SERGEI PÕLME

Biogeography and ecology of Alnus-associated ectomycorrhizal fungi – from regional to global scale
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BIOGEOGRAPHY AND ECOLOGY OF ALNUS- ASSOCIATED ECTOMYCORRHIZAL FUNGI – FROM REGIONAL TO GLOBAL SCALE
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I. LIST OF ORIGINAL PUBLICATIONS

This thesis is based by following papers that are referred in the text by Roman numerals:


Authors contribution to each publication

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2. INTRODUCTION

Ecological and biogeographical processes drive the structure and diversity of natural communities. A range of factors, including both abiotic and biotic variables, shape the distribution of organisms through their inherent ecological tolerances and differential competitive abilities. Micro- and macroorganisms are often involved in symbiotic associations that may constrain the distribution of one or both of the partners. Due to methodological restrictions, microbial biogeography has long relied on theoretical arguments over empirical data. Rapid developments of molecular tools in the recent decades have, however, enabled us to shed light in microbial biogeography and diversity at the global scale. Accumulating evidence suggests that microbes do exhibit biogeographic patterns, but these do not necessary coincide with the patterns observed in macroorganisms (patterns such as latitudinal or altitudinal gradient in diversity; Bryant et al., 2008; Fierer et al., 2011, Queloz et al., 2011; Bahram et al., 2012). Furthermore, microbial divisions and kingdoms strongly differ in their ecology and dispersal and could be subject to different ecological factors.

Mycorrhizal fungi are widespread soil-inhabiting microorganisms that display a number of mutualistic benefits from associations with most terrestrial plants (Smith & Read, 2008). Among mycorrhizal types, ectomycorrhizal (EcM) symbiosis, which appears in many ecologically and economically important trees, represents potentially one of the most prominent and ecologically crucial mutualistic associations in terrestrial habitats. It has been estimated that total EcM fungal richness could be at least 20 000 species (Rinaldi et al., 2008). Current evidence suggests that species richness of EcM fungi appears to have a unimodal relationship with latitudinal gradient (Tedersoo & Nara, 2009; Tedersoo et al., 2012) and it declines along the altitudinal gradient (Bahram et al., 2012). The first of these patterns is different from the widely observed pattern of greater species richness in tropical rather than temperate zones (Hillebrand, 2004). Similarly to macroorganisms, both of these diversity gradients were largely ascribed to climatic variables, particularly the mean annual temperature and precipitation. The unimodal relationship between EcM fungal richness and latitudinal gradient could be partly ascribed to temperate origin of EcM—the distribution of many EcM fungal lineages is restricted to the temperate ecosystems, which is consistent with the evolutionary history of host plants (Tedersoo & Nara, 2009; Tedersoo et al., 2010; Tedersoo et al., 2012). For example, the origin of the oldest known ectomycorrhizal plant family, Pinaceae, is temperate (Hibbett & Matheny, 2009). In addition to evolutionary history, high biological activity in tropical soil reduces the amount of organic matter that may result the impoverishment of niches for soil microorganisms, potentially reducing EcM fungal diversity (Wardle, 2002).

The EcM community structure is affected by a number of biotic and abiotic factors such as climate (O’Dell et al., 1999), successional stage (Nara et al., 2003), interspecific interactions of EcM (Koide et al., 2005), edaphic factors (Aponte et al., 2010) and dispersal limitation at various scales (Peay et al.,...
Studies examining autocorrelation at local scales have demonstrated that most of the EcM taxa show patchiness up to 3 meters (Lilleskov et al., 2006; Bahram et al., 2010). Host species identity has also been increasingly shown to influence the structure and richness of EcM fungal assemblages at a range of taxonomic levels, as large proportion of EcM fungi exhibit host preference (Ishida et al., 2007; Morris et al., 2008; Tedersoo et al., 2008; Bahram et al., 2012). However, it often remains unknown whether the host effect is related to phylogenetic distance among them or driven by random processes independent of evolutionary links, i.e. the effect of a species per se. For example effects of host tree on EcM communities may be mediated by indirect factors such as differences in leaf litter, carbon production or modification of soil properties (Conn & Dighton, 2000; Dickie et al., 2006; Aponte et al., 2010). Such taxonomic sampling effect is ubiquitous in ecology and largely accounts for increase in biodiversity and function of soil animals and fungi with raising richness of host or substrate (Cardinale et al., 2006). *Alnus* and its mycobionts could serve as a suitable model system to address host effect.

