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XIX CICLO

VARIATIONS OF THE ECTOMYCORRHIZAL COMMUNITY IN HIGH MOUNTAIN NORWAY SPRUCE STANDS AND CORRELATIONS WITH THE MAIN PEDOCLIMATIC FACTORS

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DATA CONSEGNA TESI

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CONTENTS

The experimental works described in this thesis are part of scientific papers submitted or to be submitted to international journals.

CHAPTER 2: Scattolin L., Montecchio L., Agerer R., 2006. The ectomycorrhizal community structure in high mountain Norway spruce stands. Submitted to "Trees - Structure and Function".

CHAPTER 3: Scattolin L., Montecchio L., 2006. The ectomycorrhizal vertical distribution in the top soil of Norway spruce stands. In preparation for "European Journal of Forest Research".

CHAPTER 4: Scattolin L., Montecchio L., Taylor AFS 2006. Sampling methods to assess the ectomycorrhizal communities: still inaccurate tools to describe the underground complexity. In preparation for "Mycorrhiza".

Chapter 1.
General introduction

1.1 Introduction

In natural and semi-natural ecosystems, symbioses at the level of complex mutually beneficial associations between identifiably different organisms play fundamental roles (SMITH and READ 1997; BUSCOT et al. 2000).

The term *symbiotismus* was used by FRANK (1877) to describe a regular coexistence of dissimilar organisms. In time, this term was used to describe the associations, not necessary mutualistic (i.e.: parasitism), between two organisms (DEBARY 1879).

Plants cooperate with many micro-organisms in the rhizosphere to form mutualistic associations. One of the best example is the mycorrhizal symbiosis between plants and fungi: the location of the fungal symbionts on the root and its hyphal connections with the soil substrates guarantee that the fungus can influence the adsorption of soil derived nutrients, supporting plants with mineral nutrients and other services and it receives, in turn, carbon as photosynthate from the autotrophic plants (SMITH and READ 1997). Mycorrhizal associations are common in almost all ecosystems and 80% of all land plants associate with these mutualistic soil fungi (VAN DER HEIJDEN and SANDERS, 2002). *Indeed, mycorrhizae, not roots, are the chief organs of nutrient uptake by land plants* (SMITH and READ 1997).

1.2 Types of mycorrhizal symbioses

Mycorrhizae are highly evolved, mutualistic associations between soil fungi and plant roots. The partners in this association (Tab. 1) are members of the fungus kingdom (Basidiomycetes, Ascomycetes and Zygomycetes) and most vascular plants (HARLEY and SMITH 1983, KENDRICK 1992, BRUNDRETT 1991).

Among the various types of mycorrhizal symbioses, arbuscular endomycorrhiza (AM), ectomycorrhiza (ECM) or ericoid associations are found on most annual and perennial

plants. About two-thirds of these plants are symbiotic with AM glomalean fungi. Ericoid mycorrhizae are ecologically important, but mainly restricted to heathlands. While a relatively small number of plants develop ECM, they dominate forest ecosystems in boreal, temperate and mediterranean regions. In the different mycorrhizal associations, hyphal networks are active metabolic entities that provide essential nutrient resources (e.g. phosphate and amino acids) to the host plant. These nutrient contributions are reciprocated by the provision of a stable carbohydrate-rich niche in the roots for the fungal partner, making the relationship a mutualistic symbiosis. The ecological performance of mycorrhizal fungi is a complex phenotype affected by many different genetic traits and by biotic and abiotic environmental factors. Without doubt, anatomical features (e.g. extension of the extramatrical hyphae) resulting from the development of the symbiosis are of paramount importance to the metabolic (and ecophysiological) fitness of the mature mycorrhiza (AGERER 2001).

Type	**VAM**	**ECM**	**Ectendo-**	**Arbutoid**	**Mono-tropoid**	**Ericoid**	**Orchid**
Septate hyphae	- (+)	+ -	+ -	+	+	+	+
Hyphae in cells	+	-	+	+	+	+	+
Hyphal coils	+ -	-	-	-	-	+	+
Arbuscules	+	-	-	-	-	-	-
Mantle	-	+ (-)	+ (-)	+	+	-	-
Hartig net	-	+	+	+	+	-	-
Vesicles	+ -	-	-	-	-	-	-
Plants	Vascular plants	Gymnosperms & Angiosperms		Ericales	Mono-tropaceae	Ericales	Orchid-aceae
Chlorophyll	+	+	+	+ -	-	+	+ -
Fungi	Zygo-Glomales	Most Basid-, but some Asco- and Zygo-				Asco-(Basid-)	Basid-

Notes: - = absent, + = present, (+)= sometimes present, (-)= sometimes absent, +- = present or absent, Basid- = Basidiomycetes, Asco- = Ascomycetes, Zygo = Zygomycetes

Table 1. Key differences between mycorrhizal association types (modified from BRUNDRETT 1999; HARLEY and SMITH 1983).

1.2.1 Ectomycorrhizal symbiosis

Ectomycorrhizal (ECM) association is the predominant form of mycorrhizal in boreal and temperate forest trees. This symbiosis has evolved repeatedly over the last 130-180 Myr and has had major consequences for the diversification of both the mycobionts and their hosts. Ectomycorrhizal fungi mainly belong to the Basidiomycetes, even though many species are found within the Ascomycetes and Zygomicetes. The first mycorrhizal associations must have been derived from earlier types of plant-fungus interactions, such as endophytic fungi in the bryophyte-like precursors of vascular plants (WILKINSON, 2001). Ectomycorrhizal symbioses have a different host range allowing formation of ectomycorrhiza on a limited set of trees and shrubs; nevertheless, a given species of ectomycorrhizal fungus is usually able to establish a mutualistic symbiosis on a broad range of species, even if highly specific interactions are present (e.g. *Suillus grevillei* - *Larix decidua*). In temperate and boreal forests, up to 95% of the short roots form ectomycorrhizae (SMITH and READ, 1997). Ectomycorrhizae have a helpful impact on plant growth in natural and agroforestry ecosystems. Fundamental to the success of these symbioses is the switch of nutrients between the symbionts: the fungus gains carbon from the plant while plant nutrient uptake is mediated via the fungus. In addition, the establishment of the symbiosis is required for the completion of the fungal life cycle (i.e. formation of fruiting bodies).

Ectomycorrhizal structure is characterized by the presence of a dense web of fungal hyphae forming a pseudoparenchymatous tissue ensheathing the root: the Hartig net of intercellular hyphae and the outward network of hyphae exploring the soil and gathering nutrients. The mantle of fungal tissue surrounding the host lateral roots varies from the characteristic pseudoparenchymatous tissue to a rather open-wefted arrangement of hyphae (AGERER 1991). Development of a mature mantle proceeds through a programmed series of events, starting from fungal hyphae originating from a soil propagule or an older mycorrhiza which penetrate into the root cap cells and grow through them. Backwards from the tip the invasion of root cap cells proceeds inwards until the hyphae reach the epidermal cells. This morphogenesis of ectomycorrhiza includes a series of complex ontogenic processes in symbionts: switching off the fungal growth mode, initiation of lateral roots, aggregation of hyphae, arrest of cell division in ensheathed roots, radial elongation of epidermal cells. These steps directed by complex

programmes of cellular development are accompanied by new metabolic organizations in fungal and plant cells and lead to the completed functioning symbiotic organ as an extended function of the root system where the extramatrical hyphae, the mantle and the Hartig net are dynamic metabolic units that grant essential nutrient resources (e.g. nitrogen, phosphate) to the host plant (VARMA and HOCK 1994, SMITH and READ1997, ALLEN 1991).

Family	***Genera***
Betulaceae	*Alnus, Betula, Carpinus, Ostrya, Ostryopsis*
Caesalpiniaceae*	*Anthonotha, Afzelia, Berlinia, Brachystegia, Eperua, Gilbertiodendron, Intsia, Isoberlinia, Julbernardia, Microberlinia, Monopetalanthus, Tetraberlinia*
Casuarinaceae*	*Allocasuarina (Cassuarina)*
Cistaceae	*Helianthemum, Cistus, Tuberaria*
Corylaceae	*Corylus*
Cyperaceae	*Kobresia (herb)*
Dipterocarpaceae	*Anisoptera, Dipterocarpus, Hopea, Marquesia, Monotes, Shorea, Vateria*
Ericaceae	*Cassiope*
Euphorbiaceae*	*Marquesia, Uapaca, Ampera, Poranthera*
Papilionaceae* (Fabaceae)	*Gastrolobium, Gompholobium, Jacksonia, Mirbelia, Oxylobium, Pericopsis*
Fagaceae	*Castanea, Castanopsis, Fagus, Nothofagus, Quercus*
Gnetaceae	*Gnetum*
Meliaceae	*Owenia*
Mimosaceae*	*Acacia*
Myrtaceae*	*Allosyncarpia, Agonis, Angophora, Baeckea, Eucalyptus, Leptospermum, Melaleuca, Tristania*
Nyctaginaceae*	*Neea, Pisonia*
Pinaceae	*Abies, Cathaya, Cedrus, Keteleeria, Larix, Picea, Pinus, Pseudolarix, Pseudotsuga, Tsuga*
Polygonaceae*	*Polygonum*
Rhamnaceae*	*Pomaderris, Trymalium*
Rosaceae*	*Dryas*
Salicaceae	*Populus, Salix*
Tiliaceae	*Tilia*

Table 2. Families and genera of plants with typical ectomycorrhizal associations. *Families with many VAM plants. Excluded families that appear in some lists, but have not been well documented or have atypical associations: *Aceraceae, Aquifoliaceae, Asteraceae, Bignoniaceae, Campanulaceae, Brassicaceae, Caprifoliaceae, Caryophyllaceae, Cornaceae, All Ferns, Goodenaceae, Lauraceae, Myricaceae, Oleaceae, Plantanaceae, Rubiaceae, Saxifragaceae, Stylidiaceae, Thymeliaceae, Ulmaceae, Vitaceae*. (modified from BRUNDRETT 1999).

In addition to absorbing and transferring nutrients, minerals and water from the external environment into the plants, many ECM fungi are able to degrade recalcitrant organic sources (SMITH and READ 1997) and some are also involved in the dissolution of soil minerals (LANDEWEERT et al. 2001) to get access to nutrients and minerals. They can also confer on their plant hosts protection against heavy metal toxicity (BRADLEY et al. 1982, VAN TICHELEN et al. 2001, ADRIAENSEN et al. 2004) and invasion by root pathogens (STENSTRÖM et al. 1997, DUCHESNE et al. 1989, MORIN et al. 1999).

The ectomycorrhizal symbioses are therefore crucial for the composition and function of all terrestrial ecosystems. For this reason, interpretation of these fungus-plant interactions should provide a key to a better understanding of ecosystem functioning and biodiversity.

Since climatic changes and human activities significantly influence our natural ecosystems, the importance of studies on the biodiversity and species composition of ECM fungi in forests are increasing. A decisive future challenge is to establish sampling protocols that can accurately determine ECM diversity. In fact, sampling effort and strategy highly influence ECM community structure (TAYLOR 2002). Morphological and anatomical characterization of the ectomycorrhizae (anatomotyping) helps to raise sample sizes and gives more data on the spatial and temporal distribution of different species (AGERER 1987-2002, 2001, 1991). Morphological data are also needed for the establishment of functional ecological groups, while for studies of the probably most dynamic part of the ECM community, (the external mycelium) molecular markers are essential (PENNANEN et al. 2001). Recently, AGERER (2001) suggested that the exploration types of ECM mycelia might mirror their ecological function.

A better understanding of the spatial and temporal dynamics of ECM communities in the field, supported by suitable sampling methods, and a deeper knowledge of ecological features of ECM species (not only as single units in a community, but also as part of functional groups) will be of great importance to future ecological studies and applications in forest management.

1.3 Ectomycorrhizal community diversity in relations to the abiotic environment

Trees and their associated ectomycorrhizal fungi have a significant influence on the soil ecosystems. The ECM symbiosis may be regarded as an adaptation to conditions of low mineral nutrition availability and situations where nutrient inputs are pulsed (SMITH and READ 1997). The nutrient status of soil have a recognized important role in determining ECM fungal structure (ERLAND and TAYLOR 2002) and it is well known that ECM species vary they ability to acquire nutrients from soil (THOMSON et al. 1994, LEAKE and READ 1997), with different efficiency.

However, the capacity in determining the influence of individual edaphic factors upon ECM community structure is strictly connected to the fact that very few components may change independently from all the others: very frequently, in fact, the environmental variables, most notably soil characteristics, are closely linked one to each other. For example, variations in ECM community were ascribed to shifts in soil pH (DIGHTON and SKEFFINGTON 1987, AGERER et al. 1998), but important connections are known to be present between soil pH and many other pedological variables, such as heavy metal and aluminium availability (MARSCHNER 1995).

The very different physical-chemical situations present in a soil forest contributes to create a spatial patch of heterogeneous niches which, according to BRUNS (1995), is involved in the maintenance of high ECM fungal diversity.

As far as we are aware, few studies have examined the microspatial distribution of individual ECM species in relation to these soil factors.

Among the studies focused on soil organic matter and spatial heterogeneity, FRANSSON et al. (2000) showed that, in Norway spruce stands in North Sweden, some species differed in their preference for the mineral and organic soil horizons: *Cenococcum geophilum* and *Tylospora fibrillosa* mycorrhizae were mainly found in the organic and mineral horizons, respectively, while mycorrhizae of *Piloderma* weren't associated to any particular soil. YANG et al. (1998) demonstrated a relationships between accumulating organic matter and mycorrhizal diversity of *Larix kaempferi* on a lava flows, while CONN and DIGHTON (2000) demonstrated that size and composition of litter patches under Pine Barrens affected the distribution of the ECM species. AGERER and GÖTTLEIN (2003) have demonstrated that differences in small scale distribution

may be associated to various abilities to make diffrenet nutrient sources available. Moreover, in a recent paper, BAIER et al. (2006) showed spatial niche differentiation in a Norway spruce plantation in the Bavarian Alps, with association of *Cenococcum geophilum* and *Sebacina* spp. to organic horizons and association of the genera *Lactarius*, *Craterellus* and *Tomentella* to mineral A-horizon.

The general features present in the studies focused on soil moisture are that where soils are more dried out, the ECM community is affected by a lower diversity and an increasing proportion of root tips colonized by *C. geophilum* (FOGEL 1980, PIGOTT 1982).

A further parameter considered in these ecological studies is soil pH, even if this could be considered the most complex as very related to many other soil features. The distribution of ECM morphotypes in soil closest to the base of very old *Fagus sylvatica* (L.) trees demonstrated (KUMPFER and HEYSER 1986) how the acid stem flow, creating a soil pH gradient with higher values with increasing the distance from the base, determined an inverse percentage of root tips colonized by *C. geophilum*.

An important and actual factor, inherent the global climate change, that very few studies (as far as we know not in the field) has taken into account for its effects on ECM community, is the temperature. In a microcosm, significant variations in the number of root tips of seedling colonized by *Piloderma croceum* and *Paxillus involutus* were studied by ERLAND and FINLAY (1992).

The essence of the researches above reported, together with all the ones not mentioned but very important as well, underlines the fact that investigations in this ecological context is really currently alive, mirroring the fundamental demand of knowledge in these aspects belonging to the soil ecology and directed to forest management. Nevertheless, the cause that influence the development and maintenance of the diversity in ectomycorrhizal community and the possibility to use ectomycorrhizal fungi as sensitive indicators of forest ecosystem responses to environmental factors and their variation continue to be a matter of debate difficult to be interpret.

1.4 Aim of the thesis

The main goal of this thesis, linked to "Dinamus - Humus and forest dynamics" project, funded by the "Fondo per i progetti di ricerca della Provincia autonoma di Trento", in co-operation with the Centre for Alpine Ecology (TN, Italy), was to verify the possibility to integrate the parameters generally used in forest soil descriptions with a biological indicator as the ectomycorrhizal community could act, being characterized by the interactions among soil variables, such as physical and chemical soil features, and dynamics of the surrounding forest.

To determine the influence of environmental features on ECM community, soil bedrock pH, exposure, humus forms and their chemical-physical properties were taken into account as the most representative and influencing factors in soil ecological dynamics.

Moreover, a particular attention was directed to the sampling design strategy, actually unstandardized.

1.5 Thesis structure

The thesis is composed by three chapters presenting the edaphic factors considered as potentially best influencing the ectomycorrhizal community in an old high mountain Norway spruce stands in northern Italy (chapter 2 and 3) and the sampling strategy applied discussed (chapter 4).

Each chapter is based on a paper submitted to, or in preparation for, an international peer-reviewed journal, then followed by a general discussion (chapter 5).

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Chapter 2.
The ectomycorrhizal community structure in high mountain Norway spruce stands

- Submitted the 14th Dec. 2006 by Scattolin L, Montecchio L., Agerer R. to *Trees - Structure and Function* -

Abstract

The species composition of ectomycorrhizal (ECM) fungal communities can be strongly influenced by abiotic and biotic factors, which determine interactions among the species such as resource partitioning, disturbance, competition, or relationships with other organisms.

