clear-cutting (light grey) or by seed trees (dark grey) and the average EMF community in nine old natural stands. Shapes represent different regions.

3.2 Retention trees enable local survival of fungi

EMF communities close to permanent retention trees differed from communities in the surrounding harvested areas, but not from those in old natural stands (Fig. 5, Paper II).

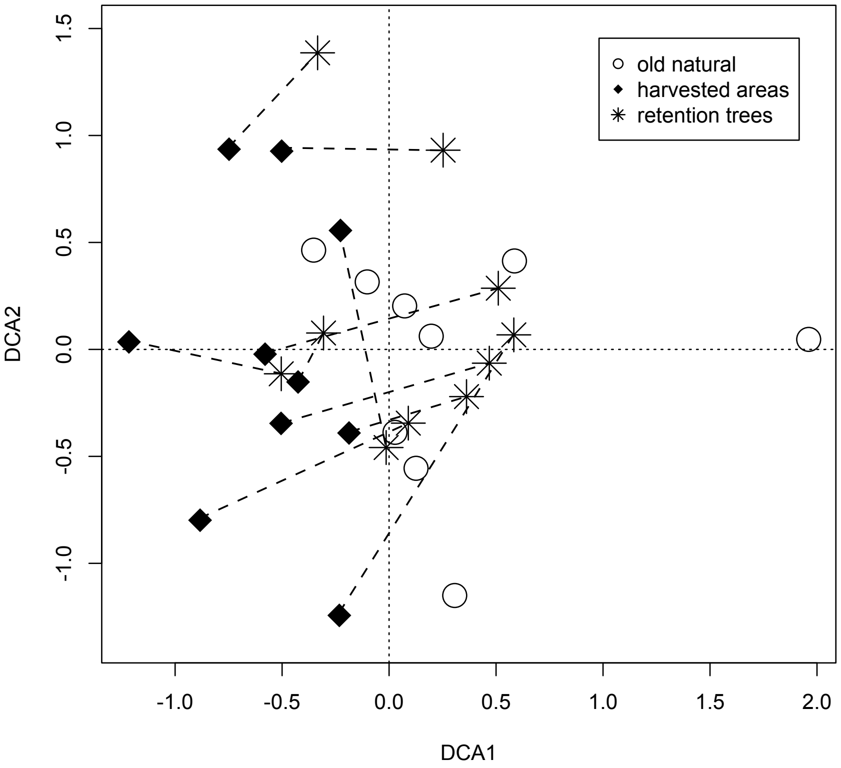

Figure 5. Detrended correspondence analysis (DCA) illustrating the composition of ectomycorrhizal fungal communities close to permanently retained trees in ten 10-30 year-old stands (stars), in the surrounding harvested areas (rhombs), and in nine old natural stands (circles). Dashed lines connect retention trees and surrounding areas in the same stand.

3.3 Tree growth relates to soil fungal community composition

Tree growth was correlated with soil fungal community composition as well as with temperature and stand age, with variation more or less equally distributed among these predictors (Fig. 6, Paper III). However, the direction of causality between fungi and tree growth could not be established. The fungal communities, which correlated with high tree growth, were characterised by high abundances of EMF and *Agaricomycetes,* and low abundances of root-associated ascomycetes. Fungal communities related to high tree growth also correlated positively with soil pH, temperature and domination of spruce.

