The new method 'micromapping', a means to study species-specific associations and exclusions of ectomycorrhizae

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Ectomycorrhizae (ECM) are obligate symbiotic associations between higher fungi and most tree species of the temperate and boreal forests, and of some tree families in tropical areas. As the anatomical features of these symbiotic organs are very diverse and suggested to improve tree growth differently efficient, their frequency and natural distribution in the soil, as well as the differentiation and amount of their substrate exploiting extramatrical mycelia, are of special ecological interest. The soil with its heterogeneous assemblage of micro-niches certainly provokes ectomycorrhizal fungi to compete for such microsites. We therefore applied the method 'micromapping' to record the ECM in their natural position with the following question in mind: Do indicators exist for an exclusion of or an association with other ectomycorrhizal species or not? Thoroughly excavated and carefully cleaned ectomycorrhizae of the O_F horizon of a *Picea abies* stand were drawn in their natural position on perspex plates of 5 x 5 cm mapping area (McMp) with ink of different colours. They were afterwards removed and specified. Following scanning of the McMp, a special computer program was applied to analyse their distribution. The spatial relations of the ECM were calculated according to the 'growing grid method'. The preliminary results suggest that the ECM of *Russula ochroleuca* and *Piceirhiza internicrassihyphis* show no common occurrence within short distances. This possibly applies also for *Russula ochroleuca* in comparison to *Piceirhiza cinnbadiosimilis,* for *Elaphomyces granulatus* in comparison to *Xerocomus badius,* and *Lactarius decipiens* in comparison to *Piceirhiza cinnbadiosimilis*. *Cortinarius obtusus* with *Piceirhiza internicrassihyphis,* and *Piceirhiza internicrassihyphis* with *Xerocomus badius,* indicate, however, rather high values of common occurrence. Due to the small number of replications, the standard deviations are high. More detailed investigations are therefore necessary before definite conclusions can be made. This method, however, apparently provides a useful tool to analyse spatial relations of ECM in the soil. Possible reasons for exclusions and associations of ECM are briefly discussed.

Most tree species of montane, temperate and boreal forests have ectomycorrhizae, symbiotic associations of primarily Hymenomycetes (Basidiomycota and Ascomycetes (Ascomycota) with roots (HARLEY & HARLEY 1987, SMITH & READ 1997). Ectomycorrhizal symbiosis is regarded as advantageous for fungi and trees because fungi obtain carbohydrates from the trees, which in turn are supplied with water and nutrients by the fungi (SMITH & READ 1997). Whereas the transfer area between the partners is rather uniform in structure (AGERER 1991a) the contact of the fungi with the soil can be highly diverse and appears to be also functionally diversified, as could be concluded from the anatomical differentiation. Extramatrical mycelium fulfils the essential role of exploration and exploitation of the nutrient resources of the soil and their transport to the ectomycorrhizal mantle (READ 1995). Rather limited knowledge exists whether there are special ecological microniches in the soil for morphologically different ectomycorrhizae. READ (1992) mentions that a rather high proportion of ECM is not in direct contact with the soil, but are primarily formed in pores between soil particles. This situation is particularly true for hydrophobic ectomycorrhizae. Hydrophilic members, which are quite frequently provided with only a rather limited and diffuse extramatrical mycelium, are often squeezed between litter and have therefore close contact with the substrate (READ 1992, UNESTAM & SUN 1995, AGERER 2001).

The distribution of ECM in natural soils depends upon the distribution of roots, availability of fungal inocula and whether fungi spread by extended mycelial networks or primarily by germinating spores (NEWTON 1992). Soil conditions are of similar importance, either for species composition (ALEXANDER & FAIRLEY 1983) or morphotype frequency (ALEXANDER & FAIRLEY 1983, ANTIBUS & LINKINS 1992, YANG et al. 1998). The ectomycorrhizal mycelium scavenges for nutrients (READ 1992). As its volume (JONES, DURALL & TINKER 1990), type

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of differentiation (AGERER 1995), and extension into the soil (RAIDL 1997, AGERER 2001) can vary considerably, its effects on tree nutrition can hence differ, too (JONES, DURALL & TINKER 1990, READ 1992). However, the amount of the extramatrical mycelium itself can be changed by nutrient availability of the soil (JONES, DURALL & TINKER 1990, ARNEBRANT 1994). In addition, spatial heterogeneity in the soil may create a mosaic of fungal colonisation (OZINGA, VAN ANDEL & MCDONNELL-ALEXANDER 1997) and coexisting species can influence the surrounding soil patches differently (JONES, DURALL & TINKER 1990, READ 1992). Studies on spatial distribution of ECM have already been performed but mostly in larger communities or even on a large scale (DAHLBERG, JONSSON & NYLUND 1996, GARDES & BRUNS 1996, ERLAND et al. 1999). Species frequency, however, can also be influenced by fertilisation (e.g. WALLANDER & NYLUND 1992, FRANSSON, TAYLOR & FINLAY 2000) or atmospheric conditions. For example, elevated CO_2 concentrations can favour some species against others (REY & JARVIS 1997).

Differences in anatomy and morphology of ectomycorrhizae can certainly be regarded as ecologically important, and differences in their structure could influence their behaviour to neighbouring species. Particularly, due to their manifold occurrence in small spaces, ECM could be expected to experience competition as well as benefits from associations with other species. Indeed there is some preliminary evidence that ECM may be influenced in their distribution and frequency by neighbouring species. First suggestions that ectomycorrhizal fungi can exclude one another in their occurrence are from studies on fruitbodies. For example, as shown by AGERER & KOTTKE (1981), the fruitbody areas of *Russula ochroleuca* (Pers.) Fr. and *R. fellea* Fr. do not overlap. The same could be found for *R. vinosa* Lindbl. as well as for *R. fellea* and *R. vinosa*. Similar results were obtained by MURAKAMI (1987) for several additional *Russula* species. MATSUDA & HIJII (1998) found evidence that a *Russula* sp. occurred exclusively, or was overlapping or independent with *Inocybe cincinnata* (Fr.: Fr.) Quél., *Strobilomyces confusus* Sing. or *Russula ochroleuca,* respectively. A few studies on ectomycorrhizae have already concluded that the frequency of some species depends on the presence of other species (SHAW, DIGHTON & SANDERS 1995, THURNER & PÖDER 1995, TIMONEN, TAMMI & SEN 1997). An influence by extramatrical mycelia was demonstrated by FRANCIS & READ (1994) and by WU, NARA & HOGETSU (1999). Ectomycorrhizae might also be influenced by saprotrophic fungi (SHAW, DIGHTON & SANDERS 1995, LINDAHL et al. 1999, BAAR & STANTON 2000), but such competition studies are beyond the boundaries of the present contribution.

The present study maps ECM in their natural position and compares their distribution in relation to neighbouring morphotypes, with the specific aim to gain a better understanding of ectomycorrhizal distribution within the soil. Morphotypes are differentiated anatomically into anatomotypes. Anatomotypes are very likely related to species or species groups of fungi.