To verify whether ectomycorrhization of the root tips and composition of the ECM community in Norway spruce vary according to site features and if ECM species peculiar to these environmental variables can be detected, 10 comparable stands differing in bedrock pH and exposure were selected and studied.

The results demonstrated that tips vitality and ectomycorrhization degree do not change significantly either on the same tree, or among trees growing in the same stand, whereas they differ greatly with bedrock pH and exposure.

ECM species composition revealed a significant connection with the 2 environmental features, with a few species significantly associated to them.

The results suggest that pH/exposure patterns play a primary role in the adaptive selection of ECM species constituting the consortium.

Key words: Ectomycorrhizal community, soil, bedrock, exposure, Norway spruce.

2.1 Introduction

The species composition of an ectomycorrhizal (ECM) community has been demonstrated to be the result of a complex and dynamic sequence of interactions mainly influenced by the characteristics of the plant and fungal species forming an ECM, the interactions between an ECM and the other fungal symbionts of the same plant, by the biotic and abiotic mycorrhizospheric features, structure of the plant community, site features and cultural techniques (HARVEY et al. 1987; GEHRING et al. 1998; GOODMAN and TROFYMOW 1998; O'DELL et al. 1999; CONN and DIGHTON 2000; LILLESKOV et al. 2001; DICKIE et al. 2002; ROSLING et al. 2003; KALDORF and RENKER 2004; MONTECCHIO et al. 2004; DICKIE and REICH 2005; SMITH and READ 1997; ALLEN 1991; PFLEGER and LINDERMAN 1994; SIMARD and DURALL 2004; COURTY et al. 2006).

The vitality and ectomycorrhization degrees of the root tips, and ECM richness and evenness, can therefore be associated to many environmental variables and their interactions.

Supposing that the functional activity of an ECM consortium as a whole can have similar efficiency in comparable forests growing in different sites, the main goal of the research, performed in 10 comparable Norway spruce forests, was to verify if the tips vitality and composition of the ECM consortium can be associated to main site features such as bedrock pH and exposure.

2.2 Materials and methods

2.2.1 Stand selection and sample collection

The research was performed in 2003 and 2004 in 10 monoculture and coeval [165(±10)-year-old] Norway spruce [*Picea abies* (L.) Karst.] stands growing in the Province of Trento (northern Italy), randomly selected among the most representative spruce forests in the Province [podzolization and brunification soil processes (ISSS et al. 1998), climatic and site features, sylvicultural treatments, productivity].

In order to distinguish these forests, the information available from the official Province of Trento forest management database and the existing literature (Provincia Autonoma di Trento 2001; SBOARINA and CESCATTI 2004) were organized through ArcExplorer software (ESRI Institute Inc., ArcExplorer, 2.0.800-version, Redlands, USA).

In these selected forests, using the same methods, 6 sub-types differing in bedrock pH [(H_2O), (A=4.3±0.3; B=5.4±0.3; C=7.6±0.4)] and exposure (N=0°±22.5°; S=180°±22.5°) were located, and among which, six 100x100 m plots were randomly selected, at least 10 km apart (AN1, AS1, BN1, BS1, CN1, CS1).

In 2004, in order to verify whether the results can be extended to sites with the same features independently of their geographical location, 4 more plots (AN2, AS2, CN2, CS2) were selected, each one at least 15 km distant from its "twin" (i.e. AN1-AN2).

In each plot, after a phytosanitary investigation, 4 healthy spruces, undamaged by climatic events, with fully-developed crown, mean age 155-175 yrs, diameter (breast height, $d_{1.30}$) 75 ±5 cm and separated by at least 15 m from the nearest tree, were randomly selected and coded. In AN1, AS1, BN1, BS1, CN1 and CS1 sites, four different trees were sampled in 2003 and 2004 in the same plots, in order to avoid temporal correlations.

In July 2003 and 2004, from each selected spruce and along the four main cardinal directions (N, E, S, W) 6 cylindrical soil cores (Ø 18 mm, h 35 cm) were collected (100, 150, 200, 250, 300, 350 cm from the collar) and stored in sealed plastic pipes at +4 ±1 °C in the dark.

The distances from the collar were chosen to check the composition of the ECM community in different sections of the rhizosphere, including both the part beneath the canopy projection (until 200-250 cm from the collar) and the one outside this. In accordance with previous investigations by the authors in the same sites, the sampling depth was chosen to include in every core the part of the root system in which root tips are denser (0-30 cm).

2.2.2 Laboratory observations and data analyses

Rootlets in every core were carefully cleaned in tap water and, among those with Ø<2 mm and the apical tip undamaged and fully-developed, 10 apexes were randomly

chosen and classified as “non vital” (NV, scurfy surface and easily detachable cortex, with or without remnants of ECM mantle), “vital non-mycorrhizal” (NM, well-developed, turgid and inflated tip, mantle lacking), or “vital ectomycorrhizal” (EM, as above, but with ECM mantle), according to MONTECCHIO et al. (2004).

The relative abundance of NV, NM and EM was calculated in each sample (NVa=NV/10, NMa=NM/10; EMa=EM/10).

By means of both dissecting and compound microscopes connected to digital cameras, 10 vital ectomycorrhizae in each sample were separated into anatomotypes and coded, recording colour, type of ramification and features of mantle surface, type of outer, middle and inner mantle, and chemical reactions. These analyses were completed within 12 days after sampling.

Type of emanating hyphae, rhizomorphs, cystidia, laticifers, and chemical reactions were observed later, after preserving in FEA solution (formalin: ethanol 70% : acetic acid = 5 : 90 : 5) according to AGERER (1991).

Ectomycorrhizal anatomotypes were classified through the available literature (GOODMAN et al. 1996; AGERER 1987-2002; CAIRNEY & CHAMBERS 1999; AGERER & RAMBOLD 2004-2005; HAUG et al. 1992). All specimens were preserved in FEA solution and stored in the herbarium of the TeSAF Dept., University of Padova.

The relative abundance of each ECM species in each sample was calculated both related to 10 tips independently of their mycorrhization (R.a. of the species x=n of tips of species x/10 tips) and to 10 ectomycorrhizal tips (R.A. of the species x=n of tips of species x/10 ectomycorrhizal tips).

The Kruskal-Wallis non-parametric test ($P<0.05$, Statistica, StatSoft Inc., Tulsa, OK, USA) was used to verify statistical differences in NVa, NMa, EMa both among samples from the same tree and among trees from the same site, and to verify possible differences between sites. In this last case, the averages of the relative abundances were calculated of NV, NM, EM in each site (i.e. NVA= [(∑ NVa1 + NVa2 +… + NVa96)/ 96]), where 96 is the number of all the samples in one site.

All the significant differences found in at least one pair of samples or in one pair of sites by the Kruskal-Wallis test ($P<0.05$), were then identified through the Mann-Whitney U-Test ($P<0.05$; Statistica, StatSoft Inc., Tulsa, OK, USA).

As ECM present patchiness at distances of between 0 and 17 m (LILLESKOV et al. 2004), and as the autocorrelation among sampling points could influence community structure, the Mantel Test was performed to test the null hypothesis of no relationships among samples (10 ECM tips in a soil core) from the same tree (MC-CUNE and GRACE 2002). The Sørensen similarity index was used to create the similarity matrix: *2a/(2a+b+c)*, where *a*= number of shared species, *b*= number of species unique to plot 1 and *c*= number of species unique to plot 2 (IZZO et al. 2005). The Mantel Test (P<0.01, number of permutations=10000) compared species dissimilarity matrix and linear distance matrix between sampling points belonging to the same plant, using the XLSTAT-Pro Program (http://www.xlstat.com). If the Mantel Test couldn't exclude a spatial correlation in a tree, it was excluded from the subsequent analyses.

Relations among environmental variables (acid, basic and intermediate bedrock pH; North and South site exposures; N, E, S, W cardinal directions and distance of sampling points from the trees) and species abundance of ectomycorrhizae were analysed by means of Multivariate Ordination Techniques (JONGMAN et al., 1995) using CANOCO (software for Canonical Community Ordination, 4.5 Version).

Detrended Correspondence Analysis (DCA; HILL and GAUCH 1980) was performed to obtain estimates of gradient lengths in standard deviation units. The detrending by segments method was applied with data not subjected to any transformations and, according to TER BRAAK and ŠMILAUER (2002), unimodal (DCA and CCA) analyses were performed. Canonical Correspondence Analysis (CCA) was then done, scaling with a focus on inter-species distances and using a bi-plot scaling type, according to TER BRAAK and ŠMILAUER (2002).

The environmental variables, listed in decreasing order of the variance they explain singly [lambda-1 (λ_1)] and considered in addition to the variance explained by the covariables, when present, but ignoring the other environmental variables (marginal effect), were studied by means Forward selection of Environmental variables.

This modality also investigates the conditional effects, with "lambda-A" (λ_A) values as both the additional variance the variable explains (given the variables already included), and the increase in the sum of all the canonical eigenvalues of the ordination (when the variable is added to the environmental variables already included). *P*-values

indicate the significance level from the Monte Carlo permutation test (P<0.05), according to TER BRAAK and ŠMILAUER (2002).

To better understand the correlations between environmental variables and ECM species, a Redundancy Analysis (RDA) was performed, with species scores divided by standard deviation and focus scaling on inter-species correlations, according TO TER BRAAK and ŠMILAUER (2002).

2.3 Results

2.3.1 Stand selection and sample collection

The most widespread spruce forests in the Province of Trento are located at 1620-1870 m a.s.l., with mean annual rainfall of 1060 mm, mean annual temperature +4 °C (SBOARINA and CESCATTI 2004) and 30-40% slope. Spruce is the dominant species, with a frequency higher than 85% (in mass), mixed mainly with *Abies alba* Mill., *Larix decidua* Mill., *Pinus cembra* L., and *Fagus sylvatica* L., managed as hollow cutting, with a growing stock of 295 m^3 /ha and a current annual increment of 4.8 m^3 /ha. The forests were classified (DEL FAVERO 2004) as high mountain Norway spruce stands on siliceous substrate of xeric and mesic soils (A and B sites, respectively) and on carbonate substrate (C sites). The main features of the 10 plots selected in 2003 and 2004 are reported in Table 1.

Site	**N coord** (Gauss-B., Km)	**E coord** (Gauss-B., Km)	**Forestry District**	**Exp.**	**Soil process**
AN1	5.127.901	1.638.049	Malé	N	Podzolization
AN2	5.132.109	1.659.234	Cles	N	Podzolization
AS1	5.122.408	1.640.742	Tione	S	Podzolization
AS2	5.114.603	1.679.950	Pergine	S	Podzolization
BN1	5.130.772	1.711.970	Cavalese	N	Podzolization
BS1	5.132.856	1.710.898	Fiera Primiero	S	Podzolization
CN1	5.146.540	1.704.852	Cavalese	N	Brunification
CN2	5.132.109	1.648.424	Malé	N	Brunification
CS1	5.151.385	1.666.915	Cles	S	Brunification
CS2	5.093.707	1.683.540	Pergine	S	Brunification

Table 1. Main features of the 10 plots selected in 2003 and 2004 (PAT 1999).

2.3.2 Laboratory observations and data analyses

Analyses of samples collected in 2003 and 2004 demonstrated that NVa, NMa and EMa, both among samples collected beneath the same tree (different directions and distances from the collar), and among the 4 trees of the same site, never differ significantly (Kruskal-Wallis Test, $P<0.05$), whereas the NVA, NMA and EMA value distributions (Table 2) are significantly different among sites (Kruskal-Wallis Test, $P<0.05$).

	2003 2004				2004		
	NVA	**NMA**	**EMA**		**NVA**	**NMA**	**EMA**
site				**site**			
AN1	0.044 0.062	0.075 0.021	0.881 0.917	**AN2**	0.125	0.015	0.860
AS1	0.079 0.123	0.004 0.044	0.917 0.834	**AS2**	0.103	0.016	0.881
BN1	0.035 0.112	0.035 0.041	0.929 0.847	**-**			
BS1	0.042 0.149	0.010 0.043	0.947 0.808	**-**			
CN1	0.060 0.083	0.073 0.036	0.885 0.880	**CN2**	0.148	0.015	0.837
CS1	0.050 0.112	0.065 0.066	0.948 0.822	**CS2**	0.125	0.040	0.835

Table 2. Mean values of not vital tips (NVA), vital but not mycorrhizal tips (NMA), ectomycorrhizal tips (EMA), in different sites and years.

This result is better explained by the Mann-Whitney U-Test ($P<0.05$), done on all the possible pairs of sites, in each sampling period, which detected the significant differences shown in Tables 3 and 4.

	NVA 2003 2004					**NMA 2003 2004**					**EMA 2003 2004**				
	AS1	**BN1**	**BS1**	**CN1**	**CS1**	**AS1**	**BN1**	**BS1**	**CN1**	**CS1**	**AS1**	**BN1**	**BS1**	**CN1**	**CS1**
AN1	0.019 0.000	n.s. 0.001	n.s. 0.000	n.s. n.s.	n.s. 0.000	0.000 0.032	0.007 n.s.	0.000 n.s.	n.s. n.s.	n.s. 0.000	n.s. 0.000	0.022 n.s.	0.002 n.s.	n.s. 0.001	n.s. 0.000
AS1		0.004 n.s.	0.009 0.016	n.s. 0.001	0.018 n.s.		0.038 n.s.	n.s. n.s.	0.000 n.s.	0.009 0.036		n.s.	0.033 0.016	0.019	n.s. n.s.
BN1			n.s. 0.003	n.s. n.s.	n.s. 0.000			n.s. n.s.	0.013 n.s.	n.s. 0.006			n.s. n.s.	0.001 0.039	n.s. n.s.
BS1				n.s. n.s.	n.s. 0.002				0.000 n.s.	0.037 0.019				0.000 n.s.	0.049 n.s.
CN1					n.s. 0.013					n.s. 0.012					n.s. 0.013

Table 3. *P*-levels, Mann-Whitney U-Test ($P<0.05$) on pairs of AN1, AS1, BN1, BS1, CN1, CS1 sites, in 2003 and 2004.

	NVA 2004			NMA 2004			EMA 2004		
	AS2	**CN2**	**CS2**	**AS2**	**CN2**	**CS2**	**AS2**	**CN2**	**CS2**
AN2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
AS2		0.001	0.050		n.s.	0.003		0.003	0.003
CN2			n.s.			n.s.			n.s.

Table 4. *P*-levels, Mann-Whitney U-Test ($P<0.05$) on pairs of AN2, AS2, CN2, CS2 sites, in 2004.

Characterization of the ECM tips during both years of investigation showed the presence of 27 ECM types (16 with R.a.>1%) in AN1, AS1, BN1, BS1, CN1 and CS1 sites, and 28 (17 with R.a.>1%) in AN2, AS1, CN2 and CS2 sites. Among these, 24 were ascribed to a fungal species [*Albatrellus ovinus* (Schaeff.) Kotl. and Pouzar, *Amanita muscaria* (L.) Pers., *Amphinema byssoides* (Pers.) J. Erikss., *Boletus edulis* Bull., *Cenococcum geophilum* Fr., *Chroogomphus helveticus* (Singer) M.M. Moser, *Cortinarius obtusus* (Fr.) Fr., *C. odorifer* Britzelm., *Elaphomyces granulatus* Fr., *Hebeloma velutipes* Bruchet, *Hydnum rufescens* Pers., *Hygrophorus olivaceoalbus* (Fr.) Fr., *Inocybe appendiculata* Kühner, *Lactarius badiosanguineus* Kühner and Romagn., *L. deterrimus* Gröger, *L. scrobiculatus* (Scop.) Fr., *Piloderma croceum* J. Erikss. and Hjortstam, *Russula acrifolia* Romagn., *R. densifolia* Secr. ex Gill., *R. ochroleuca* (Pers.) Fr., *R. xerampelina* (Schaeff.) Fr., *Sarcodon imbricatus* (L.) P. Karst., *Tricholoma sulphureum* (Bull.) P. Kumm., *Tuber puberulum* Berk. and Broome] and 4 to a non-identified ECM described in detail on spruce [*Piceirhiza nigra* (BERG and GRONBACH 1988), *P. oleiferans* (WALLER et al. 1993), *P. spinifera* (WEISS 1988) and *P. stagonopleres* (BEENKEN and AGERER 1996)]. The list of species with their R.a. in the 10 sites and for both years is reported in Table 5.

The distribution of the ECM consortium in all sites revealed a classic exponential trend, according to TAYLOR (2002), with a gradual variation in relative abundance changing from a few very frequent ECM species to others, poorly represented.

