The impact of liming on ectomycorrhizal fungal communities in coniferous forests in Southern Sweden

Rasmus Kjøller, Karina E. Clemmensen

SKOGSSTYRELSEN

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Foreword

The Swedish Forest Agency has worked with measures to counteract soil acidification for more than 15 years. Based on knowledge and experience gained an action programme was presented in 2001. The programme focused on countermeasures for acidification caused by pollution, compensation for nutrient removal with extraction of harvest residues, and management strategies adjusted to sustainable forestry. The implementation of the programme was proposed to contain a preparatory phase of three years followed by an action phase of ten years. The preparatory phase aimed at investigating and solving issues identified in the action programme. Further, large scale liming was suggested to develop tools for practical implementation.

Late 2004 the government permitted the Swedish Environmental Protection Agency to allocate SEK 10 million for measures to counteract acidification of forest soils according to the preparatory phase in the action programme. The Swedish Forest Agency developed, in co-operation with the Swedish Environmental Protection Agency, a project plan spanning from 2005 to 2007. In this plan, the study presented in this report was outlined.

Liming may affect flora and fauna. Ectomycorrhizal fungi have been found to be particularly sensitive. In the present report, a study aiming at 1) a review of results from previous studies of lime and ash addition effects and 2) an additional field study to monitor if three tonnes of lime per hectare have caused any significant effects 17 years after liming is presented.

The authors are solely responsible for the report and the views in it. Consequently, it is not an expression of the views of the Swedish Forest Agency.

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Karin Hjerpe
Project manager

Summary

- Ectomycorrhizal fungi are mutualistic symbionts of most boreal forest trees including spruce and pine and they are essential to tree nutrition by exchanging nutrients taken up from the soil with carbon derived from plant photosynthesis.

- In this report studies on effects of liming and ash addition on ectomycorrhizal fungal communities with focus on Scandinavian coniferous forests are reviewed. In addition, we report a newly conducted field study where changes in the belowground ectomycorrhizal fungal community were recorded in plots limed with 3 tonnes of dolomitic limestone per hectare 17 years prior to the study.

- The reviewed studies including the present field study clearly demonstrated that attempts to counteract acidification of forest soils by liming will influence mycorrhizal communities both aboveground and belowground, although only minor effects have been documented with doses less than 2 tonnes per hectare. In the conducted field study, 40, 59 and 51 ectomycorrhizal fungal species were identified at the sites O2, P2 and R2, respectively. Within all three sites only about 25% of the species overlapped between the limed and the reference plots. In general, the most abundant species were found in both limed and reference plots, and 60-70% of the root tips at each site were colonised by species occurring in both limed and reference plots. At the other hand, by summing up all significant root tip increases and decreases at each site, we estimated that 33% of the individual root tips became colonised by a different fungal species as a consequence of liming. Across all three sites, fungal species belonging to the species groups *Tylospora*, *Elaphomyces* (Hjorttryffler) and Pezizales (Skålsvampar) became significantly more frequent in limed plots, while species belonging to *Russula* (Kremlor) and *Lactarius* (Riskor) decreased in frequency. These results corroborate the results of several of the reviewed studies. At individual sites, several more species including *Amphinema*, *Piloderma*, *Inocybe* and *Hygrophorus* sp. were also significantly influenced by liming.

- Liming generally did not reduce species richness but increased the relative frequencies of the 1-2 most abundant species and shifted the detected assembly of rare species. In the reviewed studies – including the present field study – it has not been possible to conclude whether any species were extinct from or invaded the limed areas because of the low frequency of many species. However, it is reasonable to expect that the relative frequencies of the rare species are affected to the same degree as frequencies of the common species. This could potentially lead to species extinctions if sensitive species occur as geographically isolated populations - in other words - liming could lead to a loss of biodiversity. The larger the limed area - the higher the risk of causing species extinctions. Thus, if a fixed percentage forested area is limed regionally, this risk would be minimized if the limed plots are kept as small as possible.

- We do not know much about the functional redundancy among the vast number of ectomycorrhizal fungal species, or whether changed relative frequencies (or loss) of particular species would be a thread to the overall function of the forest

ecosystems. The present review points to particularly two qualitative shifts in ectomycorrhizal functioning that seem to be a risk if specialized ectomycorrhizal species are affected: first, a shift from primarily organic N mobilization and uptake to primarily inorganic N uptake, and, second, a loss of weathering capacity of the ectomycorrhizal community. Both of these functional shifts potentially have important impacts on tree nutrition and forest nutrient cycling, particularly if they are maintained in the long term.

1. Introduction

To revitalize acidified Southern Swedish forests lime and wood ash additions have been suggested as possible ameliorating treatments. The Swedish Forest Agency coordinates an extensive experimental research programme on the effects of liming and nutrient compensation in Swedish spruce forests. The main part of the programme concerns effects of addition of 3 ton crushed dolomitic limestone per hectare on trees, soils and water chemistry, and a range of soil parameters have been followed intensively since the lime was added to the forest floors in 1990-1991 (reports from IVL, Institutet för Vatten- och Luftvårdsforskning). However, only limited amount of data exists on how liming has influenced the symbiotic ectomycorrhizal (ECM) fungi within these forests.

Ectomycorrhizal fungi are mutualistic symbionts of most boreal forest trees including spruce and they are essential to tree nutrition by exchanging nutrients taken up from the soil with carbon derived from plant photosynthesis. Ectomycorrhizal fungal communities are highly diverse often with 20-50 species within a few hundred square meters (e.g. Dahlberg *et al.*, 1997; Jonsson *et al.*, 2000). Because of differences in how the individual fungal species take up nutrients from the soil (Leake & Read, 1997) any change in soil chemistry, e.g. liming, is likely to change the community composition of these fungi.

Ectomycorrhizal associations consist of the mycorrhizal root tip, where fungal tissue covers the tip of the tree root, and the external mycelium radiating from the mycorrhizal root (Smith & Read, 1997). Sometimes a fungal sporocarp (fruit body) is formed as well as an extensive external mycelial phase. Sporocarps may be formed belowground (e.g. truffles) or aboveground (e.g. boletes and chanterelles). The mycorrhizal root tip is the main place for interactions between the tree and fungus whereas the external mycelium has the primary contact with the surrounding soil environment. Typically close to 100% of the fine roots are colonised by ECM fungi which means that most nutrients taken up by the forest trees enter via the root-mycorrhizal interface. ECM fungi vary widely both functionally and structurally, e.g. with respect to exoenzyme production (Leake & Read, 1997), weathering capacity (Wallander, 2000), development of external mycelium (Agerer, 2001) and tolerance to high soil nutrient levels (Arnolds, 1991). The species composition of an ECM community is therefore tightly linked with both ECM fungal biomass and ECM functioning. Thus, decreased importance of particular mycorrhizal fungi and their replacement by fungi with different ecological functions with liming could have significant implications for forest vitality and sustainability.

It is well documented that ECM community composition can change in response to environmental perturbations such as increased N deposition or fertilization (Wallenda & Kottke, 1998; Cairney & Meharg, 1999; Lilleskov *et al.*, 2001; Lilleskov *et al.*, 2002a). Also, some studies have shown changes in ECM species richness or community composition with additions of lime or ash (e.g. Kårén & Nylund, 1996; Jonsson *et al.*, 1999; Taylor & Finlay, 2003). However, treatment doses, time elapsed since treatment and the methodology used to quantify ECM communities have varied widely among these studies. Thus, there is still an urgent

need for field studies on responses of ECM communities to addition of lime, especially in the perspective of using lime as an ameliorating treatment to compensate soil acidification (Taylor & Finlay, 2003).

The aims of this study are:

- To review earlier results from studies of liming and ash addition effects on ectomycorrhizal fungal communities with focus on Scandinavian spruce forests.

- To conduct an additional field study to monitor if liming with 3 tonnes of dolomitic limestone per hectare (the dose currently under consideration for large-scale liming in Sweden) 17 years prior to the investigation have caused significant effects on ectomycorrhizal fungal communities in Southern Swedish spruce and pine forests.

- To summarize the conclusions of the review and the conducted field study into recommendations - from a mycorrhizal point of view - on the future use of lime as a counter measure to soil acidification in Swedish spruce forest.

2. Literature review

This literature review first briefly summarises knowledge of the natural and human-accelerated soil acidification processes and the mitigating effects of liming and wood ash application on forest soils. Then it concentrates on documented effects of lime and ash additions on mycorrhizal fungal communities and discusses their causes and their consequences for forest vitality.

2.1. Soil acidification and mitigating treatments

Liming experiments and practices were started already in the beginning of the 20th century with the aim to improve forest production in Northern Europe (Lundström *et al.*, 2003). As most forest soils mature they naturally get more acidic because of accumulation of organic matter and humus in the soils. Liming with calcite ($CaCO_3$) or dolomite ($CaMg(CO_3)_2$) was intended to enhance decomposition rate and nutrient availability through increasing soil pH to a level more favourable for biological activity. With the recognition of the harmful effects of man-caused atmospheric acid deposition (mainly NO_x and SO_2 derived acids) in the early 1980's, liming and wood ash additions have been tested as mitigating treatments to counteract accelerated soil acidification and forest decline (Huettl & Zoettl, 1993).

Decreased tree vitality with atmospheric acid deposition in central Europe has been hypothesized to be linked to Al toxicity in acidic base-poor soils and to Mg deficiency in damaged trees (Huettl & Zoettl, 1993). When pH of a forest soil decrease, the amount of inorganic Al in soil solution increase because Al^{3+} is released from organic complexes due to equilibrium processes. Hydrogen ions also exchange with base cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) on the soil particles until they are depleted of base cations, i.e. base saturation becomes low. Thus, high concentrations of Al^{3+} and H^+ ions lead to a decreased proportion of exchangeable base cations in soil solution because of leakage of these ions from the soil. It has been suggested that decreased tree growth with acidification is probably a result of nutrient deficiencies (Lundström *et al.*, 2003). Also, high concentrations of Al with acidification have been assumed to affect tree growth directly through cell-toxic effects of Al^{3+}.

The harmful effects of acid deposition on forests depend both on the acid deposition load and on the ability of a specific forest site to assimilate the excess acid. Acid deposition has the most pronounced effects at sites with low soil cation exchange capacity, and the molar ratio between base cations and inorganic Al has been used to estimate critical loads[1] of acidity for different forest sites across Europe and NE USA (e. g. Akselsson *et al.*, 2004). Although the acid deposition load in the Nordic countries is lower than in central Europe, the impact of acid deposition on coniferous forest soils is significant because most soils are developed from gneisses and granites with low cation exchange capacity which make them sensitive to acidification (Lundström *et al.*, 2003). However, an extensive

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[1] The critical load is defined as: 'a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified elements of the environment do not occur according to present knowledge' (Akselsson *et al.*, 2004).

evaluation including 80 sites in SW Sweden did not find any correlation between soil acidification and forest production (Nyberg *et al.*, 2001).

Ectomycorrhizal fungal communities are most likely affected by both natural and man-made acidification of forest floors. Natural soil acidification probably contribute to the succession of ECM fungal communities observed as forests mature, although this is also related to factors such as changes in organic matter content and quality and physiological changes in host plants (Visser, 1995; Cairney & Meharg, 1999). Some field studies with experimental acid irrigation have found changes in ECM fungal communities (e.g. Qian *et al.*, 1998) whereas others showed no significant change (Agerer *et al.*, 1998). Thus the degree to which ECM fungal communities adjust to soil acidification may depend on the specific conditions of a forest site. It has been hypothesized that ECM fungi can weather mineral particles from the inside and thus are not much affected by increasing H^+ and Al^{3+} concentrations in the surrounding soil solution, and that this could explain the lack of correlation between forest production and soil acidification (van Breemen *et al.*, 2000; Lundström *et al.*, 2003).

Field experiments have generally shown that liming generate the anticipated effects in of increased pH (0.2-2 pH units), cation exchange capacity, base saturation and Ca/Al ratio in the upper soil layers (Huettl & Zoettl, 1993; Kreutzer, 1995; Lundström *et al.*, 2003; Uggla *et al.*, 2003). However, effects which can be regarded as less good for the soils nutrient conservation such as increased concentrations and leaching of nitrate and dissolved organic C and N (e.g. Kreutzer, 1995; Andersson *et al.*, 1999), decreased N and C contents of the humus layer and acidification of the mineral subsoil have also been found (Kreutzer, 1995; Lundström *et al.*, 2003; Uggla *et al.*, 2003).

2.2. Methodological considerations concerning the reviewed field studies

Quite a few field studies on lime and ash effects on ectomycorrhizal fungal communities have been conducted (Table 1-3). However, it should be kept in mind that they are often not directly comparable because their methodologies differ highly particularly in three aspects:

First, liming types (dolomite/calcite/mixtures, grain sizes) and doses (equivalent to 0.033-8 t Ca ha^{-1}) as well as periods of time that have elapsed since the treatments were applied (1-30 yr) differ among studies (Table 2). Also specific site conditions (e.g. initial nutrient status and acidity of the soils) differ among experimental forests.

Second, in the belowground studies there seem to be a trade-off between the total number of root tips screened in a study and the taxonomic resolution of the ectomycorrhizal typing, i.e. the number of ECM types/species identified (Table 1). In some studies with presumably high taxonomic precision such as the studies by Mahmood *et al.* (2002) and by Johnsson *et al.* (1999) relatively few root tips were screened (a few hundreds) due to methodological limitations. Because of the inherently high spatial and temporal variation in ECM communities (e.g. Antibus & Linkins, 1992; Lilleskov *et al.*, 2004) this may have limited their chance of detecting changes. On the other hand, studies with thousands of root tips screened may

be more robust to spatially variable ECM communities but may loose important information on effects on single species because several species are often pooled in ECM morphotypes when using macroscopic typing only (Kårén & Nylund, 1996).

Table 1. Methodology, total numbers of root tips screened and total numbers of ectomycorrhizal types distinguished in the reviewed studies of lime and ash effects on ectomycorrhizal communities.

Study	Method[1]	Total number of tips screened	Total number of ECM types or species found
Present study, 2007	Molecular typing, ITS sequencing	969	109
Taylor & Finlay, 2003	Morpho- and anatomotyping	4582 (Horröd)	45 (Horröd)
		998 (Hasslöv)	24 (Hasslöv)
Mahmood *et al.*, 2002	Molecular typing, ITS-RFLP	270	20
Bakker *et al.*, 2000	Morphotyping	100000	5
Jonsson *et al.*, 1999	Molecular typing, ITS-RFLP	225	16
Qian *et al.*, 1998	Morpho- and anatomotyping	2839	14
Wallander *et al.* 1997	Morphotyping	n.a.	6
Kårén & Nylund, 1996	Morphotyping	60000 (Skogaby)	20 (Skogaby)
		18000 (3 sites)	16 (3 sites)
Andersson & Söderström, 1995	Morphotyping	n.a.	6
Lehto, 1994	Morphotyping	n.a.	4
Antibus & Linkins, 1992	Morphotyping	n.a.	8
Erland & Söderström, 1991a	Morphotyping	n.a.	6
Lehto, 1984	Morphotyping	n.a.	7

[1]Morpho- and anatomotyping refers to the use of macroscopic (typically 5-64x magnification) /or microscopic (>100x magnification) characters when assigning individual root tips to taxons. See section 2.3.1. above for a more detailed explanation.

n.a. = not available

Third, different sampling strategies may have affected the descriptions of ECM communities and how they were affected by treatments (Taylor, 2002; Lilleskov *et al.*, 2004; Koide *et al.*, 2005a). Geographical scales of the studies vary. About half of the studies in Table 2 were done in replicated experiments in forests of < 1 – 2 ha (Andersson & Söderström, 1995; Kårén & Nylund, 1996; Wallander *et al.*, 1997; Jonsson *et al.*, 1999; Mahmood *et al.*, 2002; Taylor & Finlay, 2003), whereas some of the studies were made using different forest stands sometimes separated by > 100 km as replicates (Lehto, 1994a; Kårén & Nylund, 1996; Bakker *et al.*, 2000; Present study, 2007). The latter strategy may include additional variation in ECM communities which may reduce the chance of detecting treatment effects although it holds the potential to reveal more general effects on ECM communities. The studies have different vertical sampling strategies. It has been shown that ECM fungal species differ in their preference for the organic and mineral soil layers, with some species exclusively found on roots in either the organic or mineral soil horizon (e. g. Rosling *et al.*, 2003). Descriptions of the

pling strategy is the importance of how the ECM root tips are sampled; as few bulk samples or multiple individual tips. ECM fungal communities are generally species-rich with a few common species and a large number of rare species, but with the species non-randomly distributed (Peter *et al*., 2001; Taylor, 2002; Koide *et al*., 2005a; 2005b).

Most studies of ectomycorrhizal fungal communities show a strong relationship between number of root tips analysed and number species found, which means that it is important that the same number of root tips are analysed when comparing ECM fungal communities e. g. between treatments (Taylor, 2002). Many of the studies of liming effects (Lehto, 1994a; Kårén & Nylund, 1996; Taylor & Finlay, 2003), however, are based on a specific number of bulk samples with all mycorrhizal tips analysed, and in cases where fine root density is affected by the treatment this may have led to differences in the number of roots analysed and, thus, the number of species found (Table 2). In some of the reviewed liming studies this bias has been overcome by sampling a specific number of mycorrhizal tips randomly across a site (Jonsson *et al*., 1999; Mahmood *et al*., 2002; Present study, 2007). When the mycorrhizal tips are sampled individually across a site, as in the present field study, this latter strategy can also overcome the problem, that for some ECM fungal species, multiple root tips from a single soil sample are not independent because of clumped distribution of the species (Koide *et al*., 2005a). Thus, in comparison with the bulk sample strategy where a large number of root tips are analysed per sample, the latter strategy with a high number of individual samples decrease the chance of detecting species of low abundance in a single sample but it increases the chance of detecting species that are common but with a patchy distribution across a site (Koide *et al*., 2005a). Finally, the timing of the sampling effort in the studies varies. Most of the aboveground studies involves several sampling occasions over each season, whereas most of the belowground studies are based on a single sampling event. Very little is known about the life-cycle traits and seasonal fluctuations of ECM fungal species on roots, but it is likely that important information is lost by merely looking at snapshots of the communities.

