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Microfungal community structure from forest soils in Northern Thrace Region, Turkey

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Abstract - The soil fungi in the pure stand of oak (*Quercus petraea*), beech (*Fagus orientalis*), and pine (*Pinus nigra*) were investigated by the dilution plate method at Yıldız Mountain in Thrace region. The mycobiota, as well as the number of isolates per plate, was determined at various soil depths. Principal component analysis of the soil profiles indicated that there was variation in mycobiota composition and the variation was attributed to differences among the ecosystems. When comparing conifer and hardwood soils, using Sorenson's similarity index, fungal community composition corresponded more closely between the hardwood stands than with the conifer stand. Fungal community composition appears to be influenced by the organic compounds entering soil from plant litter.

Key words: fungal community, soil profile, conifer-hardwood forest.

INTRODUCTION

Soil microfungi play a crucial role in nutrient cycling in forest ecosystems. They decompose complex organic matter and release mineral nutrients for plant uptake (Killham, 1994). Microbial communities are controlled primarily by plant species composition, soil type, seasonal variability in water, temperature and availability of organic substances (Mc Culley and Burke, 2004). Species composition of soil microfungal communities has been shown to be influenced by vegetation (Christensen, 1989; Saetre and Bååth, 2000). Species of tree would affect fungal community structure by altering edaphic and microclimatic conditions.

Many studies on soil microfungi and their interactions with environment have been performed on surface soils in Turkey (Hasenekoğlu, 1982; Asan and Ekmekci, 1994). However, relatively few studies have concentrated on the vertical distribution of fungi in soil profile in Turkish forest sites. Obviously, any explanation of the variation in vertical distribution of soil fungi must be based on an understanding of the factors that control the abundance, species composition, and activity of soil microfungi.

The purpose of this study was to determine the vertical distribution of microfungi in soil profiles from Oriental beech (*Fagus orientalis* Lipsky), Sessile oak [*Quercus petraea* (Mattuschka) Lieb.] and Austrian pine (*Pinus nigra* Arnold) forest sites. It is hypothesized that distinct fungal communities would be associated with three forest types, reflecting changes in the three species. Although climate and edaphic conditions are the primary regional controls of microfungi community structure, their influence on the vertical distribution of microfungi is largely unknown. The contribution to the knowledge of mycobiota of Turkish forest soils is another purpose of this study.

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MATERIALS AND METHODS

Site description. The research area is Demirköy vicinity (41º 51′ N, 27º 46′ E) situated on Northern Thrace in Turkey. The Northern Thrace forms a mountain barrier along the Black Sea. The climate of this region is humid mesothermal type, characterised by warm summers. The warmest temperature occurs in July with mean temperature of 20.7 ºC. January is the coldest month with mean temperature of 1.6 ºC. The annual mean precipitation is 817.9 mm. The principal geological formations are metamorphic rock (Mica schist) and granite. The stands (about 70 years old) are dominated by beech (*Fagus orientalis*), oak (*Quercus petraea*), and pine (*Pinus nigra*). Prevalent ground layer species consisted of *Erica arborea* L, *Erica verticillata* Forsk, and *Rhododendron ponticum* L.

Sampling and isolation methods. Three sites were selected to take soil samples. At each site three holes, about 1.5 m^2 was excavated to a depth of 1.2 m. The soil profiles were divided into three different soil horizons, namely A1 (humus stained topsoil), A2 (pale brown eluviation zone), and B (dark brown illuviation zone). Samples from mineral soil were collected after removal litter (Brown, 1958). Soil samples were extracted laterally from one side of the exposed profile using a clean trowel sterilized with 70% alcohol. Samples from the middle of each recognizable horizon were taken and placed separately in clean polyethylene bags.