ECM	2003 2004						2004			
	AN1	**AS1**	**BN1**	**BS1**	**CN1**	**CS1**	**AN2**	**AS2**	**CN2**	**CS2**
A. ovinus	- 0.007	- 0.019	- 0.033	0.044 0.109	0.131 -	0.015 -	0.014	0.020	-	0.028
A. muscaria	0.045 0.027	- 0.052	0.030 -	- -	0.050 0.097	- -	0.012	-	0.107	-
A. byssoides	- -	- 0.097	0.060 0.108	0.112 0.004	0.002 0.054	0.130 -	-	-	0.085	0.140
B. edulis	- 0.002	- 0.009	- 0.022	- 0.001	- -	- 0.006	0.002	0.012	0.014	0.008
C. geophilum	0.223 0.230	0.238 0.428	0.298 0.470	0.418 0.37	0.472 0.414	0.421 0.246	0.372	0.390	0.288	0.255
C. obtusus	0.060 0.021	- -	- -	- -	- -	0.099 -	0.024	0.007	0.003	0.004
C. odorifer	- -	- -	- 0.016	0.108 -	0.002 -	0.080 0.013	-	-	-	0.029
C. helveticus	- -	- -	- -	- -	- -	- -	-	0.037	-	0.005
E. granulatus	- 0.016	0.087 0.014	0.029 0.019	0.025 0.063	- 0.078	0.016 0.098	0.043	0.010	0.024	0.028
H. velutipes	0.013 0.100	- 0.125	0.044 -	- 0.035	0.014 0.078	-	0.088	-	0.029	-
H. rufescens	- -	- -	0.092 -	0.062 0.033	- 0.028	- -	-	-	-	0.030
H. olivaceoalbus	0.021 0.054	0.004	0.009 -	- 0.004	- 0.017	- -	0.047	-	0.012	-
I. appendiculata	- 0.006	- 0.035	- 0.074	- 0.026	- 0.022	- 0.007	0.003	0.006	0.017	0.032
L. badiosanguineus	0.346 0.171	0.071 0.037	0.009 0.013	0.024 0.063	0.066 0.033	0.040 0.071	0.033	0.010	0.027	0.012
L. deterrimus	0.012 0.010	- -	0.012 -	- 0.022	- 0.010	- -	0.119	-	0.008	-
L. scrobiculatus	- -	- 0.014	- -	- -	- -	- 0.002	-	0.012	0.001	-
P. oleiferans	- -	0.006 0.013	- 0.005	- 0.007	0.008 -	0.030 -	0.009	0.110	-	0.037
P. nigra	- -	- 0.068	0.081 -	0.002 0.081	0.141 0.048	0.044 -	-	-	0.244	0.122
P. spinifera	- -	- 0.014	- -	- -	- -	- -	-	-	-	0.012
P. stagonopleres	- -	- -	- 0.080	0.046 0.044	- -	0.073 -	-	-	-	0.038
P. croceum	0.266 0.239	0.129 -	- -	- -	- -	- 0.085	0.107	0.180	-	-
R. acrifolia	- 0.004	0.004 -	- -	0.046 0.007	- -	0.004 0.134	0.035	0.035	-	-
R. densifolia	- -	- 0.015	- -	- -	- -	- 0.006	-	-	0.005	-
R. ochroleuca	0.015 0.023	0.451 0.066	0.339 0.014	0.113 0.101	0.099 0.120	0.048 0.310	0.078	0.079	0.096	0.196
R. xerampelina	- 0.008	- -	- -	- -	- -	- 0.005	0.008	0.013	-	-
S. imbricatus	- 0.011	- -	- 0.005	- -	- -	- 0.001	0.003	0.079	0.015	0.018
T. sulphureum	- -	- -	- 0.125	- -	- -	- -	-	-	-	0.006
T. puberulum	- -	0.013 0.005	- -	- 0.025	0.014 -	- 0.014	-	-	0.022	-

Table 5. Relative abundances (R.a.) of ECM in different sites and years.

The Mantel Test demonstrated a spatial correlation in one tree in BS1 (2003), BN1, CN1 and CS1 (2004), and in two trees in AS1. These spruces were excluded from the following statistical analyses.

DCA showed gradient lengths between 3 and 4 and demonstrated that the eigenvalues of axis 1 (horizontally) and 2 (vertically) are 0.490 and 0.378, respectively. Fig. 1 reports the scatter plot of the ECM species and the sampling points belonging to the 6 types of sites. It displays 13.5% of the inertia (=weighted variance) in species abundances, and 60.3% of the variance in both the weighted average and class totals of species with respect to the environmental variables. Environmental correlation is 0.796 for axis 1 and 0.496 for axis 2. The species reported on the external portion of the diagram (i.e.: *C. obtusus*, *C. odorifer*, *P. nigra*, *A. byssoides*, *A. muscaria*, *L. deterrimus*, *R. acrifolia*, *T. sulphureum*) are the most rare while, approaching the centre of the diagram, the richness of the ubiquitous species (unrelated to the ordination axes, bimodal or in some other way not fitting a unimodal response model) increase (TER BRAAK and PRENTICE 1988). The diagram also shows that the sites are arranged with an acid to basic bedrock pH gradient (left to right), and South to North exposure (above-below). The inter-set correlations of axis 1 with acid (A) and subacid (B) bedrock pH are -0.78 and 0.60 respectively, of axis 2 with North (N) and South (S) exposure they are -0.46 and 0.46, respectively.

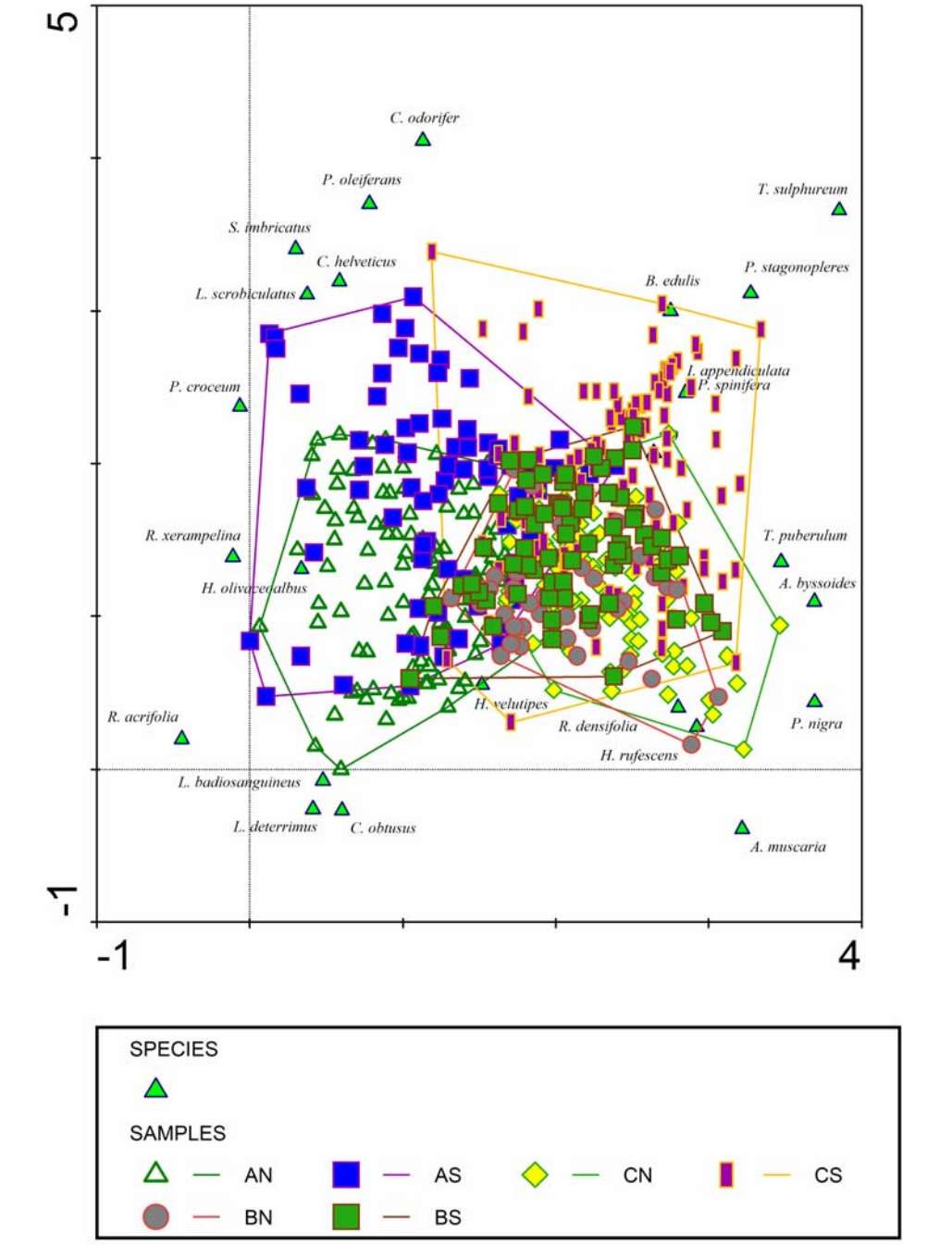

Figure 1. Scatter diagram of species and sites from Detrended Correspondence Analysis displaying the positions of ECM species and sampling points belonging to different types of site (AN, AS, CN, CS, BN, BS), in the plotted ordination plane.

In CCA, the eigenvalues of axis 1 and axis 2 are 0.325 and 0.199, respectively; the eigenvalue of axis 3 (not shown) is 0.085. Fig. 2 reports the bi-plot of species and environmental variables, displaying 8.2% of the inertia (=weighted variance) in

abundances, and 80.8% of the variance in both the weighted averages, and class totals of species with respect to the environmental variables.

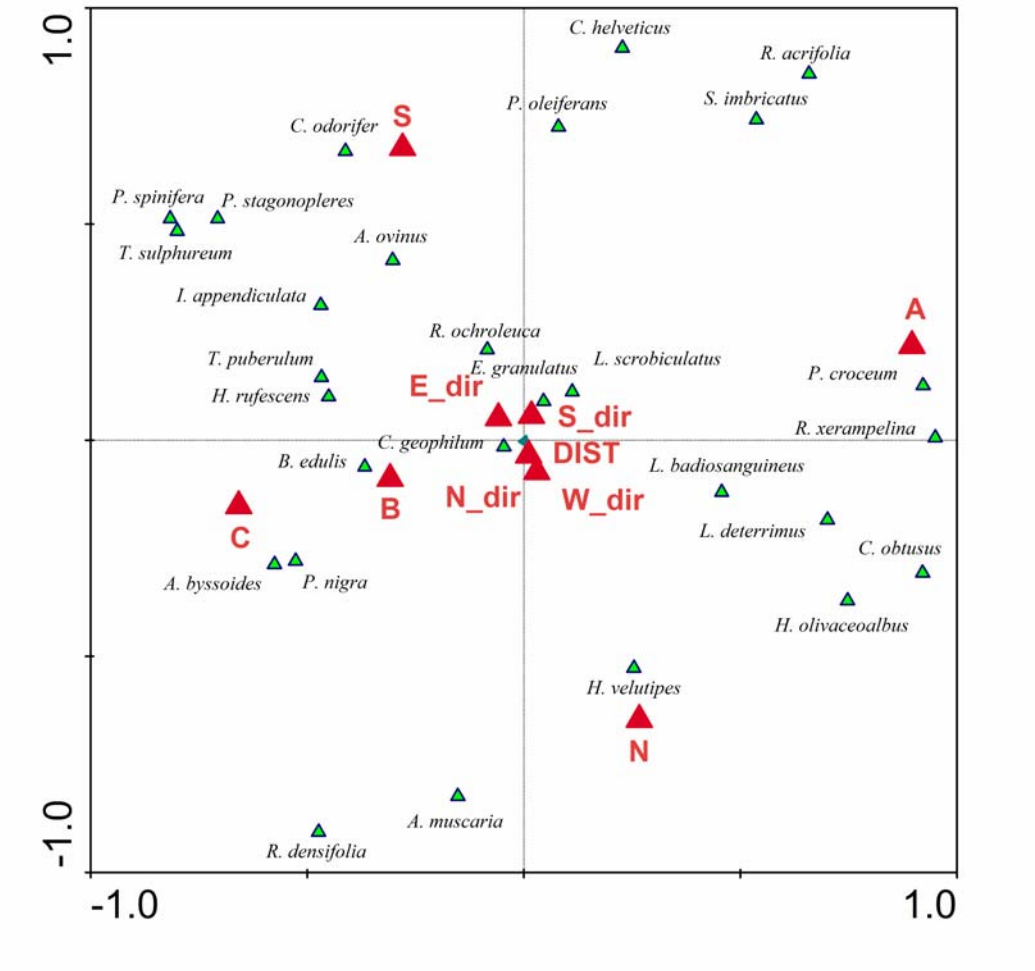

Figure 2. CCA diagram of ECM species and environmental factors: North (N) and South (S) site exposure; acid (A), subacid (B) and basic (C) bedrock pH; N, E, S and W sampling direction (_dir), and soil core distance (DIST) from each tree sampled in 2003 and 2004.

The first gradient, with eigenvalues >0.30, indicates strong gradients (TER BRAAK and VERDONSCHOT 1995), and a high significance of the first axis and all the canonical axes is present when subjected to the Monte Carlo permutations test.

As shown in the bi-plot, the first axis represents the bedrock pH variable, and the second the exposure variable. The ECM associated to acid bedrock pH are concentrated

in the right area, while the ones associated to subacid and basic bedrock pH are in the left. ECM associated to South exposure are in the upper part of the diagram, while those associated to North exposure are in the lower part. The distance between species points in the bi-plot scaling (with a focus on species distances) approximates the chi-square between the species distribution. The inter-set correlations of axis 1 with acid bedrock pH (A) is 0.79, with subacid (B) is -0.61, of axis 2 with North exposure (N) is -0.66, with South (S) is 0.66.

Comparing the first eigenvalue of both DCA and CCA analyses (0.49 and 0.32, respectively), and as in both DCA and CCA the species-environment correlations of the first axis result as higher, all the environmental variables together are able to explain the main variation of the ECM distribution incompletely (TER BRAAK 1986).

The marginal effects in CCA demonstrate that the variables better explaining the model are A and C (acid and basic bedrock), and N and S (North and South exposure), respectively λ_1= 0.31, 0.22, 0.22, 0.22. The conditional effects, showing the environmental variables in order of their inclusion in the model, demonstrate that the most useful features to explain the model are A (λ_A=0.31, *P*=0.002), N (λ_A=0.20, *P*=0.002), and C (λ_A=0.10, *P*=0.002).

Sampling directions N_dir and S_dir resulted as being less significant (respectively, λ_A=0.01, *P*=0.034 and λ_A=0.01, *P*=0.042).

ECM distribution studied by RDA demonstrates that the highest correlations are: *P. croceum* with bedrock pH acid (0.55), *H. velutipes* with N exposure (0.37) and N sampling direction (0.12), *A. byssoides* with basic pH (0.41), *H. rufescens* and *A. ovinus* with subacid pH (0.22 and 0.21, respectively), *C. odorifer* and *L. scrobiculatus* with S sampling direction (0.10), and *E. granulatus* with E sampling direction (0.10).

2.4 Discussion

To verify the possible involvement of environmental variables on tips vitality and the ectomycorrhizal community, 10 comparable plots were investigated, representative of the typical high mountain Norway spruce forests in the Trento Province (northern

Italy), but differing in bedrock pH and exposure (pH A=4.3±0.3; B=5.4±0.3; C=7.6±0.4; exposure N, S).

The ECM community showed low, not significant spatial autocorrelations among samples collected at different distances and directions from the plant, probably also related to high variability in the community composition at plant level.

The results mainly demonstrated that tips' vitality and ectomycorrhization degree strongly differ with the 6 pH/exposure combinations.

ECM species that mostly resulted as related to bedrock pH and exposure were: *P. croceum*, *R. xerampelina*, *R. acrifolia* and *S. imbricatus* (acid bedrock pH), *A. byssoides* and *P. nigra* (basic pH), *H. velutipes* (North exposure), *C. odorifer* and *P. oleiferans* (South exposure).

Unfortunately, although investigations on ECM consortia with respect to their ecological roles are of crucial importance, little is still known about the dynamics of ectomycorrhizal communities in forest ecosystems (LINDERMAN 1988; PERRIN and ESTIVALET 1989; HORTON and BRUNS 2001; MONTECCHIO et al. 2004; BRUNS 1995).

The results of the study anyway strengthen the hypothesis that marked changes in the ectomycorrhizal community depend on the selective pressure of some environmental features (AGERER 2001), suggesting that bedrock pH and exposure, interfering with wider environmental factors involving root system development and plant nutrition (i.e. plant growth, distribution and density of fine roots, moisture availability, litter evolution, type and availability of nutrients), play a crucial role on both tips' turn-over and ectomycorrhizal status, in accordance with well-known colonisation strategies (TAYLOR and ALEXANDER 1989; BERNIER and PONGE 1994; GRAYSTON and CAMPBELL 1996; LILLESKOW and BRUNS 2001; SHI et al. 2002).

pH/exposure patterns could therefore play a primary role in the adaptive selection of species or functional groups, both directly, acting on the tolerance of the fungal species (or genotypes), and indirectly, through dynamics involving plant nutrition, where nutrient availability and translocation could be essential (ALLEN 1991; DEACON and FLEMING 1992; DICKIE et al. 2002; TEDERSOO et al. 2003).