2.3. Lime and wood ash effects on ectomycorrhizal fungal communities

2.3.1. Effects on abundance

All of the reviewed studies showed that high (close to 100%) ectomycorrhizal colonization of the fine root tips is maintained irrespective of the lime or ash doses added or the time elapsed since the treatment (Table 2). Even a very high liming dose of 20 tonnes calcite per hectare (corresponding to 8 t Ca ha^{-1}) did not affect total colonization of *Pinus resinosa* (Antibus & Linkins, 1992). Lime and ash additions generally appear to stimulate fine root development in the upper-most soil layers (Huettl & Zoettl, 1993; Persson & Ahlström, 1994; Majdi & Viebke, 2004). Most of the studies in the present review report no change in fine root biomass or total root tip numbers per volume of soil (Erland & Söderström, 1991; Antibus & Linkins, 1992; Lehto, 1994a; Kårén & Nylund, 1996; Horröd site in Taylor & Finlay, 2003) (Table 2). A few of the experiments representing a wide range of liming doses (corresponding to 0.5- 2 t Ca ha^{-1}), however, found

increased absolute root tip numbers and increased abundance of ECM roots with liming (Lehto, 1984; Bakker *et al*., 2000; Hasslöv site in Taylor & Finlay, 2003) (Table 2).

Fact Box – Definitions of terms used to describe ectomycorrhizal fungal communities

Species richness refers to the number of species or types in a community.

Community structure is commonly described by rank-abundance curves, i.e. the number of tips colonized (y-axis) of single ECM types ranked according to their abundance (x-axis). The community structure describes the abundance of common types relative to rare types in a community.

Community evenness describes the relative abundance of all species found in a given area. Areas with many equally abundant species have more even communities than areas with a few dominant and many subdominant species.

Diversity of a community is described by different diversity indices, e.g. the Shannon-Wiener Diversity Index or Hill's Evenness Index. Diversity is normally regarded high in communities with high species richness and high evenness in relative abundance of species.

Community composition or species composition of a community refers to the actual species/types present in a community as well as their relative abundances. Note that community composition can change without changes in any of the other community characteristics. The ECM fungal community can be described using four different structures: above- and/or belowground sporocarps, belowground colonised root tips, extraradical mycelium or persistent propagules (spores and sclerotia) in the soil. The two latter structures have not yet been used to describe effects of forest liming on ECM communities and we focus here on the first two:

The Aboveground (sporocarp) community is the cumulative number, biomass or frequency of ECM fungal fruiting structures formed aboveground (see Table 3 for studies considering liming and ECM aboveground communities). This measure ignores non-fruiting ECM species as well as species fruiting belowground and is likely to miss inconspicuous aboveground species e.g. the important ECM forming groups of fungi with resupinate fruiting bodies (e.g. Thellephoroid and Athelioid fungi). On the other hand, large areas of forest floor can be surveyed in aboveground community studies and as fungal species are defined from their fruiting structures the taxonomic resolution is optimal (if the taxonomy is resolved). The correlation between the aboveground sporocarp and the belowground root tip ECM communities has been demonstrated many times to be weak (Gardes & Bruns, 1996; Dahlberg *et al*., 1997; Peter *et al*., 2001).

The belowground (root tip) community is quantified either as the number, biomass or frequency of root tips belonging to individual species (see Table 2 for studies considering liming and ECM below ground communities). Taxon recognition of colonised root tips can be achieved in different ways and often several of these are used in combination (Agerer, 1991; Horton & Bruns, 2001).
Morphotyping describes the use of gross morphology i.e. colour, branching pattern, size, texture, presence and position of external mycelium and rhizomorphs or other features which can be recognized under a dissection microscope. **Anatomotyping** is an extension of morphotyping adding microscopically examination of fungal mantle tissues to the features described above. While morphotyping is known to lack resolution within some fungal groups (i.e. lumping species together) anatomotyping is thought to approach the same resolution as that derived from fungal fruiting structures. In **molecular typing**, fungal-specific DNA sequences are amplified from extracted colonised root tips and compared with similar sequences from reference material. This may be a direct comparison of the DNA sequences with reference sequences (as in this study) or an indirect comparison where obtained sequences are first cut with restriction enzymes to produce taxon specific restriction patterns (RFLP) that are compared with reference material. The indirect comparison has less resolution than the direct method because only presence/absence of the specific recognition sites of the enzymes is compared. Un-matched sequences are annotated with a working name typically indicating in which fungal genera, family, order etc. that the sequence belongs. The favoured DNA marker for identifying ECM fungi is the Internal Transcribed Spacer (ITS) region located within the ribosomal gene family as this marker discriminates taxa equally well as the morphological sporocarp-defined fungal species concept.

Table 2. Field studies of effects of liming and wood ash addition on fine roots and ectomycorrhizal root tip communities.

Stand (age) Region (site) Replication[1]	Dose Type[2]	Yrs after treatment	Horizon sampled	Effects on fine roots	Effects on ECM roots	Effects on ECM fungal communities	Reference
Picea abies and *Pinus sylvestris* (40-60) S Sweden 3 stands	3 t ha^{-1} dolomite	16	Organic and mineral (-15 cm)	n.a.	n.a.	Richness unchanged Community structure less even Shift in community composition: ↑ *Tylospora asterophora, Tuber* sp. 1, *Amphinema byssoides* (two sites). Clades: *Tylospora* and allied, *Elaphomyces*, Pezizales ↓ *Lactarius rufus*. Clades: Russulaceae, Unknown ecm B	Present study
P. abies (50) S Sweden (Hasslöv) 3 replicates	8.75 t ha^{-1} dolomite	15	Organic	Increased no. tips sample^{-1}	Colonization unchanged (100%)	Richness and structure unchanged Major shift in community composition: ↑ spp. with resupinate sporocarps + spp. associated with more nutrient rich sites (e.g. tomentelloid fungi, *Amphinema byssoides*, *Piceirhiza nigra, Tuber puberulum*) ↓ *Russula ochroleuca, Tyllospora fibrillosa*	Taylor & Finlay, 2003
	1.55 t ha^{-1} dolomite	12	Organic	Increased no. tips m^{-1} root	Colonization unchanged (100%)	Minor community changes No sign effects on single types	Jonsson *et al.*, 1999
	8.75 t ha^{-1} dolomite	12	Organic	Increased no. tips m^{-1} root	Colonization unchanged (100%)	Effects on community composition: ↓ (nonsign.) *Russula ochroleuca, Tyllospora fibrillosa*	Jonsson *et al.*, 1999
P. abies (80) S Sweden (Horröd) 3 replicates	3.25 t ha^{-1} crushed lime	4	Organic	Unchanged tip no. sample^{-1}	Colonization unchanged (100%)	Richness reduced Changed structure Loss of rare species	Taylor & Finlay, 2003
	4.28 t ha^{-1} wood ash	4	Organic	Unchanged tip no. sample^{-1}	Colonization unchanged (100%)	No change in richness or community structure	Taylor & Finlay, 2003
P. abies (40) SW Sweden (Torup) 3 replicates	3 t ha^{-1} wood ash	7	Organic (-6 cm)	Unchanged tip no. m^{-1} root	Colonization unchanged (100%)	Minor changes No sign effects on single types	Mahmood *et al.*, 2002
	6 t ha^{-1} wood ash	7	Organic (-6 cm)	Unchanged tip no. m^{-1} root	Colonization unchanged (100%)	Minor changes No sign effects on single types	Mahmood *et al., 2002*

Table 2 continued

Stand (age) Region (site) Replication	Dose[1]	Yrs after treatment	Horizon	Effects on fine roots	Effects on ECM roots	Effects on ECM fungal communities	Reference
Quercus spp. (15-76) France/Netherlands 10 stands	0.8-1.6 t ha^{-1} CaO (added as CaCO$_3$)	1-27	Organic and mineral (-60 cm)	Increased length Unchanged tip no. m^{-1} root	Increased number ha^{-1}	Effects on community composition: ↓ smooth types ↑ *Cenococcum geophilum* ↑ types w/ extensive mycelia	Bakker *et al.*, 2000
P. abies (80) S Germany (Höglwald) 1 stand	4 t ha^{-1} dolomite	7	Organic and mineral (-30 cm)	n.a.	Colonization unchanged (100%)	Effects on community composition: ↓ *Tylospora fibrillosa, Russula ochroleuca* ↑ *Piceirhiza nigra, Tuber puberulum, Amphinema byssoides*	Qian *et al.*, 1998 Taylor & Brand, 1992
Pinus sylvestris seedlings grown in field soil in lab S Sweden (Farabol) 3 replicates	6 t ha^{-1} CaCO$_3$ (0.5 t ha^{-1} yr^{-1} for 12 yrs)	7-19	Organic	n.a.	n.a.	Effects on community composition: ↑ yellow smooth type ↑ types w/ inorganic N uptake ↓ types w/ organic N uptake	Wallander *et al.*, 1997
P. abies (30) SW Sweden (Skogaby) 4 replicates	48:43:218:46:75 kg ha^{-1} P:K:Ca:Mg:S	3, 4, 5	Organic	Unchanged biomass m^{-2}	No effect on EM biomass	Minor community changes ↓ white type	Kårén & Nylund, 1996
P. abies (50-75) SW Sweden 3 stands	25:62:33:12: 54 kg ha^{-1} P:K:Ca:Mg:S	3-4	Organic	n.a.	No effect on EM biomass	Minor community changes ↓ orange type	Kårén & Nylund, 1996
P. abies seedlings grown in 50 yr stand SW Sweden (Öringe) 4 replicates	3.8 t ha^{-1} CaCO$_3$	5, 6	Organic	Unchanged tip number seedling^{-1} Reduced no. root tips m^{-1} root	Colonization up (after 5 yr), colonization down (6 yr)	Effects on community composition: ↓ *Paxillus involutus*, white type ↑ *Piceirhiza nigra*, brown type	Andersson & Söderström, 1995
P. abies (60) S Finland 2 stands	2 plus 4 t ha^{-1} dolomite	30 and 12	Organic (-1 cm)	Unchanged biomass cm^{-3}	n.a.	Effects on community composition: ↑ types w/ external mycelium ↓ smooth types, *Piloderma croceum*	Lehto, 1994a
Pinus resinosa (60) USA, MA (Harvard forest) 1 stand	~20 t ha^{-1} lime (0.4 t ha^{-1} month^{-1} for 4 yrs)	2, 3	Organic and mineral (-10 cm)	Unchanged tip number sample^{-1}	Colonization unchanged (100%)	Unchanged diversity Effects on community composition: ↑ *Piloderma bicolor*, grainy brown type (cf. *Piceirhiza nigra*)	Antibus & Linkins, 1992

Table 2 continued

Stand (age) Region (site) Replication	Dose[1]	Yrs after treatment	Horizon	Effects on fine roots	Effects on ECM roots	Effects on ECM fungal communities	Reference
P. sylvestris seedlings in 40 yr stand S Sweden (Torrmyra) 4 replicates	5 t ha^{-1} lime	1½	Organic	Increased no. root tips m[-1] root Total tip number unchanged	n.a.	Effects on community composition: ↑ pink type	Erland & Söderström, 1991
	7.5 t ha^{-1} ash	1	Organic	Reduced no. root tips m[-1] root Total tip number unchanged	n.a.	No sign effects on single types	Erland & Söderström, 1991
P. sylvestris (7-35) S Finland 4 stands	2 plus 4 t ha^{-1} dolomite	10 and 1-2	Organic (-1 cm)	Increased biomass of short roots cm^{-3}	No change in total EM tips Increased hyphal length in humus	Effects on community composition: ↑ type w/ external mycelium, *Cenococcum geophilum* ↓ three types, incl. *Piloderma croceum*	Lehto, 1984

[1]The Number of replicates refers to the number of replicated blocks in experiments set up in a randomized block design within one stand. The number of stands is given in experiments where each stand contained one limed and one untreated area.

[2]Treatment agents are: Dolomite: $CaMg(CO_3)_2$, containing 22% Ca; Calcite/lime: $CaCO_3$, containing 40% Ca; CaO: containing 71% Ca. Treatments were applied as single additions unless otherwise described.

n.a. = not available

Although none of the reviewed studies reported decreased belowground mycorrhizal colonization or biomass with liming three out of the four studies of aboveground biomass showed large reductions of at least 40-50% in production of ECM sporocarps (Wiklund *et al.*, 1995; Brandrud *et al.*, 2001; Brandrud *et al.*, 2003) (Table 3). This was in spite of the relatively low liming doses of 0.2-3 tonnes per hectare added and relatively short time periods elapsed since treatment in these studies. Several earlier surveys of aboveground ECM communities found the same trend of decreased production with liming (Wästerlund, 1982; Agerer, 1989; Jacobsson, 1993). This inhibitory effect of liming on aboveground biomass is similar to what have been found with elevated levels of nitrogen deposition (Arnolds, 1991). This discrepancy between drastic effects on aboveground ECM fungal structures but little effects on ECM colonization and biomass on fine roots could have several explanations relating to changed C allocation within the fungal-plant symbiosis and different sampling strategies for above- and belowground communities, as discussed in detail by Kårén and Nylund (1996). However, the discrepancy is likely also related to the well-documented difference in community composition of root tip and sporocarp communities (Gardes & Bruns, 1996; Dahlberg *et al.*, 1997; Peter *et al.*, 2001). For instance, Kårén and Nylund (1996) estimated that *Cortinarius* spp. accounted for 30% of the reduction in sporocarp production but only decreased their relative abundance on roots by 2% with liming, as discussed in more detail below (section 2.3.2.).

2.3.2. Effects on community richness and structure

Most of the field studies reviewed in Table 2 and 3 found no significant effects of liming on richness of ECM fungal communities. Reduced species richness after liming, however, was observed in a few studies with high species resolution and relatively large numbers of samples surveyed (Brandrud *et al.*, unpublished; 2001; Taylor & Finlay, 2003). In a Norway spruce stand in Southern Sweden exposed to 3.25 tonnes lime per hectare 4 years previously, belowground richness was reduced from a total of 27 morphotypes in control areas to 16 morphotypes in limed areas (Taylor & Finlay, 2003) (Table 2). This reduction was mainly due to a loss of rare ECM types as the community structure changed towards an increased dominance of common species. In another study, liming with 3 tonnes dolomite per hectare in a Scots pine stand in Norway reduced species richness of the aboveground ectomycorrhizal sporocarp community from 25-26 species in control areas to 10-14 species in limed areas within 3 years after the lime application (Brandrud *et al.*, unpublished; 2001). In the present field study we found no effects on species richness but the community structure at all three sites changed towards increased frequency of 1-2 abundant species on the expense of 4-5 species of medium relative frequencies, i.e. the evenness of the communities decreased.

2.3.2. Effects on species composition

Most of the field studies showed changes in species composition of the ECM fungal communities with liming, with increased relative abundance of some ectomycorrhizal species or types on the expense of others (Table 2 and 3). In the three studies reporting decreased production of aboveground sporocarp communities, the decrease was partly attributed to major decreases in the

Table 3. Effects of liming on ectomycorrhizal sporocarp communities in coniferous forests.

Stand (age) Region (site)	Dose[1]	Yrs after treatment	Effects on EM fungal communities	Reference
Pinus sylvestris, Picea abies Norway (Gjerstad)	3 t ha^{-1} dolomite (0.2-2 mm)	(0, 1, 2) 6	Production reduced Richness unchanged ↓ *Cortinarius* (reduced 90%), *Russula* (reduced 80%) ↑ *Lactarius quieticolor, Russula*	Brandrud *et al.*, 2003
P. sylvestris Norway (Suldal)	3 t ha^{-1} dolomite (0.2-2 mm)	(0, ½, 1½) 2½	Production reduced 40-50% Decreased richness ↓ 75-80% red. *Cortinarius* spp.	Brandrud *et al.*, unpublished; Brandrud *et al.*, 2001
P. abies (80) S Germany (Höglwald) 1 replicate	4 t ha^{-1} dolomite	6	No major effects on production, diversity or community composition ↓ *Russula ochroleuca*	Agerer *et al.*, 1998
P. abies (30) SW Sweden (Skogaby) 4 replicates	48:43:218:46 :75 kg ha^{-1} P:K:Ca:Mg:S	3, 4, 5	Production reduced 50% Community composition affected: ↓ *Boledus edulis, Lactarius necator, L. rufus, L. theiogalus, Cortinarius brunneus*	Wiklund *et al.*, 1995

production of *Cortinarius* species. Brandrud *et al.* (2001; 2003) reported 75-90 % reductions in sporocarp production by *Cortinarius* species 2½-6 years after liming with 3 tonnes dolomite per hectare in two coniferous forests in Norway (Table 3). A lower liming dose of 218 kg Ca per hectare (equivalent to the Ca content of 1 tonne dolomite) combined with additions of P, K, Mg and S to a Norway spruce stand in Southern Sweden reduced sporocarp production in almost all of the common species including *Cortinarius brunneus* (Wiklund *et al.*, 1995). Below-ground in the same experiment, Kårén & Nylund (1996) observed significantly decreased frequency (from 3.4 to 1.2 % of ECM tips) of a white morphotype probably including *C. brunneus*. They calculated that fungal species included in this morphotype could account for 30% of the reduction in sporocarp production with liming at the site. The present field study found no such reduction in root tip frequency of *Cortinarius* spp. with liming. Actually, the most common *Cortinarius* species, *Cortinarius fennoscandicus*, showed a tendency towards increased abundance in limed plots.