Materials and methods

Isolation of ectomycorrhizae and production of micromaps

Steel frames (Fig. 1) 12.5 x 9.5 cm (inner size) and 5 cm deep with sharp lower rims were used to take soil monoliths in an area of a pure Norway spruce (*Picea abies* (L.) Karst.) stand within a mixed spruce/beech (*Fagus sylvatica* L.) forest. The whole organic layer (O_F, O_H, exclusive of the loose litter layer) and parts of the A_h horizon were included (KUNTZE et al. 1981). Humus type is a mor on dystric Cambisol derived from pleistocene loess over tertiary sediments (KREUTZER & BITTERSOHL 1986). The O_F layer is approximately 5–10 mm thick. The steel frame together with the soil was carefully removed, wrapped in aluminium foil and stored at 4 °C until processing. Four monoliths were taken concurrently, as this provided enough material to be analysed at a single time. New monoliths samples were collected throughout spring and autumn 1999 and spring 2000. Because no comparison of the ectomycorrhizal dynamics was intended, but only the assemblage of ECM within the monoliths were to be investigated, the variable collection times were supposed not to have a general impact on the results.

The steel frame with the soil was completely covered by water in a deeper tray (8 cm) for soaking. To prevent loss of soil, the lower opening of the frame was covered with a perspex plate fitting exactly into the rectangle of the frame (Fig. 1). The upper surface of the monolith was covered by a narrow-edged perspex frame stringed with a nylon grid of 10 mm width (Fig. 1). This frame fitted exactly into the iron frame and could be fixed onto the surface of the soil monolith by squeezing it into the steel frame. This device was necessary to fix the roots and ECM in their natural position during cleaning. Fine forceps and needles were used to remove all organic particles of the complete O_F-layer. In addition, a modified spray gun (Geizhals), as used in orchards, was applied to remove the soil particles with a thin stream of water. Debris floating on the surface was soaked off by a 'Wet and Dry Vacuum Cleaner' (Kärcher). After completion of cleaning, the water level was adjusted to the exposed layer of the ECM, the grid frame cautiously removed, and the organic material at the margin of the soil excavator below the narrow frame discharged.

A 2 mm thin, 10 x 7 cm perspex plate (= mycorrhizal micro map, McMp) with a 10 mm grid engraved with a fine needle on the lower surface (Fig. 2), was laid on the monolith covering the exposed ECM. Two holes outside of the selected mapping area of 5 x 5 cm were used to fix the position of the McMp on the soil and to mark the exact position by long steel needles. In addition, brass weights could be stuck on the margin of the McMp to press it against the exposed ECM and to prevent them from changing position during mapping (Fig. 2). After adjustment of the McMp to areas with ECM most appropriate for mapping (ECM exposed), the water level was

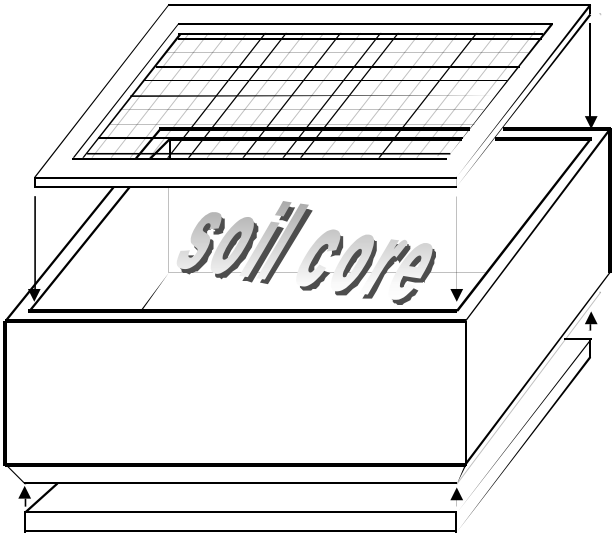

Fig. 1. Equipment for excavation of ECM. A steel frame with sharp lower rims for taking the soil core, a narrow-edged perspex frame stringed with a nylon grid of 10 mm width (above) for fixing the roots and ECM in their natural position, and a perspex plate (below) for preventing loss of soil.

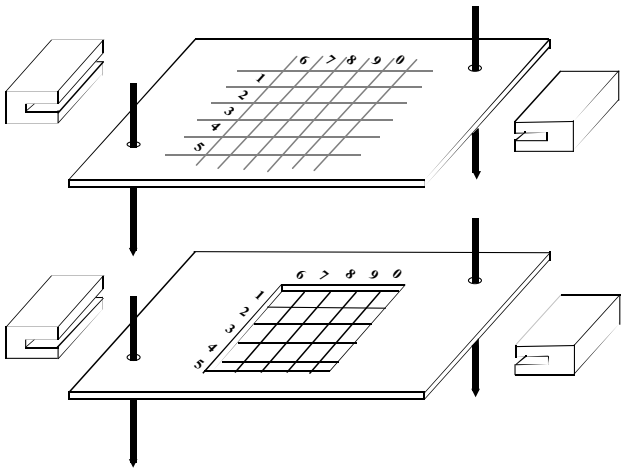

Fig. 2. Devices for documentation of the ECM. A perspex plate with a 10 mm grid engraved on its lower surface (McMp), two holes to fix its position on the soil surface by two steel needles, and brass weights that could be stuck on the margin of the perspex plate; every square obtained its own identification number, e.g. 16, 17… (above). A perspex frame with a nylon grid of 10 mm width replaced the McMp for collecting the ECM (below). The grid of the McMp and of the frame were identically positioned on the soil surface due to the position needles and the brass weights

elevated until it touched the lower surface of the McMp completely. The upper surface remained dry.

Through a dissecting microscope (magnification 6x and 12x) all ECM which appeared different in colour, surface and shape (morphotypes) were drawn on the McMp's mapping area with permanent waterproof ink (Edding) filled in isograph drawing devices (Rotring) for 0.25 mm line thickness. A different colour was used for each morphotype. After drying of the fluid, the lines obtained a final thickness of ca. 0.3 mm, a diameter approximately representative of spruce ECM (calculated after AGERER & RAMBOLD 1998: minimum value 0.18 mm, maximum value 0.9 mm, mean 0.382 ± 0.073 mm).

After having drawn all ECM of the O_F-layer in their natural position, the McMp was carefully removed, leaving the positions of the ECM and steel needles unchanged. Instead of the McMp, a second perspex frame with a nylon grid of 10 mm mesh width was stringed onto the two position needles (Fig. 2). This nylon grid frame held the threads exactly at the same positions as the McMp had its engraved lines. Therefore, all 25 squares of the nylon grid were at the same positions as were the 25 squares of the McMp. This frame was again fastened by brass weights, thus fixing the ECM with the nylon threads in their natural position. All ECM from all 25 squares could be removed and collected in small flasks. The squares of the McMp, those of the grid frame and the sample flasks were numbered identically (Fig. 2) to ensure that the ECM drawn on the McMp could be unequivocally related to their natural position within the soil.