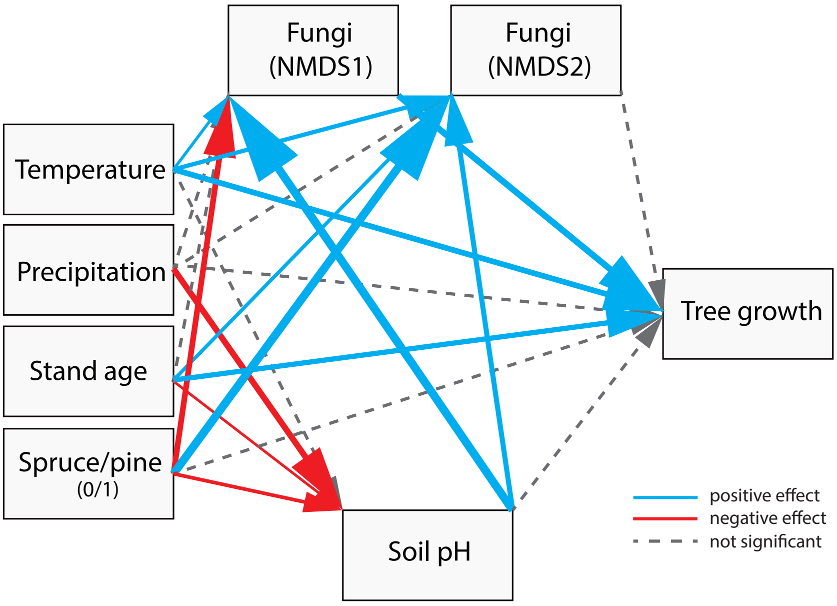

Figure 6. The result of a piecewise SEM testing soil fungal community composition (NMDS axis 1 and 2) as a predictor of tree growth and their interactions with soil pH, stand age, dominating tree species, precipitation and temperature. Arrow width corresponds to the standard estimate of each interaction and blue=positive, red=negative and dashed arrows= non-significant effects.

3.4 Forest management may impact ecosystem services

Forest management may induce shifts in the soil fungal community composition. There is a risk that such community shifts could negatively impact the ES that fungi provide (Table 1, Paper IV). However, there is uncertainty regarding the extent of functional redundancy of fungal communities.

Table 1. Expert assessments of potential consequences of Swedish forest management activities on ecosystem services provided by fungi in forests in a 100-year perspective. Direction of arrows (up, down, forward) illustrates positive, negative or negligible effects and arrow colour indicates the percentage of responding experts agreeing on the effect.

	soil fertility	soil carbon sequestration	tree biomass productivity	edible mushrooms	fungi & recreation	fungal biodiversity
clear-cutting	↘	↘	↘ →	↘	↘	↘
retention of trees & dead wood	↗	↗	↗	↗	↗	↗
change of tree species	→	→	→	→	↘ →	→
fertilisation	↗	↘	↗	↘	↘	↘
shortened rotation time	↘ →	↘	→	↘ →	↘	↘

↗ positive
↘ negative
→ negligible

→ 80-100%
→ 60-79%
→ 40-59%

4 Discussion

4.1 To leave or not to leave trees at harvest?

EMF communities were altered for several decades by tree harvesting (paper I and II), which may be explained by priority effects – species that establish early after a disturbance have a competitive advantage over species arriving later (Kennedy, Peay and Bruns 2009; Peay et al. 2012). The importance of a few species potentially surviving harvest as a dormant spore bank in the soil is uncertain (Glassman et al. 2015).

Permanent retention trees enabled local survival of some EMF but communities in seed tree regenerated stands did not differ from replanted clear-cuts (paper I and II). These results are similar to previous observations (Jones et al. 2008; Kranabetter, De Montigny, and Ross 2013; Sterkenburg 2016). The seemingly restricted influence of retained trees for EMF community composition indicates that soil chemistry changes following harvesting might have a larger effect than tree continuity on the composition of EMF communities post-harvest. This is supported by previous observations, which highlighted soil fertility as a principal driver of fungal community composition in boreal forest soils (Sterkenburg et al. 2015; Clemmensen et al. 2015).

EMF communities are composed of relatively few dominant species and many rare ones (Horton and Bruns 2001). Many EMF species are too infrequent to be captured by our study designs, simply because the probability of finding rare species at levels suitable for statistical analysis is low when sampling soil randomly. Rare species often have narrow habitat requirements (Gaston 1998), and harvesting impacts on those species could be more severe than those covered in this thesis.

During the half century, in which clear-cutting has been the prevailing harvesting method in Sweden, the forest landscape has become increasingly fragmented (Dahlberg, Genney, and Heilmann-Clausen 2010). Inability to account for the forest fragmentation within the work of this thesis introduces uncertainty as to whether older regenerated stands are a good representation of future harvesting effects.

Tree harvesting seems to induce long-term changes in the composition of EMF, which cannot be compensated for by retaining scattered trees. If aiming to conserve EMF communities similar to those in old natural forests it therefore seems more efficient to retain intact undisturbed forest patches.

4.2 Opening the fungal black box

The composition of soil fungal communities seems to relate to tree growth in Swedish boreal forests (Paper III). Fungi have for long been viewed upon as part of a “black box” of soil processes, in which the players are considered of minor importance (Allison and Martiny 2008). Cases in which the fungal community composition influences ecosystem process rates have been suggested to be a result of priority effects and historical contingency – when the current community composition is determined by the precise sequence of prior events (Peay, Kennedy, and Talbot 2016). The history of low intensity fires occurring in Nordic boreal forests (Kuuluvainen and Aakala 2011) supports these explanations.