Indeed, soil fungi are known to be biogeochemical agents that can influence weathering through physical and chemical processes (BANFIELD et al. 1999; STERFLINGER 2000). Direct weathering and nutrient uptake by ECM fungi colonising mineral particles has

also been suggested as a possible pathway for element uptake by forest trees (LANDEWEERT et al. 2001).

Although the quantitative importance of fungal weathering in plant nutrition remains controversial (SVERDRUP et al. 2002), the rate and composition of the ECM community could change in terms of contributing to tree nutrition. According to COURTY et al. (2005), this aspect could engage a variety of mechanisms for mobilizing nutrients from the soil, involving enzymatic degradation of macromolecules, metal complexation and mineral weathering.

From a functional point of view, we may therefore suppose that the fungal communities adapted to different sites can, on the whole, mobilize nutrients essential to trees in a comparable way. For instance, assuming that similar amounts of nitrogen are available to the trees in the 10 investigated stands, it can be expected that when organic nitrogen sources in the soil profile are unsatisfactory, two different ECM consortia distinctive of acid and basic plots could play an active role in making N available from ammonium and nitric ions, respectively, using different strategies.

Understanding the ecological features determining this "adaptive diversity" in ECM communities is of major importance (DAHLBERG 2001), also for assessing ecosystem resilience within the context of global climate change.

Seasonal investigations are in progress on the enzymatic and functional features of the peculiar ectomycorrhizae reported above.

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Chapter 3.

The ectomycorrhizal community in the top soil of Norway spruce stands

- Paper in preparation by Scattolin L. and Montecchio L.
for *European Journal of Forest Research* -

3.1 Introduction

Composition of ectomycorrhizae community at the root system level merits special attention because of its functional significance for forest trees (PETER et al. 2001).
Differences in the biodiversity of ectomycorrhizal anatomotypes within sites have been attributed primarily to edaphic factors such as soil acidity and soil organic matter quality (FERRIS et al. 2000; KOIDE et al. 1998; VAN DER HEIJDEN et al. 1999).
In a forest ecosystem humus form, litter quality, mycorrhizal diversity and abundance as well as nutrient uptake by the mycorrhizal anatomotypes are closely linked together.
As many studied stated that the composition of a ectomycorrhizal community depends on edaphic factors such as soil pH humus and litter type (YANG et al., 1998; KOIDE et al., 1998; VAN DER HEIJDEN et al., 1999; CONN and DIGHTON 2000; FERRIS et al., 2000; RUMBERGER MD et al., 2004;), the aims of the study were (1) to assess the vitality and ectomycorrhization of root tips in organic and mineral humus layers and (2) to relate the ECM species distribution to both the main physical and chemical soil features, and the root system's distribution.

3.2 Materials and methods

3.2.1 Study sites and plots establishment

The research was performed in four high mountain Norway spruce [*Picea abies* (L.) Karst.] stands growing in the Province of Trento (northern Italy) randomly selected among the most representative spruce forests in the Province (soil processes, climatic and site features, sylvicultural treatments, productivity).

In order to distinguish these forests, in June 2005, the information available from the official Province of Trento (PAT) forest management database and the existing literature (PROVINCIA AUTONOMA DI TRENTO 2001; Sboarina and Cescatti 2004) were organized through ArcExplorer software (ESRI Institute Inc., ArcExplorer, 2.0.800-version, Redlands, USA).

The 4 selected forests [5.122.408 ÷ 5.151.385 N, 1.638.049 ÷ 1.704.852 E (Gauss-Boaga), located in the towns of Malé, Tione, Cavalese and Cles, respectively A, B, C, and D] were located at 1,620-1,870 m a.s.l., with mean annual rainfall of 1,060 mm, mean annual temperature +4 °C (Sboarina and Cescatti 2004), podzolization and brunification soil processes (ISSS et al. 1998) and 30-40% exposure. Spruce was the dominant species, with a frequency higher than 85% (in mass), mixed mainly with *Abies alba* Mill., *Larix decidua* Mill., *Pinus cembra* L., and *Fagus sylvatica* L., managed as hollow cutting, with a growing stock of 295 m^3 /ha and a current annual increment of 4.8 m^3 /ha (PAT 2001).

In each forest, one 100x100 m plot (A1, B1, C1, D1), with trees having a mean age of 155-175 yrs, were selected. In each plot, after a phytosanitary investigation 4 spruces healthy, undamaged by climatic events, with fully-developed crown, diameter (breast height, $d_{1.30}$) 75 ±5 cm and separated by at least 15 m from the nearest tree, were randomly selected and coded.

To avoid seasonal variabilities (SITTING 1999, BAIER et al. 2006), samples below described were taken in two times in the 2005 growing season (July and September), each time from two different spruces per site.

From each spruce, along the maximum slope direction (up, down) and along the one perpendicular to it (iso dx, iso sx), 8 soil samples including every humus horizon characterizing the humus forms present (10x20 cm, h 75 cm) were collected 150 and

300 cm from the collar (below and outside the canopy projection). Each of them was accurately separated in 4 subsamples (OF, OH, A and B humus horizons, according to Jabiol et. al 1995; Zanella et al. 2001) and every subsample was vertically divided in 2 portions (10x10 cm). Every portion was then stored in sealed plastic pipes at +4 ±1 °C in the dark.

3.2.2 Laboratory observations and data analyses

Rootlets in every horizon were carefully cleaned in tap water and, among those with Ø<2 mm and the apical tip undamaged and fully-developed, 10 apexes were randomly chosen and classified as "non vital" (NV, scurfy surface and easily detachable cortex, with or without remnants of ECM mantle), "vital non-mycorrhizal" (NM, well-developed, turgid and inflated tip, mantle lacking), or "vital ectomycorrhizal" (EM, as above, but with ECM mantle), according to MONTECCHIO et al. (2004).

The abundancy (percentage) of NV, NM and EM tips were calculated in each horizon [i.e. NV (OH)= Σ NV tips in all the OH horizons/ tot. n. of tips in all the OH horizons x 100].

To verify the effects of different humus horizons on vitality and ectomycorrhization of root tips and because the data distribution can be considered normally distributed, as the sample size (number of horizons), according to Statistica electronic textbook (http://www.statsoft.com/textbook/stathome.html) is significantly high, the ANOVA (Statistica, StatSoft Inc., Tulsa, OK, USA) with a Tukey HSD post hoc test for unequal N (SPJOTVOLL and STOLINE 1973) was performed to compare NV, NM and EM in the 4 horizons by means of different multiple comparison.

By means of both dissecting and compound microscopes connected to digital cameras, 10 vital ectomycorrhizae in each horizon were separated into anatomotypes and coded, recording colour, type of ramification and features of mantle surface, type of outer, middle and inner mantle, and chemical reactions. These analyses were completed within 12 days after sampling.

Type of emanating hyphae, rhizomorphs, cystidia, laticifers, and chemical reactions were observed later, after preserving in FEA solution (formalin: ethanol 70% : acetic acid = 5 : 90 : 5) according to AGERER (1991).

Ectomycorrhizal anatomotypes were classified through the available literature (GOODMAN et al. 1996; AGERER 1987-2002; CAIRNEY & CHAMBERS 1999; AGERER & RAMBOLD 2004-2005; HAUG et al. 1992). All specimens were preserved in FEA solution and stored in the herbarium of the TeSAF Dept., University of Padova.

The relative abundance of each ECM species in each horizon was calculated related to ectomycorrhizal tips (i.e. R.a. of the species x(OH)=n of tips of species x in OH/ ectomycorrhizal tips in OH).

Soil chemistry analyses on N tot., C/N, RH, pH-values (1 m KCl) were performed in the Soil laboratory of the Centre for Alpine Ecology (TN, Italy): according to *Met. Uff. n. XIII.3 Suppl. Ord. G.U. n. 248 del 21.10.1999*; *Met. Uff. n. VII.1 Suppl. Ord. G.U. n. 248 del 21.10.1999* ; *Met. Uff. n. II.2 Suppl. Ord. G.U. n. 248 del 21.10.1999*; *Met. Uff. n. II.1 Suppl. Ord. G.U. n. 248 del 21.10.1999*.

As ECM present patchiness at distances of between 0 and 17 m (LILLESKOV et al. 2004), and as the autocorrelation among sampling points could influence community structure, the Mantel Test was performed to test the null hypothesis of no relationships among samples (10 ECM tips in a soil core) from the same tree (MC-CUNE & GRACE 2002). The Sørensen similarity index was used to create the similarity matrix: $2a/(2a+b+c)$, where a= number of shared species, b= number of species unique to plot 1 and c= number of species unique to plot 2 (IZZO et al. 2005). The Mantel Test ($P<0.01$, number of permutations=10000) compared species dissimilarity matrix and linear distance matrix between sampling points belonging to the same plant, using the XLSTAT-Pro Program (http://www.xlstat.com). If the Mantel Test couldn't exclude a spatial correlation in a tree, it was excluded from the subsequent analyses.

Relations among environmental variables (humus forms; OF, OH, A and B humus horizons; Up- and downstream directions, Isohypsae directions, distance of sampling points from the trees; C/N; N tot.; pH; RH) and species abundance of ectomycorrhizae were analysed by Multivariate Ordination Techniques (JONGMAN et al. 1995) using CANOCO (software for Canonical Community Ordination, 4.5 Version).

Detrended Correspondence Analysis (DCA; HILL and GAUCH 1980) was performed to obtain estimates of gradient lengths in standard deviation units. The detrending by segments method was applied and, according to TER BRAAK & ŠMILAUER (2002), unimodal (DCA and CCA) analyses were performed. Canonical Correspondence

Analysis (CCA) was then done, scaling with a focus on inter-species distances and using a bi-plot scaling type, according to TER BRAAK & ŠMILAUER (2002).

The environmental variables, listed in decreasing order of the variance they explain singly [lambda-1 (λ_1)] and considered in addition to the variance explained by the covariables, when present, but ignoring the other environmental variables (marginal effect), were studied by means of Forward selection of Environmental variables. This modality also investigates the conditional effects, with "lambda-A" (λ_A) values as the additional variance the variable explains, given the variables already included. *P*-values indicate the significance level from the Monte Carlo permutation test ($P<0.05$), according to TER BRAAK & ŠMILAUER (2002).

3.3 Results

3.3.1 Laboratory observations and data analyses

From the 128 humus profiles sampled, among the 482 horizons identified, 126 were classified as OF, 103 as OH, 126 as A and 127 as B.

Significant differences (ANOVA, $P<0.05$) were found in NV tips (Fig. 1), identified between OF and A, OF and B (Tukey HSD post hoc test, $P<0.05$); in NM tips (Fig. 2), between OF - OH and every other horizon; in EM tips (Fig. 3) between OF and A, OF and B.

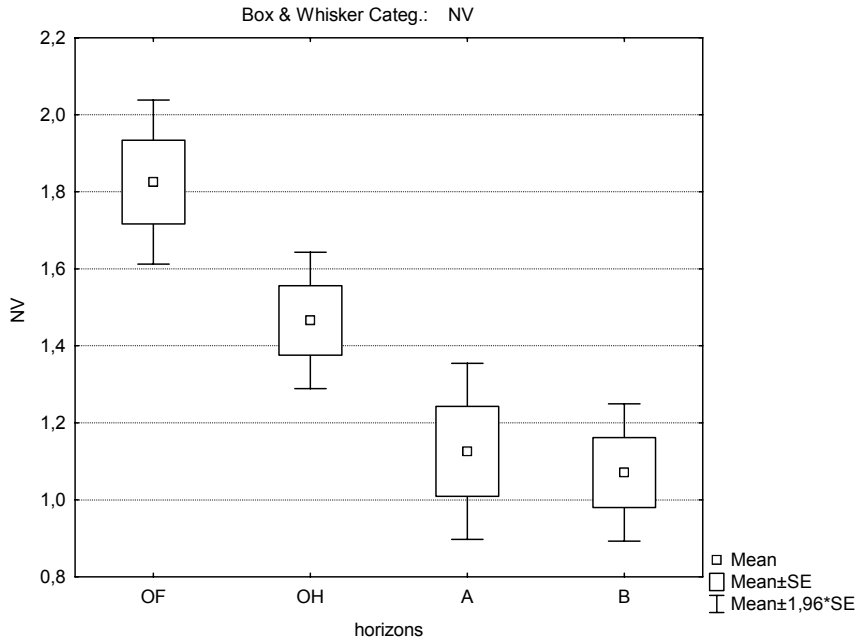

Figure 1. Box plot of non vital root tips (NV) in OF, OH, A and B humus horizons.

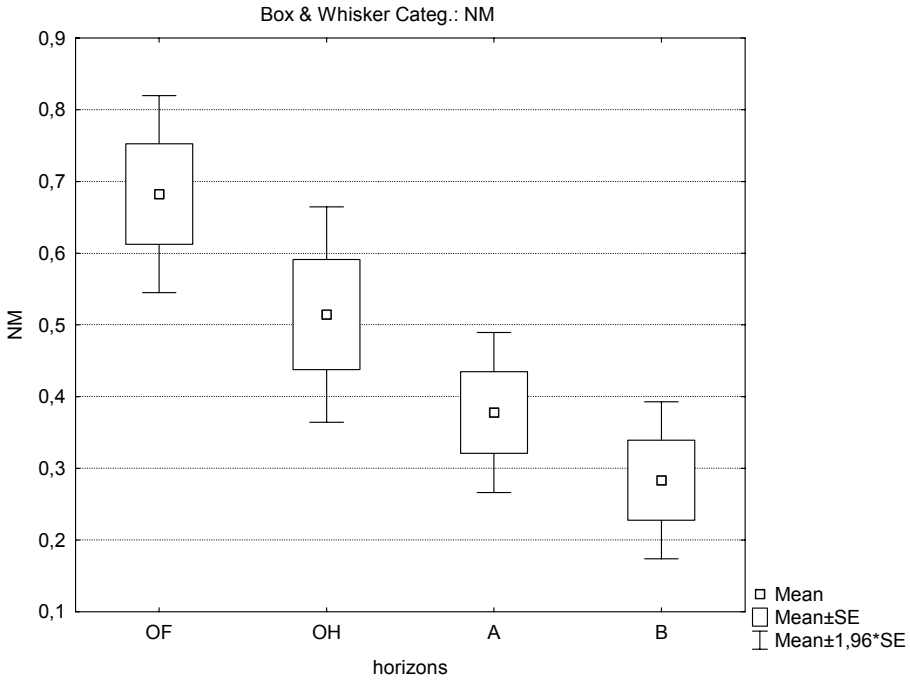

Figure 2. Box plot of non mycorrhizal root tips (NM) in OF, OH, A and B humus horizons.

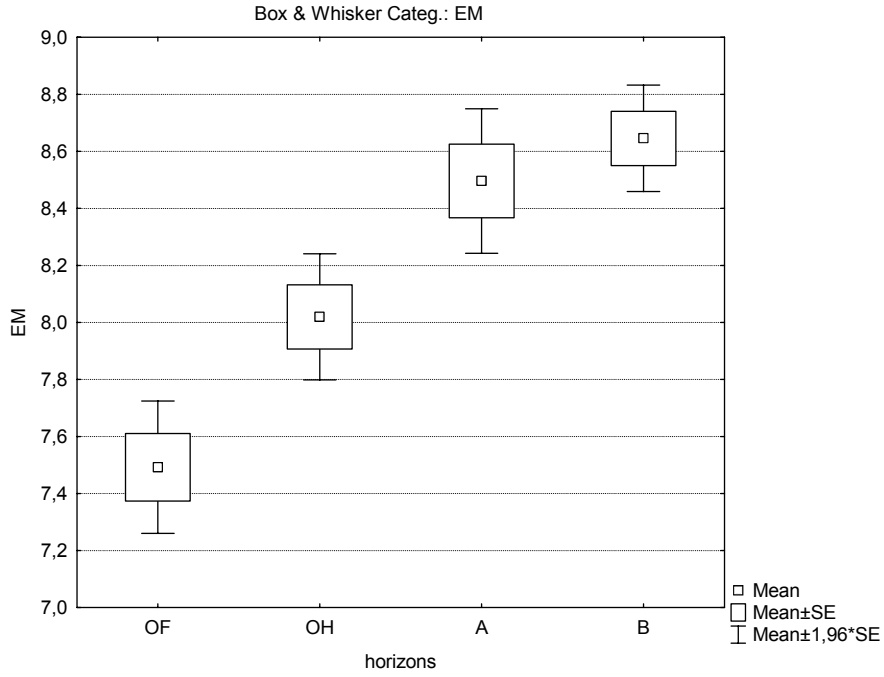

Figure 3. Box plot of non vital and ectomycorrhizal root tips (EV) in OF, OH, A and B humus horizons.