The collected morphotypes of each flask were used for anatomical studies either for determination (AGERER 1987-1998, AGERER & RAMBOLD 1998) or for a brief characterisation (AGERER 1991a). The morphotypes could therefore be differentiated into anatomotypes (= species of ectomycorrhizae). A magnified xerocopy of the original McMp allowed us to designate and name each individually drawn ECM. In some cases, morphotypes were heterogeneous and had to be divided into two anatomotypes. This was specified on the xerocopy. For each McMp, ECM were deposited as fixed voucher collections (AGERER 1991a) in Botanische Staatssammlung München (= M, HOLMGREN, HOLMGREN & BARNETT 1990). Some fresh ECM were fixed for DNA analysis (AGERER, MÜLLER & BAHNWEG 1996).

Processing of micromaps

The McMps were scanned (Hewlett Packard, Scarlett 6300C) and saved as an AdobePhotoshop5.5 file. As the evaluation for ectomycorrhizal associations is dependent on anatomotypes, the colour of additional anatomotypes included in a heterogeneous morphotype, was changed into a colour not used in this particular McMp. A total of 50 McMps was investigated during the present study. Every McMp was allocated a serial number, e.g. McMp0001, McMp0002, etc. Seventeen anatomotypes (Tab. 1) have been analysed and were numbered consecutively, and could be studied regarding their distribution. Each anatomotype of each McMp therefore received its own identification number, e.g.

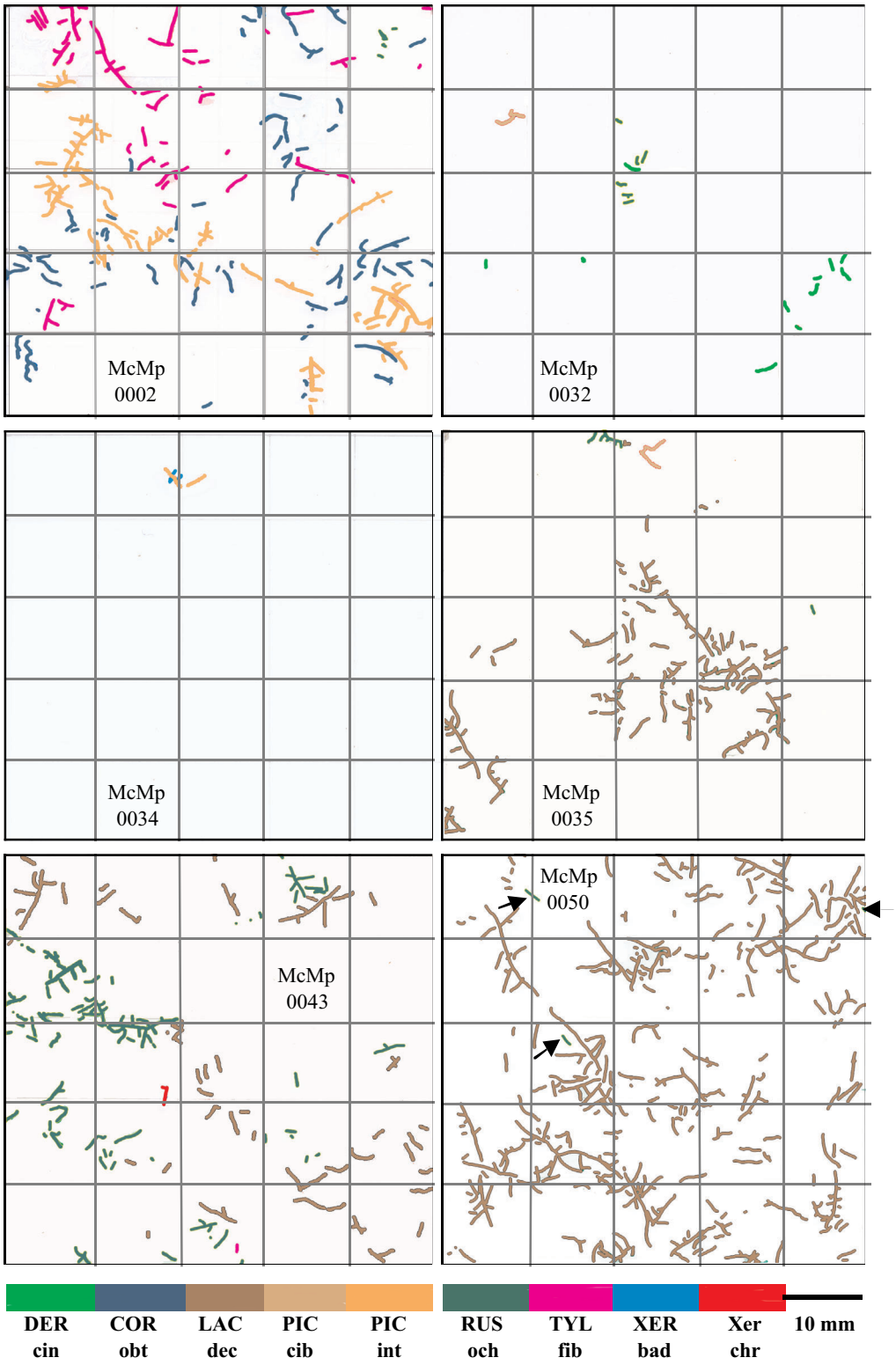

Fig. 3. Six McMps with different ectomycorrhizal densities, distributions and species compositions in their natural position. McMp0002: *Cortinarius obtusus* and *Piceirhiza internicrassihyphis* show a considerable overlap in their ectomycorrhizae; *P. internicrassihyphis* and *Tylospora fibrillosa* ECM are rather separated; few ECM of *Russula ochroleuca* occur in the upper right corner. – McMp0032: *Piceirhiza cinnbadiosimilis* takes a separate position as do most ECM of *Dermocybe cinnamomea*; *R. ochroleuca* shows a partial overlap with *D. cinnamomea*. – McMp0034: There is an intimate association between *P. internicrassihyphis* and *Xerocomus badius*. – McMp0035: *Lactarius decipiens* ECM occupy a large area; *P. cinnbadiosimilis* is close to a complex composed of a few *R. ochroleuca* ECM and one ECM of *L. decipiens*. – McMp0043: *Lactarius decipiens* ECM appear to grow in different micro-sites as compared to *R. ochroleuca; Tylospora fibrillosa* and *Xerocomus* cf. *chrysenteron* are very infrequent and within these areas. – McMp0050: Three ECM of *R. ochroleuca* are nested within *L. decipiens* (arrows). Grid width represents 10 x 10 mm.