A few other ectomycorrhizal types decreased in abundance with liming in more than one study. In the Norway spruce forest in Southern Sweden limed with 8.75 tonnes dolomite per hectare, two ectomycorrhizal species, *Russula ochroleuca* and *Tyllospora fibrillosa*, almost disappeared from the limed plots (Jonsson *et al.*, 1999; Taylor & Finlay, 2003) (Table 2). The same two species decreased in relative abundance to about 31 and 12 % of that in the control plot 7 years after liming with 4 tonnes dolomite per hectare in an upland Norway spruce stand in S Germany (Taylor & Brand, 1992; Qian *et al.*, 1998). *R. ocroleuca* and *T. fibrillosa* are common in nutrient poor Norway spruce forests, and they made up 15 and 45%, respectively, of the mycorrhizal root tips in control plots at the Swedish site and 32 and 29%, respectively, of mycorrhizal tips in the control plot at the German site. In the present field study the Russulaceae clade, including *Lactarius* spp. and *Russula* spp., made up 25 and 30% of the root tip communities in the

organic and mineral horizons, respectively, in the reference plots. In the limed plots, however, the relative frequency of Russulaceae was reduced to 5 and 7.5% in the two horizons. *Lactarius rufus* contributed strongly to this reduction in relative frequency of Russulaceae in all sites.

Although the relatively high liming dose (8.75 t dolomite ha^{-1}) did not change richness of the ectomycorrhizal community in the Norway spruce forest studied by Taylor and Finlay (2003), the composition of the community was almost completely changed. Control and limed areas had only 3 species in common out of the 12 and 15 species found in control and treated areas, respectively. Liming resulted in an increased abundance of species that produce inconspicuous resupinate sporocarps including *Amphinema byssoides* and most of the tomentelloid fungi recorded (Taylor & Finlay, 2003). Liming effects on these mycorrhizal fungi may generally have been underestimated in conducted field studies. The tomentelloid species generally form brown ectomycorrhizas that are not possible to distinguish using macroscopic features alone. In some studies unidentified brown mycorrhizas made up most of the community (Kårén & Nylund, 1996). Also, the contribution by tomentelloid fungi to the communities studied using molecular typing, e.g. in the studies by Jonsson *et al.* (1999), may have been underestimated because of low success rate of DNA amplification within the tomentelloid fungi (Taylor & Finlay, 2003). Furthermore, due to the inconspicuous nature of their sporocarps these fungal species may generally have been overlooked in sporocarp surveys.

Taylor and Finlay (2003) also noted that some of the mycorrhizal types, *Amphinema byssoides* and *Tuber puberulum*, that thrived after the high liming dose were species that are normally associated with more nutrient-rich or calcareous soils. The two species were not recorded in control plots but made up 38 and 9%, respectively, of the mycorrhizal root tips in the limed plots. Similarly, *Piceirhiza nigra* was not present in control plots but made up 20% of the community in the limed plots. In the present field study, *Amphinema byssoides* and the unidentified *Tuber* sp. 1 also increased in relative abundance from < 1% in reference plots to about 2.5% and 7% in limed plots, respectively. All three ectomycorrhizal types also showed large increases in abundance in the German spruce forest limed with 4 tonnes dolomite per hectare (Taylor & Brand, 1992; Qian *et al.*, 1998).

The only study reporting no effects on community composition with liming is the one by Jonsson *et al.* (1999) where the effect of adding 1.55 tonne dolomite per hectare to a Norway spruce stand was analysed 12 years later. This application rate may be below the critical load for the belowground ectomycorrhizal community at this site. Kårén and Nylund (1996) also concluded that in the short-term (<5 years) the use of moderate amounts of N-free fertilization (with a Ca content corresponding to that in 0.15-1 tonnes dolomite) is not likely to drastically affect dominant ectomycorrhizal fungi in roots. Also, the critical load for sporocarp communities in nutrient-poor coniferous forests in Western Norway seems to be below 2 tonnes dolomite per hectare according to a field study of short-term effects (T. E. Brandrud, personal communication; Brandrud *et al.*, 2001).

Contrary to liming, moderate levels of ash additions (3-7.5 tonnes ha^{-1}) mostly showed only minor changes in the ECM communities on roots (Erland & Söderström, 1991; Mahmood *et al.*, 2002; Taylor & Finlay, 2003) (Table 2). This is despite of the fact that wood ash addition generate some of the same effects on

forest soils as liming, e. g. increased pH and N availability (Palmberger & Egnell, 2006). However, only four shorter-term (1-7 years after ash additions) studies are included in the present review (Table 2) and more long-term studies of ectomy-corrhizal responses to ash application are needed to fully assess the effects of wood ash on ectomycorrhizal communities.

2.4. Suggested mechanisms behind community changes

The conducted studies have suggested various factors as the primary driving forces behind observed effects of liming on ectomycorrhizal communities. Below, the most often suggested drivers are discussed separately although they are diffi-cult to separate in field studies and probably work in combination in most cases.

2.4.1. Increased level of inorganic N in soil

Several studies have pointed to increased mobilization of inorganic N after liming as the primary driving force behind reduced production of ectomycorrhizal sporo-carps and shifts in communities of ectomycorrhizal fungi. Most boreal forests are considered N limited (Tamm, 1991), and it is well documented that increased N inputs to forest ecosystems through pollutant deposition or N fertilization affect ectomycorrhizal communities both aboveground (Arnolds, 1991; Wiklund *et al.*, 1995; Lilleskov *et al.*, 2001) and belowground (Wallenda & Kottke, 1998; Cairney & Meharg, 1999; Lilleskov *et al.*, 2002a). The field observation, that lim-ing decreases the abundance of species adapted to N limited conditions like many *Cortinarius* species (Brandrud *et al.*, 2003) while species associated with more rich sites increases (Taylor & Finlay, 2003) support this hypothesis. Also, effects on ECM fungal communities were generally stronger in more nutrient-poor sites than in richer sites (Brandrud *et al.*, 2003).

One mechanism responsible for the community changes could be differential di-rect effects of increased inorganic N concentrations in soils on ectomycorrhizal fungi. In a microcosm study Arnebrant (1994) found that ectomycorrhizal fungal isolates growing in symbiosis with pine seedlings varied in their sensitivity to inorganic nitrogen additions. Mycelial growth was reduced to 80% of the growth in the control in one of the *Paxillus involutus* isolates, to 30% in a *Suillus bovinus* isolate and to 3% in an unidentified isolate. In another study with pine seedlings growing in symbiosis with isolates of *P. involutus*, Wallander *et al*. (1999) showed that isolates with higher NH_4^+ uptake were reduced more in growth at high N levels than isolates with lower NH_4^+ uptake. They concluded that low rates of N uptake may enable some ectomycorrhizal fungi to avoid stress induced by elevated levels of nitrogen in their surroundings. Thus ectomycorrhizal fungi probably have different tolerance levels to inorganic N in their surroundings, and direct effects of inorganic N concentration can potentially change the competitive balance between ectomycorrhizal species through differential effects on their my-celial growth rates and colonization capacities (Lilleskov *et al.*, 2001; 2002a; 2002b).

It is possible, however, that ectomycorrhizal fungal communities also are indi-rectly affected by increased N availability through effects on tree nutrition. Stud-ies including saprotrophic species in aboveground sporocarp surveys show that saprotrophs are affected by liming to a lesser extent than ectomycorrhizal species

and in some cases they are even promoted (Andersson & Söderström, 1995; Wiklund *et al.*, 1995). Given that the ectomycorrhizal fungi, contrary to saprotrophic fungi, are dependent on the exchange of carbohydrates with their host for their growth, this points to symbiosis-mediated effects of liming on the ectomycorrhizal fungi. This could be through decreased allocation of carbohydrates to the roots because of a greater carbon demand of the growing shoots when nutrient limitation is relieved (e.g. Kårén & Nylund, 1996). Alternatively, it is rather the ectomycorrhizal fungi which fix a larger part of the received C into amino acids in order to assimilate the extra N taken up from the soil to avoid toxic effects. Therefore less C is allocated to growth of the external mycelium and production of sporocarps (Wallander, 1995). In any case however, changed C allocation may affect ectomycorrhizal fungi differently, i.e. in relation to their exploration for nutrients and new roots to colonize, and thus lead to changes in their community composition both above- and belowground.

The study by Wallander *et al.* (1997) where *Pinus sylvestris* seedlings were grown in soil collected from a forest liming experiment (Table 2) suggested that ectomycorrhizal fungi that preferentially took up N in inorganic forms were promoted by liming whereas isolates primarily utilizing organic N became less abundant. The higher uptake of ammonium by individuals from the limed soil was confirmed in pure culture studies. In a later study by Wallander *et al.* (2002) there was no clear effect of liming on the uptake of N from an organic source among different ectomycorrhizal fungal isolates. An unidentified 'silvery white' morphotype making up 27% of the ectomycorrhizal community on the seedling roots in the earlier study (Wallander *et al.*, 1997), however, was reported to reduce its uptake of organic N after liming. These results point to a potentially important shift in ectomycorrhizal functioning after liming that is probably caused by increased inorganic N availability and changed ectomycorrhizal community composition after liming (Wallander *et al.*, 1997). In support of such mechanistic relationship between N availability and ectomycorrhizal functioning, Taylor *et al.* (2000) found that low N deposition rates favoured fungal species with high capacity to utilize organic N while the relative abundance of such species decreased with increasing N deposition along a N deposition gradient in Europe. A similar relationship was also found by Lilleskov *et al.* (2002b) along a N deposition gradient in Alaska. Although not fully confirmed these studies suggest that ECM fungi that preferentially take up inorganic N are likely to increase in abundance with liming probably because of increased N availability in the soil.

2.4.2. Increased soil pH

Some of the field studies listed in Table 2 pointed to increased pH as the main driving force behind liming-induced changes in ectomycorrhizal fungal communities. In their study of aboveground sporocarp communities in a spruce forest in Southern Germany Agerer *et al.* (1998) found that soil organic horizon pH may be an important determinant of ectomycorrhizal community composition although they reported no major adverse effects of liming on the communities (Table 2). However, liming in this experiment also caused a significant increase in the concentration of nitrate in the soil solution (Kreutzer, 1995), which could have partly explained the observed pH effect (Agerer *et al.*, 1998). Belowground, pH was found to explain part of the shift within the smooth ectomycorrhizal morphotypes

after liming in the study in central European *Quercus* forests by Bakker *et al.* (2000) (Table 2). This was mainly due to increased abundance of *Cenococcum geophilum* with increasing pH. Lehto (1994a; 1994b) also suggested that altered ionic strength and pH rather than Ca^{2+} concentration affected the ectomycorrhizal communities associated with Norway spruce after liming. The present field study also identified pH as the primary force leading to community shifts, although available N should be measured directly rather than estimated using soil C/N ratio to rule out that increased N availability played a role in changing ECM fungal communities at the studied sites.

In support of these field observations and in parallel to the laboratory studies of N effects on mycelial growth, pH of the growth medium appears to have differential effects on the growth of ectomycorrhizal fungal isolates in pure culture (Hung & Trappe, 1983). Different ectomycorrhizal fungal species produce varying types and amounts of enzymes exuded to their surroundings, and the activities of these exo-enzymes have been shown to have different pH optima (Leake & Read, 1997). For instance Bending and Read (1995) found that activities of proteases involved in the mobilization of N from humus declined above pH 4.5. Thus the effects of increased pH on enzyme production probably vary among fungal species and individuals, which in turn can lead to changed growth in individual ectomycorrhizal fungi and to the observed changes in composition of ectomycorrhizal communities. However, one of the conclusions in an earlier report on effects of forest liming on ectomycorrhizal fungal communities was that the growth and infectivity of ECM fungal species generally have a broad pH optimum of pH 4-7, and that the typical pH increase from pH 4 to 5 in itself was not likely to have caused observed community shifts (Erland & Andersson, 1996).

2.4.3. Increased levels of other nutrients

The availability of a range of other nutrients like different heavy metals and macro- and micronutrients are concomitant with changes in soil pH or in some cases change independently of changes in pH. Kårén and Nylund (1996) suggested that increased P availability had a larger effect on mycorrhizas than the other applied nutrients in their Skogaby experiment, leading to decreased mycelial growth and the observed reduced production of sporocarps (Tables 2 and 3). Neither N availability (NH_4-N and NO_3-N pools, net N mineralization) nor pH increased significantly in this experiment (see references in Kårén & Nylund, 1996). However the N uptake by the aboveground parts of the trees in the Skogaby experiment was promoted, hence indirect N effects of improved tree nutrition on mycorrhizas could not be ruled out.

It has been documented that ectomycorrhizal fungi can improve plant access to nutrients like P, K, Ca and Mg derived from weathering of primary minerals in soils (Landeweert *et al.*, 2001). In the immediate vicinity of roots, root-induced weathering takes place and ectomycorrhizal fungi can enhance this process by exudation of low molecular weight organic acids such as oxalic acid and citric acid. The external ectomycorrhizal mycelia furthermore are able to tightly enclose mineral particles and penetrate microsites within the particles which are inaccessible to plant roots and protected from bulk soil solution phenomena (van Breemen *et al.*, 2000). Different ectomycorrhizal fungi however show large variation in their uptake capacity both from solubilised and poorly soluble sources of

e.g. P (Burgess *et al.*, 1993; Wallander, 2000). Therefore, it could be hypothesized that different fungi have different tolerance levels to changes in factors affecting weathering processes, such as soil pH, nutrient availabilities and changed C allocation belowground (Landeweert *et al.*, 2001). However the current knowledge of the weathering capacity of different mycorrhizal fungi and their responses to forest management practises is still very limited.

2.4.4. Altered competitive situation

An even less well studied, but potentially important mechanism influencing ectomycorrhizal fungal community changes, is the changed competitive situation in a limed soil. The liming-induced increased frequency of decomposer fungi (Andersson & Söderström, 1995) and increased microbial biomass and activity (Smolander *et al.*, 1994; Anderson, 1998) suggests increased competition from saprotrophic fungi and bacteria after liming. This could potentially affect different ectomycorrhizal fungi to varying extents.

Finally, Brandrud *et al*. (2003) hypothesized that the increased abundance of a few species adapted to poor conditions (*Cantharellus cibarius* and *Russula sardonia*) could be related to some disturbance induced by the liming treatment, as these species are typical for sites with disturbance of the humus layer.

2.5. Possible consequences for ecosystem processes

With a decline in species richness or a change in composition of ectomycorrhizal communities, functions important for forest health and sustainability could be altered. Particularly two qualitative shifts in ectomycorrhizal functioning seems to be a risk if specialized ectomycorrhizal species are lost or reduced in abundance: first, a shift from primarily organic N (and P) mobilization and uptake to primarily inorganic N uptake, and, second, a decrease in weathering capacity of the ectomycorrhizal community. Both of these functional shifts potentially have important impacts on tree nutrition and forest nutrient cycling, particularly if they are maintained in the long term.

Many of the conducted studies suggested reduced growth of the external mycorrhizal mycelium as the primary mechanism responsible for community changes. Ectomycorrhizal mycelia probably contribute substantially (same magnitude as leaf plus root litter) to the input of organic material to forest floors (Godbold *et al.*, 2006). Thus the often reported reductions in mycelial development with liming might have important implications for C and N allocation and retention and for the accumulation of organic matter in forest floors.

3. Field study

3.1. Methods

3.1.1. Study sites

Ectomycorrhizal communities were sampled 14-16th August 2007 at the SKO-KAL-sites O2, P2 and R2 in South-Western Sweden (Fig. 1). One limed and one reference plot was sampled at each site (six plots in total). A limed plot with the same age as the reference plot was chosen at each site (Table 4). For detailed descriptions of the sites see Uggla et al. (2003).

3.1.2. Sampling of mycorrhizal root tip communities

In each of the six plots, samples were taken with regular intervals of 2 m thereby covering the entire plot (in total 576 soil samples, 96 per plot). Samples were taken by inserting a 2-cm diameter soil corer to a depth of 15 cm, roughly corresponding to the depth to which the soil chemistry was changed due to the liming treatment (Uggla *et al.*, 2003). Each soil core was split into a top organic part and a below mineral part (1152 split soil samples in total) typically 5 and 10
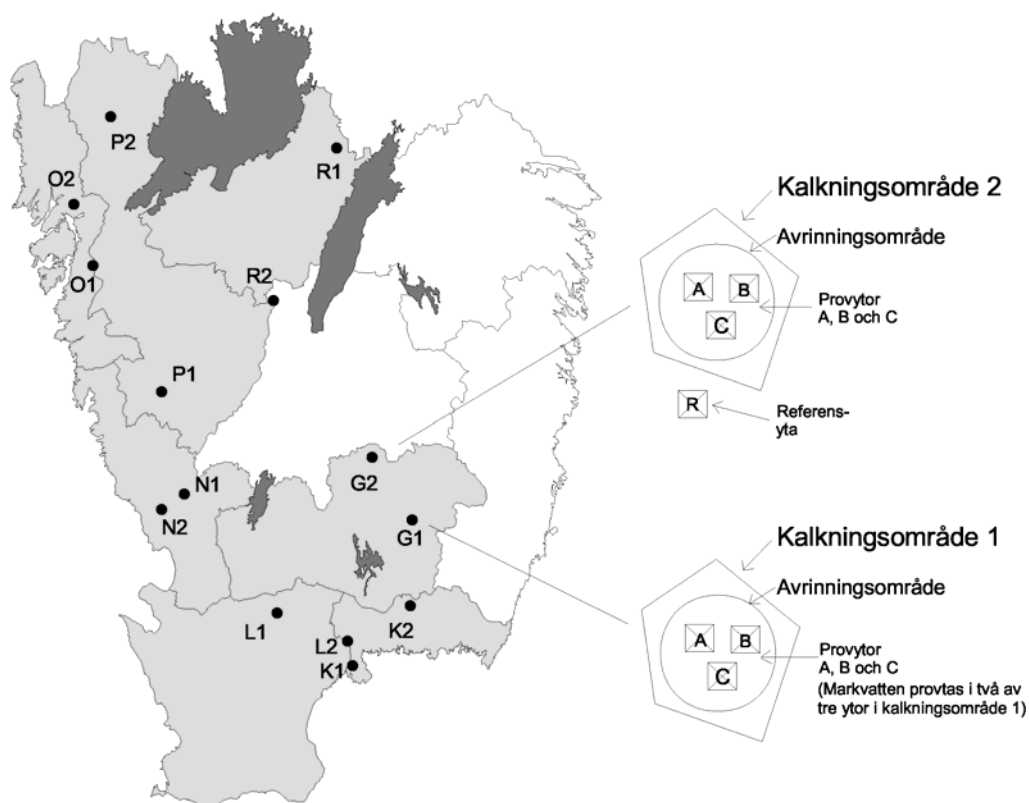
Figure 1. The 14 limed areas in the liming effect programme. Plots A, B and C refer to 77, 57 and 37 years old (+/- 5 years) stands, respectively. Samples for determining mycorrhizal fungal communities were taken in sites O2, P2 and R2. O2 is a pine stand while P2 and R2 are spruce stands. Map courtesy of the Swedish Forest Agency.

cm deep. Cores were sealed in plastic bags immediately after collection and stored at 4°C until processed. In the laboratory, roots were washed out of the soil and collected on a 1-mm sieve. Two random mycorrhizal root tips from each soil sample were selected for molecular identification as follows: The washed roots were spread out in a 9- or 15-cm Petri dish (depending on the size of the root sample) and the first healthy looking mycorrhizal root tip observed under a dissection microscope were picked. Thereafter the Petri dish was repositioned and a second root tip was picked. In total 2304 root tips were sampled, 384 from each plot. The remainder of the roots were stored at 20°C and kept as reserve. No attempts were made to score the total ectomycorrhizal colonization percent or the total number of root tips in each sample. Collected roots were transferred to and stored in RLT lysis buffer included in the MagAttract 96 DNA Plant Kit (QIAGEN, Hilden, Germany). All soil samples were processed within four weeks after collection.