Fungal communities, which correlated with high tree growth, were characterised by high abundances of EMF and *Agaricomycetes* and low abundances of root-associated ascomycetes (Paper III). Ascomycetes seem to be more stress tolerant but to have lower decomposing capacities than basidiomycetes (Sterkenburg et al. 2015). Some EMF species may, by supplying trees with nutrients, directly favour tree growth, while others may be more parasitic (van der Heijden Bardgett, and van Straalen 2008; Näsholm et al. 2013; Baskaran et al. 2016). *Agaricomycetes* include both EMF and saprotrophs with capacity to decompose complex organic compounds (Floudas et al. 2012; Kohler et al. 2015). Fast decomposition results in mineralisation of nutrients, which may indirectly favour tree growth (van der Heijden Bardgett, and van Straalen 2008; Kyaschenko et al. 2017b).

Soil fungal communities related to high tree growth were also characterised by high soil pH, temperature and domination of spruce (Paper III). From these results it seems that decreasing environmental stress enables colonisation of soil fungi with high capacities of nutrient mineralisation, which may in turn benefit tree growth. However, since the causal direction of the fungi – tree growth interaction could not be established, the underlying mechanisms of these trends remain uncertain. Hence, increased knowledge on species-specific functional traits of fungi will improve predictions of boreal forest productivity.

4.3 Resilient forests – the fungal perspective

Fungi are major providers of ES in forests and management induced shifts in the composition of fungal communities may therefore have undesired consequences for these ES (Paper IV). Impacts of the current Swedish forest management on fungal ES are complex. Tree biomass productivity, soil fertility, fungal biodiversity, and recreation may be negatively impacted, while soil carbon sequestration and production of some edible mushrooms may respond positively to the current management (Paper IV). The results of Papers I-III, which indicate that the composition of soil fungal communities correlates with tree growth and is altered by clear-cutting, support potential negative effects of intensive harvesting on tree biomass productivity.

Even though the relative importance of fungi for the delivery rates of ES should be further evaluated, there is a risk that the current management do not ensure resilient forests. If aiming for sustainable exploitation of forest products, forest management may therefore benefit from including considerations of potential impacts on fungal communities.

Biodiversity is today stated in Swedish legislation as a goal of forest management in parallel with biomass production (Beland Lindahl et al. 2017). However, the full range of ES provided by forests is seldom considered in forest management (Mori, Lertzman, and Gustafsson 2017). A proposed way to ensure resilient ecosystems while still meeting demands of goods is to aim for multiple ES simultaneously (Bender, Wagg, and van der Heijden 2016). In the case of forestry, this may be impractical on the scale of single stands. However, even if individual stands would deliver few ES, forest management could potentially be adapted for simultaneous delivery of multiple ES within a landscape. Hopefully, viable populations of fungi and other forest organisms could thereby be maintained, which would contribute to forest resilience.

5 Conclusion and future outlook

The DNA-based methods used in this thesis have enabled identification of fungal species and thereby increased our understanding in interactions between fungi, forest management, and ecosystem services (ES). However, we are still at the beginning of understanding the mechanisms of ecosystem processes, in which fungi take part. Emerging techniques based on whole genomes, RNA and enzymes will most probably provide increased resolution in descriptions of the variation in functional traits among fungal communities. Hopefully, it will also get easier to simultaneously study traits and interactions of multiple organisms. The importance of fungi relative to other organisms for the delivery of ES and how such relationships vary with context may be useful for development of forest management at local scales.

During this thesis, the first steps were taken to incorporate DNA characterisation of fungal communities in national monitoring of forest soils. If this monitoring can continue and more organisms are included, national-scale assessments of the status of soil dwelling organisms as well as of the processes in which they take part will most probably be improved. Possibly, as sample processing gets increasingly automated and sequencing costs decrease, DNA-based characterisation of soil organisms can in the future also become a realistic part of forest management planning.

This thesis provide examples of the wide range of ES that are delivered by fungi in forests but also indicates that intensive management may alter fungal communities leading to undesired effects on these ES. Opinions differ regarding what ES should be the priority of forest management. Personally, I perceive the debate regarding the Swedish forest management rather polarised, either promoting biodiversity or wood production. I hope that we can move towards a creative discussion, in which multiple ES are considered. I believe that this is needed in order to ensure the resilience of boreal forests.