Tab. 1: ECM compared in this study, their exploration type (EXTY) and hydrophilic/ hydrophobic (HY) features: *C* = Contact exploration type; *SD* = Short Distance exploration type; *MDs* = Medium-Distance smooth exploration type; *MDf* = Medium Distance fringe exploration type; *LD* = Long Distance exploration type. – *ho* = hydrophobic; *hi* = hydrophilic. (according to AGERER 2001, UNESTAM & SUN 1995, and unpubl. data)

Sp. No.	Akronym	EXTY	HY	Name of ectomycorrhiza
-04	CORobt	MDf	*ho*	*Cortinarius obtusus*
-05	DERcin	*MDf*	*ho*	*Dermocybe cinnamomea*
-06	ELAgra	*SD*	*hi*	*Elaphomyces granulatus*
-02	LACdec	*MDs*	*hi*	*Lactarius decipiens*
-03	PICcib	*MDs*	*ho*	*Piceirhiza cinnbadiosimilis*
-20	PICnif	*C*	*hi*	*Piceirhiza nigripunctiformis*
-09	PICint	*C/SD/MDs*	*hi*	*Piceirhiza internicrassihyphis*
-18	PICsub	*LD*	*ho*	*Piceirhiza subtilis*
-19	PICnip	*CT/SD*	*hi*	*Piceirhiza nigripunctata*
-01	RUSoch	*C*	*hi*	*Russula ochroleuca*
-11	TOMsp1	*C/SD*	*hi*	*Tomentella* sp. 1
-12	TOMsp2	*SD*	*hi*	*Tomentella* sp. 2
-13	TOMsp4	*SD*	*hi*	*Tomentella* sp. 4
-17	TOMsp5	*SD*	*hi*	*Tomentella* sp. 5
-14	TYLspe	*SD*	*hi*	*Tylospora fibrillosa*
-15	XERbad	*LD*	*ho*	*Xerocomus badius*
-16	XERchr	*LD*	*ho*	*Xerocomus* cf. *chrysenteron*

McMp0002-01, McMp0002-04, McMp0032-01, McMp0032-03, etc. (Fig. 3).

A special program for Windows operating systems was developed (Seifert & Grote, unpubl.) to analyse the projection area of each ECM in each square of each McMp and to analyse their distribution patterns. The analysis is pixel-based, but can also be expressed in units of covered area (= projection area). This program requires a separate bitmap file for each anatomotype of every individual McMp, i.e. of each identification number (e.g., McMp0002-01, McMp0002-04, McMp0002-09, McMp0002-14; Figs. 2, 3). An analysis of single species distribution could be applied, which is based on the coefficient of dispersion (FISHER, THORNTON & MAC KENZIE 1922). This index is particularly suitable to evaluate the degree of contagiousness and could be applied using number and standard deviation of ECM-pixel per grid (Sn^2 / n). Since the values obtained depend on grid size, it is recommended to investigate several grid sizes according to the investigation method proposed by GREIG-SMITH (1983), which we will discuss in a forthcoming investigation. The grid widths 2.5 mm, 5 mm, 7.5 mm, 10 mm, 12.5 mm, and 15 mm were used for final comparison of ECM occurrence. Broader grid sizes were not suitable for evaluation purposes, because too many ECM locations are pooled together to allow different distribution patterns to be distinguished. Apart from the total projection area and the degree of contagiousness per ECM, the relation between any two species of ECM is determined by evaluating their 'spatial relation'-value (IR).

IR = nSd / nS
IR: spatial relation - Index
nSd: number of squares with two species
nS: total number of squares

'Spatial relation' is a number that is calculated as the number of squares, in which both species occur, divided by the total number of squares, and it thus can vary between 0 and 1. It should be noted that this number also depends strongly on the dimensions of the squares, because the chance of two species occurring in the same square increases with square size.

For final comparisons, the grid-widths 2.5 mm, 5 mm and 7.5 mm were applied as the most predicative (Figs. 4, 5).

Studied ectomycorrhizal material

All determinations of ectomycorrhizae were performed with DEEMY (AGERER & RAMBOLD 1998) and AGERER (1987-1998), hence all names have to be considered under the concept of these publications. Therefore, it should be taken into account that, under a given fungal species name, additional species could have formed ECM of identical structure and might thus all be included in a single anatomotype. However, of all ECM which have been designated in the present study by a fungal species name, fruitbodies have been reported near the studied plot (GRONBACH 1988, AGERER, TAYLOR & TREU 1998) and were found again within the area where the soil cores had been taken. The identity of some ECM has been confirmed by comparison of their restriction fragment length polymorphisms of ITS regions of nuclear ribosomal DNA with

that of fruitbodies; using the following restriction enzymes, *Alu*I, *EcoR*I, *Hinf*I, and *Taq*I (AGERER, MÜLLER & BAHNWEG 1996). In particular, ECM were subjected to DNA analysis when anatomical identification was ambiguous (see below).

Collection data of ECM: Germany, Bayern, district Aichach-Friedberg, between Odelzhausen and Mering, in the forest Höglwald near Tegernbach; close to the forest road near Zillenberg. Leg. R. Agerer, det. R. Agerer (all in M):