Table 4. Study site details.

Site	Forest type	Stand age	Dose	Year	Plot replication	Plot size	Comment
O2, Munkedal	Pine, secondary forest	60	3 ton/ha, once	1991	n=1	30X30 m	Treated and reference plots are located in the same forest stand
P2, Bäckefors	Spruce	40	3 ton/ha, once	1991	n=1	20X20m	Treated and reference plots are located 500 m apart
R2, Sandhamn	Spruce	40	3 ton/ha, once	1991	n=1	20X20m	Both treated and reference plots contain charcoal from fire event

3.1.3. Molecular identification of mycorrhizal fungal root tips

DNA was extracted from root tips using the MagAttract Kit following the instruction included except that only half the volumes were used in all steps. The internal transcribed spacer (ITS) region was PCR amplified with the fungal-specific primer combination ITS1F and ITS4 (White *et al*., 1990; Gardes & Bruns, 1993). PCR conditions included 35 cycles of amplification and an annealing temperature of 55°C. PCR products were run on 1.5% agarose gels and all PCR products which appeared only to have one band (i.e. only one fungus colonising the root tip) and of a sufficient intensity were sequenced. The PCR products were purified using the NucleoFast 96 PCR clean-up kit from Macherey-Nagel (Düren, Germany) following the instructions included.

In total 1135 PCR products (Table 5) were sequenced at Macrogen Inc. (Seoul, Korea) with the forward primer ITS1F. Chromatograms were analyzed using vector NTI Advance 10 software (Invitrogen Corp, Carlsbad, CA) and sequences were collected into the BIOEDIT Sequence Alignment Editor Version 6 (Hall, 1999). Sequences were identified and assigned into ITS sequence-defined species

(hereafter referred to as species) in the following way: First, all sequences were compared to the sequences in GenBank by BLAST searches. Thereafter the

Table 5. PCR and sequencing success

Site	Plot	Soil horizon	Number of samples processed	PCR products selected for sq/double bands or no or weak PCR	ECM sq success* /non ECM sq or sq failures
O2	Treated	Top	192	109/83	83/26
		Bottom	192	104/88	80/24
	Reference	Top	192	88/104	75/13
		Bottom	192	72/120	59/13
P2	Treated	Top	192	101/91	78/23
		Bottom	192	79/113	71/8
	Reference	Top	192	106/86	85/21
		Bottom	192	109/83	94/15
R2	Treated	Top	192	111/81	100/11
		Bottom	192	95/97	90/5
	Reference	Top	192	83/109	78/5
		Bottom	192	78/114	76/2
All sites/plots/horizons			2304	1135/1169	969/166

*from selected PCR products

sequences were sorted accordingly in BIOEDIT and sequences belonging to the same fungal clades were aligned to identify species.

ITS sequences were assigned with matching species names if appropriate BLAST matches were found. A match was considered appropriate if several GenBank sequences of different origin were identical or almost identical to the query sequence. If no appropriate match was found the sequence was assigned with a higher level taxonomic name followed by a number. Some sequences clearly were of non-ectomycorrhizal fungal origin and were not included in the dataset. If sequences did not match any known taxonomic groups but did correspond to several other environmental sequences obtained from ectomycorrhizal tissues they were included in the dataset.

Datasets were constructed both for the occurrence of the 107 identified species and for the occurrence of 20 fungal clades (Appendix 1 and 2). In the latter dataset species were merged into phylogenetically defined fungal clades which in most cases corresponded to fungal genera (Tedersoo *et al*., 2003). The rationale for merging ectomycorrhizal species into clades is that many functional traits are conserved within clades e.g. fungal exploration type (Agerer, 2001) and nutrient

acquisition preferences (Leake & Read, 1997; Taylor *et al.*, 2000; Lilleskov *et al.*, 2002b). By merging species into clades some of the variation caused by stochastic founder effects of individual species is reduced. For sequences belonging to Atheliaceae (*Pilodorma*, Tylospora, *Amphinema*, *Byssocorticium* and some un-assigned species), the assignment into two clades were based on a phylogenetic analysis of a MAFFT aligned dataset (Katoh *et al.*, 2002). The phylogenetic analysis was performed with PAUP software (Svofford, 1998).

3.1.4. Soil chemistry

Soil environmental parameters were measured in 2007 in soil samples from all six plots as described in Uggla (2003) i.e. 15 soil samples were taken along two diagonals within each plot and a mean was calculated for each plot (30 samples in total). The following parameters were measured: depth of the organic horizon, pH, base saturation, Mg^{++}, Ca^{++}, titratable acidity, H^+, Al^+, cation exchange capacity, Na+, humidity and total C and N contents (Unpublished data from SLU, samples collected and analysed by SLU on behalf of the Swedish Forest Agency).

3.1.5. Data treatment and statistical analyses

To overcome the difference in numbers of successful sequences obtained from each plot/horizon data were standardized pr. sampling unit (i.e. plot or top or bottom horizon within a plot). This was done by calculating the relative frequency in percent of each species or clade out of the total number of successful sequences obtained from each plot/horizon.

Species and clade accumulation curves, which relate the number of species and clades detected to the number of samples (= individual root tips) taken, were calculated for each of the six plots using PC-ORD software (McCune & Mefford, 1999). This software simultaneously calculates first-order and second-order jacknife estimates of true species richness within the area sampled. To describe community structure, rank-abundance diagrams with relative frequencies of species plotted against the species in rank order were produced for each plot.

The compositions of the ECM fungal communities in relation to liming and soil horizon were analyzed by two different methods: Detrended Correspondance Analysis (DCA) and Permutational Multivariate Analysis of Variance (PERMANOVA). Datasets were pruned for species or clades detected only once or twice. For DCA analysis, the default program parameters were used (rescaling threshold=0; number of segments=26) in PC-ORD (McCune & Mefford, 1999). Correlations between community structure and environmental soil data were also simultaneously tested in the DCA analysis. For all the measured soil data the mean value for each plot and horizon was used. Because our bottom horizon was approximately taken to 10 cm depth below interface between the organic and mineral horizons the means of the soil data from horizon 0-5 and 5-10 were used. The PERMANOVA analysis implemented liming treatment and soil horizon as two factors and the three sites as "replication within cells". This analysis tests whether the two factors have significant effects on the observed communities using a permutation method (Anderson, 2005).

Relative frequencies of the 19 clades and of the 13 species occurring at all three sites were analyzed using treatment and horizon as main factors in two-way analyses of variance (ANOVAs) with type III sums of squares in the GLM procedure with the SAS 8.0 package (Statistical Analysis System Institute, 1999). The interaction between treatment and horizon was included in all models and site (corresponding to a block effect) was included in the ANOVAs if $p<0.20$ for this factor to account for variation caused by site-related differences in relative frequencies. Significant ANOVA results were evaluated using Tukey's HSD multiple comparisons of means test. All variables were arcsine-transformed and tested for homogeneity of variances (Levene's test) prior to analyses. The frequency of species only occurring at one or two sites was compared between limed and reference plots at individual sites using an X^2 test with Yates' correction.

3.2. Results

3.2.1. General description of the ectomycorrhizal communities

In total 107 ectomycorrhizal species were identified from the three sites (Appendix 1 and 2). Only 13 species were common for all three sites, 17 species were found at two sites and the remaining 77 species were identified at one site only (Appendix 1 and 2). Forty, 59, and 51 species were identified at the three sites O2, P2 and R2, respectively. Within each of these sites (O2, P2 and R2) only 11, 13, and 12 species overlapped between the limed and the reference plots (Table 6). 21, 21, and 19 species overlapped between the top and the bottom horizons at each site (O2, P2 and R2). Note that at all sites many species were detected only in a single sample and are therefore only found at one specific site/plot/horizon combination (Appendix 1 and 2). In general, the most common species were found in both limed and reference plots at each site. Therefore taking a root tip view of the species distributions shows that between 60-70% of the root tips at each site were colonised by species occurring in both limed and reference plots (Table 6).

Table 6. Overlap of species between limed and reference plots at each site.

Site	Root tip view (%)	Species view (%)
In common O2	66.7	27.5
Only in limed plot O2	14.5	37.5
Only in reference plot O2	18.9	35.0
In common P2	70.4	22.0
Only in limed plot P2	17.1	39.0
Only in reference plot P2	12.5	39.0
In common R2	58.7	23.5
Only in limed plot R2	27.6	52.9
Only in reference plot R2	13.7	23.5

The species sampling intensity curves indicate that although the accumulation of new species as a function of number of samples analysed began to level off there

is still more species to be found in all plots if more root tips were sampled (Fig. 2a). The first- and second-order jacknife estimates (Fig. 2) indicates that 69-80% or 60-78%, respectively, of the true species richness have been sampled in the individual plots. The curves depicting the accumulation of new clades as a function of sampling intensity indicates that most of the clades present were detected at sites O2 and R2 (Fig. 2b). For the most species rich site (P2) more clades would be expected if more samples were taken.

The rank abundance graphs indicate that at all sites the ectomycorrhizal fungal community structure in limed plots deviated from the community structure in reference plots (Fig. 3a, b). The species with medium relative abundance were less abundant in the limed than in the reference plots, probably because the 2-3 most abundant species became more dominant in the limed than in the reference plots making the limed communities less even. The long 'tail' of rare species was the same in reference and limed plots.

a)
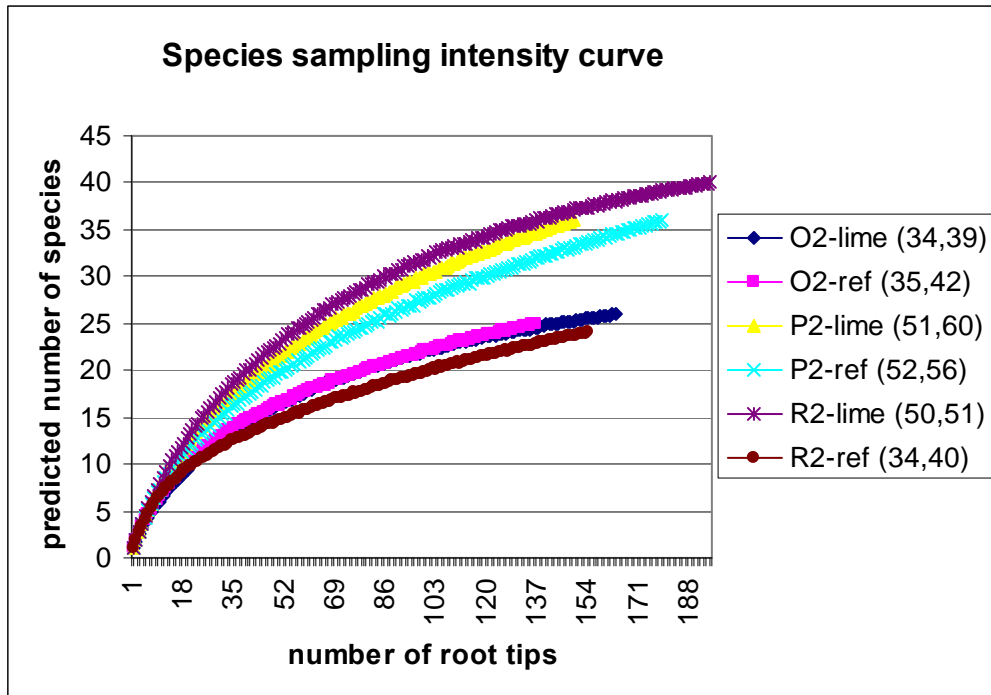
b)
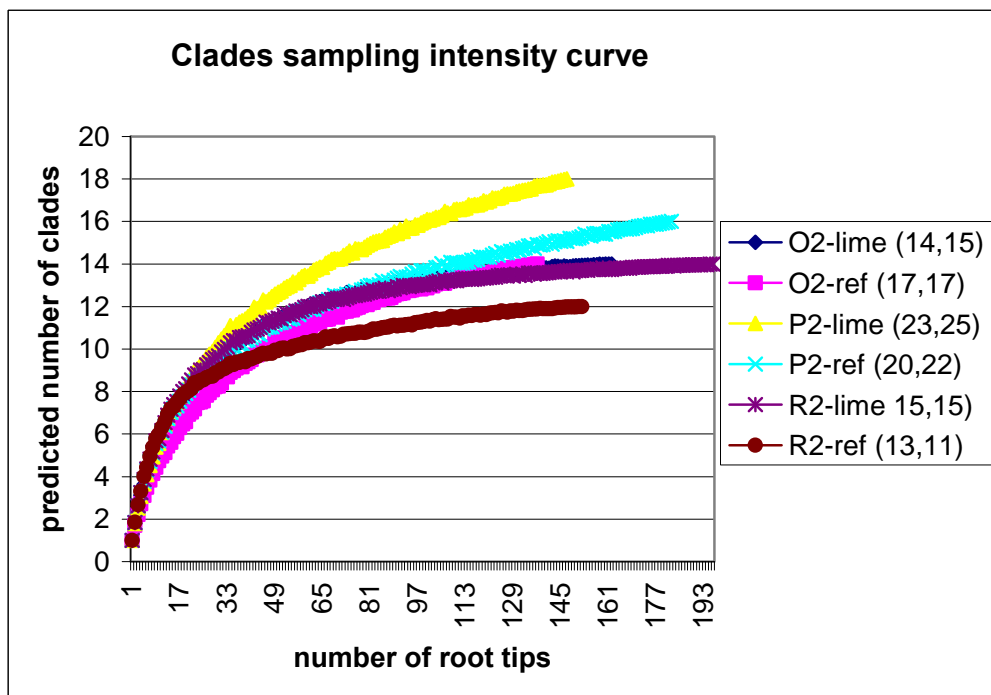
Figure 2 – species (a) and clade (b) area curves. Predicted number of species or clades detected as a function of samples taken for each of the six plots. The numbers in parenthesis after the plot designations shows first- and second-order jacknife estimates of true species richness within the areas sampled.