Much of the early work about host effects on EcM assemblages focused on the genus *Alnus* (Molina, 1979; Molina, 1981). EcM fungal communities of other boreal and temperate ecosystems are more diverse compared to *Alnus*, which associates with greater proportion of host specific fungi (Kennedy & Hill, 2010). Around 50–60 species of EcM fungi are documented as ectomycorrhizal symbionts of *Alnus* world-wide (Pritsch et al., 1997; Kennedy & Hill, 2010; Rochet et al., 2011) and at least the basidiomycetes are strongly specific to their host tree genus (Molina et al., 1992; but see Kennedy et al., 2011). Based on a representative collection of fruit-body specimens, Rochet et al. (2011) argued that a few EcM fungal MOTUs (molecular operational taxonomic unit) partition hosts at the subgenus level in France. Hereafter the EcM fungal MOTUs are treated as species level taxa. While most of the *Alnus*-associated fungal MOTUs have been recorded only once, a few of the most common species are distributed both in Europe and North and South America (Becerra et al., 2005; Kennedy & Hill, 2010; Kennedy et al., 2011). Together with EcM fungi, *Alnus* forms tetrapartite association involving arbuscular mycorrhizal fungi (AM) and actinorrhizal bacteria. However, infection of AM is usually scant in mature trees (S. Põlme et al., unpublished) and EcM fungi are thought to play a major role in scavenging soil nutrients (Chatarpaul et al., 1989; Yamanaka et al., 2003). For *Alnus*, both fungal and actinobacterial root symbionts are obligatory and beneficial for obtaining atmospheric nitrogen and soil mineral nutrients, respectively (Yamanaka et al., 2003; Benson & Dawson, 2007). Although *Frankia* actinobacteria are always present in healthy *Alnus* stands, actinorrhizal symbiosis is facultative for the actinobacteria that are ubiquitous free-living soil organisms (Benson & Dawson, 2007; Chaia et al., 2010).

First known fossil records of *Alnus* dates back to late Tertiary (Chen & Li, 2004) and origin of genus is considered to be East Asia where the highest level
of endemism occurs (Navarro et al., 2003). Donoghue and Smith (2004) compared continental disjunction patterns of major plant clades and concluded that Beringia has been primary path between Old and New world. Fossil records support the hypothesis that Alnus has spread from Eurasia multiple times using both the Beringian and North Atlantic land bridges (Furlow, 1979). Today, Alnus comprises approximately 28–44 species that are widely distributed in the boreal and temperate zone of the Northern hemisphere and extends rapidly further south along with the Andes in South America (Chen & Li, 2004). Such wide geographical range of host-symbiont association could potentially provide a good model system for addressing biogeography, co-evolution and host specificity of microbial taxa (Yamanaka et al., 2003; Anderson et al., 2009; Pokharel et al., 2011; Rochet et al., 2011).

2.1. Aims and hypothesis

In this thesis, I focus on Alnus-associated ectomycorrhizal fungi because of their manageable richness, wide geographical distribution and similar habitat in wet soils. This thesis aims to disentangle the relative effects of ecological and climatic factors on the community structure and species richness of Alnus-associated EcM fungi at the regional and global scale. The following hypotheses were postulated:

1) since Alnus mycota seems to be distinct in broad geographical area despite environmental and spatial variation, we hypothesized that Alnus species and their phylogenetic relationships account for the strongest predictor of EcM fungal community composition at the intrageneric level at the global scale (III);

2) based on results of global metastudy (Tedersoo et al., 2012), we hypothesized that Alnus EcM fungal species richness is largely determined by mean annual temperature and precipitation at the global scale (III);

3) since edaphic variables have crucial importance in microbial ecology we hypothesized that at regional scale, soil conditions, particularly pH and limiting phosphorus, drive the EcM community structure and species richness rather than geographical and genetic variables (I);

4) global biogeography of Alnus EcM communities reflects ancient migration routes of host (III).

In addition, our aims were to

6) describe the morphological and anatomical features of EcM types in poorly investigated Argentinean Alnus forests (II);

7) investigate EcM mycota of Estonian Alnus communities (I).
3. MATERIAL AND METHODS

3.1. Sampling design

Study sites in *Alnus* habitats were arbitrary selected to meet certain criteria of each study. Each study site harbored at least six host trees situated at minimum 10 meters apart to secure statistical independence between individual samples (Lilleskov et al., 2006). From around each individual tree, one soil core (15*15 cm to 10 cm depth) including *Alnus* roots were collected with a spade or a sharp knife. In studies I and II, study sites were larger and more than six soil cores per plot were sampled (see details from particular articles). In total we sampled 97 study sites of *Alnus* habitats (Fig. 1) from all continents where *Alnus* is distributed, except Africa where a single species (*A. glutinosa*) inhabits a narrow area in Northwest part of continent. Out of ca. 28–44 valid species our sampling covered 22 *Alnus* species from all three subgenera — *Alnobetula, Clethropsis* and *Alnus* (Navarro et al., 2003).