Cortinarius obtusus Fr.: McMp0003, 28. 6. 1999 (RA12773); McMp0004, 28.6.1999 (RA12776); McMp0011, 7.10.1999 (RA12818); McMp0015, 7.10.1999 (RA12822); McMp0014, 7.10.1999 (RA12824); McMp0017 (R48), 7.10.1999 (RA12825); McMp0016(R28), 7.10.1999 (RA12826; RA12825); McMp0027, 22.10.1999 (RA12900); McMp0045, 8.4.2000 (RA12933); McMp0044, 8.4.2000 (RA12934); McMp0046/47, 8.4.2000 (RA12936). - RFLPs of the ECM RA12818, RA12822, RA12824, RA12825, RA12826, RA12900, RA12933, RA12934, and 12936 were compared with those of fruitbodies RA13079, RA13080 and revealed as being identical. – *Dermocybe cinnamomea* (L.: Fr.) Wünsche: McMp0026, 7.10.1999 (RA12890); McMp 0025, 7.10.1999 (RA12892); McMp0029, 22.10.1999 (RA 12903); McMp0028, 22.10.1999 (RA12904); McMp0032 (Q40), 4.12.1999 (RA12910). – *Elaphomyces granulatus* Fr. : McMp0018(R68), 7.10.1999 (RA12828); McMp0020(R37), 7.10.1999 (RA12829). – *Lactarius decipiens* Quél.: McMp 0008, 28.6.1999 (RA12782); McMp0009, 28.6.1999 (RA12783); McMp0025, 7.10.1999 (RA12891); McMp0026, 7.10.1999 (RA12889; RFLPs identical with those of fruitbodies RA12964, RA12966); McMp0027(R49), 22.10.1999 (RA12901; RFLPs identical with those of fruitbodies RA12964, RA12966); McMp0031, 22.10.1999 (RA12906; RFLPs identical with those of fruitbodies RA12964, RA12966); McMp0030, 22. 10. 1999 (RA12907); McMp0036, 4. 12. 1999 (RA12913; RFLPs identical with those of fruitbodies RA12964, RA12966); McMp0035, 4. 12. 1999 (RA 12914); McMp0040, 3. 4. 2000 (RA12923; RFLPs identical with those of fruitbodies RA12964, RA12966); McMp0042, 3. 4. 2000 (RA12931); McMp0043, 3. 4. 2000 (RA12928); McMp0050(Q50), 8. 4. 2000 (RA12940; RFLPs identical with those of fruitbodies RA12964, RA12966); McMp0050 (Q18), 8. 4. 2000 (RA12941; RFLPs identical with those of fruitbodies RA12964, RA12966); McMp0050(Q58), 8.4.2000 (RA12942; RFLPs identical with those of fruitbodies RA12964, RA12966). – *Piceirhiza cinnbadiosimilis,* unpubl.: McMp0029(R19), 22.10.1999 (RA12902); McMp0028 (R49), 22.10.1999 (RA12905); McMp0032(Q26), 4.12.1999 (RA12909); McMp0035(Q18), 4.12.1999 (RA12915). – *Piceirhiza nigripunctiformis*, unpubl.: McMp0012, 7.10.1999 (RA12819). – *Piceirhiza internicrassihyphis* (Agerer, in prep.): McMp0015, 7.10.1999 (RA12821); McMp0014, 7.10.1999 (RA12823); McMp0033(Q56), 4.12.1999 (RA12911). – *Piceirhiza subtilis* (HAUG & PRITSCH 1992): McMp0048, 8.4.2000 (RA12937). – *Piceirhiza nigripunctata* (Agerer, in prep.): McMp0048, 8.4.2000 (RA12938); McMp0049, 8.4.2000 (RA12939). – *Russula ochroleuca* (Pers.) Fr.: McMp0019, 7.10.1999 (RA12827); McMp0040, 3.4.2000 (RA12925); McMp0042, 3.4.2000 (RA12932). – *Tomentella* sp. 1, unpubl.: McMp0003, 28.6.1999 (RA12770). – *Tomentella* sp. 2, unpubl.: McMp0003, 28.6.1999 (RA 12771). – *Tomentella* sp. 4, unpubl.: McMp0013, 7.10.1999 (RA12820). – *Tomentella* sp. 5, unpubl.: McMp0039, 3.4.2000 (RA12922); McMp0044, 8.4.2000 (RA12935). – *Tylospora fibrillosa* (Burt) Donk: McMp0003, 28.6.1999 (RA 12774); McMp0007, 28.6.1999 (RA12777); McMp0041, 3.4.2000 (RA12927; RFLPs identical to those published by EBERHARDT, WALTER & KOTTKE 1998 for strain 1w10). – *Xerocomus badius* (Fr.) Kühn.: Gilb.: McMp0001, 28.6.1999 (RA12769); McMp0022(R27), 7.10.1999 (RA12830; RFLPs identical with those of fruitbodies RA12893); McMp 0021(R67), 7.10.1999 (RA12831). – *Xerocomus* cf. *chrysenteron* (Bull.: St. Amans) Quél.: McMp0023, 7.10.1999 (RA 12888); McMp0038(Q18-19), 3.4.2000 (RA12919; RFLPs identical with those of fruitbodies RA12896, RA12946); McMp0039, 3.4.2000 (RA12921); McMp0040, 3.4.2000 (RA12924; RFLPs identical with those of fruitbodies RA12896, 12946); McMp0043, 3.4.2000 (RA12929; RFLPs do not fit to either fruitbody tested above); McMp0042, 3.4.2000 (RA12930; RFLPs do not fit to either fruitbody tested above).

Collection data of fruitbodies: Germany, Bayern, district Aichach-Friedberg, between Odelzhausen and Mering, in the forest Höglwald near Tegernbach; in the forest near Zillenberg. Leg. R. Agerer, det. R. Agerer (all in M):

Cortinarius obtusus: 14.10.2000 (RA13079, RA13080). – *Lactarius decipiens*: 9.9.2000 (RA12964, RA12966). – *Xerocomus badius*: 22.10.1999 (RA12893). – *Xerocomus chrysenteron*: 22.10.1999 (RA12896), 12.8.2000 (RA12946).

Results

Seventeen different ectomycorrhizal anatomotypes were isolated and could be determined in part to species level due to anatomical features (Tab. 1). Only a small portion of the 50 McMp had the same anatomotype combinations, hence, depending upon the compared species, only 2 to 7 repetitions could be used to compare the distribution. Fourteen of the 50 McMp contained only a single anatomotype and could therefore not be used for statistical treatments of exclusion and association reactions. Particularly *Cortinarius obtusus* formed extended ectomycorrhizal patches (data not shown).

The following combinations and repetitions could be used for the analyses:

7 times: *Russula ochroleuca* vs. *Lactarius decipiens*.

4 times: *Russula ochroleuca* vs. *Cortinarius obtusus*. – *R. ochroleuca* vs. *Xerocomus* cf. *chrysenteron*. – *Cortinarius obtusus* vs. *Piceirhiza internicrassihyphis*.

3 times: *Russula ochroleuca* vs. *Piceirhiza cinnbadiosimilis*. – *R. ochroleuca* vs. *Dermocybe cinnamomea*. – *R. ochroleuca* vs. *Tylospora fibrillosa*. – *R. ochroleuca* vs. *Xerocomus badius*. – *Lactarius decipiens* vs. *Dermocybe cinnamomea*. – *Piceirhiza cinnbadiosimilis* vs. *Dermocybe cinnamomea*. – *Cortinarius obtusus* vs. *Tylospora fibrillosa*. – *Piceirhiza internicrassihyphis* vs. *Tylospora fibrillosa*. – *P. internicrassihyphis* vs. *Xerocomus badius*.

2 times: *Russula ochroleuca* vs. *Elaphomyces granulatus*. – *R. ochroleuca* vs. *Piceirhiza internicrassihyphis*. – *Lactarius decipiens* vs. *Piceirhiza cinnbadiosimilis*. – *L. decipiens* vs. *Xerocomus* cf. *chrysenteron*. – *Elaphomyces granulatus* vs. *Xerocomus badius*.

Russula ochroleuca and *Piceirhiza internicrassihyphis* show no common occurrence in the evaluated grids (Figs. 4a-c). However, this combination was only found twice. *Russula ochroleuca* and *Piceirhiza cinnbadiosimilis* have no common occurrence in 2.5 mm and 7.5 mm grid width, though they are

found together in 5 mm and in 10 mm grids (not shown); they are recorded together three times. Although a high standard deviation is apparent, *Russula ochroleuca* and *Xerocomus badius* have a rather high value of common occurrence in the 2.5 mm grid; this combination was recorded three times. The differences in comparison to the other anatomotypes level off in wider grids (Figs. 4a-c).

Elaphomyces granulatus and *Xerocomus badius*, recorded only twice together, do not occur in the three tested grid-widths (Figs. 5a-c). A very low association show *Lactarius decipiens* and *Xerocomus* cf. *chrysenteron* as well as *Piceirhiza cinnbadiosimilis* and *Dermocybe cinnamomea*; these combinations are recorded twice and three times, respectively. *Cortinarius obtusus* with *Piceirhiza internicrassihyphis* and *Piceirhiza internicrassihyphis* with *Xerocomus badius* have, in all grid widths, high values of co-occurrence, but particularly high values are evident in the smallest grids. The former combination is recorded four and the latter three times.