Characterization of the ECM tips showed the presence of 31 ECM types. Among these, 27 were ascribed to a fungal species [*Albatrellus ovinus* (Schaeff.) Kotl. and Pouzar, *Amanita muscaria* (L.) Pers., *Amphinema byssoides* (Pers.) J. Erikss., *Boletus edulis* Bull., *Cenococcum geophilum* Fr., *Chroogomphus helveticus* (Singer) M.M. Moser, *Cortinarius obtusus* (Fr.) Fr., *C. odorifer* Britzelm., *Elaphomyces granulatus* Fr., *Hebeloma velutipes* Bruchet, *Hydnum rufescens* Pers., *Hygrophorus olivaceoalbus* (Fr.) Fr., *Hygrophorus pustulatus* (Pers.) Fr., *Inocybe appendiculata* Kühner, *Lactarius badiosanguineus* Kühner and Romagn., *L. deterrimus* Gröger, *L. scrobiculatus* (Scop.) Fr., *Piloderma croceum* J. Erikss. and Hjortstam, *Pisolithus tinctorius* (Mich.: Pers.) Coker & Couch, *Ramaria largentii* Marr & D. E. Stuntz, *Russula acrifolia* Romagn., *R. densifolia* Secr. ex Gill., *Sarcodon imbricatus* (L.) P. Karst., *Tricholoma sulphureum* (Bull.) P. Kumm., *Tuber puberulum* Berk. and Broome] and 4 to a non-identified ECM described in detail on spruce [*Piceirhiza nigra* (BERG and GRONBACH 1988), *P. oleiferans* (WALLER et al. 1993), *P. spinifera* (WEISS 1988) and *P. stagonopleres* (BEENKEN and AGERER 1996)]. The list of species with their R.a. in the 4 humus horizons is reported in Fig. 4. The A horizon showed the highest number of ECM species (27), while the other are rather similar: OF and OH with 22 species, B with 21. *C. geophilum*, present in all the horizons, is clearly the dominant species in organic

layers, especially in OF (52.7%), with a progressive decreasing trend. *R. acrifolia*, present as well in all the horizons, progressively increasing its abundance from organic to mineral horizons, is the dominant species in B (23.2%).

The data considered in the following analyses belong to the 16 spruces selected, as the Mantel Test excluded any spatial correlation in each of them.

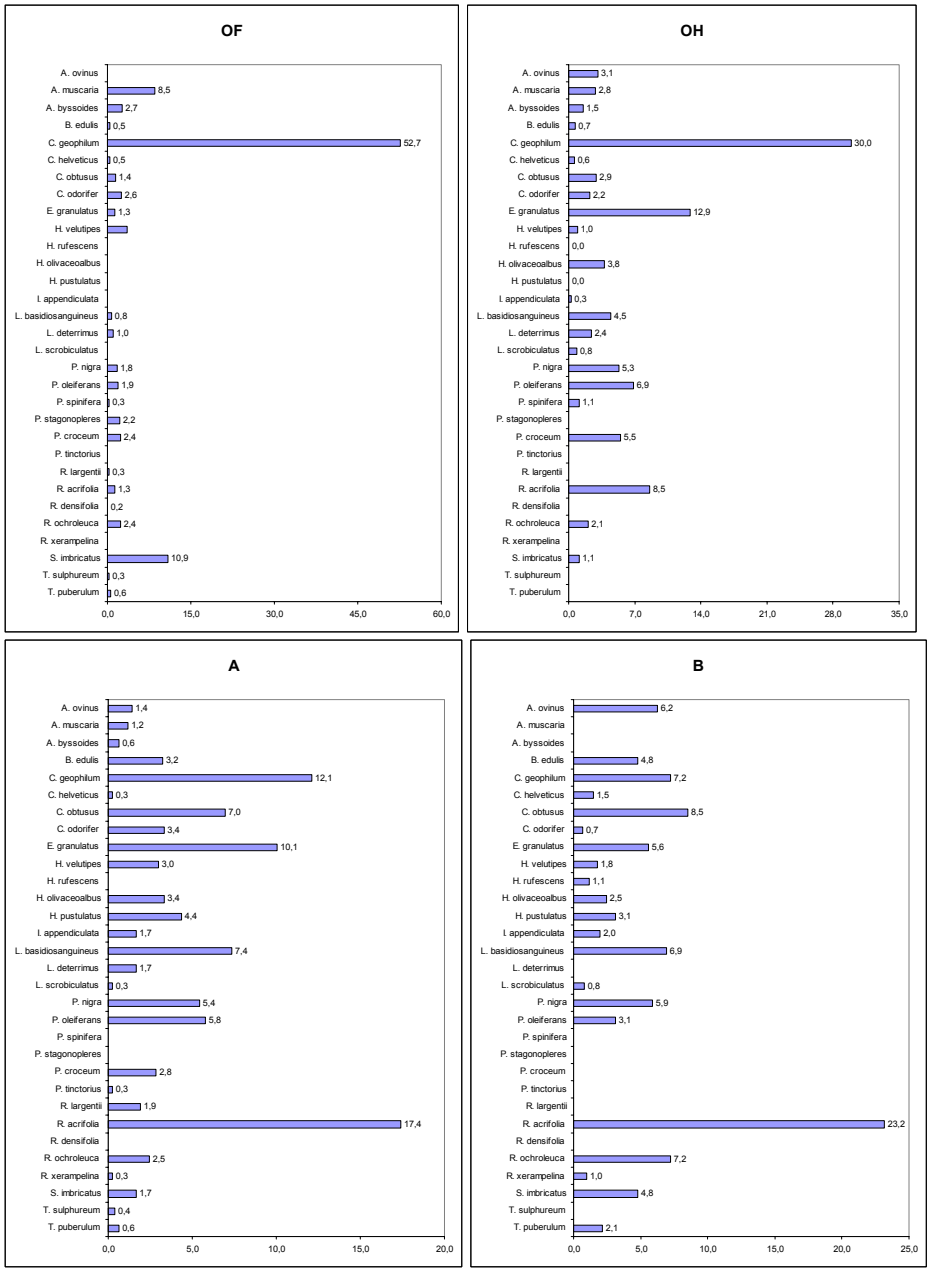

Figure 4. Abundances of the 31 ECM, expressed as the total number of ECM species root tips in the 4 soil horizons.

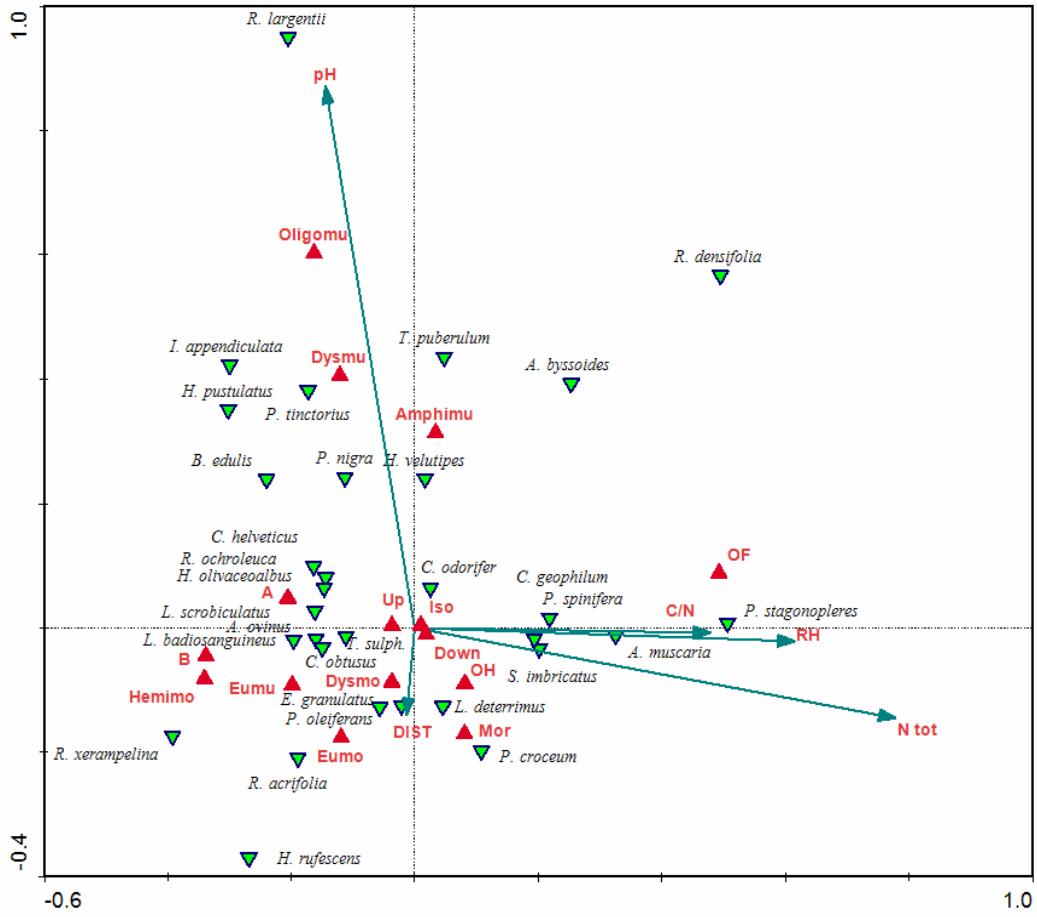

Figure 5. CCA bi-plot of the 31 ECM species and 20 environmental variables: 483 cases (horizons), 15 qualitative [Up-, Downstream (Up, Down) and Isohypsae (Iso) directions, Amphimull (Amphimu), Dysmoder (Dysmo), Dysmull (Dysmu), Eumoder (Eumo), Eumull (Eumu), Hemimoder (Hemimo), Mor, Oligomull (Oligomu) humus forms; OF, OH A, B horizons] and 5 quantitative [Distance (DIST), N tot., C/N, moisture (RH), pH] variables.

By means of the canonical coefficients among variables and axes, we inferred that the first axis is defined by N tot., RH (respectively, correlation coefficient=0.78 and 0.62) and by the humus horizons (in particular, OF is the most correlated with this axis, correlation coefficient=0.85). The second axis is defined by pH gradient (correlation coefficient=0.87). The intra-set correlations of axis 1 with OF, N tot., C/N, RH was

0.85, 0.78, 0.47, 0.61, respectively. The intra-set correlations of axis 2 with pH, Amphimull, Oligomull, Mor was 0.87, 0.45, 0.43, -0.51. ECM species mainly associated to organic horizons, high values of N tot, RH and C/N were in the right area of the diagram; those associated to basic pH, Oligomull and Dysmull were concentrated in the upper part of the diagram, while the ones associated to acid pH and Mor are in the lower part. The distance between species points in the bi-plot scaling (with a focus on species distances) approximated the chi-square distance between the species distributions. The ECM that resulted associated to OF and N tot. are: *Piceirhiza stagonopleres, Amanita muscaria, Cenococcum geophilum*, to acid pH: *Hydnum rufescens, Russula acrifolia, R. xerampelina*, to basic pH: *Ramaria largentii*, *Russula densifolia*, to Dysmull: *Pisolithus tinctorius, Hygrophorus pustulatus*, to Mor: *Lactarius deterrimus, Piceirhiza oleiferans*. *Piloderma croceum* was associated both to Mor and acid pH, *Tuber puberulum* both to basic pH and Dysmull, *Inocybe appendiculata* to basic pH and Dysmull. The marginal effects in CCA demonstrated that the variables better explaining the model are OF, N org. and pH (respectively λ_1= 0.28, 0.26, 0.24), the conditional effects, showing the environmental variables in order of their inclusion in the model, demonstrated that the most useful features to explain the model are OF (λ_A=0.28), pH (λ_A=0.25), N org (λ_A=0.14), Dysmull (λ_A=0.10), Mor (λ_A=0.11), Oligomull (λ_A=0.09), C/N (λ_A=0.09), OH (λ_A=0.09), Dysmoder (λ_A=0.07), Amphimull (λ_A=0.08), A (λ_A=0.07); (*P*=0.002).

Sampling distances and the up- , downstream and isohypsae directions of sampling weren't relevant in explaining the variability of the model.

Comparing the first eigenvalues of both DCA and CCA analyses (0.75 and 0.35, respectively), and considering that in CCA the species-environment correlations of the first axis resulted as high, apparently the measured environmental variables are not sufficient to explain the major variation among species distribution (TER BRAAK 1986).

3.4 Discussion

This study, having the main aim to relate the ECM species distribution to both the main physical and chemical soil features, and the root system's architecture/distribution/morphology, demonstrated that, in the 10 Spruce forests investigated, the frequency of both not vital tips and vital, not mycorrhized tips is decrease with deepness, while the ectomycorrhizal tips are mainly increase.

From a qualitative point of view, the ECM richness (number of species) is rather similar in all the horizons, except the A, probably because this horizon is very different in its composition due to different structures of organic and mineral aggregations, according to BRÊTES et. al 1992, JABIOL et. al 1995 and ZANELLA et. al 2001.

Analysing the ECM at species level, a significant difference arose taking into account the different humus layers and soil properties. Probably due to the high fluctuations in moisture and temperature, the upper organic horizons were dominated by *C. geophilum,* known to be able to tolerate water and thermic stresses, while *Russula* and *Lactarius* species demonstrated to be more present in mineral horizons, according to BRAND (1991) and AGERER (2006).

Considering the variables taken into account, (OF, pH, N tot, Dysmull, Mor, Oligomull, C/N and OH), the consortium *Hydnum rufescens*, *Piloderma croceum*, *Russula acrifolia* and *R. xerampelina* characterized acidic environments (pH=2.4-4.2), *Ramaria largentii*, *R. densifolia*, *Inocybe appendiculata* and *Tuber puberulum* basic pH (4.1-6.9); *Piceirhiza stagonopleres*, *Amanita muscaria* and *Cenococcum geophilum* were mainly present in the OF horizon and where the total N was higher; *Pisolithus tinctorius*, *T. puberulum*, *I. appendiculata*, *Hygrophorus pustulatus* characterized Dysmull, while *Piloderma croceum*, *Lactarius deterrimus* and *Piceirhiza oleiferans* were associated to Mor.

The composition of these consortia were maintained independently from the portion of the root system considered: it didn't changed from the part below the crown to the one outside it, nor along the slope line or along the isohypsa, demonstrating the resilience of the consortium to insolation and to litter thickness.

The research, performed relating all the above listed variables, including different humus forms, to single trees, demonstrated different preferences of ECM groups for the soil layers and their features, including moisture and available nitrogen.
An understanding of the functional role of the sinlge consortia and the ecological features determining this "adaptive diversity" in ectomycorrhizal communities is thus of major importance for assessing the resilience of ecosystems.

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Chapter 4.

Sampling methods to assess the ectomycorrhizal communities: still inaccurate tools to describe the underground complexity

- Paper in preparation

by Scattolin L, Montecchio L., Taylor A. for *Mycorrhiza* -

4.1 Introduction

The investigations of the ectomycorrhizal (ECM) community in recent years showed an increasing interest both on ecological and phytosanitary aspects (DAHLBERG 2001, ERLAND and TAYLOR 2002; AKEMA and FUTAI 2005).

In this respect, it is of particular importance to distinguish the different ectomycorrhizal species and to determine their distribution in a plant community.

In the early stages, these investigations were performed through sporocarp inventories and most of available information on the ecology of ectomycorrhizal fungi in forest ecosystems is mainly derived from those inventories (HAAS 1932-33; ROMELL 1938; COOKE 1953; VOGT et al. 1992). In this approach, however, only that part of the ectomycorrhizal community forming large, conspicuous sporocarps, can be easily monitored. The detection of other ectomycorrhizal species as the ones forming subterranean sporocarps and the asexual fungi, depending on the skillfulness of the investigator, may be underestimated.

Furthermore, sporocarp production depends on many factors, as for instance on environmental conditions (overall moisture and temperature; LANGE 1978, EVELING et al. 1990), that can considerably differ in different years. That is why it is necessary to monitor the same site in different years (VOGT et al. 1992). Although a positive correlation has been identified between the number of sporocarps and mycorrhized tips

per soil volume unit (JANSEN 1991), the relationship between sporocarp production and the degree of root system ectomycorrhization is still unknown.

The direct observation of root samples gave more accurate data, by means of the characterization of the ECM in a root system through detailed morphological and molecular approaches (INGLEBY et al. 1990, AGERER 1996, GOODMAN et al. 1996; CAIRNEY & CHAMBERS 1999; AGERER & RAMBOLD 2004-2005; HAUG et al. 1992). In this way, the ectomycorrhizal features can be classified and the anatomotype linked to the fungal species.