References

Allison S. D. and Martiny J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities, *PNAS 105*, 11512–11519.

ArtDatabanken (2015). Rödlistade arter i Sverige (Red-listed species in Sweden, in Swedish). *ArtDatabanken SLU*, Uppsala.

Baskaran P., Hyvönen R., Berglund S. L., Clemmensen K. E., Ågren G. I., Lindahl B. D., et al. (2016). Modelling the influence of ectomycorrhizal decomposition on plant nutrition and soil carbon sequestration in boreal forest ecosystems, *New Phytologist 213 (3),* 1452-1465.

Beland Lindahl K., Sténs A., Sandström C., Johansson J., Lidskog R., Ranius T., et al. (2017). The Swedish forestry model: more of everything? *Forestry Policy and Economics 77,* 44-55.

Bender S. F., Wagg C., and van der Heijden M. G. A. (2016). An Underground Revolution: Biodiversity and Soil Ecological Engineering for Agricultural Sustainability. *Trends in Ecology and Evolution 31,* 6, 440-452.

Blackwell M. (2011). The fungi: 1, 2, 3…5.1 million species? *American Journal of Botany 98(3),* 426–438.

Boa E. (2004). Wild edible fungi, A global overview of their use and importance to people, Non-wood forest products. *Food and agriculture organization of the united nations*, Rome.

Boddy L., Frankland J. C., and van West P. (2008) Ecology of Saprotrophic Basidiomycetes. *Elsevier*, Amsterdam.

Bonan G. B. and Shugart H. H. (1989). Environmental Factors and Ecological Processes in Boreal Forests. *Annual Review of Ecology and Systematics 20*, 1-28.

Bowman S. M. and Free S. J. (2006). The structure and synthesis of the fungal cell wall. *BioEssays 28*, 799-808.

Butenschoen O., Poll C., Langel R., Kandeler E., Marhan S., and Scheu S. (2007). Endogeic earthworms alter carbon translocation by fungi at the soil–litter interface. *Soil Biology and Biochemistry 39,* 2854–2864.

Clemmensen K. E., Bahr A., Ovaskainen O., Dahlberg A., Ekblad A., Wallander H., et al. (2013). Roots and Associated Fungi Drive Long-Term Carbon Sequestration in Boreal Forest. *Science 339*, 1615-1618.

Clemmensen K. E., Finlay R. D., Dahlberg A., Stenlid J., Wardle D. A., and Lindahl B. D. (2015). Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests, *New Phytologist 205*, 1525–1536.

Clemmensen, K.E., Ihmark, K., Brandström-Durling, M. and Lindahl, B.D. (2016). Sample preparation for fungal community analysis by high-throughput sequencing of barcode amplicons in Martin, F., Uroz, S., (Eds.), Microbial Environmental Genomics. *Springer*, New York, NY, USA, 61-88.

Cline, E.T., Ammirati, J.F., and Edmonds, R.L. (2005). Does proximity to mature trees influence ectomycorrhizal fungus communities of Douglas-fir seedlings? *New Phytol. 166,* 993–1009.

Cole G. T. (1996). Chapter 73: Basic Biology of Fungi in Medical Microbiology, 4th edition. *University of Texas Medical Branch*, Galveston.

Dahlberg A., Genney D. R., and Heilmann-Clausen J. (2010). Developing a comprehensive strategy for fungal conservation in Europe: current status and future needs. *Fungal Ecology 3,* 50–64.

De Silva D. D., Rapior S., Sudarman E., Stadler M. Xu J., Alias S. A., et al. (2013). Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. *Fungal Diversity 62*, 1–40.

Douhan G.W., Vincenot L., Gryta H., and Selosse M-A. (2011). Population genetics of ectomycorrhizal fungi: from current knowledge to emerging directions. *Fungal Biol. 115,* 569–597.

Fedrowitz, K., Koricheva, J., Baker, S.C., Lindenmayer, D.B., Palik, B., Rosenvald, R., et al. (2014). Can retention forestry help conserve biodiversity? A meta-analysis. *Journal of Applied Ecology 51,* 1669-1679.

Ferguson B. A., Dreisbach T. A., Parks C. G., Filip G. M., and Schmitt C. L. (2003). Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. *Can. J. For. Res. 33,* 612-623.