Discussion

The assertion of the value 'spatial relation' can best be discussed with *Russula ochroleuca* ECM. This species shows combinations with nine different species of two up to seven repetitions. In general, *R. ochroleuca* ECM could be found together with 11 species (anatomotypes) in the 50 McMp studied and in two further McMp it was the exclusive species. It occurs generally together with almost all other frequent anatomotypes. The next frequent combinations were those of *Tylospora fibrillosa* with 9, *Piceirhiza internicrassihyphis* with 8, and *Cortinarius obtusus* with 7 species. None of the species combinations reached the high number of repetitions as those with *R. ochroleuca*.

Several grid widths have been checked. The most informative are 2.5 mm, 5 mm and 7.5 mm (Figs. 4, 5). The other widths are too wide for an interpretation as to whether ectomycorrhizae exclude other species within short distances or are associated with them. The higher the grid width the higher the possibility that species, usually not growing close together, are found as being associated. For example, ectomycorrhizae of *Russula ochroleuca* appear combined with *P. internicrassihyphis* but only from the 17.5 mm wide grid (data not shown). High standard deviations are a key characteristic of these studies. This is due to the yet low number of replications.

Provided that the high standard deviation allows for a consistent conclusion, it appears that *Russula ochroleuca* and *Piceirhiza internicrassihyphis* exclude another (Fig. 4). This possibly also applies for *Russula ochroleuca* and *Piceirhiza cinnbadiosimilis* (Fig. 4a), as well as for *Elaphomyces granulatus* and *Xerocomus badius* (Figs. 5a-c). The common occurrence of *R. ochroleuca* and *P. cinnbadiosimilis* in the 5 mm grid and their presence in wider grids, but not in the 7.5 mm grid, is due to a methodological problem. Since 50 mm (the dimension of a McMp) is not divisible by 7.5 mm a marginal stripe of 5 mm is excluded from the analysis (Fig. 3, McMp 0035). Only one of the three replicates analysed suggested a spatial relation between these two species, and this McMp showed the marginal position of these ectomycorrhizae.

Associations between species are suggested by *Russula ochroleuca* and *Xerocomus badius* at closest distances (Fig. 4a), *Cortinarius obtusus* and *Piceirhiza internicrassihyphis* (Figs. 5a-c), and perhaps also between *Piceirhiza internicrassihyphis* and *Xerocomus badius* (Figs. 5a-c). All other combinations neither hint at an association nor an exclusion.

Several reasons for exclusions or associations of ectomycorrhizae in natural soils must be considered. Exclusion might be caused simply by soil heterogeneities already present before colonisation of the micro-site. Differential demands of ectomycorrhizae for soil conditions are well known. YANG et al. (1998) found a correspondence between the type and frequency of ectomycorrhizae and litter accumulation. MENGE, GRAND & HAINES (1977) reported on the influence of N-fertilisation on the composition of ectomycorrhizal types, an observation supported several times since (ALEXANDER & FAIRLEY 1983, ARNEBRANT & SÖDERSTRÖM 1992, ARNEBRANT 1996, TAYLOR & READ 1996, RUNION et al. 1997, FRANSSON, TAYLOR & FINLAY 2000). Furthermore, lime influences the associations of morphotypes (ERLAND & SÖDERSTRÖM 1990, ANTIBUS & LINKINS 1992). Moreover, it was recently shown that ectomycorrhizae of *Lactarius decipiens* are significantly correlated to different soil ion concentrations, including K, Mg and pH (Agerer & Göttlein, unpubl.). Soil pH is generally regarded as important for ectomycorrhizae (KUMPFER & HEYSER 1986, MCAFEE & FORTIN 1987, ERLAND & SÖDERSTRÖM 1990, VAN DER HEIJDEN & VOSATKA 1999) and was repeatedly shown as crucial for fruitbody occurrence of some species (TYLER 1985, AGERER 1990). Identical preferences of different ECM for soil conditions, on the other hand, may trigger associations of different species.

The manifestation of an exclusion might be the final state of a dynamic development beginning with an apparent association and continuing with a step-by-step overgrowth and replacement of one species that formerly occupied a special niche exclusively. Such a mechanism was studied by WU, NARA & HOGETSU (1999) in rhizotrones containing thin substrate layers. They provided evidence that the extramatrical mycelium, ECM, and rhizomorphs of *Pisolithus tinctorius* (Mich.: Pers.) Coker & Couch were discoloured and later displaced by the mycelium of an unidentified ectomycorrhizal isolate. In some cases, this overgrowth resulted in ECM composed of two fungal species. However, no interaction became apparent for mycelia of *P. tinctorius* and of *Suillus luteus* (L.: Fr.) S. F. Gray, although the mycelial amounts of *S. luteus* increased conversely to the diminishing ones of *P. tinctorius*. The replacement of *P. tinctorius* by the unknown ectomycorrhizal mycelium was explained by possibly different affinities to the host tree or to the soil conditions provided, which could be differently appropriate for the tested fungal species. In our studies, the close association of *Cortinarius obtusus* and