a)
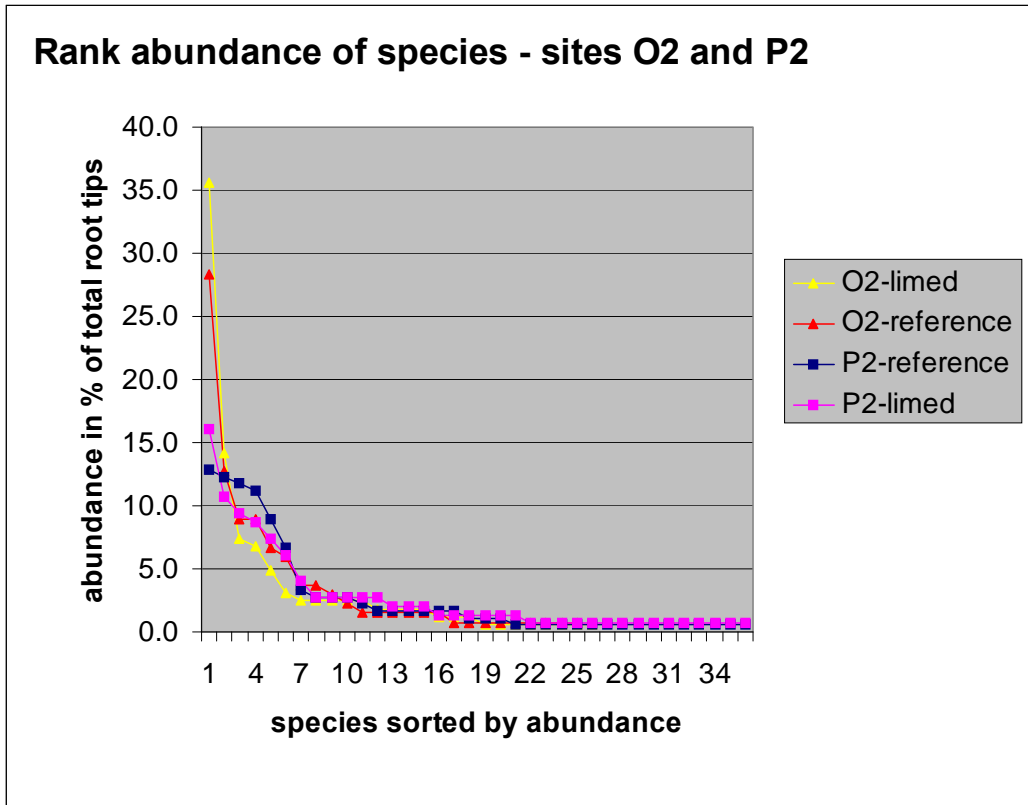
b)
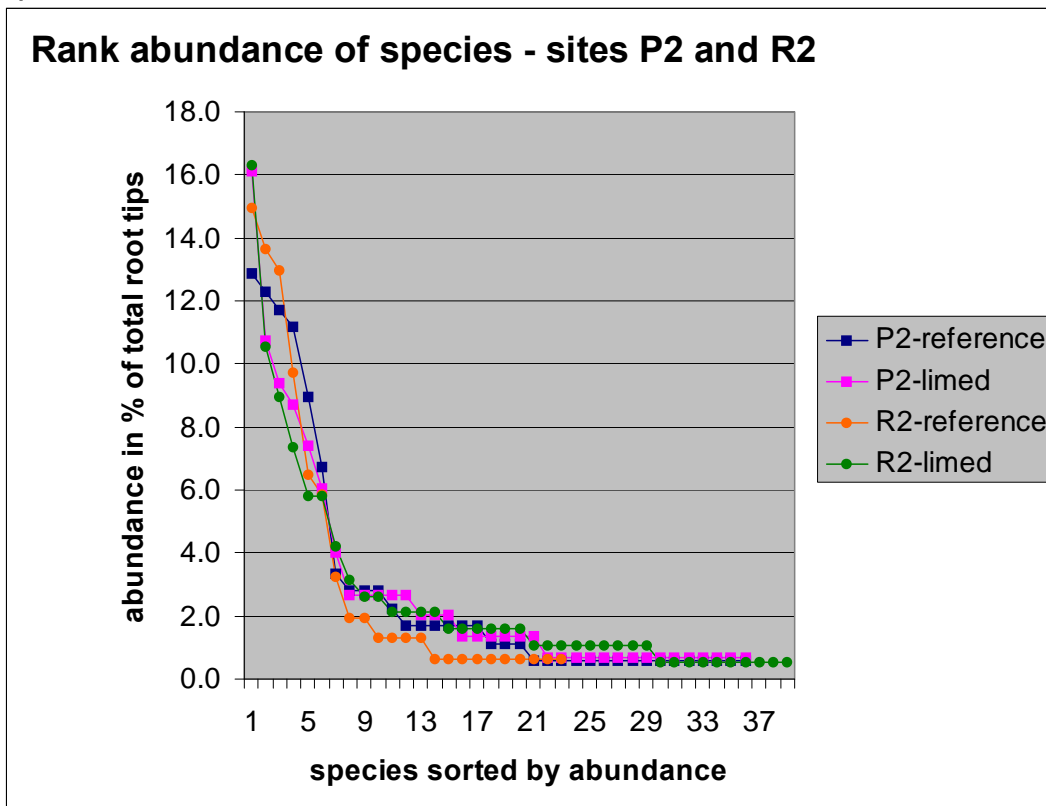
Figure 3 – rank abundance curves for the six plots. Abundances expressed as percentage of the total number of successfully amplified root tips of individual species are shown as a function of species ranked from the most abundant to the left to the less abundant to the right. For better visualization only four curves are plotted in each graph. a) O2 and P2 data, b) P2 and R2 data.

3.2.2. Liming effects on the ectomycorrhizal community

The PERMANOVA test using relative species frequencies as response variables did not show any significant effects of neither liming nor horizon (P=0.12 and P=0.70 respectively). Using relative frequencies of clades as response variables, horizon still did not influence the community assembly (P=0.97) while liming had a significant effect (P=0.016). Merging *Russula* and *Lactarius* into a single clade made the effect of liming even more significant (P=0.007).

The ordination results based on detrended correspondence analysis (DCA) clustered the three sites relatively close together both when using relative frequencies of species (Fig. 4a) and relative frequencies of clades (Fig. 4b). It was also evident that the two spruce communities (P2 and R2) were more similar to each other than to the pine site (O2). The communities from the different horizons were scattered across the DCA plot both when using species or clade data, while the DCA plot calculated from the clade data did cluster the two treatments in separate spheres of the plot: the limed plots in the top/middle part and the reference plots in the lower part, the spruce reference plots to the left and the pine reference plots to the right (Fig. 4b).

Using frequency of clades as variables the ordinated communities along axis 2 correlated significantly (P<0,05) with the three different pH measures and with base saturation (Fig 5b). Similarly using species as variables, the communities along ordination axis 2 correlated significantly with pH (Fig 5a) and axis 3 correlated with Aluminium concentration (not shown).
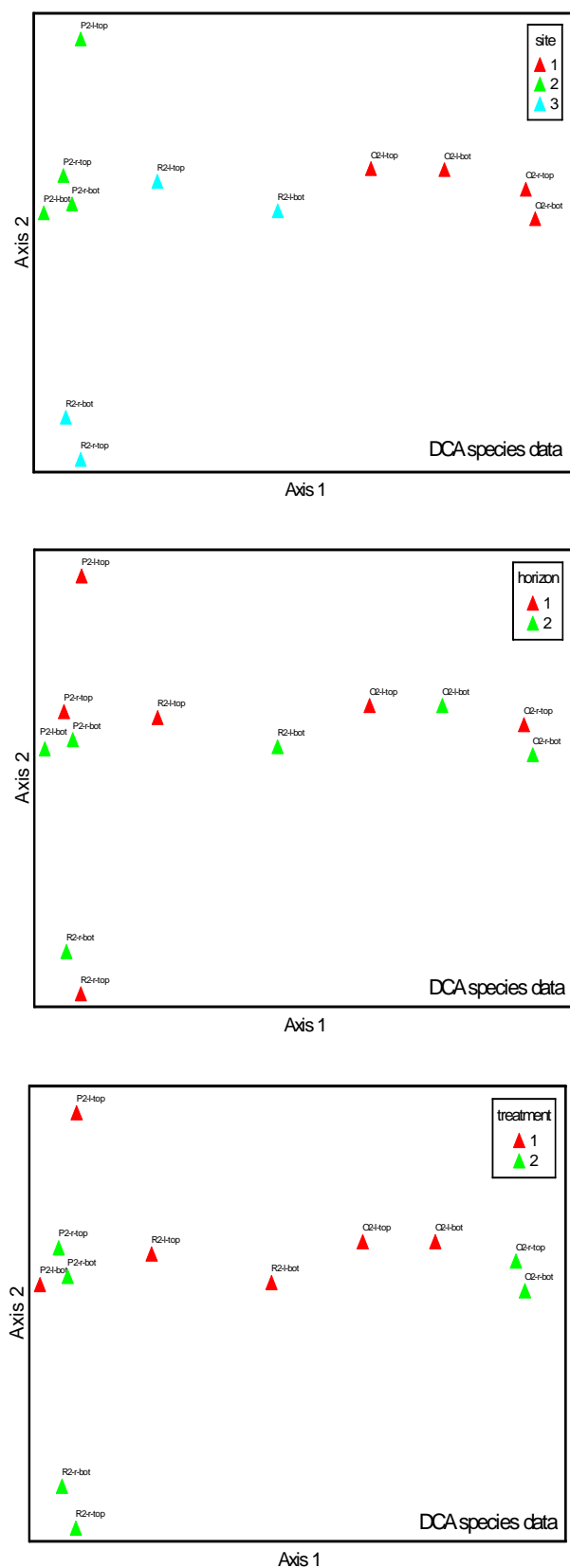
*Figure 4a – DCA with different overlays. Detrended correspondance analysis (DCA) of the 12 diffe-
rent plot/horizon-specific ectomycorrhizal fungal communities. DCA run with species as variables.
The three figures are the same but have been colour coded with sites, horizons or treatments,
respectively. Species only occurring in one or two samples were excluded before analysis. Sites 1,
2 and 3 are O2, P2 and R2 respectively; horizons 1 and 2 are the top organic and below mineral
horizons respectively; treatments 1 and 2 are limed and reference plots respectively.*
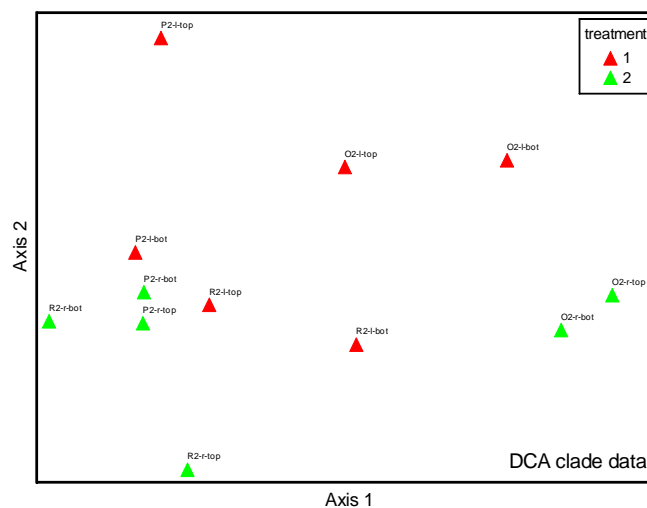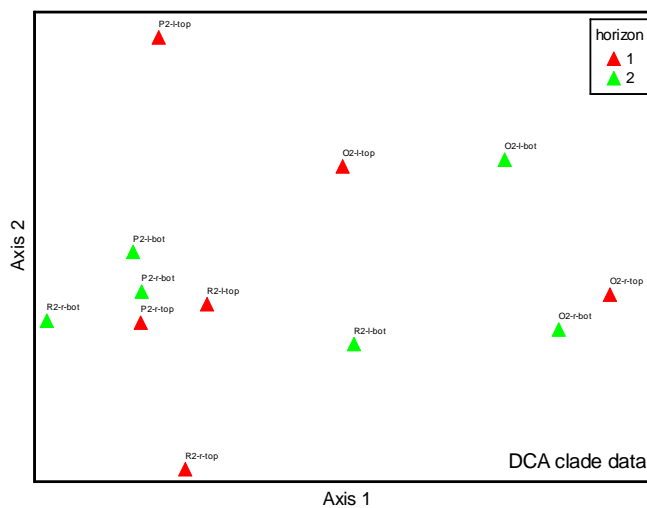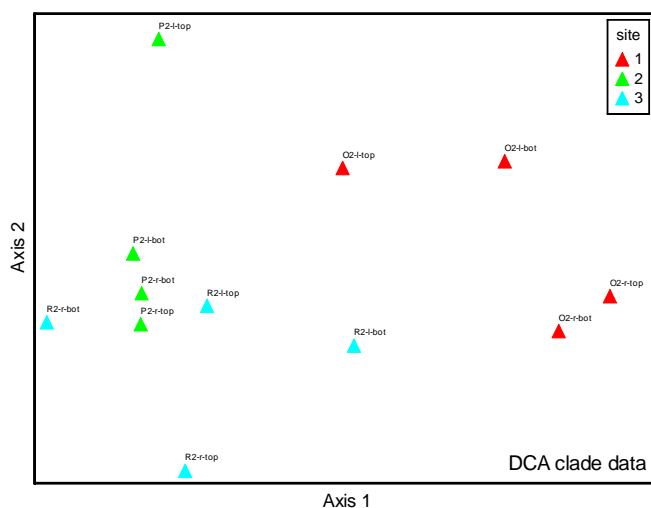
Figure 4b – DCA with different overlays. Detrended correspondance analysis (DCA) of the 12 different plot/horizon specific ectomycorrhizal fungal communities. DCA run with clades as variables. The three figures are the same but have been colour coded with sites, horizons or treatments, respectively. Clades only occurring in one or two samples were excluded before analysis. Sites 1, 2 and 3 are O2, P2 and R2 respectively; horizons 1 and 2 are the top organic and below mineral horizons respectively; treatments 1 and 2 are limed and reference plots respectively.

a)
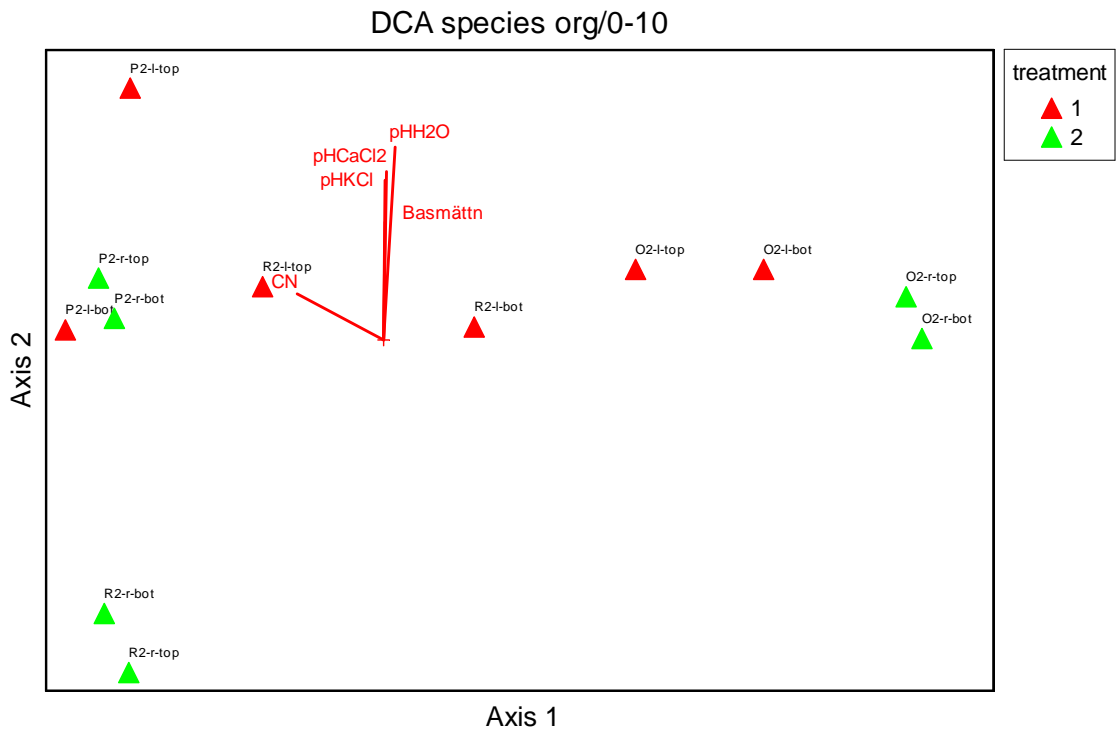
b)
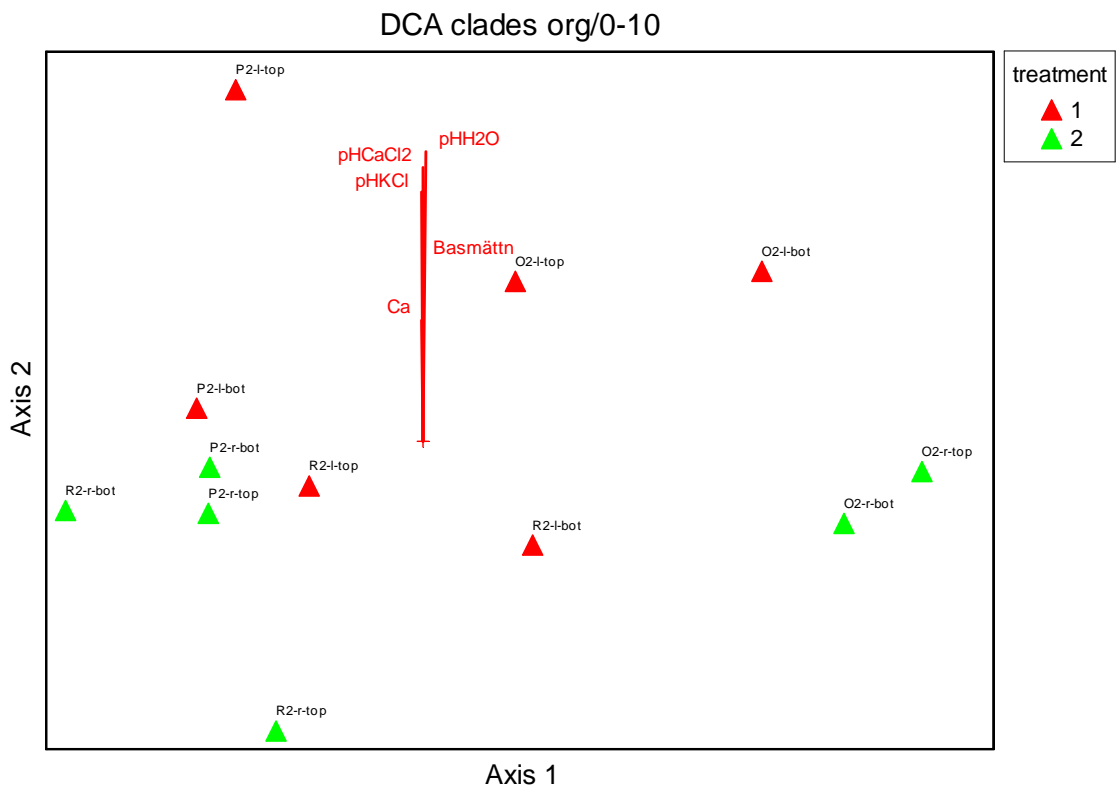
Figure 5 – DCA with vectors. Detrended correspondence analysis (DCA) of the 12 different plot/horizon-specific ectomycorrhizal fungal communities correlated with soil data. The length and direction of the vectors points towards correlations between fungal communities and environmental parameters. The longer the vector, the stronger the correlation with either of the axis. Only environmental variables with a correlation coefficient (r2) above 0.2 are shown. a) DCA based on frequencies of clades. b) DCA based on frequencies of species. Treatment 1 and 2 are limed and reference plots respectively.