![Figure 1](image)

**Figure 1.** 1A) map of study sites indicating number of host species/number of sites studied in the region; 1B) approximate *Alnus* distribution in the present day; a) hypothetical origin of the genus; b) possible migration route via Bering land bridge; c) possible migration route via North Atlantic land bridge.
Soil samples including *Alnus* roots were stored in the plastic bags until processed within 48 hours after collection. Tree roots were separated from soil and carefully cleaned under tap water. Ectomycorrhizal morphotypes were distinguished under stereomicroscope based on colour and roughness of mantle, presence of emanating hyphae and rhizomorphs (Fig. 2), except for study II where the more specific comparative anatomical protocol of Agerer (1991) was followed. All different morphotypes from each study site were stored in CTAB buffer (1% cetyltrimethylammonium bromide, 100 mM Tris-HCL (pH 8.0), 1.4 M NaCl, 20 mM ethylenediaminetetraacetic acid) and subjected to further molecular analyses. In order to analyse soil properties at each site, ca 50 g of rhizosphere soil was pooled from the six soil core samples (I, III). Concentrations of total soil nitrogen (N), exchangeable phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and soil pH were measured. In addition to soil properties we evaluated host age at each site, using available data of habitats or advice of local experts. Geographical coordinates and altitude were recorded using a GPS Garmin 60CSx (Garmin International Inc., Olathe, KS, USA).

![Figure 2](image1.png)

**Figure 2.** Selection of ectomycorrhizal root tips associated with *Alnus glutinosa*: a) *Cortinarius alnetorum*; b) *Inocybe* sp. (white); c) *Tomentella aff. ellisii*; d) *Lactarius* sp.
3.2. Molecular analysis

At least one mycorrhizal root tip from each morphotype per soil core was subjected to DNA extraction. Fungal genomic DNA was extracted using QIAGEN DNeasy 96 Plant Kit for study I and III, or QIAGEN DNeasy Plant Mini kit for study II following the manufacturer’s instructions in both cases. Fungal rDNA Internal Transcribed Spacer (ITS) region was amplified with a forward primer ITSOF-T (5’-actggtcatttagaggaatt -3’) in combination with reverse primers LB-W (5’-cttttcacctctccgagc -3’) or TW13 (5’-ggctcctttcaagg -3’) or primers ITS1F (5’-cttgcctttagaggaattcgaag -3’) and ITS4B (5’-caggaggacttgaacgctcgcg -3’) (Gardes & Burns, 1993) in study II. Detailed PCR protocols are described in particular articles. Primers TrnC (5’-cgaaatatgtagacgctacg -3’) and TrnD (5’-gggaagtcgaggttcgaaac -3’) were used to amplify plant plastid trnL region from mycorrhizas in study I and III, in order to confirm host identity of rare fungal species (occurring once or twice). PCR products were separated by electrophoresis through a 1.5% agarose gel in 0.5 × TBE buffer (45 mM Tris Base, 45 mM Boric Acid, 1 mM EDTA (pH 8.0)), visualized under UV light and purified using Exo-Sap enzymes (Sigma, St. Louis, MO, USA) in I and III, or a QIAquick PCR purification kit (QIAGEN, Hilden, Germany) in study II.

Sequencing of fungal DNA was performed with primers ITS5 (5’-gggatgtagacgctcagcgcg -3’) and ITS4 (5’-tcctccgcttattgatatgc -3’) in I and III or ITS1F and ITS4B (II). In studies I and III, sequences were assembled, checked, trimmed and manually corrected using Sequencher 4.10.1 software (GeneCodes Corp., Ann Arbor, MI, USA). Sequences were confirmed to belong to EcM lineages (cf. Tedersoo et al., 2010) or Alnus host trees by the use of blastN searches against the International Sequence Databases (INSD) or UNITE (Abarenkov et al., 2010). In study III, for each fungal lineage the ITS sequence of suitable outgroup taxa was downloaded from INSD and aligned automatically using MAFFT 6 (Katoh & Toh, 2008). In study II, the alignments were performed with ClustalW (http://www.ebi.ac.uk/clustalw/). In study I, 97% ITS region identity was used as a barcoding threshold except in the genus Alnicola, where molecular species were identified based on a neighbour-joining tree comprising both the generated root tip and previously deposited voucheded fruit-body sequences downloaded from the INSD. In study III, Maximum Likelihood (ML) and fast bootstrap analyses were performed applying default settings in RAxML (Stamatakis et al., 2008) as implemented in the Cipres web portal in order to create phylograms of all Alnus-associated fungal genera. These phylograms were used to distinguish fungal MOTUs based on combination of branch length and bootstrap support values (Fig. 3). In study II, a Neighbour joining analysis was performed in 4.0d81 PAUP (Swofford, 2002), to demonstrate the phylogenetic affinities of Alnus-associating Tomentella spp.
Figure 3. Delimitation of *Inocybe* species based on ITS phylogram.
3.3. Data analyses