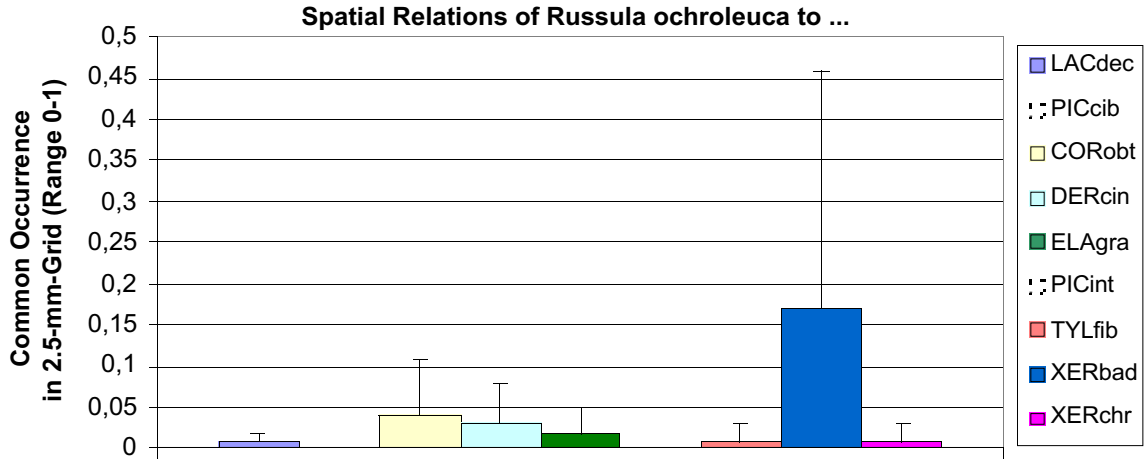

Fig. 4a

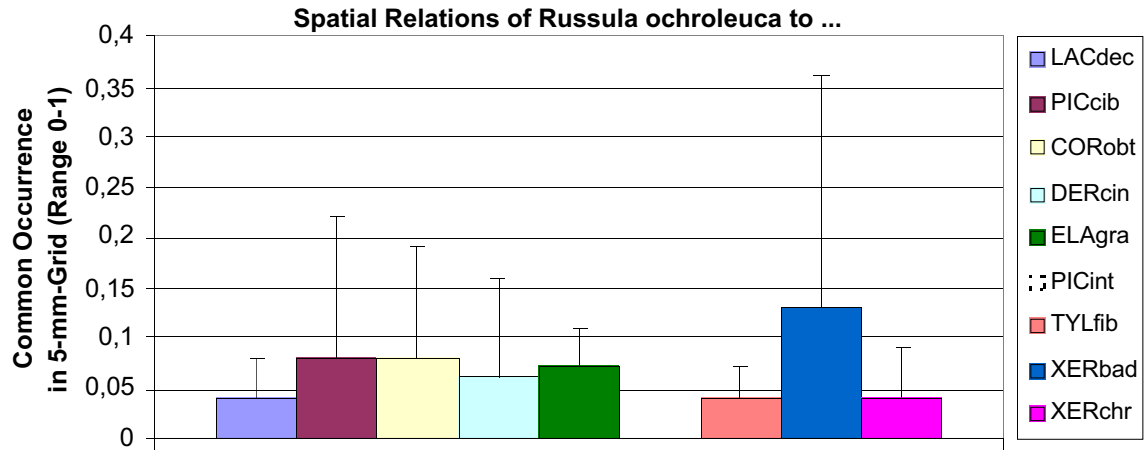

Fig. 4b

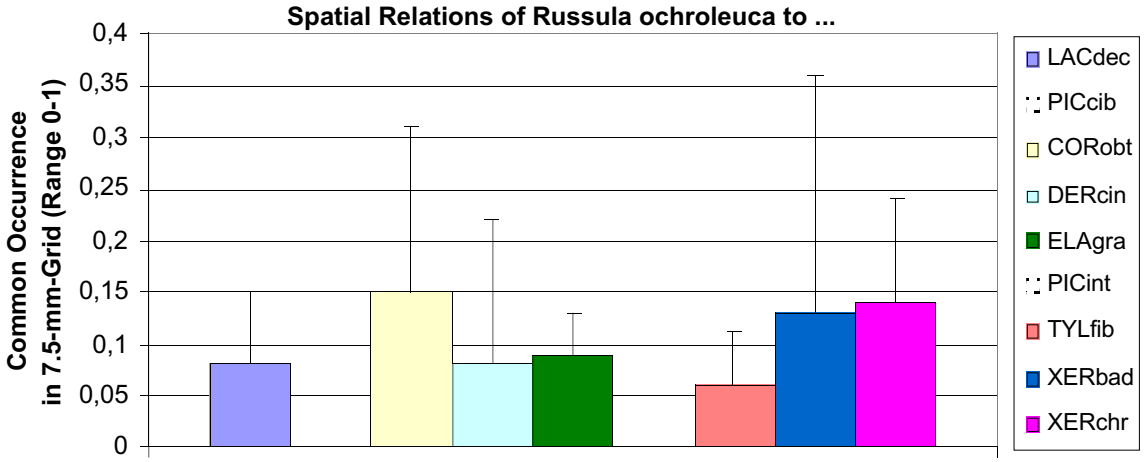

Fig. 4c

Fig. 4. Spatial relations between *Russula ochroleuca* and different species in grid widths of 2.5 mm (a), 5 mm (b) and 7.5 mm (c). For further explanations see text, abbreviations of species names are given in table 1.