3.2.3. Liming effects on single ectomycorrhizal species and clades

Five clades responded significantly to the liming treatment (Fig. 7a, Table 7a). Three clades, Tylospora and allied, *Elaphomyces* and Pezizales, increased their relative frequencies, and two clades, Russulaceae (with *Russula* and *Lactarius* summed) and Unknown ecm B, decreased their relative frequencies. The *Russula* and *Lactarius* clades also individually decreased (not shown). The results of the 2-way ANOVAs on the single species mirrored the clade results as *Tylospora asterophora* (in the Tylospora and allied clade) and *Tuber* sp. 1 (in the Pezizales clade) increased their frequencies and *Lactarius rufus* decreased in frequency (Fig. 7b, Table 7b). Only a single species, *Cenococcum geophilum*, differed significantly between the two soil horizons sampled as this species was more abundant in the top organic layer than in the lower mineral horizon (Fig. 7b, Table 7b). A significant interaction between the effects of liming and horizon was found for *Tylospora asterophora* (and the *Tylospora* and allied clade) because the increased frequency of this species was confined to the organic horizon (Table 7b). Besides these significant differences in species frequencies across all three sites many species showed striking differences between limed and reference plots but only at a single or at two sites, e. g. *Amphinema byssoides* increased strongly with liming at the two *Picea* sites. Significant changes of species frequencies between limed and reference plots at each single site are indicated on figure 6a-c.

3.2.4. Soil chemistry

The soil environmental parameters responded in the following way: pH, base saturation, Mg^{++} concentration (except for site P2 where no Mg^{++} was included in the treatment), and Ca^{++} concentration increased with liming, while titratable acidity and H^+ and Al^+ concentrations decreased with liming. Cation exchange capacity, Na+ concentration, humidity and C/N ratio did not change with liming.

a)
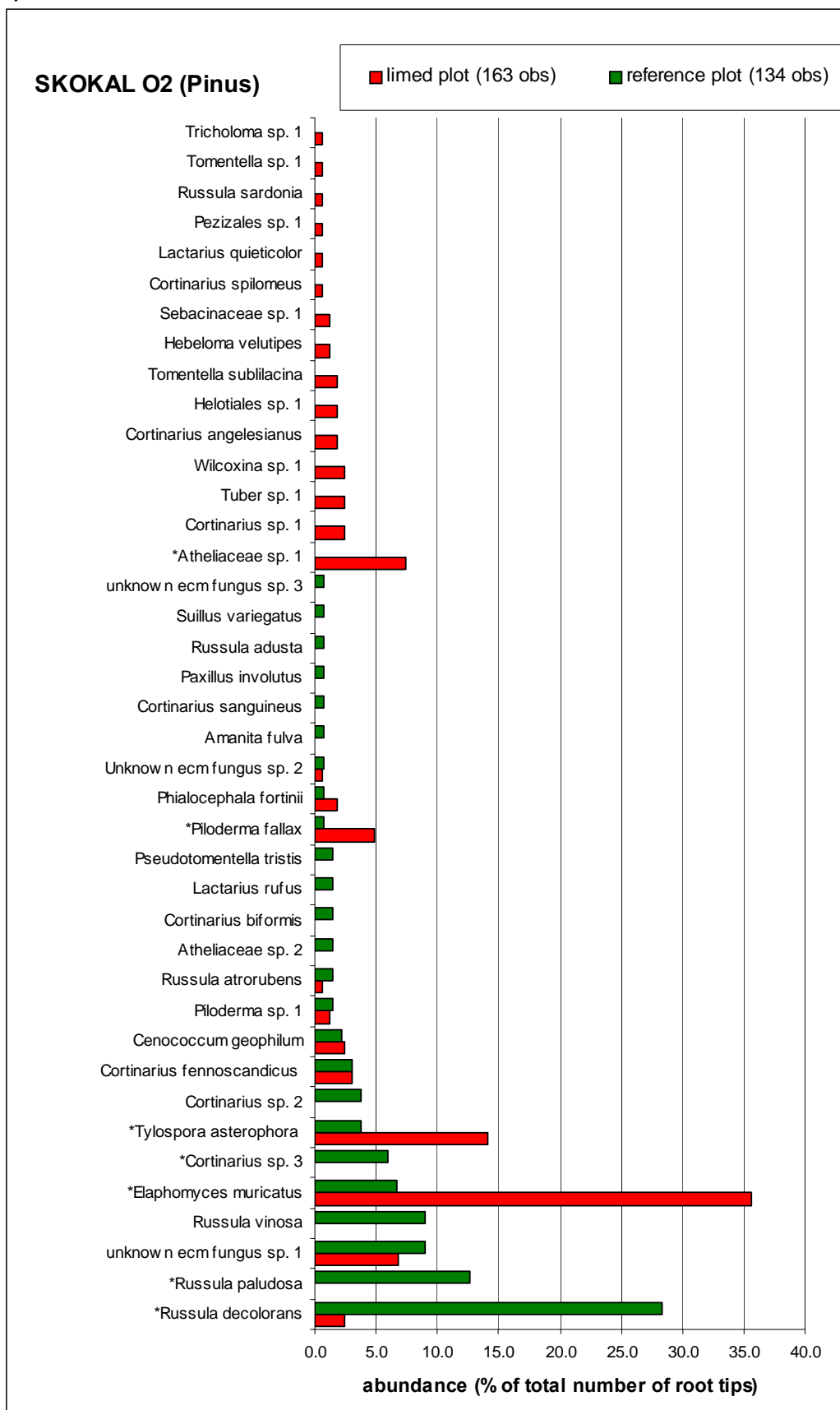
Figure 6a – community plots at SKOKAL site O2, limed and reference comparisons. The relative frequency of ectomycorrhizal fungal species in limed and reference plots. The frequency of species marked with * differ significantly between the limed and reference plot (X2 test).

b)
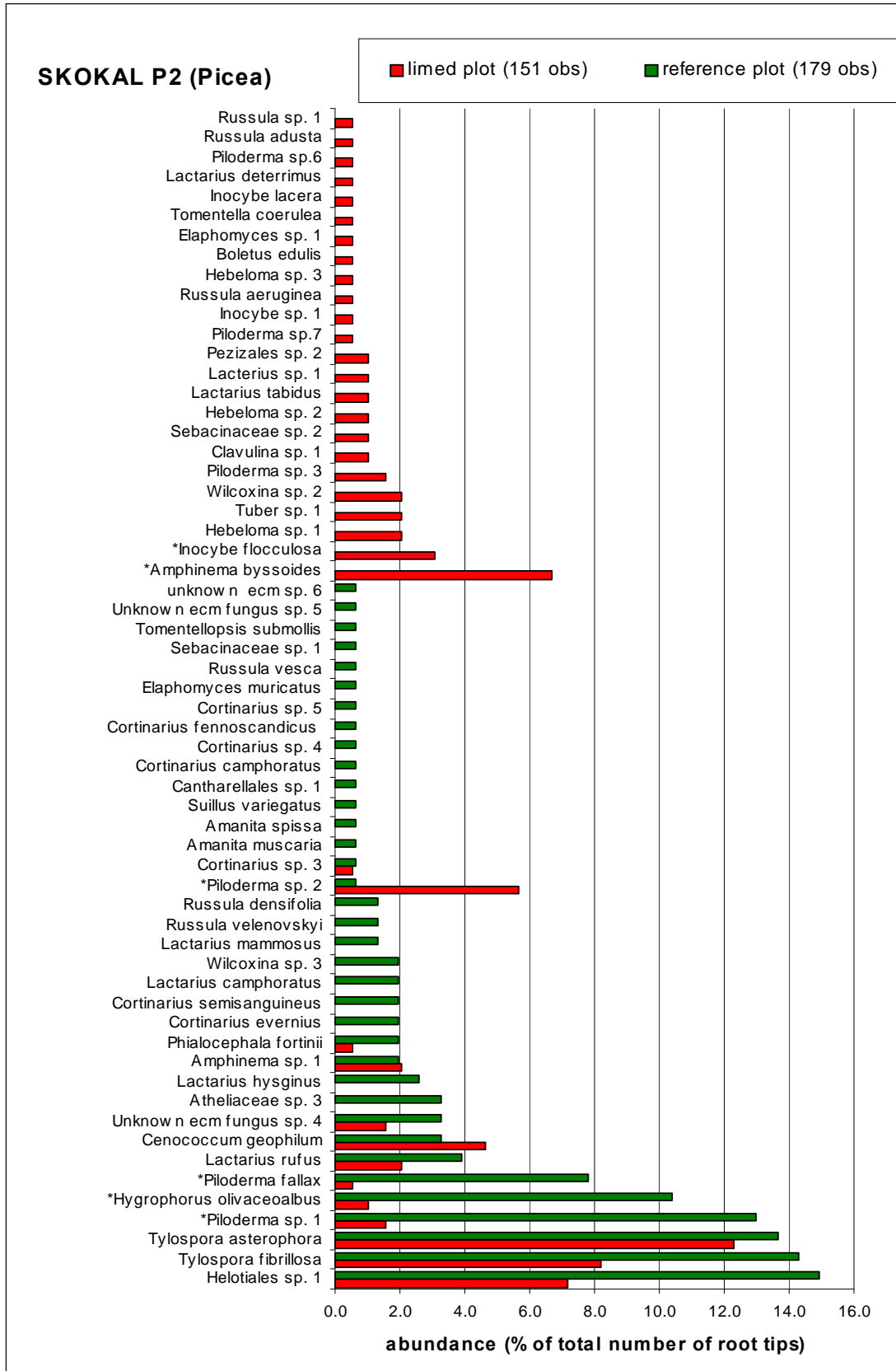
Figure 6b – community plots at SKOKAL site P2, limed and reference comparisons. The relative frequency of ectomycorrhizal fungal species in limed and reference plots. The frequency of species marked with * differ significantly between the limed and reference plot (X2 test).

c)
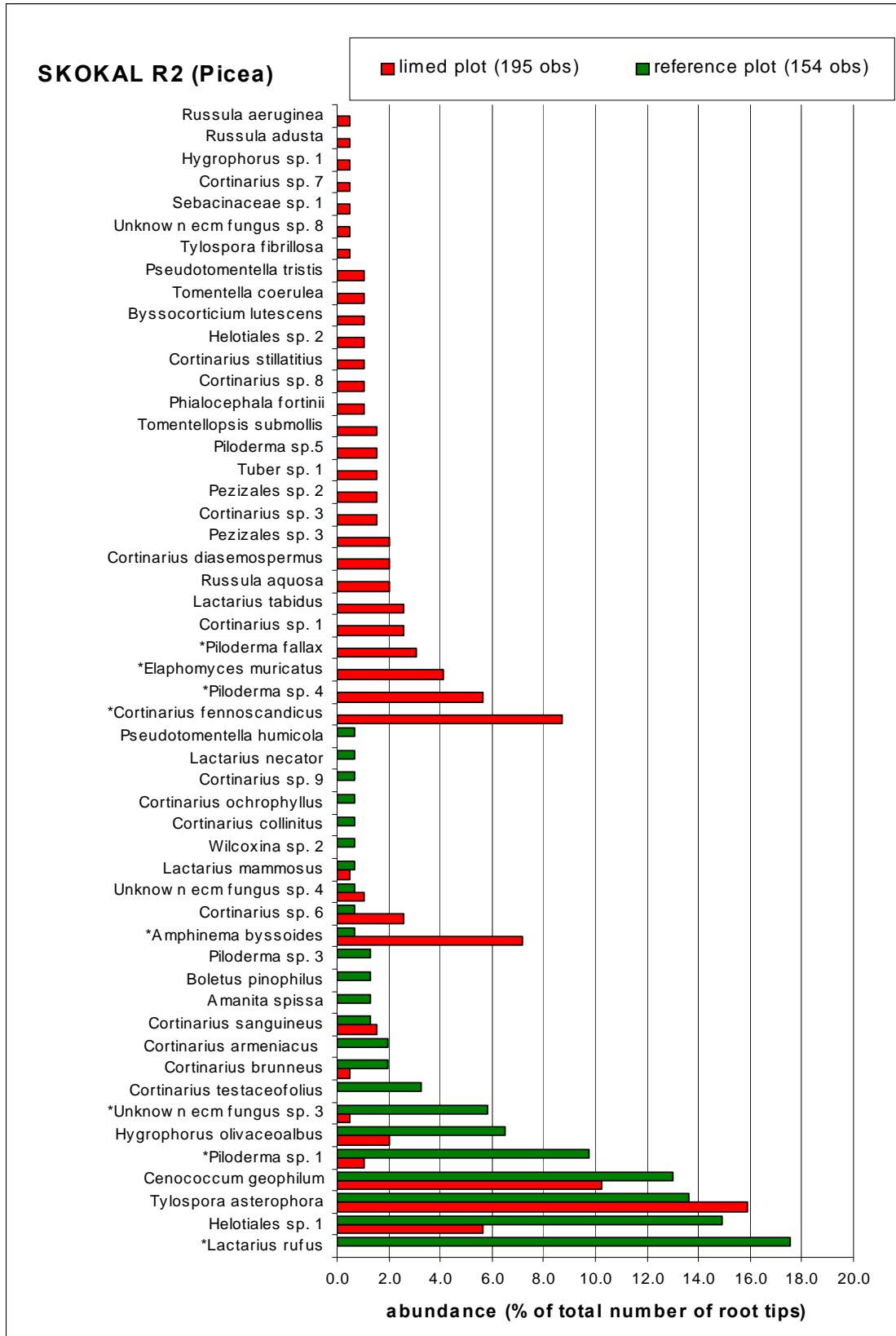
Figure 6c – community plots at SKOKAL site R2, limed and reference comparisons. The relative frequency of ectomycorrhizal fungal species in limed and reference plots. The frequency of species marked with * differ significantly between the limed and reference plot (X2 test).

a)

SKOKAL, all sites, clades
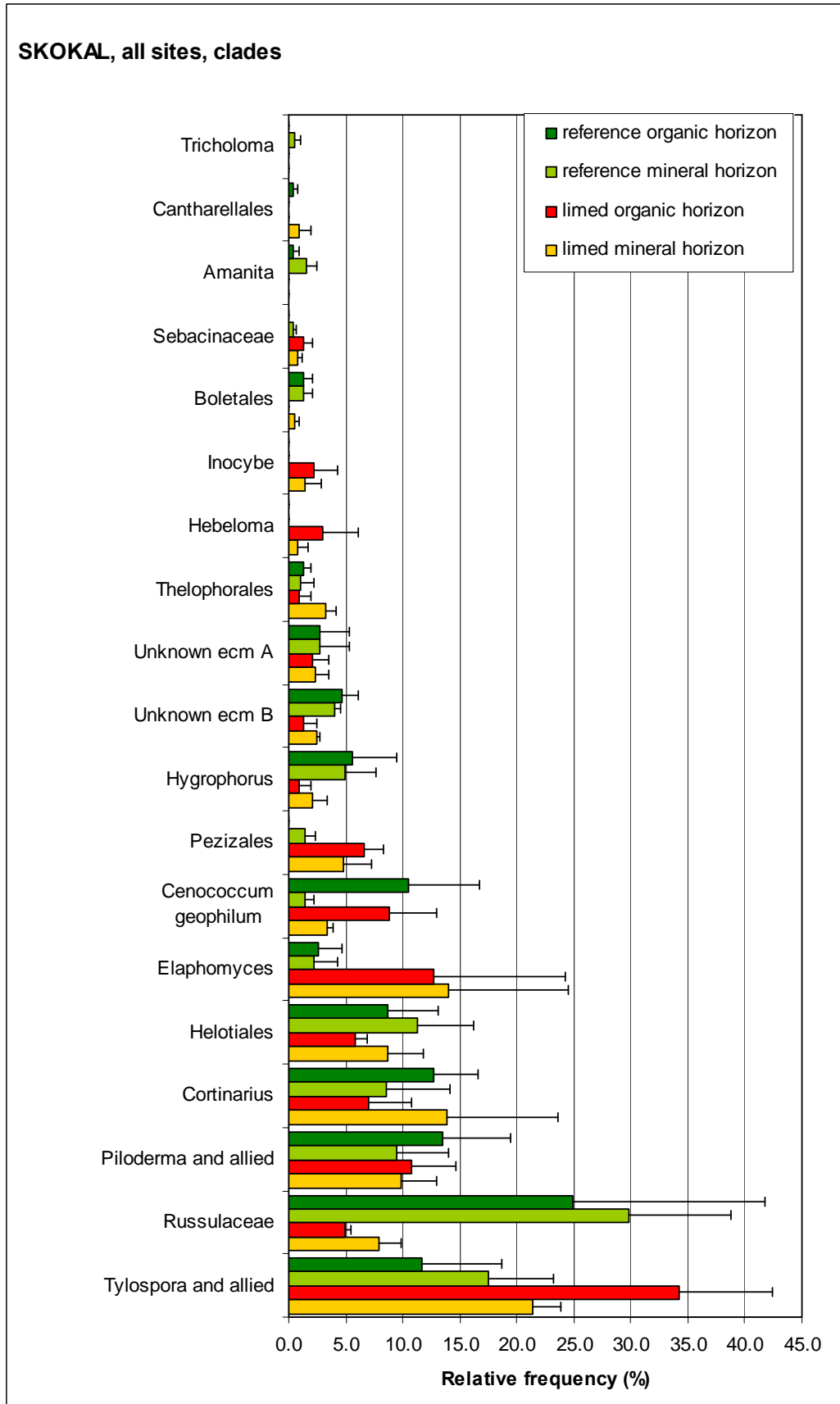
Figure 7a – consensus communities across the three sites, 2-factor figures. The relative frequency of ectomycorrhizal fungal clades in limed or reference, organic or mineral horizons. Bars are means across the three sites with standard errors.