Finlay R. D., Wallander H., Smits M., Holmström S., van Hees P., Lian B., et al. (2010) The role of fungi in biogenic weathering in boreal forest soils, *Fungal Biology Reviews 23,* 101-106.

Fisher M. C., Henk D. A., Briggs C. J., Brownstein J. S., Madoff L. C., McCraw S. L., et al. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature 484,* 186-194.

Floudas D., Binder M., Riley R., Barry K., Blanchette R.A., Henrissat B. et al. (2012). The paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science 336,* 1715–1719.

Framstad E., de Wit H., Mäkipää R., Larjavaara M., Vesterdal L., and Karltun E. (2013). Biodiversity, carbon storage and dynamics of old northern forests. *TemaNord 507,* Nordic Council of Ministers, Rosendahls-Schultz Grafisk, Denmark.

Gaston, K.J. (1998). Rarity as double jeopardy. *Nature 394,* 229–230.

Glassman S.I., Peay K.G., Talbot J.M., Smith D.P., Chung J.A., Taylor J.W., et al. (2015). A continental view of pine-associated ectomycorrhizal fungal spore banks: a quiescent functional guild with a strong biogeographic pattern. *New Phytologist 205,* 1619–1631.

Gómez-Baggethun E., de Groot R., Lomas P. L., and Montes C. (2010). The history of ecosystem services in economic theory and practice: From early notions to markets and payment schemes. *Ecological Economics 69*, 1209–1218.

Grace J. B., Scheiner S. M., and Schoolmaster D. R. Jr. (2015). Chapter 8: Structural equation modelling: building and evaluating causal models, in Fox G. A., Negrete-Yankelevich S., and Sosa V. J. (Eds.) Ecological Statistics: Contemporary and Application, first edition. *Oxford University Press*, Oxford.

Gunderson L. and Holling C. S. (2001). Panarchy: understanding transformations in human and natural systems. *Island Press,* Washington (DC).

Heilmann-Clausen J., Barron E. S., Boddy L., Dahlberg A., Griffith G., Nordén J., et al. (2014). A Fungal Perspective on Conservation Biology. *Conservation Biology* 0, 1–8.

Henriksen S. and Hilmo O. R. (2015). Norsk rødliste for arter 2015. *Artsdatabanken*, Norge.

Horton T. R. and Bruns T. D. (2001). The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology 10 (8),* 1855-1871.

Ihrmark K., Bödeker I.T.M., Cruz-Martinez K., Friberg H., Kubartova A., Schenck J., et al. (2012). New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol Ecol. 82 (3),* 666-677.

Jones M. D., Durall D.M., Cairney J.W.G. (2003). Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. *New Phytologist 157*, 399–422.

Jones M. D., Twieg B. D., Durall M. D., and Berch S. M. (2008). Location relative to a retention patch affects the ECM fungal community more than patch size in the first season after timber harvesting on Vancouver Island, British Columbia. *Forest Ecology and Management 255,*1342–52.

Jones M. D. (2017). Chapter 11: Integrating Ectomycorrhizas into sustainable management of temperate forests, in Johnson N., Gehring C. and Jansa J. (Eds) Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage. *Elsiver Inc,* 187-211.

Kallis G., Gómez-Baggethun E., and Zografos C. (2013). To value or not to value? That is not the question. *Ecological Economics 94*, 97–105.

Karlsson C. and Öhrlander G. (2004) Naturlig föryngring av tall (Natural regeneration of Scots pine, in Swedish). Skogsstyrelsen, Rapport 4/2004, *Skogsstyrelsens förlag*.

Kennedy P. G., Peay K. G. and Bruns T. D. (2009) Root tip competition among ectomycorrhizal fungi: are priority effects a rule or an exception? *Ecology 90,* 2098–107.

Kohler A., Kuo A., Nagy L.G., Morin E., Barry K.W., Buscot F., et al. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nat. Genet. 47,* 410–417.

Kranabetter J. M. (2004). Ectomycorrhizal community effects on hybrid spruce seedling growth and nutrition in clearcuts. *Canadian Journal of Botany 82*, 983-991.

Kranabetter J. M., Durall D. M., and MacKenzie W. H. (2009) Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest, *Mycorrhiza, 19,* 99–111.

Kranabetter J. M., De Montigny L., and Ross G. (2013) Effectiveness of green-tree retention in the conservation of ectomycorrhizal fungi. *Fungal Eco*logy *6,* 430–438.