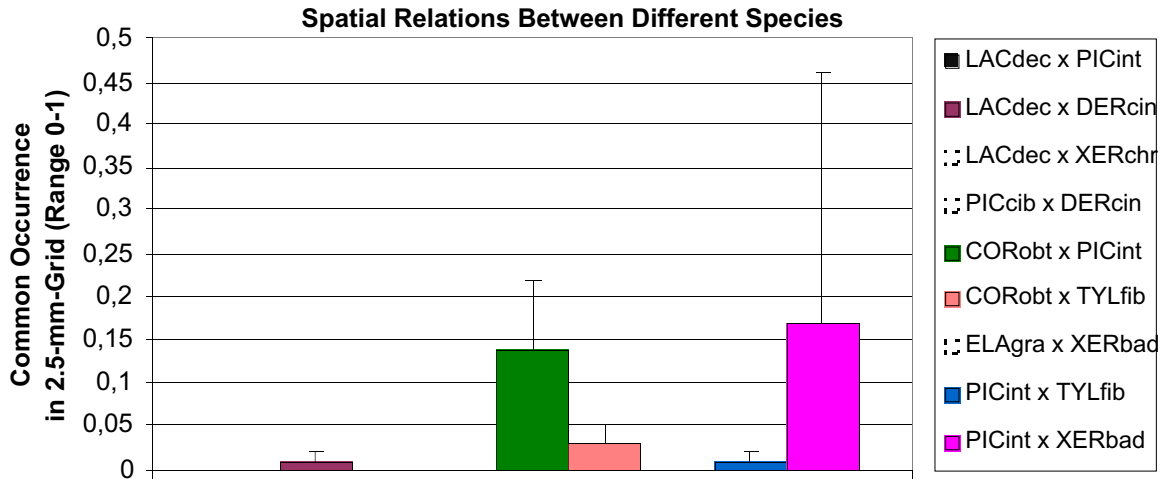

Fig. 5a

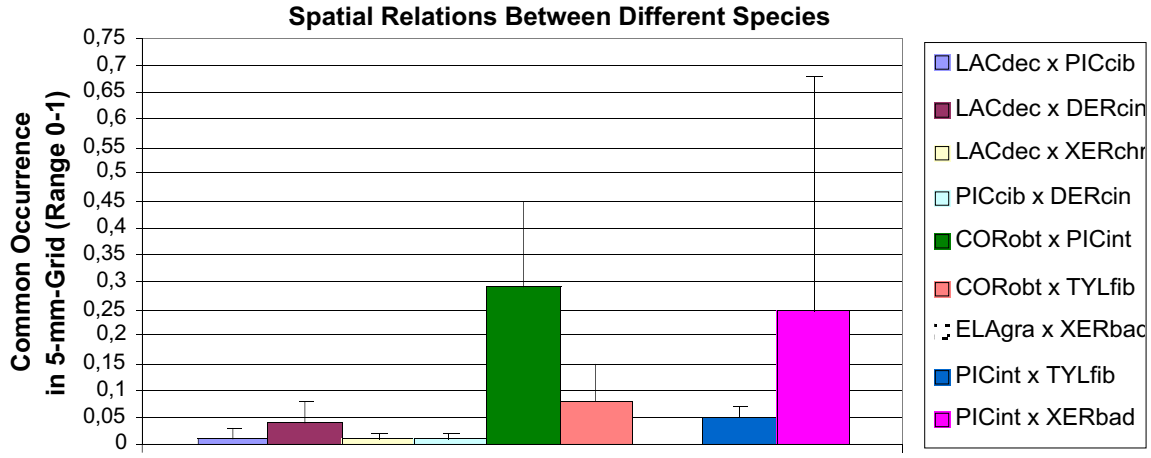

Fig. 5b

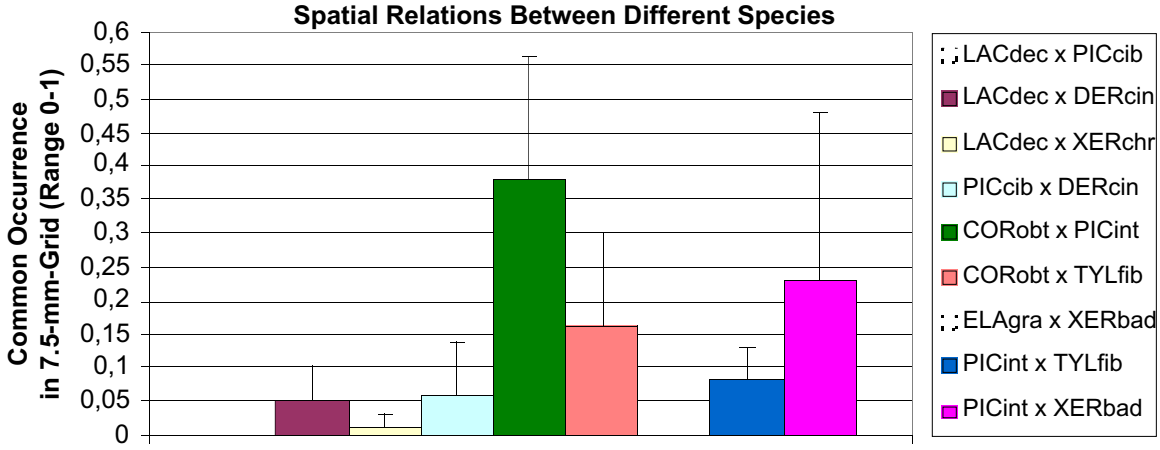

Fig. 5c

Fig. 5. Spatial relations between different species in grid widths of 2.5 mm (a), 5 mm (b) and 7.5 mm (c). For further explanations see text, abbreviations of species names are given in table 1.

Piceirhiza internicrassihyphis had, in few cases, also the appearance of a replacement reaction, since the typical white hyphae and rhizomorphs of *C. obtusus* grew on the brown mantle of *P. internicrassihyphis*. Sometimes also emerging root tips were occupied by *C. obtusus*. In addition, DAHLBERG, JONSSON & NYLUND (1997) obtained evidence for an exclusion of ECM, since they consistently found exclusively either *Tylospora fibrillosa* or *Piloderma croceum* Erikss. & Hjortst. ECM in 1.5 x 1.5 cm soil cores of their study plot. Species-specific and even genotype-dependent competition patterns were published by TIMONEN, TAMMI & SEN (1997) for *Suillus bovinus* (L.: Fr.) O. Kuntze and *S. variegatus* (Swartz: Fr.) O. Kuntze.

A reason for an association of different species can be a need for a one-sided or mutual enhancement of growth. A stimulation of hyphal growth is known for some species of Gomphidiaceae (AGERER 1996, OLSSON et al. 2000, AGERER 2001) by ECM and rhizomorphs of *Suillus* spp. and *Rhizopogon* spp., as hyphae of Gomphidiaceae can be frequently observed within the mantle, rhizomorphs and cortical cells of *Suillus* and *Rhizopogon* ECM. Also their ectomycorrhizae can sometimes be closely associated with those of *Suillus* and *Rhizopogon* (AGERER 1992). Furthermore, cultures of *Gomphidius roseus* (Fr.) P. Karst. could only be obtained, when fruitbody tissue of *G. roseus* was laid in close vicinity to *Suillus bovinus* fruitbody explants (AGERER 1991b). In an experiment by SHAW, DIGHTON & SANDERS (1995), who squeezed roots and inoculum between walls of glass tubes and terylene cloth, *Lactarius rufus* (Scop.) Fr. was seen to stimulate the colonisation of roots by *Suillus bovinus* and *Paxillus involutus* (Batsch) Fr. With *Laccaria laccata* (Scop.: Fr.) Berk. & Br., however, the ECM formation was suppressed for both species. The replacement reactions were basically explained by different growth rates of the ectomycorrhizal mycelia.

As a further possibility to prevent growth of different ectomycorrhizae in close proximity may be the formation of antifungal substances, directed against competitors. Such a capability has been proven in pure culture systems against parasitic fungi (MARX & DAVEY 1969, CHAKRAVARTY & HWANG 1991, BRANZANTI, ROCCA & ZAMBONELLI 1994). Different ectomycorrhizal fungi have not been tested in this respect and not at all conclusively in natural substrates.

Very limited interpretations can be attempted, based on the data of the present investigations, regarding distribution of hydrophilic and hydrophobic ECM (according to UNESTAM & SUN 1995) and their exploration types (according to AGERER 2001). Further studies have to show whether the impression is right that preferably hydrophobic and hydrophilic ECM are associated in comparison to ECM identical in that character. Fig. 4 suggests an association of the hydrophilic species *Russula ochroleuca* with the hydrophobic *Xerocomus badius*. Fig. 5 indicates the same relation between the hydrophobic *Cortinarius obtusus* and the hydrophilic *Piceirhiza internicrassihyphis,* and between *P. internicrassihyphis* and the hydrophobic *Xerocomus badius*. The present preliminary study suggests that the two hydrophilic species, *R. ochroleuca* and *P. internicrassihyphis,* possibly exclude one another (Fig. 4). *Elaphomyces granulatus* and *X. badius* (Fig. 5). which are hydrophilic and hydrophobic, respectively, appear as not being associated. In Fig. 3, McMp0002, McMp0032, McMp0034, and McMp0043 indicate again the above-mentioned relations between hydrophilic and hydrophobic ECM.

An association of a contact exploration type ECM (*Russula ochroleuca*) with a long distance exploration type (*Xerocomus badius*) is indicated in Fig. 4. *Cortinarius obtusus* (medium distance fringe) and *Piceirhiza internicrassihyphis* (medium distance smooth) belong to different exploration types, as do the possible associates *P. internicrassihyphis* and *X. badius*. Such a distribution would make ecological sense as the exploitation sites of these species differ, although the ECM grow closely together. The exploiting sites of a hydrophobic long distance exploration type, like *X. badius*, are the remote distal ends of the rhizomorph branches (RAIDL 1997) whereas the exploiting sites of the hydrophilic smooth exploration type, like *Piceirhiza internicrassihyphis*, are in the proximity of the ECM. Hence, in spite of their close neighbourhood, they can indeed occupy different ecological niches.

In summary, although there is preliminary evidence that ectomycorrhizae are not evenly distributed in the soil and they possibly indicate association with and exclusion of different species, much more detailed studies have to be performed. Definite reasons for uneven distribution patterns of ectomycorrhizae are still unknown. Future studies should focus on the distribution of heterogeneous micro-sites caused by patchy distribution of organic material and nutrients. Micro-scale analyses are hence needed. The method 'micromapping' could provide a basis for such studies.

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