b)
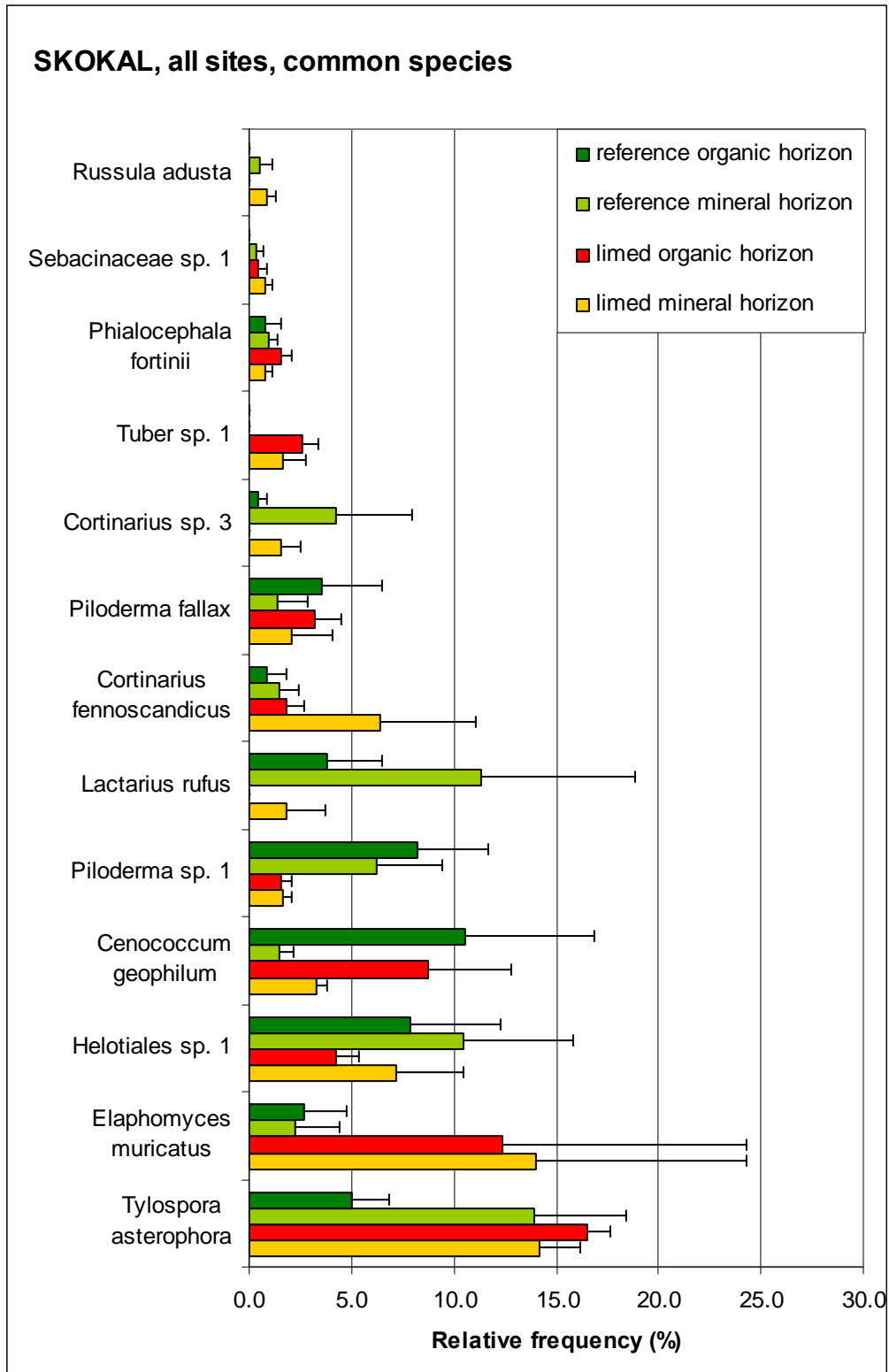
Figure 7b – consensus communities across the three sites, 2-factor figures. The relative frequency of ectomycorrhizal fungal species in limed or reference, organic or mineral horizons. Only species occurring at all three sites are included. Bars are means across the three sites with standard errors.

Table 7a. Results of two-way ANOVAs on relative frequencies of ectomycorrhizal fungal clades at the SKOKAL sites. Arrows indicate increase or decrease in frequency in response to liming.

Clade	Liming	Horizon	Lime x Horiz
Tylospora and allied	**0.007 ** ↑**	0.714	**0.037 ***
Russulaceae	**0.040 * ↓**	0.530	0.907
Piloderma and allied	0.903	0.489	0.611
Cortinarius	0.729	0.894	0.314
Helotiales	0.745	0.298	0.772
Elaphomyces	**0.041 * ↑**	0.891	0.719
Cenococcum geophilum	0.562	**0.027 ***	0.361
Pezizales	**0.015 * ↑**	0.832	0.160
Hygrophorus	0.122	0.064	0.545
Unknown ecm B	**0.036 * ↓**	0.300	0.202
Unknown ecm A	0.641	0.901	0.908
Thelophorales	0.439	0.404	0.179
Hebeloma	0.215	0.678	0.678
Inocybe	0.138	0.863	0.863
Boletales	0.114	0.644	0.611
Sebacinaceae	0.097	0.841	0.460
Amanita	0.060	0.348	0.348
Cantharellales	0.732	0.732	0.149
Tricholoma	0.347	0.347	0.347

Table 7b. Results of two-way ANOVAs on relative frequencies of ectomycorrhizal species found at all three SKOKAL sites. Arrows indicate increase or decrease in frequency in response to liming.

Species	Liming	Horizon	Lime x Horiz
Tylospora asterophora	**0.024 * ↑**	0.142	**0.041 ***
Elaphomyces muricatus	0.072	0.914	0.579
Helotiales sp. 1	0.683	0.461	0.947
Cenococcum geophilum	0.562	**0.027 ***	0.361
Piloderma sp. 1	0.093	0.625	0.583
Lactarius rufus	**0.048 * ↓**	0.189	0.649
Cortinarius fennoscandicus	0.323	0.411	0.752
Piloderma fallax	0.781	0.302	0.925
Cortinarius sp. 3	0.490	0.122	0.929
Tuber sp. 1	**0.003 ** ↑**	0.389	0.389
Phialocephala fortinii	0.411	0.754	0.341
Sebacinaceae sp. 1	0.263	0.299	0.996
Russula adusta	0.597	0.073	0.597

*p<0.05 **p<0.01

3.3. Discussion

3.3.1. The effect of liming on overall community structure

The present study showed that liming with 3 tonnes of dolomite changed the community of ectomycorrhizal fungal associates with pine and spruce. Liming led to a less even community structure (rank abundance curves) and to significant changes in both the community as such (PERMANOVA and DCA analysis) and in the abundance of specific species or groups of closely related species (clades). Site-related differences in species presence and abundances were large particularly between the two spruce sites and the pine site. Thus, more significant effects were found when merging closely related species into clades and thereby reducing the noise caused by these site differences. At individual sites, only between 22 and 28% of the species detected overlapped between the limed and reference plots but liming did not change the total number of species in the communities. In that respect, the present results are similar to the results of Taylor and Finlay (2003) obtained from a spruce stand near Hasslöv (Skåne) more South in Sweden than the sites in the present experiment. They observed an even more extreme species replacement, only 12.5% species overlap between limed and unlimed plots, but like in our study communities richness was unaffected. The limed plots in Hasslöv were treated with 8.75 tonnes dolomite per hectare which is almost three times the dose used in the present study which may explain the greater response found by Taylor and Finlay (2003). However, since most species only occurred on very few root tips (1/3 of the species only on a single root tip) the low species overlap observed exaggerates the treatment effect. A more realistic representation of the community change on root-tip level is to sum up all significant root tip increases and decreases from the pair-wise comparisons at each site (Fig. 6). Doing this, we estimate that 33% of the individual root tips became colonised by a different fungal species in response to liming. This number may be overestimated because it is assumed that limed and reference plots had an identical species composition before the liming took place, which may not be correct. On the other hand, the many species with low abundance and therefore non-significant distributions between limed and reference plots are not included in this estimate but may in reality just as often as the more abundant species be significantly influenced by liming.

3.3.2. The effect of liming on fungal species and clades

Concerning changes of individual species and clades the results from the present study are generally in good accordance with previous studies: The increase of the two Atheliaceae species, *Amphinema byssoides* and *Tylospora asterophora* because of liming, is similar to the results by Taylor and Finlay (2003). Increased abundance of *Amphinema byssoides* with liming was also found in some earlier studies (Taylor & Brand, 1992; Veerkamp *et al.*, 1997). Taylor and Finlay (2003) also found an increased abundance of *Elaphomyces* sp. with liming although not as dramatic as seen at the pine site (O2) in the present study. The increase of *Tuber* cf. *puberulum* with liming observed in other studies (Taylor & Brand, 1992; Taylor & Finlay, 2003) may be consistent with the increase in ectomycorrhizal fungi belonging to the order Pezizales (which includes the genus *Tuber*) seen in the present study. In accordance with the results of the present study, several earlier studies found that the abundance of *Russula* and *Lactarius* species decreased

by liming (Wiklund *et al*., 1995; Agerer *et al*., 1998; 8.75 t ha-1 in Jonsson *et al*., 1999; Taylor & Finlay, 2003). Also, some studies based on morphotyping noted that ectomycorrhizal types with smooth mycorrhizal root tips decreased in abundance with liming while types with well-developed external mycelia increased in abundance (Lehto, 1994a; Bakker *et al*., 2000). These changes may very well reflect decreased abundance of *Lactarius* and *Russula* species as they are known to form mycorrhizal tips with smooth mantles.

The increase of a yet unidentified *Tomentella* sp., *Piceirhiza nigra*, with liming (Taylor & Brand, 1992; Antibus & Linkins, 1992; Taylor & Finlay, 2003) was not mirrored by the present study as none of the four *Tomentella* species detected were abundant (see below). Similarly we do not in the present study corroborate the decrease in *Tylospora fibrillosa* with liming as found in some earlier studies (8.75 t ha-1 in Jonsson *et al*., 1999; Taylor & Finlay, 2003). In the present study *Tylospora fibrillosa* was abundant only at one of the sites (P2) and was found in roughly equal frequencies in limed and unlimed plots. Several studies have demonstrated that aboveground sporocarp biomass especially of *Cortinarius* species decreases following liming (Wiklund *et al*., 1995; Brandrud *et al*., 2001; Brandrud *et al*., 2003). This response of *Cortinarius* species was not seen on the belowground community in the present study as the frequencies of *Cortinarius* spp. were similar in limed and reference plots. Actually, the most common *Cortinarius* species, *Cortinarius fennoscandicus*, showed a tendency (though not significant) towards increased abundance in limed plots.

3.3.3. Possible causes for the observed community changes

The experimental liming treatments of Southern Swedish coniferous forests has generally and within our plots realized many of the effects anticipated by the Swedish Forest Agency (Uggla *et al*., 2003; Unpublished data from SLU, samples collected and analysed by SLU on behalf of the Swedish Forest Agency). The effects include increases in soil pH, base saturation and associated decreases in H^+ and Al^+ concentrations. Additionally, liming have increased Ca^{++} and Mg^{++} concentrations (dolomite which contains Mg^{++} is the liming agent used most often but note that at site P2 no Mg^{++} was added). The change in the ectomycorrhizal community along the ordination axis 2 of the DCA analysis in the present study correlated significantly with an increase in pH and base saturation and axis 3 correlated with decreased Al^+ concentration. Axis 1 most likely represents some unknown factor separating pine and spruce forests.

In the literature, liming effects on ectomycorrhizal communities is often ascribed to an increase in available nitrogen (see section 2.4.1). The impact of nitrogen availability and N-quality on ectomycorrhizal communities is well known (Lilleskov & Parrent, 2007) but within the experimental 3-tonnes liming treatments co-ordinated by the Swedish Forest Agency there is apparently no increase in available nitrogen as reflected by the unchanged C/N ratio. The forest floor C/N ratio has been shown to be a good predictor of nitrate leaching (Gundersen *et al*., 1998) and therefore presumably also of N-availability. However, more direct measurements of N availability and N cycling within the treatments administrated by the Swedish Forest Agency are needed to confirm if N availability is indeed unaffected by the liming treatments. The lack of a liming effect on N availability is also indirectly supported by the fact that no increasing in growth (or the oppo-

site) has been recorded for the host trees after liming (Anderson & Hildingsson, 2004). The lack of effects on *Cortinarius* frequencies in the present study also agrees with this, as this genus is highly sensitive to increases in N availability (Wiklund *et al*., 1995; Lilleskov *et al*., 2001; Brandrud *et al*., 2001; 2003). Finally, the highly significant drop in frequency of *Lactarius rufus* found in the present study would also be counter intuitive if N availability was increased with liming. *Lactarius rufus*, and other *Lactarius* spp., are often classified as nitrophilic fungi and generally increase in abundance with increased N availability (Taylor *et al*., 2000; Lilleskov *et al*., 2002b; Kjøller and Nielsson, unpublished).

The significant changes in the ectomycorrhizal community documented in the present experiment must therefore be caused by changes in other parameters than N availability, most likely on changes in pH or base saturation (or mediated effects not accounted for in the measured soil parameters). Lilleskov (2002a) also noted that the increase in *Amphinema byssoides* with liming and in nurseries more likely was an effect of increased pH rather than N as this species shifted from being the second-most abundant species to being extinct along a transect with increasing N deposition (Lilleskov *et al*., 2002a). *Tuber* species and other ectomycorrhizal species within Pezizales are also known for increasing in abundance with higher pH (Pasioni & Comandini, 1999; Tedersoo *et al*., 2006). pH changes can both alter the function of the fungi, e.g. their nutrient uptake over the membrane and the activity of secreted enzymes (Deacon, 2006), as well as affect multiple interconnected soil chemical parameters such as ion concentrations and base saturation. Therefore it is extremely difficult to ascribe the observed changes in the fungal communities to specific causes. Future experiments aiming at manipulating these factors individually would help to unravel the mechanistic explanations for the observed community changes, although it will probably be impossible to manipulate all factors independently in the field.

3.4. Implications and recommendations

3.4.1. Loss of ectomycorrhizal functionality with liming?

In the present experiment, liming did not reduce species richness or the number of ectomycorrhizal clades. As functional trait diversity to some extent is correlated with phylogenetic diversity the limed communities can still be regarded as highly diverse. The ectomycorrhizal fungal communities in the limed areas can probably still provide a diverse range of ectomycorrhiza-mediated services for their host trees. Previous studies have also uniformly showed that no reduction in mycorrhizal colonization is observed and that root tip biomass is mostly either unchanged or stimulated with liming (see Table 2 and references therein). The unaffected tree growth in the limed areas in comparison with the unlimed areas (Anderson & Hildingsson, 2004) also suggests that the general functionality of the mycorrhizal community is intact as the major part of nutrient uptake of trees presumably pass through their mycorrhizal partners (Smith & Read, 1997).

A note of caution however is needed. We know very little about the functional characteristics of individual ectomycorrhizal species and this only for a very limited number of species. Therefore, it is premature to state whether crucial mycorrhiza-mediated functions have been lost or not as a consequence of the de-

creases and increases and even replacements of species observed with liming. In the present experiment it is worrying that the abundance of Russulaceae is reduced 80% from being the most dominant clade to a much lower frequency. Species within Russulaceae are, like in the reference plots in the present experiment, often a dominant fungal constituent of ectomycorrhizal communities (Horton & Bruns, 2001; Dahlberg, 2001). However, we do not know exactly what the functional characteristics of Russulaceae species are in comparison with other mycorrhizal groups.

Ectomycorrhiza is associated with not just one but a multitude of beneficial effects for the host plants e.g. improved inorganic N and P uptake, acquisition of nutrients bound in organic material otherwise not accessible for plants, storage of nutrients in the fungal mantle structure for release in periods where uptake activities are otherwise low and weathering of rocks and protection of roots against pathogens (Smith & Read, 1997). Different ectomycorrhizal species and clades definitely differ in their efficiency of the above mentioned functions i.e. some are efficient in taking up mineral nutrients while others are providing nutrients stored in organic material (Leake & Read, 1997; Taylor *et al*., 2000; Lilleskov *et al*., 2002b), but our knowledge is patchy. Furthermore, the species which have been characterized for their functional performances are not a representative subset of all ectomycorrhizal fungi because they are the species which can withstand manipulations in either pure culture or in microcosms. Attempts to culture many ectomycorrhizal fungal species including extremely important components of ectomycorrhizal communities such as *Russula* and *Cortinarius* spp. have failed. Still, many more careful laboratory experiments comparing functional performances of different ectomycorrhizal fungi seem to be at least one important way forward in order to match functional traits to the phylogenetic diversity of ectomycorrhizal fungi. As more data on functional traits of various ectomycorrhizal fungal species and clades accumulates, trait-based analysis of whether traits differ more between treatments e.g. between limed and reference plots than within treatments will be possible (Ackerly & Cornwell, 2007). Other important future directions for tying functional and phylogenetic ectomycorrhizal diversity together were recently reviewed by Koide *et al*. (2007). Last but not less important is the collection of a large research-community driven global dataset tying fungal community data with environmental parameters. Such databases will allow the development of predictive models of ectomycorrhizal fungal community assemblies in relation to environmental parameters (Lilleskov & Parrent, 2007).

3.4.2. Loss of ectomycorrhizal fungal biodiversity with liming?

Over 1000 ectomycorrhizal Basidiomycota species have been described from the Nordic countries (Koljalg *et al*., 2005). Including the Ascomycota species and the numerous "cryptic" species within several of the ectomycorrhizal Basidiomycota clades (e.g. within Sebacinaceae, *Cortinarius* and *Tomentella*) the number may very well be twice as high. As discussed above, liming does not seem to reduce species richness but may cause species displacements or replacements. Species replacements with liming in theory might lead to species extinctions if sensitive species occur as geographically isolated populations. Thus, the larger the limed area the higher the risk of causing species extinctions, and if a fixed percentage forested area is limed regionally, our prediction would be that the smaller the

limed plots - the lower the risk of loosing locally isolated species. However, our knowledge of ectomycorrhizal species distributions is mostly based on sporocarp surveys which are biased towards large epigeous species and are often rather imprecise in describing the belowground communities (Gardes & Bruns, 1996; Dahlberg *et al.*, 1997; Horton & Bruns, 2001). Even with future field studies adding to the present knowledge of species abundance and distribution across Southern Swedish coniferous forests we believe that it is very likely that the community profiles will still look like that in the present study and numerous earlier studies, i.e. with a long tail of rare species. In this light it also seems highly possible that some of these rare species would be lost in response to liming or other management-related or natural disturbances. However, as discussed above, we do not know enough about the functional redundancy among ectomycorrhizal fungal species, and concluding whether loosing some of these species would be a thread to the function of the forests is premature.