Furthermore, as researches in this field are still young and up to now little is known, only a small amount of mycobionts can be correctly classified morphologically, and often many of them remain unidentified and named temporarily with a special nomenclature (AGERER et al. 1996).

In recent years, molecular approaches improved the correct fungal symbiont classification (ERLAND 1995; GARDES & BRUNS 1996; BUÉE et al. 2005), and the number of determined ectomycorrhizae is therefore rapidly increasing.

In this way, through microscopical observation, virtually every mycorrhizal rootlet can be accurately studied, counting tips and distinguishing them between alive and dead, ectomycorrhizal and not. The number of ectomycorrhizal anatomotypes and their distribution can be also assessed, deriving interesting parameters such as tips vitality, probability to find vital ectomycorrhizae, and frequencies and distribution of ECM species, all of them useful in the most of the ecological studies involving ECM populations and their dynamics.

The availability of sampling methods easily applicable and statistically validated, are therefore of main importance. In fact, the accuracy characterizing these studies should lead to a detailed representation of the structure of an ECM community, despite its variations with the tree species, age, phenology and health status, and the local environmental features.

Hypothesising that the main limiting factors in sampling design are plant age and health, the soil volume explored by roots, the soil features, the sampling period, and the spatial distribution of the ECM species, the aim of this study was to verify the possibility to tune a sampling method able to describe with enough accuracy the ECM

community in a widely studied forest tree with a superficial, dense root system as Norway spruce [*Picea abies* (L.) Karst.] is, observing the smallest number of root tips. With this goal, the experiment was set up in an easily identifiable phenological phase on mature, healthy individuals growing in 2 different and common forest soils, and the sampling design was characterized by small samples and random tip observations in order to consider small scale soil heterogeneity (MAGURRAN 1988, DAHLBERG et al. 1997, HORTON & BRUNS 2001, BRUNS 1995, TAYLOR 2002; LILLESKOV et al. 2004, KOIDE et al. 2005, KOLJALG et al. 2005).

4.2 Materials and methods

4.2.1 Stand selection, sample collection and ECM identification

The research was performed in 4 coeval [165(±10) yrs-old] Norway spruce (*Picea abies* [L.] Karst.) stands randomly selected among the most representative (Provincia Autonoma di Trento 2001) spruce forests located in the Province of Trento (northern Italy). Two stands were on podzols and two on cambisols.

In each stand, a 100m x 100m plot was randomly selected, and in each of these plots 4 healthy spruce trees were identified. These trees were chosen as they were undamaged by climatic events, with fully developed crown, with a dbh of 75 ±5 cm and separated by at least 15 m from the nearest tree,. From each of the 4 trees and for each main cardinal direction (N, S, E, W), 2 cylindrical soil cores (Ø 18 mm, h 35 cm) were collected in June 2005. Soil cores were collected at 100 and 350 cm from the collar: these positions were determined from a previous analysis (SCATTOLIN, unpublished), which demonstrated that only the samples collected between 100 and 350 cm from the collar of each plant (in a sampling design with a sampling points every 50 cm and with a maximum distance of 600 cm from the collar of each plant) were characterized by significant differences from each other in ECM species composition (Chi-squared tests; $P<0.05$).

The apical tip of each selected fragment was classified as “non vital” (NV, scurfy surface and easily detachable cortex, with or without remnants of ECM mantle), “vital

non-mycorrhizal" (NM, well developed, turgid and inflated tip, mantle lacking), or "vital ectomycorrhizal" (EM, as above, but with ECM mantle), according to MONTECCHIO et al. (2004).

All vital ectomycorrhizae were separated into anatomotypes and classified using the available literature (GOODMAN et al. 1996, AGERER 1987-2002, CAIRNEY & CHAMBERS 1999, AGERER & RAMBOLD 2004-2005, HAUG et al. 1992). All specimens were preserved in FEA solution and stored in the herbarium of the TeSAF Dept., University of Padova.

Further details on plot description, sample collection and ECM identification are reported in chapter 2.

4.2.2 Statistical analyses

4.2.2.1 Theoretical distribution of ECM populations and theoretical sampling size

The relative abundances of each ECM species were calculated as the number of vital tips ectomycorrhized by each species on the total number of vital and ectomycorrhizal tips.

According to TAYLOR (2002), their theoretical detection limits were applied calculating the probability (p) to find a known species in a sample of a given size, the probability of not finding a species (A) on one alive and ectomycorrhizal root tip (p=1–x), the probability of not finding the same species (A) on a given number (y) of alive and ectomycorrhizal root tips [$p=(1-x)^y$], the probability of finding a species (A) on a given number of alive and ectomycorrhizal root tips [$p=1-(1-x)^y$], where x is the proportion of the species A in the community.

The relative abundance (%) data for each species were then ranked; x, y were then calculated as the number of ECM tips that needed to sampled in order to obtain the observed the ECM population in each site (P=0.95).

Diminution of the number of tips observed in each sampling point

As first step, the hypothesis of reducing the number of samples observed (96/site) by means of repeated Chi-squared tests (P<0.05) was considered, verifying if there were

significant differences between relative abundances of each ECM species present in couples of: a) site; b) tree; c) sampling directions; d) distances from the collar. As, excluding very few cases, all these tests were highly significant and, moreover, as the few not significant weren't systematically present in all the site, the number of sampling points wasn't decreased, while the number of tips studied in each sampling points was reduced randomly excluding an ECM tip from each sample, to verify if there were differences in the species composition (richness and relative abundance) of the population of each site.

The diminution of the ectomycorrhizal tips observed was calculated according to Chi-Square tests [$(O-E)^2/E$ index], where O=observed frequencies, and E=expected frequencies], ($P<0.05$), between each ECM relative abundance in both a plan with 10 measures (= number of tips observed in each sampling point) and in a plan with decreasing measures, randomly eliminating only ectomycorrhizal tips, to have a more shrunk and significant result regarding the description of our ECM population.

The decrease of ECM tips had been progressively repeated in each sample until the test ($P<0.05$) resulted significant, limiting the least number of tips that should be observed in that sampling point. Subsequently, the relative abundances of each ECM species in each site by that new sample size were calculated.

Moreover, as it's known that Chi-square test value, in correspondence of degree of freedom (df)=n-1 (n=number of ECM species) and $p=0.05$ is $F_{(\infty;95)}$, and that Chi-square test is significant ($p<0.05$) if its value $F_0<F_{(\infty;95)}$, ECM species giving a high contribute to the significance of every Chi-square tests were empirically detected dividing Chi-square value (F_0) for n, and obtaining a reference value (k); in correspondence of $(O-E)^2/E$ values higher than k, the ECM giving a high contribute to the Chi-square test significance were detected.

4.2.2.3 MDI index

To interpret the type of distribution of the 4 ECM populations, the data belonging to the ECM relative abundances in the 4 areas were subjected to Morisita's Index of Dispersion (MID), (MORISITA 1959; SOKAL & ROHLF 1981).

This test was chosen because it has the advantage to be enough independent by the number of samples, density of the population studied, and sampling size (KREBS 1989).

4.3 Results

4.3.1 Identification of ECM

Characterization of the ECM tips showed the presence of 15 ECM types in site AS, 12 ECM in BN, 16 in CN and 12 in CS.

Among these, 25 anatomotypes were ascribed to a species [*Cenococcum geophilum* Fr., *Piloderma croceum* J. Erikss. and Hjortstam, *Lactarius badiosanguineus* Kühner and Romagn., *Amphinema byssoides* (Pers.) J. Erikss., *Hebeloma velutipes* Bruchet, *Cortinarius obtusus* (Fr.) Fr., *Russula ochroleuca* (Pers.) Fr., *Albatrellus ovinus* (Schaeff.) Kotl. and Pouzar, *Amanita muscaria* (L.) Pers., *Hygrophorus olivaceoalbus* (Fr.) Fr., *Russula acrifolia* Romagn., *Tuber puberulum* Berk. and Broome, *Elaphomyces granulatus* Fr., *Sarcodon imbricatus* (L.) P. Karst., *Inocybe appendiculata* Kühner, *Boletus edulis* Bull., *Russula xerampelina* (Schaeff.) Fr., *Lactarius scrobiculatus* (Scop.) Fr., *Russula densifolia* Secr. ex Gill., *Chroogomphus helveticus* (Singer) M.M. Moser], *Russula ochroleuca* (Pers.) Fr., *Lactarius deterrimus* Gröger, *Hydnum rufescens* Pers., *Cortinarius odorifer* Britzelm., *Tricholoma sulphureum* (Bull.) P. Kumm., and 4 were classified as *Piceirhiza nigra* (BERG and GRONBACH 1988), *P. spinifera* (WEISS 1988), *P. stagonopleres* (BEENKEN and AGERER 1996) and *P. oleiferans* (WALLER et al. 1993).

4.3.2 Theoretical distribution and sampling size of ECM populations

A rank list of the ECM species founded in AS, BN, CN and CS sites are reported in Tab 1-4. The theoretical detection of each ECM species in each site, at 4 different sample size (10, 20, 50 and 100 tips), is reported in Fig. 1-4, with horizontal lines representing the 95% of the theoretical detection (TAYLOR 2002).

ECM	Relative Abundance (%)	rank order
C. geophilum	39.038	1
P. croceum	18.034	2
P. oleiferans	10.938	3
S. imbricatus	7.905	4
R. ochroleuca	7.858	5
C. helveticus	3.701	6
R. acrifolia	3.528	7
A. ovinus	1.996	8
R. xerampelina	1.310	9
B. edulis	1.235	10
L. scrobiculatus	1.180	11
L. badiosanguineus	1.054	12
E. granulatus	0.963	13
C. obtusus	0.669	14
I. appendiculata	0.584	15

Table 1. ECM found in AS site: relative abundances and rank order.

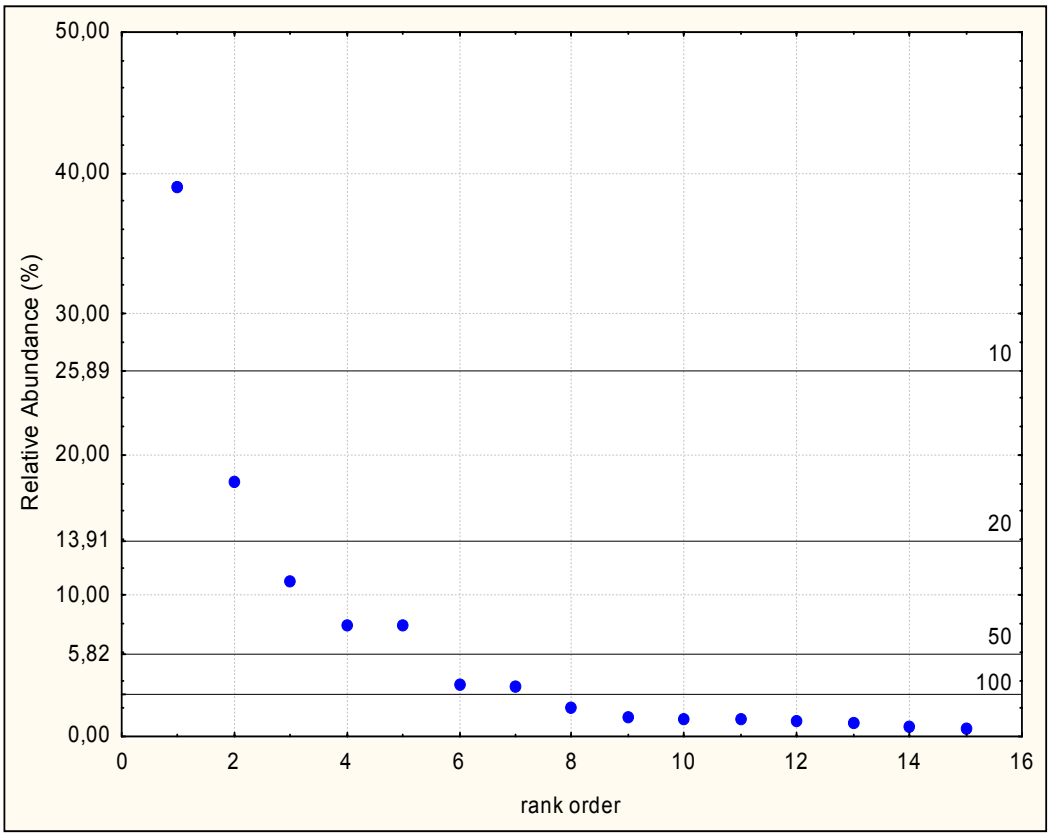

Figure 1. Rank abundance plot of the ECM in AS site, according to TAYLOR (2002).

ECM	Relative Abundance (%)	rank order
C. geophilum	41.433	1
R. ochroleuca	12.001	2
A. muscaria	9.655	3
E. granulatus	7.827	4
H. velutipes	7.797	5
A. byssoides	5.412	6
P. nigra	4.831	7
L. badiosanguineus	3.341	8
H. rufescens	2.814	9
I. appendiculata	2.219	10
H. olivaceoalbus	1.665	11
L. deterrimus	0.998	12

Table 2. ECM found in BN site: relative abundances and rank order.

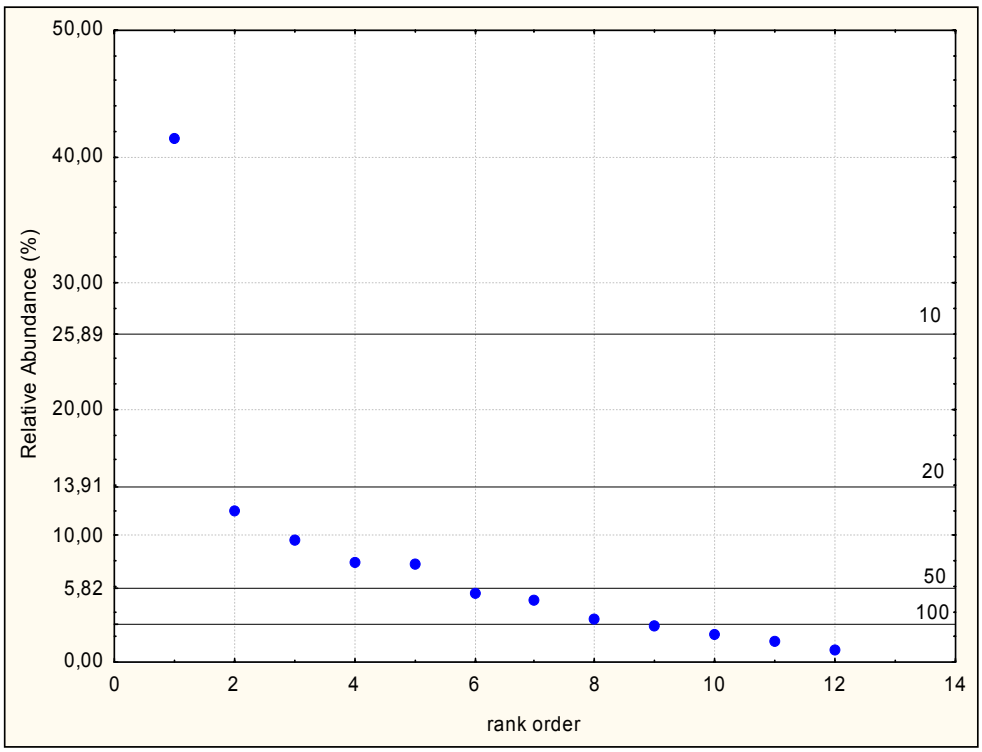

Figure 2. Rank abundance plot of the ECM in BN site, according to TAYLOR (2002).