Soil samples were stored in a refrigerator at 4 ºC for up to 72 h prior to use. Isolation of fungi from the soil samples was accomplished using the suspension plating technique (Bills, *et al*., 2004). Commonly a 10^{-4} suspension, made from 25 g soil (dry weight), yielded the desired abundance of colony forming units (CFU) per plate. Peptone dextrose agar with Rose-Bengal and streptomycin medium was used for isolation and counts of fungi (Martin, 1950). Five Petri dishes were parallel inoculated with 1 ml of the ultimate dilution. The dishes were incubated at 25-27 ºC for 6 to 7 days. CFU were enumerated and fungi were individually transferred to Potato dextrose agar slants. At a suitable age, the isolates were sorted according to their appearance of cultural characteristics. The identification of fungal isolates was made on various media such as Malt extract agar (MEA), Potato dextrose agar (PDA), Czapek yeast extract agar (CYA), Czapek dox agar (CA), 25% Glycerol nitrate agar (G25N). The measurements of the fungal structures were performed in lactophenol.

Identification of the isolates was carried out according to Raper and Fennell (1965), Ellis (1971), Pitt (1979),Gerlach and Nirenberg (1982), Nelson *et al*. (1983), Domsch *et al*. (1993), Barnett and Hunter (1998). Reference strains identified to species level have been deposited in the Culture Collections of KUKENS (WDCM101), Centre for Research and Application of Culture Collections of Microorganisms.

The additional larger soil samples were collected in each soil profile for determination of pH, particle size distribution, moisture content, and organic carbon. The soil samples were air dried and sieved by 2 mm sieve. The pH was measured in a 1:2.5 mixture of soil and distilled water using a glass electrode. Soil moisture was determined gravimetrically. Particle size distribution of soils was measured by Bouyoucos hydrometer method (Bouyoucos, 1962). Total organic carbon was estimated using Walkley-Black wet oxidation method (Walkley and Black, 1934). All measurements were made in three different soil horizons (A1, A2, and B) and replicated thrice.

Data analysis. To compare the fungal community of different soils, Sorenson's similarity index (Ss) was used (Krebs, 1989):

$$Ss = 2a / 2a + b + c$$

where a is the number of species found both in samples A and B, b is the number of species found only in sample B (not in sample A), and c is the number of species found only in sample A (not in sample B). Coefficients may vary between 0, when the two communities have no species in common, and 1, when they have all species in common.

Principal components analysis (PCA) was performed to compare the soil horizons and tree species with respect to their fungal species composition. R-technique, calculated from a correlation matrix based on the Pearson correlation coefficient was used.

Paired *t* tests were performed to determine the effect of soil depth on fungal abundance calculations were done by using SPSS 8.0.

RESULTS

Soil characteristics

The pH values of soil horizons were low, but the pine stand soil was slightly acid, being 5.01, in the B horizon (Table 1). Organic matter contents are compared in Table 1. In the A1 horizons, organic matter content of Pine soil was around 4.79% and lower than in Oak and Beech soils. Organic matter was significantly higher in the A1 horizon than in the subsurface soils. The finest textured soils occurred at Beech stand. Soil moisture mirrored these textural trends, with highest soil moisture corresponding to the finest soils.

Fungal numbers

In order to facilitate quantitative comparison among forest sites, the numbers of soil fungi from different soil horizons are shown in the Fig. 1. The number of soil fungi continuously decreased with depth in all forest type. A2 horizon of beech and oak soils contained 88.6% and 81.68% less microfungi, respectively, than that in the A1 horizons. In the pine profile there was a 54.72% reduction in microfungi number from the A1 to A2 horizons. In the A1 and A2 horizons, fungal counts were significantly ($p < 0.05$) different by stand type. However, there was no significant difference ($p < 0.05$) between beech and pine forest soil in the B horizon.

Fungal community structure

Total 48 taxa were isolated during the study. Tables 2-4 give complete list of fungi isolated from each soil horizon. There was a strong dominance of *Penicillium* in the whole soil profile. In the all forest type, 11-16 *Penicillium* species accounted for 35-50% of the taxa.