3.4.3. The impact of liming in comparison with other forests management procedures or environmental changes

In the short term, clear cutting or stand-replacing fires have a tremendous impact on the structure of the ectomycorrhizal community (Baar *et al.*, 1999; Jones *et al.*, 2003). Basically, after removal of the host trees the fungal community will have to re-establish from soil borne resistant propagules and/or by re-invasion of propagules from surrounding areas. With time the ectomycorrhizal community matures and after some decades a community similar to the surrounding areas is established (Visser, 1995; K Føns and R Kjøller, unpublished). Liming is similar to the above scenarios in the sense that the effect starts at a specific point in time, but liming impose a less dramatic effect on the forest ecosystem that is left more or less functionally intact. However lime acts over many years and especially the study by Taylor and Finlay (2003) shows that liming potentially can result in an almost complete shift within an ectomycorrhizal community as also observed following clear cutting or stand-replacing fires. Soil borne resistant propagules and re-invasion from surrounding areas act to limit the risk of loosing species and thereby potentially functionality but as discussed above the larger the managed areas the higher the risk of loosing geographically isolated species. Acting in a different way is the slowly but continuously increasing amounts of anthropogenic nitrogen deposition experienced throughout large parts of the world (Galloway *et al.*, 2003). Forests have a high potential for accumulating nitrogen, but if the load gets high enough extractable soil NO_3^- will increase causing well documented changes within the ectomycorrhizal fungal community and functionality (Taylor *et al.*, 2000; Lilleskov *et al.*, 2001; Lilleskov *et al.*, 2002b; Nilsson & Wallander, 2003; Toljander *et al.*, 2006). In contrast to the various forest-management practices or natural occurring disturbances, nitrogen deposition offers no areas to where the fungal communities can escape at least at a regional scale. Together with the well-documented knowledge of diverse direct and indirect effects of nitrogen on ectomycorrhizal fungal growth, physiology and community composition the risk of causing permanent changes in ectomycorrhizal fungal communities therefore seems higher with continued N deposition than with lime or ash additions.

4. Acknowledgements

5. References

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Appendix 1. Numbers of species detected in each of the plots/horizons in the study. The numbers are based on successfully amplified sequences from single ectomycorrhizal root tips. The table is sorted by total abundance of the species.

Species	O2-lime-top	O2-lime-bot	O2-ref-top	O2-ref-bot	P2-lim-top	P2-lim-bot	P2-ref-top	P2-ref-bot	R2-lime-top	R2-lime-bot	R2-ref-top	R2-ref-bot
Tylospora asterophora	14	9	1	4	14	10	5	16	15	16	6	15
Elaphomyces muricatus	15	43	5	4	0	0	1	0	1	7	0	0
Helotiales sp. 1	3	0	0	0	5	9	7	16	4	7	12	11
Cenococcum geophilum	3	1	2	1	6	3	5	0	17	3	18	2
Piloderma sp. 1	2	0	2	0	2	1	11	9	1	1	8	7
Russula decolorans	2	2	26	12								
Lactarius rufus	0	0	0	2	0	4	2	4	0	0	7	20
Tylospora fibrillosa					9	7	14	8	1	0	0	0
Hygrophorus olivaceoalbus					0	2	11	5	2	2	3	7
Amphinema byssoides					13	0	0	0	11	3	1	0
Piloderma fallax	7	1	1	0	1	0	8	4	6	0	0	0
Cortinarius fennoscandicus	0	5	2	2	0	0	0	1	3	14	0	0
Unknown ecm fungus sp. 1	8	3	8	4								
Russula paludosa	0	0	8	9								
Cortinarius sp. 3	0	0	1	7	0	1	0	1	0	3	0	0
Piloderma sp. 2					0	11	1	0				
Atheliaceae sp. 1	12	0	0	0								
Russula vinosa	0	0	7	5								
Piloderma sp. 4									9	2	0	0
Unknown ecm fungus sp. 3	0	0	0	1					0	1	6	3
Unknown ecm fungus sp. 4					1	2	2	3	0	2	0	1
Tuber sp. 1	2	2	0	0	3	1	0	0	3	0	0	0
Phialocephala fortinii	2	1	1	0	1	0	2	1	1	1	0	0
Cortinarius sp. 1	2	2	0	0					0	5	0	0
Amphinema sp. 1					3	1	2	1				

Species	Y1	Y2	Y3	Y4	O1	O2	O3	O4	P1	P2	P3	P4
Cortinarius sp. 6									5	0	1	0
Inocybe flocculosa					5	1	0	0				
Cortinarius sanguineus									1	2	2	0
Cortinarius testaceofolius									0	0	5	0
Pezizales sp. 2					2	0	0	0	3	0	0	0
Piloderma sp. 3					0	3	0	0	0	0	0	2
Wilcoxina sp. 2					0	4	0	0	0	0	0	1
Atheliaceae sp. 3					0	0	1	4				
Cortinarius sp. 2	0	0	2	3								
Cortinarius brunneus									1	0	3	0
Cortinarius diasemospermus									2	2	0	0
Pezizales sp. 3									4	0	0	0
Russula aquosa									1	3	0	0
Tomentellopsis submollis					0	0	1	0	3	0	0	0
Lactarius mammosus					0	0	0	2	0	1	0	1
Pseudotomentella tristis	0	0	0	2					0	2	0	0
Sebacinaceae sp. 1	0	2	0	0	0	0	0	1	0	1	0	0
Hebeloma sp. 1					4	0	0	0				
Lactarius hysginus					0	0	1	3				
Cortinarius semisanguineus	0	0	1	0	0	0	3	0				
Wilcoxina sp. 1	1	3	0	0								
Cortinarius armeniacus									0	0	3	0
Piloderma sp.5									1	2	0	0
Amanita spissa					0	0	0	1	0	0	0	2
Tomentella coerulea					0	1	0	0	1	1	0	0
Russula adusta	0	0	0	1	0	1	0	0	0	1	0	0
Cortinarius evernius					0	0	1	2				
Lactarius camphoratus					0	0	1	2				
Wilcoxina sp. 3					0	0	0	3				
Cortinarius angelesianus	1	2	0	0								
Russula atrorubens	1	0	2	0								

Species	Y1	Y2	Y3	Y4	O1	O2	O3	O4	P1	P2	P3	P4
Tomentella sublilacina	2	1	0	0								
Boletus pinophilus									0	0	0	2
Byssocorticium lutescens									2	0	0	0
Cortinarius sp. 8									0	2	0	0
Cortinarius stillatitius									0	2	0	0
Helotiales sp. 2									0	2	0	0
Russula aeruginea					1	0	0	0	0	1	0	0
Clavulina sp. 1					0	2	0	0				
Hebeloma sp. 2					2	0	0	0				
Lacterius sp. 1					1	1	0	0				
Russula densifolia					0	0	0	2				
Russula velenovskyi					0	0	1	1				
Sebacinaceae sp. 2					2	0	0	0				
Suillus variegatus	0	0	1	0	0	0	0	1				
Atheliaceae sp. 2	0	0	1	1								
Cortinarius biformis	0	0	2	0								
Hebeloma velutipes	2	0	0	0								
Unknown ecm sp. 2	1	0	0	1								
Cortinarius collinitus									0	0	1	0
Cortinarius ochrophyllus									0	0	1	0
Cortinarius sp. 7									1	0	0	0
Cortinarius sp. 9									0	0	0	1
Hygrophorus sp. 1									1	0	0	0
Lactarius necator									0	0	0	1
Pseudotomentella humicola									0	0	1	0
Unknown ecm sp. 8									0	1	0	0
Amanita muscaria					0	0	0	1				
Boletus edulis					0	1	0	0				
Cantharellales sp.					0	0	1	0				
Cortinarius camphoratus					0	0	1	0				
Cortinarius sp. 4					0	0	1	0				

Cortinarius sp. 5					0	0	0	1				
Elaphomyces sp. 1					1	0	0	0				
Hebeloma sp. 3					1	0	0	0				
Inocybe lacera					0	1	0	0				
Inocybe sp. 1					0	1	0	0				
Lactarius deterrimus					1	0	0	0				
Piloderma sp.6					0	1	0	0				
Piloderma sp.7					0	1	0	0				
Russula sp. 1					0	1	0	0				
Russula vesca					0	0	1	0				
Unknown ecm sp. 6					0	0	0	1				
Unknown ecm sp. 5					0	0	1	0				
Amanita fulva	0	0	1	0								
Cortinarius spilomeus	1	0	0	0								
Lactarius quieticolor	0	1	0	0								
Paxillus involutus	0	0	1	0								
Pezizales sp. 1	1	0	0	0								
Russula sardonia	0	1	0	0								
Tomentella sp. 1	1	0	0	0								
Tricholoma sp. 1	0	1	0	0								
Lactarius rufus													
SUM	**83**	**80**	**75**	**59**	**163**	**134**	**297**	**78**	**71**	**85**	**94**	**149**

Appendix 2. Numbers of detected species summed for plots and sites. The numbers are based on successfully amplified sequences from single ectomycorrhizal root tips. The table is sorted by total abundance of the species. The clade assignments for each species is also shown.

Species	Clade assignment	O2-lime-sum	O2-ref-sum	O2-sum	P2-lime-som	P2-ref-sum	P2-sum	R2-lime-sum	R2-ref-sum	R2-sum	SUM
Tylospora asterophora	*Tylospora* and allied	23	5	28	24	21	45	31	21	52	125
Elaphomyces muricatus	*Elaphomyces*	58	9	67	0	1	1	8	0	8	76
Helotiales sp. 1	Helotiales	3	0	3	14	23	37	11	23	34	74
Cenococcum geophilum	*Cenococcum geophilum*	4	3	7	9	5	14	20	20	40	61
Piloderma sp. 1	*Piloderma* and allied	2	2	4	3	20	23	2	15	17	44
Russula decolorans	*Russula*	4	38	42							42
Lactarius rufus	*Lactarius*	0	2	2	4	6	10	0	27	27	39
Tylospora fibrillosa	*Tylospora* and allied				16	22	38	1	0	1	39
Hygrophorus olivaceoalbus	*Hygrophorus*				2	16	18	4	10	14	32
Amphinema byssoides	*Tylospora* and allied				13	0	13	14	1	15	28
Piloderma fallax	*Piloderma* and allied	8	1	9	1	12	13	6	0	6	28
Cortinarius fennoscandicus	*Cortinarius*	5	4	9	0	1	1	17	0	17	27
Unknown ecm sp. 1	Unknown ecm A	11	12	23							23
Russula paludosa	*Russula*	0	17	17							17
Cortinarius sp. 3	*Cortinarius*	0	8	8	1	1	2	3	0	3	13
Piloderma sp. 2	*Piloderma* and allied				11	1	12				12
Atheliaceae sp. 1	*Tylospora* and allied	12	0	12							12
Russula vinosa	*Russula*	0	12	12							12
Piloderma sp. 4	*Piloderma* and allied							11	0	11	11
Unknown ecm sp. 3	Unknown ecm B	0	1	1				1	9	10	11
Unknown ecm sp. 4	Unknown ecm A				3	5	8	2	1	3	11
Tuber sp. 1	Pezizales	4	0	4	4	0	4	3	0	3	11
Phialocephala fortinii	Helotiales	3	1	4	1	3	4	2	0	2	10
Cortinarius sp. 1	*Cortinarius*	4	0	4				5	0	5	9
Amphinema sp. 1	*Tylospora* and allied				4	3	7				7

Species	Group	Y1	Y2	Y3	O1	O2	O3	P1	P2	P3	Total
Cortinarius sp. 6	*Cortinarius*							5	1	6	6
Inocybe flocculosa	*Inocybe*				6	0	6				6
Cortinarius sanguineus	*Cortinarius*							3	2	5	5
Cortinarius testaceofolius	*Cortinarius*							0	5	5	5
Pezizales sp. 2	Pezizales				2	0	2	3	0	3	5
Piloderma sp. 3	*Piloderma* and allied				3	0	3	0	2	2	5
Wilcoxina sp. 2	Pezizales				4	0	4	0	1	1	5
Atheliaceae sp. 3	*Piloderma* and allied				0	5	5				5
Cortinarius sp. 2	*Cortinarius*	0	5	5							5
Cortinarius brunneus	*Cortinarius*							1	3	4	4
Cortinarius diasemospermus	*Cortinarius*							4	0	4	4
Pezizales sp. 3	Pezizales							4	0	4	4
Russula aquosa	*Russula*							4	0	4	4
Tomentellopsis submollis	Thelophorales				0	1	1	3	0	3	4
Lactarius mammosus	*Lactarius*				0	2	2	1	1	2	4
Pseudotomentella tristis	Thelophorales	0	2	2				2	0	2	4
Sebacinaceae sp. 1	Sebacinaceae	2	0	2	0	1	1	1	0	1	4
Hebeloma sp. 1	*Hebeloma*				4	0	4				4
Lactarius hysginus	*Lactarius*				0	4	4				4
Cortinarius semisanguineus	*Cortinarius*	0	1	1	0	3	3				4
Wilcoxina sp. 1	Pezizales	4	0	4							4
Cortinarius armeniacus	*Cortinarius*							0	3	3	3
Piloderma sp.5	*Piloderma* and allied							3	0	3	3
Amanita spissa	*Amanita*				0	1	1	0	2	2	3
Tomentella coerulea	Thelophorales				1	0	1	2	0	2	3
Russula adusta	*Russula*	0	1	1	1	0	1	1	0	1	3
Cortinarius evernius	*Cortinarius*				0	3	3				3
Lactarius camphoratus	*Lactarius*				0	3	3				3
Wilcoxina sp. 3	Pezizales				0	3	3				3
Cortinarius angelesianus	*Cortinarius*	3	0	3							3
Russula atrorubens	*Russula*	1	2	3							3

Species	Group										Total
Tomentella sublilacina	Thelophorales	3	0	3							3
Boletus pinophilus	Boletales							0	2	2	2
Byssocorticium lutescens	*Piloderma* and allied							2	0	2	2
Cortinarius sp. 8	*Cortinarius*							2	0	2	2
Cortinarius stillatitius	*Cortinarius*							2	0	2	2
Helotiales sp. 2	Helotiales							2	0	2	2
Russula aeruginea	*Russula*				1	0	1	1	0	1	2
Clavulina sp. 1	Cantharellales				2	0	2				2
Hebeloma sp. 2	*Hebeloma*				2	0	2				2
Lacterius sp. 1	*Lactarius*				2	0	2				2
Russula densifolia	*Russula*				0	2	2				2
Russula velenovskyi	*Russula*				0	2	2				2
Sebacinaceae sp. 2	Sebacinaceae				2	0	2				2
Suillus variegatus	Boletales	0	1	1	0	1	1				2
Atheliaceae sp. 2	*Piloderma* and allied	0	2	2							2
Cortinarius biformis	*Cortinarius*	0	2	2							2
Hebeloma velutipes	*Hebeloma*	2	0	2							2
Unknown ecm sp. 2	Unknown ecm B	1	1	2							2
Cortinarius collinitus	*Cortinarius*							0	1	1	1
Cortinarius ochrophyllus	*Cortinarius*							0	1	1	1
Cortinarius sp. 7	*Cortinarius*							1	0	1	1
Cortinarius sp. 9	*Cortinarius*							0	1	1	1
Hygrophorus sp. 1	*Hygrophorus*							1	0	1	1
Lactarius necator	*Lactarius*							0	1	1	1
Pseudotomentella humicola	Thelophorales							0	1	1	1
Unknown ecm sp. 8	Unknown ecm B							1	0	1	1
Amanita muscaria	*Amanita*				0	1	1				1
Boletus edulis	Boletales				1	0	1				1
Cantharellales sp.	Cantharellales				0	1	1				1
Cortinarius camphoratus	*Cortinarius*				0	1	1				1
Cortinarius sp. 4	*Cortinarius*				0	1	1				1

Species	Group										Total
Cortinarius sp. 5	*Cortinarius*				0	1	1				1
Elaphomyces sp. 1	*Elaphomyces*				1	0	1				1
Hebeloma sp. 3	*Hebeloma*				1	0	1				1
Inocybe lacera	*Inocybe*				1	0	1				1
Inocybe sp. 1	*Inocybe*				1	0	1				1
Lactarius deterrimus	*Lactarius*				1	0	1				1
Piloderma sp.6	*Piloderma* and allied				1	0	1				1
Piloderma sp.7	*Piloderma* and allied				1	0	1				1
Russula sp. 1	*Russula*				1	0	1				1
Russula vesca	*Russula*				0	1	1				1
Unknown ecm sp. 6	Unknown ecm B				0	1	1				1
Unknown ecm sp. 5	Unknown ecm B				0	1	1				1
Amanita fulva	*Amanita*	0	1	1							1
Cortinarius spilomeus	*Cortinarius*	1	0	1							1
Lactarius quieticolor	*Lactarius*	1	0	1							1
Paxillus involutus	Boletales	0	1	1							1
Pezizales sp. 1	Pezizales	1	0	1							1
Russula sardonia	*Russula*	1	0	1							1
Tomentella sp. 1	Thelophorales	1	0	1							1
Tricholoma sp. 1	*Tricholoma*	1	0	1							1
SUM		**163**	**134**	**297**	**149**	**179**	**328**	**190**	**154**	**344**	**969**

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Liming may affect flora and fauna. Ectomycorrhizal fungi have been found to be particularly sensitive. In the present report, a study aiming at 1) a review of results from previous studies of lime and ash addition effects and 2) an additional field study to monitor if three tonnes of lime per hectare have caused any significant effects 17 years after liming is presented

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