MOTU occurrence data of soil core samples in each study site were pooled and each site was treated as a single sampling unit. Bray-Curtis dissimilarity metric was used to calculate community distance matrices (III). All soil nutrient concentrations were logarithm-transformed prior to analyses (I, III). In addition, EcM colonization and soil nutrient proportions were arcsine-square-root transformed and species richness was square-root-transformed (I).

Estimates of the mean annual temperature and precipitation were retrieved from a high-resolution database of Earth’s surface climate (Hijmans et al., 2005) using the software ArcGIS 9.3 (ESRI, Redlands, CA, USA). The effect of geographical and phylogenetic distances derived from geographical distance matrix and host phylogeny respectively, were taken into account using Principal Coordinates of Neighbour Matrices (PCNM) — eigenvectors that are used to transform distances to rectangular data that suitable for constrained ordination or regression (Borcard & Legendre, 2002). Significant PCNM vectors (α=0.05) were forward-selected in the Packfor package of R (Dray et al., 2007) and used in the multivariate analyses (III).

3.3.1. Community structure

To address the relative importance of climatic, edaphic, spatial and biological factors on community structure of EcM fungi as based on the frequency of MOTUs in study III, we used a permutational multivariate analysis of variance as implemented in the Adonis routine of the Vegan package of R (Oksanen et al., 2007). Adonis is a nonparametric modification of multivariate ANOVA. Adonis enables to include multiple factors, covariates and their interactions in the model. Statistical significance was tested against 999 null permutations (III). In study I, the main variables accounting for changes in the EcM fungal community structure were tested for significance implementing the computer program DISTLM forward 1.3 (McArdle & Anderson, 2001) using 9999 permutations. DISTLM forward does a multivariate multiple regression based on forward selection of predictor variables, either individually or in sets, with tests by permutation. All individual variables were tested (i) separately or (ii) grouped by site, soil, host and micro-biotope before the analysis.

3.3.2. Species richness

In study III, the effects of edaphic factors and climate on species richness of EcM fungi were addressed by use of the Generalized Least Squares analysis (GLS) as implemented in the nlme package of R 2.12.1 (Pinheiro et al., 2008). In study I, the effects of site, host species, micro-biotope and soil nutrient variables on species richness per sample (i.e. species density) and EcM
colonization were studied using a mixed ANOVA with plot as a random factor nested within site and site nested within host species.

3.3.3. Biogeography

In order to evaluate similarity of fungal communities between biogeographic regions (III), statistical support of similarity in the area cladograms was tested in the Pvelust package of R (Suzuki & Shimodaira, 2006). Pvelust calculates probability values (p-values) for each cluster using bootstrap resampling techniques with 1000 repetitions.
4. RESULTS AND DISCUSSION

Phylogenetic relations among hosts accounted for strongest predictor of EcM community structure at global scale (III), while at regional scale edaphic variables, particularly soil organic matter and pH were the main determinants of community structure among two sister species (I). Ecological processes are often scale dependent (Schneider, 2001), which is also relevant in case of \textit{Alnus} mycobiont communities. \textit{Alnus} is considered to possess strongest host-genus specificity among EcM trees (Molina, 1981). It is suggested that host specificity is most pronounced at the level of \textit{Alnus} subgenus, but not between sister species (Rochet \textit{et al.}, 2011). This is supported by results of study I where redundancy analysis revealed marginal difference between \textit{A. glutinosa} and \textit{A. incana} EcM communities. However, when accounting for host evolutionary history as measured by multi-level phylogenetic relationships among 22 \textit{Alnus} species at the global scale, host explains 43\% of EcM community structure, being the most influential factor (III). Based on Estonian material, \textit{Alnus} exhibits strong reciprocal specificity with the associated mycobionts especially with basidomycetes (I), which was generally supported at the global scale (III).