Comparison of oak, beech, and pine forest soil microfungi using the coefficient of similarity (Sorenson's index) in each soil horizon are presented in Table 5. In the A1 horizons, the highest coefficient value was recorded between oak and beech soil (0.78), where they share 18 fungal species. High similarity values indicated that these stand posses species common to each other as well as their

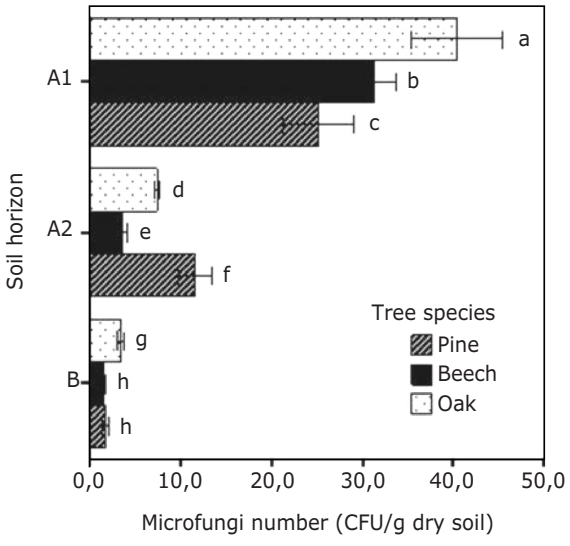


FIG. 1 - Depth profile diagram comparing fungal numbers of oak, beech, and pine stand in the different soil horizons (all bars represent mean ± s.e.). Significant differences ($p < 0.05$) among the stand type and soil horizons are represented by different letters on the graph.

TABLE 1 - Properties of soil horizons (A1, A2, and B) at three Turkish forest sites

Properties of soil horizons	Tree species		
	Sessile oak	Oriental beech	Austrian pine
pH (H_2O)			
A1	4.39 ± 0.48[a]	4.44 ± 0.47[a]	4.57 ± 0.31[a]
A2	4.61 ± 0.28[ab]	4.51 ± 0.26[b]	4.73 ± 0.38[a]
B	4.57 ± 0.29[b]	4.64 ± 0.37[b]	5.01 ± 0.28[a]
Organic matter (%)			
A1	5.97 ± 1.25[a]	6.34 ± 1.09[a]	4.79 ± 0.96[b]
A2	2.10 ± 0.36[a]	2.24 ± 0.76[a]	1.94 ± 0.23[a]
B	1.45 ± 0.27[a]	0.93 ± 0.18[b]	1.03 ± 0.12[b]
Gravimetric H_2O (%)			
A1	21.95 ± 1.37[b]	28.31 ± 1.96[a]	19.68 ± 2.27[b]
A2	16.87 ± 5.23[b]	21.65 ± 2.10[a]	18.77 ± 1.77[b]
B	20.14 ± 0.66[b]	22.19 ± 1.21[a]	19.53 ± 1.52[b]
Sand and clay (%)			
A1	74-11	51-27	78-11
A2	74-15	43-25	74-17
B	65-17	40-32	59-28
Textural class			
A1	Sandy loam	Sandy clay loam	Sandy loam
A2	Sandy loam	Loam	Sandy loam
B	Sandy loam	Clay loam	Sandy clay loam

Values are averages ± Standard deviations. Different letters indicate significant differences between within means rows (p-value ≤ 0.05)

TABLE 2 - Fungi isolated from soil under *Fagus orientalis*

Organism	Soil horizons		
	A1	A2	B
Acremonium exiguum W. Gams	X		X
Acremonium rutilum W. Gams		X	
Chaetomium anguipilium L.M. Ames			X
Mortierella sp. 1	X		
Mortierella sp. 2	X		X
Mortierella sp. 3	X		
Mucor circinelloides Tiegh.	X		
Mucor sp. 1	X	X	
Mucor sp. 2	X	X	
Mucor sp. 3	X	X	
Myrothecium sp.	X		
Oidiodendron tenuissimum (Peck)			X
Paecilomyces farinosus (Holmsk.)	X		
Paecilomyces lilacinus (Thom) Samson	X		
Paecilomyces sp.	X		
Penicillium citreonigrum Dierckx	X		
Penicillium corylophilum Dierckx	X	X	X
Penicillium griseoroseum Dierckx	X	X	
Penicillium melinii Thom	X	X	X
Penicillium montanense Christensen & Backus		X	
Penicilliumochrochloron Biourge	X		
Penicillium paxilli Bainier	X		
Penicillium restrictum J.C. Gilman & E.V. Abbott	X	X	
Penicillium simplicissimum (Oudem.) Thom	X	X	X
Penicillium vinaceum J.C. Gilman & E.V. Abbott		X	
Penicillium waksmanii K.M. Zalessky	X		
Scopulariopsis brevicaulis (Sacc.) Bainier	X	X	
Scopulariopsis brumptii Salv.-Duval	X	X	
Thysanophora penicillioides (Roum) W.B. Kendr.		X	
Trichoderma sp. 1	X	X	X
Trichoderma sp. 2	X	X	X