ECM	Relative Abundance (%)	rank order
C. geophilum	42.806	1
H. velutipes	12.521	2
A. byssoides	9681	3
P. nigra	6.766	4
R. ochroleuca	6.646	5
A. muscaria	5.219	6
L. badiosanguineus	3.744	7
I. appendiculata	3.498	8
A. ovinus	1.879	9
E. granulatus	1.377	10
R. densifolia	1.362	11
L. scrobiculatus	1.354	12
P. oleiferans	1.276	13
B. edulis	0.857	14
T. puberulum	0.543	15
H. olivaceoalbus	0.462	16

Table 3. ECM found in CN site: relative abundances and rank order

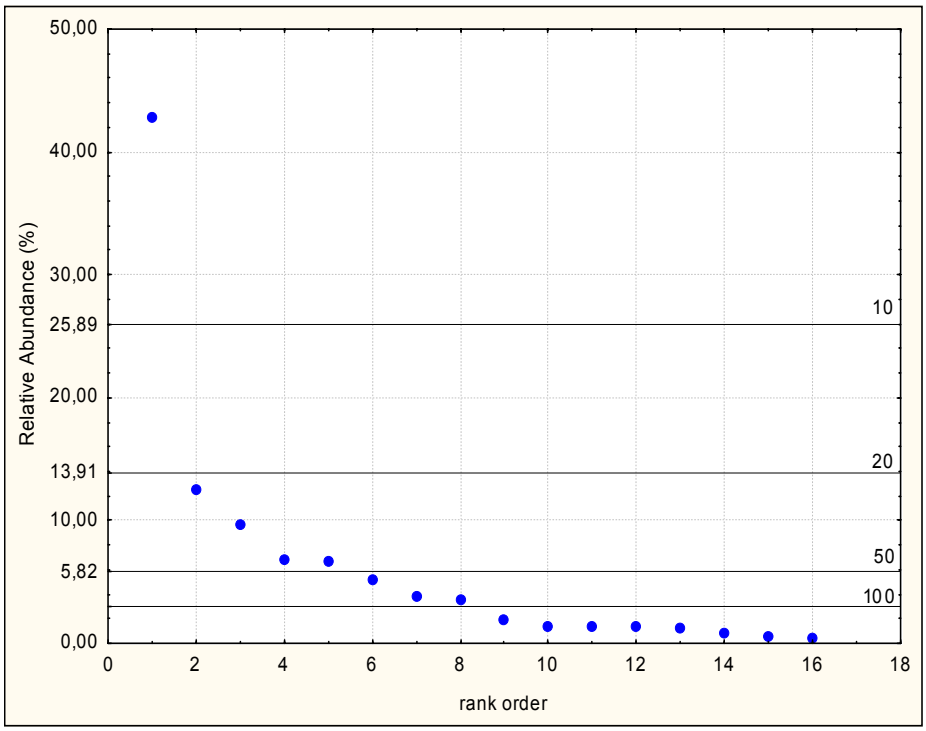

Figure 3. Rank abundance plot of the ECM in CN site, according to TAYLOR (2002).

ECM	Relative Abundance (%)	rank order
C. geophilum	25.465	1
R. ochroleuca	19.634	2
A. byssoides	14.041	3
P. nigra	12.175	4
P. stagonopleres	3.803	5
P. oleiferans	3.735	6
I. appendiculata	3.202	7
Hydnum rufescens	3.050	8
C. odorifer	2.870	9
E. granulatus	2.776	10
A. ovinus	2.763	11
S. imbricatus	1.775	12
P. spinifera	1.238	13
L. badiosanguineus	1.157	14
B. edulis	0.775	15
T. sulphureum	0.578	16
C. helveticus	0.510	17
C. obtusus	0.446	18

Table 4. ECM found in CS site: relative abundances and rank order ECM founded in CS site.

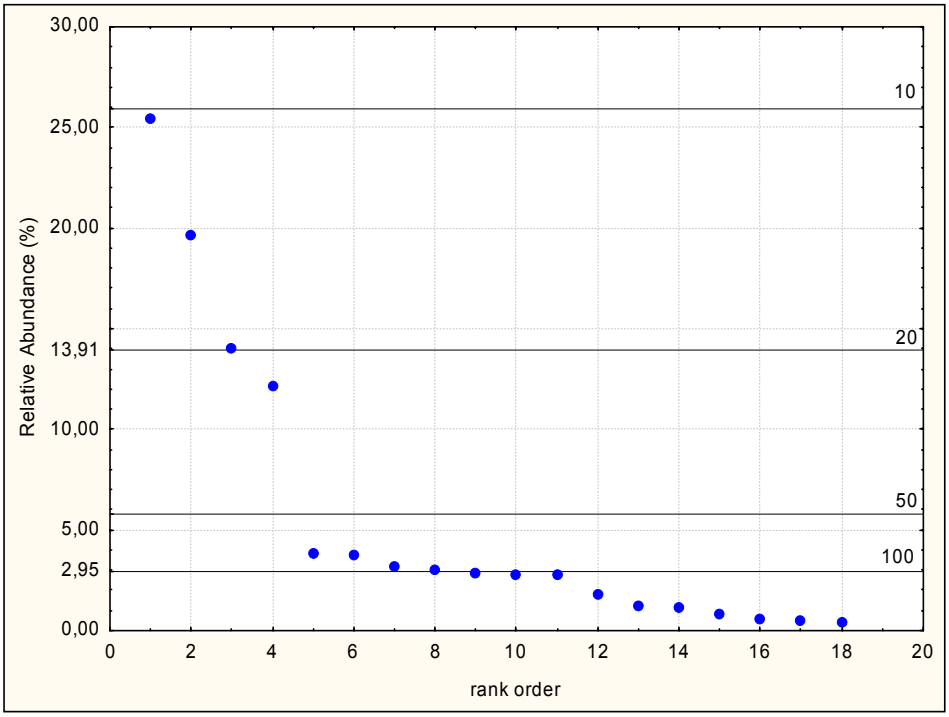

Figure 4. Rank abundance plot of the ECM in CS site, according to TAYLOR (2002).

In AS site, taking into account as a reference the relative abundance of *I. appendiculata* (0.58%), the satisfactory number of living ECM tips enough to detect the 15 ECM species observed, results to be y = 511 (p=0.95), lesser than the 846 considered. In BN site, taking into account as a reference the relative abundance of *L. deterrimus* (0.99 %), the satisfactory number of living ECM tips enough to detect the 12 ECM species observed, results to be y = 298.6 (p=0.95), lesser than the 813 considered. In CN site, taking into account as a reference the relative abundance of *H. olivaceoalbus* (0.46 %), the satisfactory number of living ECM tips enough to detect the 16 ECM species observed, results to be y = 645.6 (p=0,95), lesser than the 845 considered. In CS site, taking into account as a reference the relative abundance of *C. obtusus* (0.45 %), the satisfactory number of living ECM tips enough to detect the 18 ECM species observed, results to be y = 669.5 (p=0,95), lesser than the 802 considered.

4.3.2.1 Diminution of sampling size

For each site, only the most significant differences in couples of data (Chi-square tests, p<0.05) are shown.

AS site

The most significant difference was found through Chi-squared test (p<0.05) between relative ECM abundances observing 10 and 3 tips, respectively, at 250 cm from the collar of the plant (Tab 5).

As Chi-square test revealed to be significant (p<0.05) when lower than 23.684791, dividing this value for n=15 (n. of ECM species in this site), 1.57899 was obtained: in correspondence of $(O-E)^2/E$ >1.57899, ECM that give a high contribute to the significance of this Chi-square test were detected: *A. ovinus*, *P. oleiferans*, *S. imbricatus*, *C. helveticus* (which has the $(O-E)^2/E$ highest value = 18.277).

	10 tips/sample (E)						3 tips/sample (O)						
	DISTANCE (cm)												
	100	**150**	**200**	**250**	**300**	**350**	**100**	**150**	**200**	**250**	**300**	**350**	**(O-E) ^{2}/E**
A. ovinus	0.000	0.694	4.166	5.555	1.562	0.000	0.000	0.000	6.250	0.000	4.166	0.000	**5.555**
B. edulis	0.625	0.625	0.694	1.562	0.781	3.125	2.083	0.000	2.083	0.000	0.000	4.166	1.562
C. geophilum	36.515	37.916	44.273	42.708	36.319	36.495	44.791	35.416	46.875	39.583	22.916	28.125	0.228
C. helveticus	2.968	4.375	4.702	3.975	4.045	2.142	2.083	6.250	3.125	12.500	8.333	4.166	**18.277**
C. obtusus	0.000	0.000	0.000	0.000	3.125	0.892	0.000	0.000	0.000	0.000	6.250	0.000	0.000
E. granulatus	1.250	0.000	0.781	2.343	0.625	0.781	0.000	0.000	0.000	4.166	2.083	2.083	1.417
I. appendiculata	1.250	0.000	2.256	0.000	0.000	0.000	0.000	0.000	4.166	0.000	0.000	0.000	0.000
L. badiosanguineus	1.875	2.777	1.674	0.000	0.000	0.000	0.000	2.083	0.000	0.000	0.000	0.000	0.000
L. scrobiculatus	1.562	1.388	0.000	0.781	1.319	2.031	0.000	2.083	0.000	0000	2.083	0.000	0.781
P. oleiferans	11.056	13.836	11.748	11.059	6.232	11.696	2.083	12.500	6.250	4.166	2.083	3.125	**4.295**
P. croceum	21.153	16.076	8.506	8.975	27.378	26.116	26.041	18.750	4.166	6.250	37.500	33.333	0.827
R. acrifolia	6.562	3.819	2.976	4.687	3.125	0.000	8.333	0.000	4.166	6.250	0.000	0.000	0.520
R. ochroleuca	5.267	13.142	9.885	10.850	5.347	2.656	0.000	12.500	12.500	14.583	6.250	0,.000	1.284
R. xerampelina	0.694	1.388	0.781	0.625	1.406	2.968	0.000	0.000	0.000	0.000	0.000	4.166	0.625
S. imbricatus	9.218	3.958	7.552	6.875	8.732	11.093	14.583	10.416	10.416	12.500	8.333	20.833	**4.602**

Table 5. ECM relative abundances at different distances from the collar of the plant (cm) in AS site, considering 10 and 3 tips in each sample.In the right column: observed vs. expected RA: Chi-Square = 39.97821, df=14, p<0.000257. In evidence, $(O-E)^2/E$ values >1.57899 (= k)

BN site

The most significant difference was found through Chi-squared test (p<0.05) between relative ECM abundances observing 10 and 6 tips/sample in tree 4 (Tab 6). Chi-square test value =19.675138 (df=11, p=0.05).

As Chi-square test revealed to be significant (p<0.05) when lower than 19.675138, diving this value for n=12 (n. of ECM species in this site), 1.63959 was obtained: in correspondence of $(O-E)^2/E$ >1.63959 ECM that give a high contribute to the significant of Chi-square test were detected: *C. geophilum* and *E. granulatus* (which has the $(O-E)^2/E$ highest value =19.233) (Tab 6).

	10 tips/sample (E)				6 tips/sample (O)				
	TREE								
	1	2	3	**4**	1	2	3	**4**	**$(O-E)^2/E$**
A. muscaria	10.706	16.972	2.777	8.164	11.944	14.930	5.000	7.777	0.018
A. byssoides	18.964	0.000	1.759	0.925	19.166	0.000	2.083	1.388	0.231
C. geophilum	54.108	41.382	35.300	34.943	56.041	33.750	42.777	52.847	**9.172**
E. granulatus	0.520	1.521	7.083	22.184	0.694	1.666	6.250	1.527	**19.233**
H. velutipes	0.000	6.967	21.180	3.042	0.000	2.777	5.555	3.472	0.060
H. rufescens	0.595	4.768	2.546	3.349	0.000	4.861	2.777	3.055	0.025
H. olivaceoalbus	0.000	0.000	2.546	4.117	0.000	0.000	2.777	5.000	0.189
I. appendiculata	3.645	1.388	3.842	0.000	3.125	2.083	3.472	0.000	0.000
L. badiosanguineus	1.041	2.979	3.888	5.456	0.694	4.375	2.777	4.444	0.187
L. deterrimus	0.520	0.925	2.546	0.000	0.694	1.388	1.388	0.000	0.000
P. nigra	4.166	6.539	6.388	2.232	2.500	12.152	10.833	2.083	0.009
R. ochroleuca	5.729	16.554	10.138	15.583	5.138	22.013	14.305	18.402	0.509

Table 6. ECM relative abundances in 4 trees in BN site, considering 10 tips and 6 tips observed in each sample. In the right column: observed vs. expected RA: Chi-Square =29.64001, *df*= 1, $p<0.001805$. In evidence $(O-E)^2/E$ values >1.63959 (= k).

CN site

The most significant difference was found through Chi-squared test ($p<0.05$) between relative ECM abundances observing 10 and 4 tips/sample, in sampling points distant 3 m from the collar of the tree (Tab 7). Chi-square test value =24.995790 (*df*=15, $p=0.05$). As Chi-square test revealed to be significant ($p<0.05$) when lower than 24.995790, dividing this value for n=16 (n. of ECM considered in this site), 1.56224 was obteined: in correspondence of $(O-E)^2/E>1.56224$ ECM that give a high contribute to the significant of Chi-square test were detected: *H. velutipes*, *P. nigra*, *A. ovinus*, *A. muscaria* and *C. geophilum* (which has the $(O-E)^2/E$ highest value =10.045) (Tab 7).

	10 tips/sample (E)						4 tips/sample (O)						
	DISTANCE (cm)												
	100	**150**	**200**	**250**	**300**	**350**	**100**	**150**	**200**	**250**	**300**	**350**	**(O-E) ^{2}/E**
A. ovinus	2.962	2.173	2.158	1.342	1.408	0.704	7.291	1.562	5.208	0.000	3.645	2.083	**3.554**
A. muscaria	2.962	8.695	7.194	2.013	5.633	4.225	0.000	6.770	3.125	1.562	0.000	4.166	**5.633**
A. byssoides	19.259	7.246	7.194	12.080	6.338	5.633	16.145	4.687	2.083	12.500	5.729	12.500	0.058
B. edulis	3.703	0	0.7194	0	0	0.704	4.687	0.000	0.000	0.000	0.000	1.562	0.000
C. geophilum	45.185	44.927	43.165	36.241	41.549	41.549	45.833	52.083	41.145	40.625	61.979	36.979	**10.045**
E. granulatus	0.740	0	5.755	0	0.704	1.408	0.000	0.000	6.250	0.000	0.000	3.125	0.704
H. velutipes	10.370	13.043	14.388	13.422	11.971	15.492	10.937	14.583	15.625	11.458	4.687	12.500	**4.432**
H. olivaceoalbus	1.481	0	0	1.342	0	0	1.562	0.000	0.000	1.562	0.000	0.000	0.000
I. appendiculata	3.703	6.521	5.035	0.671	1.408	2.816	4.687	6.250	10.416	0.000	0.000	5.729	1.408
L. badiosanguineus	1.4814	7.246	0	4.0268	8.450	1.4084	2.083	7.812	0.000	4.687	11.458	3.645	1.070
L. scrobiculatus	2.222	2.173	2.158	0.671	0.704	0	2.083	4.687	2.083	2.083	1.562	0.000	1.046
P. nigra	1.481	4.347	5.755	10.738	9.859	9.859	0.000	0.000	3.125	6.250	1.562	4.687	**6.981**
P. oleiferans	0.740	0.724	0	2.013	2.112	2.816	1.562	1.562	0.000	3.125	3.125	3.125	0.485
R. densifolia	0.740	0.724	1.438	2.013	1.408	2.112	0.000	0.000	4.687	2.083	0.000	3.125	1.408
R. ochroleuca	2.962	2.173	5.035	11.409	7.042	11.267	3.125	0.000	6.250	14.062	4.687	6.770	0.787
T. puberulum	0	0	0	2.013	1.408	0	0.000	0.000	0.000	0.000	1.562	0.000	0.016

Table 7. ECM relative abundances at different distance from the collar of the plant (cm) in CN site, considering 10 and 4 tips observed in each sample. In the right column: observed vs. expected RA: Chi-Square =37.63272, df=5, p<0.001023. In evidence, $(O-E)^2/E$ values >1,56224 (= k).

CS site

The most significant difference was found through Chi-squared test (p<0.05) between relative ECM abundances observing 10 and 8 tips/sample in sampling points at W direction (Tab 8). Chi-square test value =27.587112 (df=17, p=0.05). As Chi-square test revealed to be significant (p<0.05) when lower than 27.587112, dividing this value for n=18 (n=number of ECM here considered), 1.53262 was obtained: in correspondence of $(O-E)^2/E$>1.53262 ECM that give a high contribute to the significant of Chi-square test were detected: *L. badiosanguineus*, *A. byssoides*, *P. nigra*, *P. stagonopleres*, *S. imbricatus* and *C. odorifer* (which has the $(O-E)^2/E$ highest value =9.051) (Tab 8).

	10 tips/sample (E)				8 tips/sample (O)				
	DIRECTION								
	N	**S**	**E**	**W**	**N**	**S**	**E**	**W**	**(O-E) ^{2} / E**
A. ovinus	1.909	4.513	1.851	2.777	3.505	2.810	3.538	3.141	0.04763
A. byssoides	11.789	14.019	12.739	17.615	13.870	13.022	13.824	11.410	**2.18591**
B. edulis	0.462	0.983	0.595	1.058	0.848	0.674	0.848	0.768	0.07912
C. geophilum	26.946	27.048	23.328	24.540	24.313	23.915	24.981	25.028	0.00969
C. helveticus	0.462	0.520	0.000	1.058	0.661	0.429	0.661	0.231	0.64587
C. obtusus	0.000	0.595	0.000	1.190	0.231	0.231	0.231	0.000	1.19048
C. odorifer	4.976	4.108	1.562	0.833	2.141	2.364	3.695	3.579	**9.05101**
E. granulatus	2.789	1.579	2.579	4.158	3.073	3.564	2.911	2.498	0.66270
H. rufescens	3.042	2.380	3.042	3.736	3.505	3.703	3.306	2.976	0.15481
I. appendiculata	2.372	4.546	2.967	2.921	2.835	2.571	2.976	3.025	0.00371
L. badiosanguineus	0.000	0.000	4.166	0.462	1.984	1.984	0.198	1.785	**3.77929**
P. nigra	14.976	11.491	13.969	8.263	11.970	13.417	12.905	14.856	**5.25877**
P. oleiferans	6.539	2.025	6.377	0.000	1.959	3.017	1.917	3.447	0.00000
P. spinifera	0.983	0.925	1.388	1.653	1.620	1.628	1.430	1.397	0.03972
P. stagonopleres	2.025	5.109	6.053	2.025	4.125	3.000	2.414	4.125	**2.17701**
R. ochroleuca	19.785	19.733	15.276	23.741	21.501	21.405	23.125	20.099	0.55885
S. imbricatus	0.937	0.416	1.785	3.961	0.859	1.264	1.033	0.636	**2.79078**
T. sulphureum	0.000	0.000	2.314	0.000	0.992	0.992	0.000	0.992	0.00000

Table 8. ECM relative abundances at different directions in CS, considering 10 and 8 tips observed in each sample. In right column: observed vs. expected RA: Chi-Square=28.63536, *fd*=17, p<0.038046. In evidence, (O-E) ^{2}/E values >1.53262 (= k).