Highly similar EcM morphotypes resulted in different sequences referring to cryptic radiation of closely related species (I, II, III). Host promiscuity and speciation via shifts to phylogenetically distant hosts rather than co-speciation seems to be more frequent in other groups of EcM fungi (e.g. den Bakker \textit{et al.}, 2004; Suvi \textit{et al.}, 2010; but see Wu \textit{et al.}, 2000). However, different \textit{Alnus}-associated lineages are not closely related indicating frequent host shifts after the divergence of \textit{Alnus} and \textit{Betula} (I). The important role of host structuring mycobiont community has been increasingly shown in numerous studies (Ishida \textit{et al.}, 2007), but according to the best of our knowledge this is the first time when intragenic host effect, measured by multi-level phylogenetic relationships, is tested at global scale.

Spatial structure explained roughly 10\% of EcM community composition at global and regional scales (I, III). Biological similarity typically decreases with geographical distance, known as distance decay. Distance decay has important role in ecology (Nekola & White, 2004) and is often regarded as self-evident. However, studies of ectomycorrhizal communities have controversial results — for example Queloz and colleagues (2010) demonstrated that the similarity of species assemblages of the \textit{Phialocephala fortinii} s.l.–\textit{Acephala applanata} species complex did not decrease with increasing geographical distance. Members of this complex are, however asexual root endophytes and their species context obscure, which may have blurred particular results. We demonstrated that in case of \textit{Alnus} mycobionts, distance decay is evident, but by far less influential than host phylogeny (III).

Soil calcium accounted for the strongest predictor of richness of \textit{Alnus}-associated EcM species at global scale (III) exhibiting positive effect, while soil humidity was most important at local scale (I). To best to our knowledge, study III is the first study, simultaneously accounting for edaphic variables with
unified methodology and addressing ectomycorrhizal ecology at global scale. Therefore, this unexpected outcome contrasts with the conclusions of metastudy conducted by Tedersoo and colleagues (2012), who demonstrated that global patterns of EcM species richness can be largely ascribed to mean annual temperature and precipitation. Biotic calcium availability is often a limiting factor shaping ecosystem structure and function (Beier et al., 2012) as calcium deficits may have pervasive impacts at multiple scales from organisms to ecosystems (Likens et al., 1998; DeHayes et al., 1999; Hamburg et al., 2003). The effect of liming in manipulative field studies supports our finding — calcium affected the overall fungal richness in forests of Picea and Fagus (Rineau & Garbaye, 2009). Aponte and colleagues (2010) accounted for natural calcium gradient and demonstrated that greater soil calcium concentration favours generalist EcM species over specialists. However, direct causal relationship between Alnus-associated EcM diversity and calcium concentration remains conjectural. Possibly, elevated levels of available soil calcium could enhance the role of EcM symbiosis in mineral nutrition of plants, which in turn broadens the niche for co-existence of more species. Arnott (1995) suggested that ubiquity of Ca-oxalate crystals in fungal hyphae may provide selective advantage to the organism. It has been shown that calcium uptake is limiting for many key functions of plants (McLaughlin & Wimmer, 1999) and improved physical condition of host plants may thereby enhance richness of EcM fungi (Swaty et al., 2004). In addition, Alnus and its EcM fungi might have elevated requirements of soil calcium since the presumed origin of host genus is Southeast Asia (Navarro et al., 2003), where limestone rich habitats are abundant. Moreover, unexpected results of study III might be influenced by specific ecological traits of Alnus genus. For example, Alnus species are absent from tropical lowland forests, which shortens the temperature gradient by ca 25%, just from the critical point above 20 °C, where EcM fungal richness has been suggested to decline (Tedersoo et al., 2012). Association with actinobacteria, pioneer or riparian habitats and extreme reciprocal specificity makes Alnus distinct host genera, which might strongly contribute to symbiont diversity. In particular, low within site species richness may cause low resolution blurring global patterns.