TABLE 3 - Fungi isolated from soil under *Quercus petreae*

Organism	Soil horizons		
	A1	A2	B
Acremonium kilinese W. Gams	X	X	
Acremonium rutilum W. Gams		X	
Basipetospora sp.	X		
Humicola parvispora Gambogi		X	
Mortierella sp. 1	X	X	X
Mortierella sp. 2	X	X	X
Mortierella sp. 3	X	X	
Mucor sp. 1	X	X	
Mucor sp. 2	X		
Mucor sp. 3	X		
Myrothecium sp.	X		
Paecilomyces sp.	X		
Penicillium brevicompactum Dierckx	X	X	
Penicillium citreonigrum Dierckx	X	X	
Penicillium corylophilum Dierck*x*	X	X	X
Penicillium janthinellum Biourge	X	X	
Penicillium melinii Thom	X	X	X
Penicillium montanense Christensen & Backus	X	X	X
Penicillium ochrochloron Biourge	X		
Penicillium paxilli Bainier	X	X	X
Penicillium restrictum J.C. Gilman & E.V. Abbott	X	X	X
Penicillium simplicissimum (Oudem.) Thom	X	X	
Penicillium vinaceum J.C. Gilman & E.V. Abbott	X	X	
Penicillium waksmanii K.M. Zalessky	X	X	X
Scopulariopsis brevicaulis (Sacc.) Bainier	X	X	X
Trichoderma sp. 1	X	X	X
Trichoderma sp. 2	X	X	X

TABLE 4 - Fungi isolated from soil under *Pinus nigra*

Organism	Soil horizons		
	A1	A2	B
Acremonium exiguum W. Gams			X
Alternaria tenuissima (Kunze) Wiltshire			X
Aspergillus glaucoaffinis Samson & W. Gams	X		
Cladosporium herbarum (Pers.) Link			X
Gliocladium roseum Bainier		X	X
Gliocladium vermoeseni (Biourge) Thom		X	
Humicola grisea Traaen			X
Humicola parvispora Gambogi	X		
Mortierella sp. 2		X	
Mucor sp. 3	X	X	X
Paecilomyces farinosus (Holmsk.)	X	X	X
Paecilomyces variotti Bainier		X	X
Penicillium brevicompactum Dierckx		X	
Penicilliumcitrinum Thom		X	
Penicillium citreonigrum Dierckx	X	X	
Penicillium corylophilum Dierckx	X	X	X
Penicillium decumbens Thom	X		
Penicillium janthinellum Biourge	X	X	X
Penicillium jensenii K.M. Zalessky	X		
Penicillium melinii Thom	X	X	
Penicillium montanense Christensen & Backus		X	
Penicillium paxilli Bainier	X	X	X
Penicillium purpurogenum Stoll			X
Penicillium restrictum J.C. Gilman & E.V. Abbott	X		X
Penicillium simplicissimum (Oudem.) Thom	X	X	
Penicillium variabile Sopp	X		X
Penicillium vinaceum J.C. Gilman & E.V. Abbott	X	X	
Penicillium waksmanii K.M. Zalessky	X		
Scopulariopsis brevicaulis (Sacc.) Bainier	X		X
Thysanophora penicillioides (Roum) W.B. Kendr.	X		X
Trichoderma sp. 1	X	X	X
Trichoderma sp. 2	X	X	X

TABLE 5 - Sorenson's similarity coefficients for fungal community in mineral soil horizons of oak, beech, and pine forest sites

	A1				A2				A3			
	Oak	Beech	Pine		Oak	Beech	Pine		Oak	Beech	Pine	
Oak	1	0.78	0.43	Oak	1	0.57	0.64	Oak	1	0.38	0.40	
Beech		1	0.53	Beech		1	0.47	Beech		1	0.29	
Pine			1	Pine			1	Pine			1	

own distinctive assemblages. In this horizon, oak and pine soil share only 12 fungal species and thus have the lowest similarity (0.43). In the case of A2 and B horizons, the highest similarities occurred between oak and pine forest soil community (0.64 and 0.40).