Kuuluvainen, T. and Aakala, T. (2011). Natural Forest dynamics in boreal Fennoscandia: a review and classification. *Silva Fennica 45,* 823-841.

Kyaschenko J., Clemmensen K. E., Hagenbo A., Karltun E., and Lindahl B. D. (2017a). Shift in fungal communities and associated enzyme activities along an age gradient of managed Pinus sylvestris stands. *The ISME Journal 11(4),* 863-874.

Kyaschenko J., Clemmensen K.E., Karltun E., Lindahl B.D. (2017b). Below-ground organic matter accumulation along a boreal forest fertility gradient relates to guild interaction within fungal communities. *Ecology Letters*. In press.

Kårén, O. (1997). Effects of Air Pollution and Forest Regeneration Methods on the Community Structure of Ectomycorrhizal Fungi. Doctoral thesis. *Swedish University of Agricultural Sciences.*

Kõljalg U., Nilsson R.H., Abarenkov K., Tedersoo L., Taylor A.F.S., Bahram M., et al. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology 22 (21),* 5271–5277.

Lefcheck J. S. (2016). PiecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution and systematics. *Methods in Ecology and Evolution 7,* 573-579.

Legendre P. and Legendre L. (2012). Ecological resemblance – Chapter 7. *Developments in Environmental Modelling 24*, 265-335.

Lindahl B. D., Nilsson R. H., Tedersoo L., Abarenkov K., Carlsen T., Kjøller R., et al. (2013). Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. *New Phytologist 199 (1),* 288–299.

Lindahl, B. D. and Tunlid, A. (2015). Ectomycorrhizal fungi - potential organic matter decomposers, yet not saprotrophs. *New Phytologist 205 (4),* 1443-1447.

Lindahl B. D. and Clemmensen K. E. (2017). Chapter 21: Fungal ecology in boreal forest ecosystems, in Martin F. (Ed) Molecular Mycorrhizal Symbiosis, First Edition. *John Wiley & Sons Inc*.

Luoma D. L., Stockdale C. A., Molina R., and Eberhart J. L. (2006). The spatial influence of *Pseudotsuga menziesii* retention trees on ectomycorrhiza diversity. *Can. J. For. Res. 36,* 2561–2573.

McLaughlin D. J., Hibbett D. S., Lutzoni F., Spatafora J. W., and Vilgalys R. (2009). The search for the fungal tree of life. *Trends in microbiology 17 (11),* 488-497.

MEA (2005). Millennium Ecosystem Assessment, Ecosystem and Human Well-being: Synthesis, *Island Press*, Washington DC.

Menkis A., Vasiliauskas R., Taylor A. F. S., Stenlid J., and Finlay R. (2007). Afforestation of abandoned farmland with conifer seedlings inoculated with three ectomycorrhizal fungi- impact on plant performance and ectomycorrhizal community. *Mycorrhiza 17(4),* 337-348.

Mori A. S., Lertzman K. P., and Gustafsson L. (2017). Biodiversity and ecosystem services in forest ecosystems: a research agenda for applied forest ecology. *Journal of Applied Ecology 54*, 12–27.

Nai C. and Meyer V. (2016). The beauty and the morbid: fungi as source of inspiration in contemporary art. *Fungal Biol Biotechnol 3*,10.

Nicholson M. S. (2009). Some Spiritualistic Uses of Mushrooms. *Fungi 2 (2),* 26-27.

Norgaard R. B. (2010). Ecosystem services: From eye-opening metaphor to complexity blinder. *Ecological Economics 69,* 1219–1227.

Näsholm T, Höberg P, Franklin O, Metcalfe D, Keel S. G, and Campbell C. (2013). Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? *New Phytol* 198, 214–221.

Pality O. and Shankar V. (2016). Application of multivariate statistical techinques in microbial ecology. *Molecular Ecology 25,* 1032-1057.

Peintner U., Schwarz S., Mesic A., Moreau P-A., Moreno G., and Saviuc P. (2013). Mycophilic or Mycophobic? Legislation and Guidelines on Wild Mushroom Commerce Reveal Different Consumption Behaviour in European Countries. *PLoS ONE 8 (5),* 1-10.

Petersen J. H. (2013). The Kingdom of Fungi. *Princeton University Press*, Princeton and Oxford.

Peay K. G., Schubert M. G., Nguyen N. H., and Bruns T. D. (2012). Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. *Molecular Ecology 21*, 4122–4136.