While the effect of temperature was negligible, study III did confirm the negative relationship between mean annual precipitation and species richness at global scale (Tedersoo et al., 2012), whereas plot humidity was most important determinant of richness at regional scale (I). For example, dry sites harboured three times more singletons than waterlogged sites at regional scale (I). It is suggested that anaerobic conditions in water saturated soils may cause low oxygen stress and/or competition among functional guilds of soil microbes (Tedersoo et al., 2012). Since enhanced water uptake is regarded as one of the main benefits for host plant from EcM (Marschner & Dell, 1994) with increased drought tolerance (Parke et al., 1983), water saturated soils may also reduce mycorrhizal dependence of host plants, potentially reducing EcM diversity and biomass.
The total richness of 146 species found worldwide (I, II, III) is substantially higher than previous records from *Alnus* EcM studies and can be ascribed to greater sampling effort in terms of both geographical area and number of host species. In addition to dominant fungal species that were relatively widespread and abundant across most of study sites and host species, our data indicates numerous rare species found only once or twice that had restricted geographical range and host associations. This could be partly matter of sampling intensity as for many taxa, especially microbes, asymptote may never be reached (Gotelli *et al*., 2001). However there are studies that demonstrate that even severely undersampled communities are able to detect valid diversity patterns and gradients (Kuczynski *et al*., 2010). According to extrapolation of rarefaction curve, the number of *Alnus*-associated EcM fungal species is substantially greater and certain isolated locations or rare host species may harbour several potentially endemic taxa (Rochet *et al*., 2011).

All studied species of *Alnus* seem to exhibit narrow specificity towards highly specific fungal taxa. Thus, high specificity seems to be a common phenomenon of all *Alnus* species, which distinguishes this genus from other members of the Betulaceae family. Such great, uniform specificity seems to be unique to *Alnus* among EcM plant genera. There is some evidence that the level of specificity may be quite variable among the tropical genera *Pisonia* (Nyctaginaceae; Suvi *et al*., 2010) and *Gnetum* (Gnetaceae; Tedersoo & Põlme, 2012).

In accordance with earlier studies, *Alnus*-associated EcM fungal communities were relatively uniform at the global scale (I, II, III). Most abundant and species-rich phylogenetic lineages of EcM fungi included /tomentella-thelephora (comprising 32 MOTUs in 95% of sites), /hebeloma-alnicola (22 MOTUs in 67% of sites), /cortinarius (24 MOTUs in 52% of sites), /russula-lactarius (15 MOTUs in 43% of sites), /inocybe (13 MOTUs in 21% of sites) and /genea-humaria (6 MOTUs in 21% of sites). Despite resemblance of *Alnus*-associated EcM communities at global scale, relative abundance of fungal lineages were strongly shifted in certain regions. For example /genea-humaria was dominant lineage in Iran, but only occasionally present in other regions. In addition, the /inocybe lineage was also relatively common in Iran compared with the other regions. Similarly, sampling *Alnus* communities in Mexico mountain forests ascertained high abundance of /clavulina and /sebacina species (Kennedy *et al*., 2011), which were rarely present *Alnus* symbionts in the rest of the study areas. Causes of these discrepancies remain unknown as community composition of other hosts in Iran and Mexico provides no evidence to the hypothesis that certain regional environmental conditions favour the abundance of particular EcM fungal lineages (Morris *et al*., 2009; Bahram *et al*., 2012). Moreover, it is not the case of unique host association as South America fungal communities of the same host do not resemble to Central American communities in terms of abundance of particular fungal lineages.

Remarkably, several *Alnus*-associated mycobionts that shared 100% ITS similarity occured both in Europe, Asia and America (I, II, III), which is the
The greatest natural range of EcM fungal species besides the asexual *Cenococcum geophilum* complex. Symbiont migration follows migration routes of host (Murat et al., 2004) or may occur via long distance dispersal events (Moyersoen et al., 2003). Considering strong reciprocal specificity characteristic to *Alnus* mycobionts, presence of the same EcM species in such large geographical distance is probably a result of host-symbiont co-migration. In specific biological interactions such as *Alnus* and its EcM fungi, competition among species and environmental filtering may be less strong than in communities that are saturated with species.

Comparison between fungal community similarities of different regions suggested that the host and mycobiont have co-migrated at different spatial and temporal scales (III). For example, the relative similarity of EcM community in Eastern Asia compared with Northwest America is consistent with the hypothesis that many temperate forest plant groups moved out of Asia to the new World mostly via Beringian land bridge during the last 30 Myr (Donoghue & Smith, 2004). Last glacial maximum depleted dramatically biota of Northern and Central Europe and most of the species survived in Southern refugia. After retraction of ice shield Northern Europe was re-colonized by the descendants of Southern refugia (Hewitt, 1999) resulting in high similarity between these regions.

Both, the wide distribution of symbiont species and biogeographic similarity between South and North Europe, and Asian regions with Northwest America provides evidence for strong co-migration between the mycobiont and host.
5. CONCLUSIONS

The following main conclusions and working hypotheses can be inferred from my thesis:

- Intrageneric phylogenetic relationship among *Alnus* species is the main driver of EcM community structure at the global scale, while sister species support similar fungal communities at regional scale. Cryptic radiation of EcM fungal species within the host genus seems to be more prominent compared to other host genera (I–III).