Principal components analysis (PCA) indicated two components explaining 51.36% of the total variance in the composition of the fungal community. The ordination of different horizon for the first two principal component axes is shown Fig. 2. The two axes accounted for 31.69 and 19.67% of the total variation, respectively.

There was marked variation in microfungal community composition among soils from three sites, primarily due to tree species. PC1 showed a strong separation of oak and beech forest from pine forest, which clustered close together with respect to PC1. A1 horizon of oak and beech forest site located in the third quadrants and their position indicate a strong resemblance microfungal community between oak and beech soil.

The second principal component differentiated soil horizons from pine forest from those of other two forest type (oak and beech). Soil horizons of pine forest are clustered at one end of the second axis and distant from oak and beech forest centres (Fig. 2).

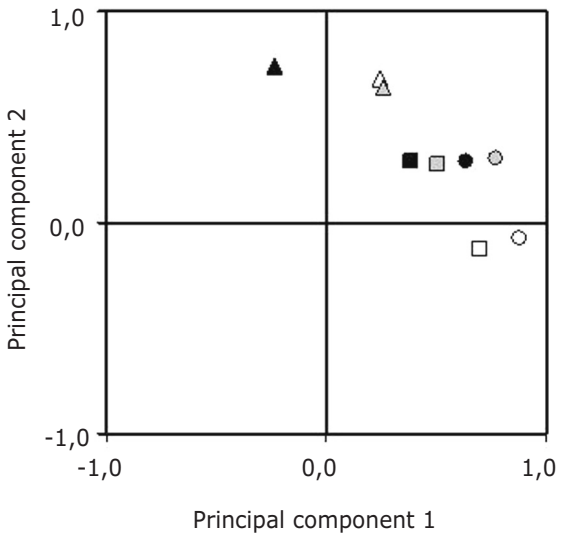


FIG. 2 - Ordination of the horizons on the first two principal components. PC1 represents 31.69% and PC2 19.67% of the total variation. Soil horizons of oak forest: ○A1, ●A2, ●B; beech forest □A1, ■A2, ■B; pine forest △A1, ▲A2, ▲B.

DISCUSSION

Fungal abundance

The number of fungi in the soil profiles continuously decreased from top to bottom in all sites (Fig. 1). Typically, soil fungal numbers have been found proportionately more numerous in the A1 horizons, as do the organic matter, and moisture. Similarly, it was noted that edaphic properties of surface soils supported more soil microbes under native forest site (Tate, 2000). There were significant differences in fungal abundance isolated from A1 horizon of each tree species (Fig. 1). Fungal abundance in pine forest was lower than that of deciduous forest. Two explanations may account for this reduced fungal abundance. First, the lower of organic matter content in A1 horizon of pine forest can reduce microfungus abundance (Behera and Mukerji, 1985). Secondly, decreases in the fungal abundance probably result from a variation in the chemical composition of pine litter. Previous works (Christensen, 1969; Jensen, 1974) were able to detect variability in microbial community depending on the type of litter entering soil. In the A2 horizon of beech, however, there was a noticeable decrease in the fungal abundance. While both organic matter and fungal abundance decrease with depth in all sites, it is interesting to note that a sudden drop in numbers occurs in the A2 horizon of beech, although the organic matter content is higher than other stand type. This is probably due to the poor aeration on fine textured soils. Gravimetric water content of beech soil mirrored these textural trends (Table 1). Fine textured soils allow relatively slow gas and water movement because of the dominance of micropores. Pore sizes also determine entrance into and occupancy by soil fungi. They usually do not enter micropores (Paul and Clark, 1996). In the pine and oak profile, there was a limited reduction in fungal abundance from the A1 to the B horizon compared with beech forest soils. These results were consistent with expectations based on the soil physical characteristics. The beech forest soil was set apart from the other two forest soils by contrasting soil condition, such as higher amount of clay, higher water content, and lower aeration (Table 1).

Fungal community structure

The results of this study suggested that *Penicillium* species were noticeably abundant and they contributed relatively high proportions of total taxa (35-50%). Similarly, Christensen *at al*. (2000) stated that *Penicillium* was a prominent genus at mid latitudes in the northern hemisphere, which was 27-49% of total fungal species.