Peay K. B. (2016). The Mutualistic Niche: Mycorrhizal Symbiosis and Community Dynamics. *Annu. Rev. Ecol. Evol. Syst. 47*, 143–64.

Peay K. G., Kennedy P. G., and Talbot J. M. (2016). Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology 14,* 434-447.

Prescott C. E. and Grayston S. J (2013). Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. *Forest Ecology and Management 309*, 19–27.

Ramette A. (2007). Multivariate analysis in microbial ecology. *FEMS Microbiol. Ecol. 62*, 142-160.

Rassi P., Hyvärinen E., Juslén A., and Mannerkoski I. (2010). The 2010 Red List of Finnish Species. *Ympäristöministeriö & Suomenympäristökeskus*, Helsinki.

Rosenvald R. and Lõhmus A. (2008). For what, when, and where is green-tree retention better than clear-cutting? A review of the biodiversity aspects. *Forest Ecology and Management 255,* 1–15.

Shah F., Nicolás C., Bentzer J., Ellström M., Smits M., Rineau F., et al. (2015). Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. *New Phytol. 4*, 1705–1719.

Skogsstyrelsen (2016). Quality of the regrowth 2015/2016. Sveriges officiella statistik, Statistiska meddelanden 1601. Skogsstyrelsen.

Smith S. E. and Read D. J. (2008). Mycorrhizal symbiosis, *Elsevier Science*, Oxford, UK.

Sterkenburg E., Bahr A., Brandström Durling M., Clemmensen K.E., Lindahl B.D. (2015). Changes in fungal communities along a boreal forest soil fertility gradient. *New Phytol. 207 (4),* 1145–1158.

Sterkenburg E. (2016). Drivers of soil fungal communities in boreal forests. Doctoral thesis. *Swedish University of Agricultural Sciences.*

Tedersoo L., Bahram M., Põlme S., Kõljalg U., Yorou N. S., Wijesundera R., et al. (2014). Global diversity and geography of soil fungi. *Science 346,* 6213, 1256688:1-10.

ter Braak C. J. F. and Verdonschot P. F. M. (1995). Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic Sciences 57 (3),* 255-235.

Twieg B.D., Durall D.M., Simard S.W. (2007). Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytol. 176,* 437–447.

van der Heijden M. G. A., Bardgett R. D., and van Straalen M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters 11,* 296-310.

Wallander H., Johansson U., Sterkenburg E., Brandström Durling M. and Lindahl B.D. (2010). Production of ectomycorrhizal mycelium peaks during canopy closure in Norway spruce forests. *New Phytologist 187,* 1124–1134.

Østergaard L. H. and Olsen H. S. (2010). Industrial applications of fungal enzymes In: Hofrichter M. (Ed.) Industrial Applications, 2nd edition, The Mycota X, *Springer-Verlag,* Berlin Heidelberg.

Acknowledgements

I want to start by thanking my supervisors for supporting me during this roller-coaster journey. Anders – tack för din positiva attityd och för att du delat med dig av tankar om forskning och om livet i största allmänhet. Björn – tack för din genuina glädje över resultat och för din förmåga att se möjliga vägar framåt. Lucy – thank you for listening to both hopes and doubts and for encouraging me to walk this path in my own way. Roger – tack för att du delat med dig av din kunskap och för din optimism inför mitt arbete.

To all Mykopat PhD students who struggled beside me during these years: thanks to Julia for an exiting collaboration, Andreas for making teaching so much fun, Erica for discussions about research and more important things, Lea for being my partner in crime during the thesis writing, Diem for introducing me to PhD life and for shared scripts and papers, Florence for your care and stories, Zaenab for laughter and magical homemade coffee, Tina G for being an excellent office mate, Hernán for fun and interesting political discussions, and to Tina P, Miguel AR, Miguel NG, Islam, Prakash, Rajiv, Sara, Hanna, Kajsa, Mudassir, Srisailam, Arpita, Georgios, Anne, Arpita, Joao, Maria, Kateryna, and Yang; thank you for dinners, fikas, discussions, and for being positive and caring, which made my life easier both inside and outside work.