- Geographical and edaphic variables have a minor effect on *Alnus*-associated EcM fungal community compared to host identity at the global scale (III), while the complex of soil variables and geographical (site) effect drives the community composition at the local and regional scales (I).

- The positive effect of soil calcium on *Alnus*-associated EcM fungal species richness contrasts with the general unimodal relationship between EcM fungal species richness and mean annual temperature (latitude). This finding also refers to the potential importance of local predictors including edaphic variables at the global scale. However, I anticipate that this contrasting pattern may result from the peculiar ecology (wet and pioneer habitats, association with actinobacteria and narrow range of EcM fungi) of the genus *Alnus* (III).

- The mean annual precipitation has a negative effect on *Alnus*-associated EcM fungal species richness at the global scale (III). Similarly, soil humidity accounts for the strongest predictor of species richness at the local scale (I).

- Although *Alnus* mycobiont communities are relatively uniform at global scale, there are certain discrepancies in particular biogeographic regions (III).

- Comparison of fungal community similarity supports the hypothesis of Beringia being the primary migration route of *Alnus* between Eurasia and America. The mycota of Northern Europe is highly similar to that of Southern Europe due to the recent origin of northern biota from southern refugia (III).
6. SUMMARY

Discipline of biogeography addresses distribution and abundance of organisms at various geographical scales. Traditionally, biogeographic research has focused on macroorganisms due to their size and straightforward species delimitation. Therefore, ecological patterns of macroorganisms are well documented, while relevant microbial data is controversial and give rise to wide debates. Microbial biogeography patterns do not necessarily coincide with patterns observed in macroorganisms. Moreover, microbial taxa and ecological guilds could be subject to different ecological factors.

Ectomycorrhizal (EcM) fungi that form mutualistic relationships with many ecologically and economically important host trees are typically regarded as microorganisms. Current metadata suggest that host identity has a key role in fungal community structure. In contrast to most macroorganisms, EcM fungal species richness exhibits unimodal relationship with latitude, which is largely ascribed to favourable climate in temperate regions as well as the evolutionary origin of major host taxa. However, to date there are no studies with unified methodology addressing EcM fungal ecology within a particular host genus at global scale. In this thesis, I aimed to disentangle ecology and biogeography of Alnus-associated EcM fungi in different spatial scales. Alnus has a wide geographical distribution range and manageable richness of fungal symbionts, which we considered favourable for addressing differences in richness and inferring biogeographic patterns. I postulated the following alternative hypothesis: 1) host species and their phylogenetic relationships account for the strongest predictor of EcM fungal community composition at the intrageneric level at the global scale; 2) at regional scale, soil conditions such as pH and phosphorus concentration drive the EcM community structure rather than geographical and host genetic distance; 3) at global scale, Alnus-associated EcM fungal species richness is largely determined by the mean annual temperature and precipitation; 4) global biogeography of Alnus EcM communities reflects ancient migration routes of the host.

The main results and conclusions are the following: 1) phylogenetic relations among hosts account for strongest predictor of EcM community structure, while geographical and edaphic variables have a relatively low impact at the global scale; 2) at the regional scale edaphic variables were the dominant determinants of EcM fungal community structure; 3) soil calcium accounted for the key determinant of Alnus-associated EcM fungal species richness at the global scale, while soil humidity was the most influential at the local and regional scales; 4) Beringia is likely to be primary migration route of Alnus from Eurasia to America rather than North Atlantic land bridge; 5) high similarity between North and South Europe probably results from recent post-glacial recolonisation.
7. SUMMARY IN ESTONIAN
Lepa juurtel ektomükoriisat moodustavate seente biogeograafia ja ökoloogia.


Lepa juurtel ektomükoriisat moodustavate seente biogeograafia ja ökoloogia. Ökoloogia uurib organismide interaktsioone ja sõltuvust keskonnast, biogeograafia uurib taksonite ja ökosüsteemide levikut ja sellest tulenevaid protsesses. Traditsiooniliselt on ökoloogia ja biogeograafia peamisteks uurimisobjektideks olud makroorganismid. Molekulsete meetodite kasutamine on näitavaks olnud, et mikroorganismide levikut ja sellest tulenevaid protsessi uurimisel on oluline. Ökoloogia ja biogeograafia peamisteoks oluld taksonite ja ökosüsteemide levikut ja sellest tulenevaid protsessi uurimisel. Ökoloogia uurib organismide ühtseid ja erinevaid levikut, biogeograafia uurib taksonite ja ökosüsteemide levikut ja sellest tulenevaid protsessi. Ökoloogia ja biogeograafia peamisteoks oluld taksonite ja ökosüsteemide levikut ja sellest tulenevaid protsessi uurimisel.