Associated with *Penicillium*, *Trichoderma*, *Mortierella*, and *Mucor* also occurred frequently. These microfungi are a group, which appear to be prominent in acid forest soils. Although *Aspergillus* spp. and *Fusarium* spp. were common in soil, they were isolated rarely in present study. *Fusarium* is a commonly encountered genus in grassland soils (Ranzoni, 1968; Gochenaur, 1970; Christensen, 1981). Given these previous studies and the known differences in vegetation, we believe this result may be related to forest soil characteristics. In addition to the effect of vegetation differences, *Trichoderma* sp., an abundant fungal species in this study, may suppress *Fusarium* sp. Some investigators also reported that *Trichoderma* sp. were predator of *Fusarium* sp. (Papavizas, 1985; Sivan *et al*., 1987). *Aspergillus* species was found very low in the present study. This is consistent with the results of Christensen and Tuthill (1985); and Klich (2002), who reported that *Aspergillus* species, which were isolated as common microfungi of tropical and subtropical soils, decreased towards higher latitudes.

Examination of similarity coefficients reveals that there was a difference among the fungal communities of different tree species (Table 5) In the A1 horizon, the highest similarity coefficient is obtained by comparing oak and beech forest. On the other hand, A1 horizon of pine stand soil and oak stand soil has the lowest similarity. Community composition of microfungi in coniferous forests is generally different from that of deciduous forests because of the chemical content of litter. Kuiters (1990) reported effects of pine on the topsoil, decrease in biological activity are mainly due to the richness of pine litter in terpenic and phenolic compounds. Further study also showed that secondary compounds found in high concentration in the pine litter, especially ferulic acids, might have fundamental effects on microbial community (Priha *et al*., 2001).

In contrast to A1 horizon, it is found that the highest similarity in subsurface soil fungi community occurs between pine and oak forest, which can be explained, in part; by chemical differences in the organic compounds that have leached downward. Depending on tree species, there are differences in the leaching of substances from litter. The waxes of the surface layer and high concentration of lignin and other polyphenolic compounds make needle litter difficult to decompose, whereas leaf litter contains more easily leached and decomposed water-soluble compounds: sugars, amino acids and aliphatic acids (Harris and Safford, 1996). It is possible that the leaching of recalcitrant substances from pine litter is lower in soil profile; otherwise, they would have been inhibitory to fungal community in subsurface soil. Most likely, this is due to the major controlling factors of soil, such as soil porosity, structure, texture, and moisture, being larger in the subsoil.

Previous works have shown that tree cover is one of the major factors determining the characteristics of forest topsoil (Berg and Wessén, 1984). It is thus considered that the differential effects of tree species on microbial community were clearly on surface soil compared to subsoil. Christensen (1969, 1981) showed that microfungal species composition in A1 horizon soil is strongly correlated with plant species composition in the overlying vegetation.

PCA analysis (Fig. 2) of soil horizons also revealed that there were differences and these differences are related to tree species. The variable loadings of the horizons formed two clusters corresponding to the ordination of the soils from three sites, indicative of distinct fungal communities at each of the tree sites. PC1 separated soil horizons from the oak and beech site from those of the pine site (Fig. 2). A1 horizon of oak and beech forest sites located in the third quadrants and corresponded with high loadings on the first component. Microfungal community composition data (PC1 and PC2) consistent with similarity index value, also suggested that variability in tree species supported different microfungi composition, at least, in top soil.

The 12 species, which were obtained in just Pine stand, were well suited this site, since that species were not encountered in oak and beech forest stands. These data would also suggest that the pine stand contained species different from other forest type, as well as common species.

We have learned about the similarities and differences in microfungal community composition of soil in Turkish forest sites. Differences in community composition detected between conifer and hardwood forests support the idea that different forest types have distinct soil microfungal communities. However, many aspects of community structure were not accounted for by the forest type only, and were likely to be related to other environmental parameters. Further research is needed to address these factors contributing to differences in the fungal communities. The data collected in this study provide a unique dataset that can be used to monitor the future changes of soil microfungi composition in the research area.

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