Results from Chi-square tests performed on these 4 sites are summarized in tab 9, where the possible reduction of the sampling size (N tips), according to results from comparing the total distribution of ECM relative abundances in each site (column General), the couples of plants (column Tree), the couples of directions (column Direction) and the couples distances (column Distance), are shown.

SITE	*p*-values Observed vs. Expected - Chi-Square test														
N tips	General	Tree				Direction				Distance (cm)					
		1	2	3	4	N	S	E	W	100	150	200	250	300	350
AS															
> 4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
3	NS	NS	NS	NS	NS	NS	0.02	NS	NS	0.009	NS	NS	0.000	0.003	0.01
BN															
> 7	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
6	NS	NS	NS	0.036	0.002	NS	NS	NS	NS	NS	NS	0.005	NS	NS	0.04
CN															
> 5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
4	NS	0.01	0.014	NS	NS	NS	NS	NS	NS	NS	NS	0,04	NS	0.001	0.03
CS															
> 9	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
8	NS	NS	NS	NS	NS	NS	NS	NS	0.038	NS	NS	NS	NS	NS	NS

Table 9. Summary of the results from Chi-square tests performed on AS, BN, CN and CS sites.

Summarizing the results, inside each sample the number of observed tips can be decreased from 10 to 4, 7, 5, and 9 in AS, BN, CN, and CS sites, respectively, corresponding to 384, 672, 480, and 864 EM (alive and ectomycorrhizal) tips, respectively.

MID results showed in AS site *Cenococcum geophilum*, *Lactarius scrobiculatus* and *Chroogomphus helveticus* as the only three ECM presenting a random distribution, while all the other ECM have an aggregated distribution (Tab 10). In BN site *Cenococcum geophilum*, instead, presents a uniform distribution and *Lactarius deterrimus* a random distribution; all the other ECM have an aggregated distribution (Tab 11).

In CN site *C. geophilum* presents once again an uniform distribution, *Albatrellus ovinus*, *Lactarius scrobiculatus*, *Russula densifolia* and *Tuber puberulum* a random distribution; all other ECM have an aggregated distribution (Tab 12). In CS site *Cenococcum geophilum* and *Inocybe appendiculata* have a uniform distribution, *Cortinarius obtusus*, *Russula ochroleuca*, *Elaphomyces granulatus*, *Sarcodon imbricatus*, *Boletus edulis*, *Piceirhiza spinifera* and *Chroogomphus helveticus* have a random distribution; all the other ECM have an aggregated distribution (Tab 13).

	Mean	Variance	Rel. Ab. (%)	Variance Rel. Ab.	MDI Id	F0	distribution
A. ovinus	0,1771	0,7157	1,99653	88,9300	19,059	4,041	aggregated
B. edulis	0,1146	0,2078	1,23553	23,7701	8,727	1,813	aggregated
C. geophilum	3,4375	3,8697	39,03811	459,8820	1,036	1,126	random
C. helveticus	0,3125	0,2803	3,70164	41,2964	0,662	0,897	random
C. obtusus	0,0521	0,1762	0,66964	28,0109	57,6	3,383	aggregated
E. granulatus	0,0833	0,1614	0,96354	22,4171	13,714	1,937	aggregated
I. appendiculata	0,0521	0,0709	0,58449	9,6385	9,6	1,362	aggregated
L. badiosanguineus	0,0937	0,2753	1,05448	32,9357	24	2,937	aggregated
L. scrobiculatus	0,1042	0,0943	1,18056	12,2271	0	0,905	random
P. croceum	1,6667	4,2667	18,03447	472,1009	1,932	2,56	aggregated
P. oleiferans	0,9375	1,3224	10,93833	197,8203	1,438	1,411	aggregated
R. acrifolia	0,2917	1,2193	3,52844	183,0891	12,19	4,18	aggregated
R. ochroleuca	0,7083	1,8086	7,85838	224,6822	3,202	2,554	aggregated
R. xerampelina	0,1146	0,1657	1,31076	23,4124	5,236	1,446	aggregated
S. imbricatus	0,6667	1,1298	7,90509	165,2094	2,048	1,695	aggregated

Table 10. ECM distributions in AS.

	Mean	Variance	Rel. Ab. (%)	Variance Rel. Ab.	MDI Id	F0	distribution
A. muscaria	0,8125	1,4592	9,6553	207,9583	1,982	1,796	aggregated
A. byssoides	0,4062	0,7069	5,4125	133,8654	2,85	1,74	aggregated
C. geophilum	3,4375	1,8276	41,4339	294,1995	0,865	0,532	uniform
E. granulatus	0,6458	1,6206	7,8274	262,2538	3,351	2,509	aggregated
H. velutipes	0,7187	2,0359	7,7976	228,0516	3,56	2,832	aggregated
H. rufescens	0,2396	0,4578	2,8150	69,4176	4,933	1,911	aggregated
H. olivaceoalbus	0,1354	0,2867	1,6659	49,7383	9,846	2,117	aggregated
I. appendiculata	0,1875	0,3434	2,2193	43,0239	5,647	1,832	aggregated
L. badiosanguineus	0,3021	0,4446	3,3416	54,3170	2,601	1,472	aggregated
L. deterrimus	0,0937	0,1069	0,9983	11,9048	2,667	1,14	random
P. nigra	0,3958	0,5575	4,8318	86,6638	2,048	1,408	aggregated
R. ochroleuca	1,0937	2,4437	12,0015	285,2059	2,127	2,234	aggregated

Table 11. ECM distributions in BN.

	Mean	Variance	Rel. Ab. (%)	Variance Rel. Ab.	MDI Id	F0	distribution
A. ovinus	0,1562	0,1543	1,8791	21,8337	0,914	0,987	random
A. muscaria	0,4479	0,6288	5,2190	86,5856	1,914	1,404	aggregated
A. byssoides	0,8437	1,1858	9,6813	155,8348	1,481	1,405	aggregated
B. edulis	0,0729	0,1315	0,8578	17,3445	13,714	1,803	aggregated
C. geophilum	3,6979	2,6551	42,8063	380,2849	0,924	0,717	uniform
E. granulatus	0,1250	0,2158	1,3773	25,2874	7,273	1,726	aggregated
H. velutipes	1,1562	2,3227	12,5219	261,5968	1,871	2,009	aggregated
H. olivaceoalbus	0,0416	0,0825	0,4630	10,1798	32	1,979	aggregated
I. appendiculata	0,2917	0,6298	3,4987	93,8407	5,079	1,159	aggregated
L. badiosanguineus	0,3334	0,4982	3,7442	63,1660	2,516	1,495	aggregated
L. scrobiculatus	0,1146	0,1236	1,3550	16,7937	1,745	1,078	random
P. oleiferans	0,1250	0,3210	1,2760	32,6309	14,545	2,568	aggregated
P. nigra	0,6250	1,2263	6,7667	132,1031	2,549	1,962	aggregated
R. densifolia	0,1250	0,1105	1,3628	13,2717	0	0,884	random
R. ochroleuca	0,5937	1,2753	6,6468	168,2410	2,846	2,107	aggregated
T. puberulum	0,0521	0,0499	0,5440	5,4580	0	0,958	random

Table 12. ECM distributions in CN.

	Mean	Variance	Rel. Ab. (%)	Variance Rel. Ab.	MDI Id	F0	distribution
A. ovinus	0,2500	0,589474	2,7633	69,6936	6,609	2,358	aggregated
A. byssoides	1,1771	2,273575	14,0410	309,6681	1,79	1,932	aggregated
B. edulis	0,0625	0,059211	0,7750	9,2328	0	0,947	random
C. geophilum	2,1250	1,647368	25,4659	220,1052	0,895	0,775	uniform
C. helveticus	0,0417	0,040351	0,5105	6,1287	0	0,968	random
C. obtusus	0,0312	0,030592	0,4464	6,2433	0	0,979	random
C. odorifer	0,2500	0,610526	2,8704	82,0869	6,957	2,442	aggregated
E. granulatus	0,2292	0,199561	2,7765	29,2795	0,416	0,871	random
H. rufescens	0,2396	0,668311	3,0506	102,7612	8,727	2,789	aggregated
I. appendiculata	0,2708	0,199561	3,2023	28,2993	0	0,737	uniform
L. badiosanguineus	0,1042	0,431140	1,1574	53,2272	31,133	4,139	aggregated
P. nigra	1,0312	2,199013	12,1755	299,2645	2,098	2,132	aggregated
P. oleiferans	0,3125	0,764474	3,7355	113,2714	5,738	2,446	aggregated
P. spinifera	0,1042	0,115351	1,2380	16,0883	2,133	1,107	random
P. stagonopleres	0,3229	0,684101	3,8033	97,0084	4,542	2,119	aggregated
R. ochroleuca	1,6146	1,839364	19,6342	278,0965	1,086	1,139	random
S. imbricatus	0,1354	0,160417	1,7754	28,2120	2,462	1,185	random
T. sulphureum	0,0521	0,260417	0,5787	32,1502	96	5	aggregated

Table 13. ECM distributions in CS.

Focalizing the attention to the more abundant and common ECM, we can see that *Lactarius badiosanguineus*, present in every site, is always characterized by an aggregated distribution; *Amphinema byssoides*, present in BN, CN and CS, is always aggregated. *Cenococcum geophilum*, instead has a uniform distribution in all the sites, excepted in AS, where it is randomly distributed.

4.4 Discussion

Fundamental ecology concepts for recording a certain proportion of individuals in community are those of the minimum sampling area and of how samples should be spatially distributed.

Because of the no-random spatial and temporal distribution of ECM species, estimates of minimum sampling areas, to have a realistic description of the ECM community, are very difficult. A universal method cannot be defined even in adult, healthy plants with superficially developed root system, collecting cores in the most ECM-rich period.

The geometrical design (4 plants, 4 directions, 6 distances from the collar) works, and can be useful in researches where *the plant* and not *the forest* is the subject (i.e. in phytopathological studies), or in mixed forest where the discrimination from tips belonging to the investigated tree species and the others are difficult.

The minimum number of tips to be observed per sample, able to characterize the ECM community, differently, changes from site to site, being constant the tree species, its age and health.

Exposition and soil pH are associated to these differences, probably because of their involvement in ECM richness, abundance and aggregation type (TAYLOR 2002, BRUNS 1995).

Besides a few of the less abundant species (*I. appendiculata*, *L. deterrimus, H. olivaceoalbus*, *C. obtusus* in AS, BN, CN and CS, repectively), other demonstrated to be important in determining the sampling size, because of their spatial distribution in relation to the sampling points detected. The results showed that *C. helveticus*, *S. imbricatus*, *A. ovinus*, *P. oleiferans* are the most limiting species in sampling size decreasing in AS site; *E. granulatus* and *C. geophilum* in BN site; *C. geophilum*, *P. nigra*, *A. muscaria, H. velutipes* and *A. ovinus* in CN site; *C. odorifer*, *P. nigra*, *L. badiosanguineus*, *S. imbricatus*, *A. byssoides*a and *P. stagonopleres* in CS site.

In forest studies concerning the ectomycorrhizal ecology, the sampling effort and strategy, having a strong influence on the perception of the ECM community structure, should be taken more into account and associated to each research to validate the study, according to the main aim of the research itself.

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Chapter 5.
General discussion

5.1 Discussion

Fitness in forest plants is maintained by both above-ground and below-ground biotic and abiotic processes (MANION 2003).

Even if the functional roles of the various groups of soil organisms are often unclear, they can provide important clues in early warning of ecosystem degradation.

In temperate and boreal forest soils, one of the most abundant and diverse communities are the ectomycorrhizal symbionts (READ 1991).

Supposing that the functional activity of an ECM consortium as a whole can have similar efficiency in comparable forests growing in different sites, the main goals of the research, performed in 10 comparable Norway spruce forests in the Trento Province (northern Italy), was to verify if the root tips vitality and composition of the ECM consortium can be associated to the main pedoclimatic factors.

The results highlighted that broad-spectrum site features (bedrock pH and exposure) and more characterizing factors (pH, OF horizon, humus forms) are highly correlated to ECM species and functional groups, allowing the distinction of ECM consortia peculiar to the environmental variables above reported.

Moreover, taking into account that the non-random spatial distribution of ECM within the soil thwarts the detection of the right composition and diversity inside communities, the importance of a sampling method able to describe the ECM community was highlighted. The effectiveness of the geometrical sampling design used in this research is theoretically confirmed and points out how the sampling design used can be useful in researches where the plant and not the forest is the subject (i.e. in phytopathological studies), or in mixed forest where the discrimination from tips belonging to the investigated tree species and the others are difficult.

The ectomycorrhizal symbiosis, real interface between roots and soil, differs according to plant species, aboveground features, physical and chemical soil properties.

This study, interpreting some of relations among symbiotic community and environmental-pedological variables, induces to hypothesize the possibility of integrating the parameters generally used in forest soil descriptions with a biological indicator as the ectomycorrhizal community *status* could act, being the direct result of the plant-soil interactions.

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Abstract - **Variations of the ectomycorrhizal community in high mountain Norway spruce stands and correlations with the main pedoclimatic factors**

The species composition of ectomycorrhizal (ECM) fungal communities can be strongly influenced by abiotic and biotic factors, which determine interactions among the species such as resource partitioning, disturbance, competition, or relationships with other organisms.

In order to determine the influence of environmental features on ECM community, soil bedrock pH, exposure, humus features and sampling points locations were taken into account as the most representative and influencing factors in these soil ecological dynamics.

In summer 2003, 2004 and 2005, in 10 [165(±10)-year-old] Norway spruce [*Picea abies* (L.) Karst.] stands located in the Province of Trento (northern Italy), root tips were collected according to an experimental sampling method designed and statistically tested on purpose.

The investigation of the ECM community composition (species richness and abundance) in relation to the main pedoclimatic factors revealed the importance of bedrock pH and site exposure as variables at a macro-scale level.

A spatial niche differentiation of ECM species and ecological ECM groups, based on similar organization and extent of the extramatrical mycelium, were mostly associated to organic layers (OF), pH and N tot variables at a vertical micro-scale level of study.

The results suggest that bedrock pH, exposure and humus dynamics play a primary role in the adaptive selection of ECM species constituting the consortium.

Riassunto – Variazioni della comunità ectomicorrizica in peccete altimontane e relazioni con i principali fattori pedoclimatici

Le specie componenti una comunità ectomicorrizica possono essere fortemente influenzate da fattori abiotici e biotici determinanti interazioni tra specie come partizione delle risorse, effetti di disturbo, competizione i relazioni con altri organismi.
Con la finalità di determinare l'influenza dei fattori ambientali sulla comunità ectomicorrizica, il pH della roccia madre, l'esposizione del versante, l'humus e la localizzazione dei punti di campionamento sono state considerati come variabili maggiormente rappresentative ed in grado di condizionare dette dinamiche ecologiche nel suolo.
Durante la stagione estiva del 2003, 2004 e 2005, in 10 peccete [*Picea abies* (L.) Karst.] altimontane della Provincia di Trento (TN, Italia), sono stati campionati apici radicali, secondo una metodologia di campionamento sperimentale e statisticamente testata per detto studio.
L'indagine sulla composizione della comunità ectomicorrizica (numero di specie e abbondanza) in relazione ai principali fattori pedoclimatici ha rivelato, ad un livello di macroscala, l'importanza del pH della roccia madre e dell'esposizione del versante.
Ad un livello di microscala, una differenza spaziale, associabile all'occupazione di differenti nicchie ecologiche, nella distribuzione delle specie ectomicorriziche e di gruppi ecologici, basati su organizzazione ed estensione di micelio extramatricale, è stata principalmente associata ad orizzonti organici (OF), pH e N tot.
I risultati indicano come il pH della roccia madre, l'esposizione del versante e le dinamiche dell'humus assumano un ruolo primario nella selezione adattatativa delle specie ectomicorriziche che costituiscono un consorzio.

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