Ektomükoriisat moodustavate seente biogeograafia ja ökoloogia. Ökoloogia uurib organismide interaktsioone ja sõltuvust keskonnast, biogeograafia uurib taksonite ja ökosüsteemide levikut ja sellest tulenevaid protsesses. Traditsiooniliselt on ökoloogia ja biogeograafia peamisteks uurimisobjektideks, kes määratletakse üheks maailma liitutud objektideks. Ökoloogia uurib organismide interaktsioone ja sõltuvust keskonnast, biogeograafia uurib taksonite ja ökosüsteemide levikut ja sellest tulenevaid protsesses. Traditsiooniliselt on ökoloogia ja biogeograafia peamisteks uurimisobjektideks, kes määratletakse üheks maailma liitutud objektideks.
ja teadaolevalt esinevad mitmed scenetaksoid ainult lepa juurtel. See on erinev
enamikest teistest ektomükoriisist moodustavatest puu perekkondadest, kus sellist
spetsialiseerumist esineb harva. Ligikaudu viimase kolme miljoni aasta jooksul
on lepp kiiresti laiendanud oma levilat Lõuna-Ameerikas, kasutades Kesk-
Ameerika ja Andide mäestikke levikukoridorina läbi troopiliste laiuskraadide.
Selline kirjeldatud laialt levinud peremees-sümombiooni kooslus on sobiv mudel
globaalseks biogeograafia ja ökoloogia uurimiseks. Minu doktoritöö peamised
testitavad hüpoteesid oled alljärgnevad: 1) globaalses skalaars mõjutab lepa
seenkoosluse struktuuri enim peremeesliikide fülogueenetiline distants;
2) regionaalises skalaars mõjutab lepa juurtel ektomükoriisat moodustavate
seente koosluse struktuuri mulla koostis, eriti pH ja fosfor; 3) globaalses skalaars
on lepaga seotud ektomükoriisat seente liigilise mitmekesisus määratud aasta
keskmise temperatuuri ja sademete hulga poolt; 4) lepaga seotud ektomükoriisa
seente biogeograafia peegeldab peremehe globaalse levikuteid.

Käesolev doktoritöö võimaldab teha mõned uusid järeldusi ja kinnitada
olemasolevaid hüpoteese. Globaalses skalaars mõjutavad peremeesliikide fülogueenetilised
distantsid 43% ulatuses lepaga seotud ektomükoriisisse teente
koosluse struktuuri. Ruumiline paikneb mõjutab seenekoosluse struktuuri
10% ulatuses ning mulla pH–1 ja aasta keskmisel temperatuuril on marginaalne
mõju. Seevastu regionaalises skalaars, määravad mulla pH ja orgaanilise aine
sisaldus, enim seenekoosluse struktuuri. Varasemad uuringud on leidinud, et
peamised mõjutegurid, mis määravad ektomükoriisisse teente liigirikkust glo-
baalises skalaars, on aasta keskmine temperatuur ja sademete hulk. Täpsemalt on
tuvastatud ektomükoriisa liigirikkuse unimodaline seos temperatuuriga ja nega-
tiivne seos sademete hulga. Meie uuringust selgus üllatuslikult, et lepaga
seotud ektomükoriisisse teente liigirikkus on enim mõjutatud ja positiivses
seoses mulla kaltsiumsisaldusega. Samas leidis kinnitust negatiivne seos ekt-
omükoriisa liigirikkuse ja sademete hulga vahel. Meile teadaolevalt oli see
esimene uuring, mis ühtselt metoodikaga adreseeris ektomükoriisa bioge-
ograafiat globaalses skalaars ja võtis ühtlasi arvesse ka mulla omadusi. Sarnaselt
globaalsele mustrile oli ka regionaalises skalaars liigirikkus negatiivses seoses
mulla veesisaldusega. Mõned lepaga seotud mükobiondid, kes esinevad
Ameerikas, Euroopas ja Aasias, jagavad omavahel 100% sarnasust ITS järjes-
tustes. Vörreldes lepaga seotud seenekoosluste liigist ja arvulist koosseisu erine-
vate mandrite ja piirkondade vahel selgus, et tõenäoliselt on Beringia mais-
maal olnud põhilise lepa levikutee Eurasia ja Ameerika vahel. Põhja ja
Lõuna Euroopa seenekooslused osutusid väga sarnaseks, mis tõenäoliselt tule-
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