Katta, Maria, and Rena: tack för all hjälp, finurliga idéer och otroligt tålamod. Utan er blir det ingen forskning gjord. Ariana – tack för ett fint samarbete och för hjälp med analyser. Chris – thanks for interesting discussions, which lead to paper III. Karina – tack för att jag fick möjligheten att undervisa och för alla värdefulla tankar. Petra – tack för viktiga samtal och för din smittande glädje. Cajsa – tack för din fantastiska illustration. Mikael – tack för att du utvecklade SCATA så att mina data kunde analyseras. Uwe – tack för hjälpen med rådatan. Erica och Karin – tack för all praktisk hjälp med fakturor och kursregistreringar. Les – tack för all datorsupport. Shahid – thank you for lab assistance. The Microbial Ecology journal club: thank you for interesting discussions which expanded my knowledge of Ecology in general and of carbon and nitrogen cycling in particular. And to all other Mykopat employees: thanks for research support and for interesting and fun discussions during workshops, conferences, fikas and parties.

Pernilla Borgström och Veera Tuovinen: tack för alla värdefulla pratstunder och för hjälpen med manuset till artikel IV. Lena Gustafsson – tack för värdefulla tankar i samband med min halvtid. Claudia von Brömssen och Ulf Olsson – tack för statistikhjälp när jag som mest behövt det. Johan Stendahl – tack för dina tankar och hjälp med data som gjorde artikel III möjlig. Ola Löfgren and Bertil Westerlund – tack för hjälp med data till artikel III. Maria Elene Johansson – tack för ditt arbete att ta fram skogar för min andra studie. Lill Eilersten – tack för hjälp med provtagning och för ett mycket väl utfört mastersarbete. Tord Snäll – tack för input på analyserna i artikel III. To all employees of the SFSI and NFI – thank you for making paper III possible. To workers of SLU that makes research possible through general maintenance – I feel so privileged to be working in such a clean and well-organised environment.

Emelie Lassén – tack för all ovärderlig hjälp med provtagning trots 30 graders värme, orimligt många myggbett och för mycket stress, och tack för att du är du. Ola Kårén – tack för dina tankar kring min första artikel samt för data till min andra studie. Jonathan S. Lefcheck – thank you for helping me to carry out and understand SEM, you made paper III so much more exiting. Christer Karlsson – tack för data från Siljansfors. Dan Broström – tack för ditt utmärkta minne om de fruktkroppsinventeringar du utförde på 90-talet. Hans Winsa och Börje Petterson: tack för data över skogar som möjliggjorde min andra studie. Hans-Olov Andersson – tack för din experthjälp med flygbilder över de skogar jag undersökte i min andra studie. Bálint Dima, Ellen Larsson and Karl-Henrik Larsson – tack you for help with species identifications. To all organisers of the courses I attended: It’s been so exiting and interesting to take part of your knowledge and to engage in discussions with you and the other students. SciLifeLab – thank you for performing the sequencing for all my studies. Formas, Tryggers och Konsul Faxes stiftelse – tack för finansiering som gjorde min avhandling möjlig.

Under de här åren har jag, utöver att doktorera, varit en del av nätverket Ingen människa är illegal. Detta engagemang har gett mig perspektiv på hur otroligt lyckligt lottad jag är som kan ägna min tid åt att åka runt i Sverige och gräva i jorden medan andra människor oroar sig för att bli deporterade till krig eller hur de ska ordna mat till sina barn. Men genom IMÄI har jag inte bara mött människor som tvingats leva under ovärdiga förhållanden, jag har också mött en känsla av sammanhållning, kärlek och omtanke som jag önskar att varje människa får uppleva. Tack till IMÄI för möten, demonstrationer,

manifestationer, flygbladsutdelningar, konserter, poesikvällar, fester, informationsträffar, debattartiklar, engagemang, hopp, glädje, delade sorger, och för att ni får mig att våga tro på en annan värld.

Ett annat sammanhang som jag är tacksam över att ha fått tillhöra under de här åren är Uppsalas Anarkafeministkör. Jag vill tacka alla medlemmar för rep, konserter, demonstrationer, skratt, möten, visdomar, kampvilja och för att ni är så otroligt bra.

I have no words to express the gratefulness I feel knowing that I have so many wonderful friends. In conversations, hugs, dances, lunches, fikas, dinners, parties, trips, nature and cultural experiences I have in your company grown as a person. So to all my very dear friends near and far: I love you, thank you for being exactly who you are. I could not have done this without you.

Familjen – Allt blir bra, men det blir det tack vare er.

Amanda – Tack för kärlek, glädje och för drömmar om framtiden.