Diversity of microfungi and yeasts in soils of the alluvial zone national park along the river Danube downstream of Vienna, Austria ("Nationalpark Donauauen")

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Mikropilze und Hefen in Auwaldböden und bewirtschafteten Böden im Nationalpark Donauauen

1. Introduction

Soil is a very species-rich habitat containing all major groups of microorganisms like bacteria, algae, protists and fungi (HAGVAR, 1998). The great majority of fungal species have at least some part of their life cycle in soil (BRIDGE and SPOONER, 2001). The soil microcommunity plays a vital role for the global element cycles and thus for life on earth, because 60–90 % of the whole terrestrial primary production is decomposed in the soil, and furthermore many waste products of human society are detoxified there (GILLER, 1996). Fungi play a fundamental role for the functioning of the ecosystem soil (DORAN and PARKIN, 1994, 1996; HAWKSWORTH et al., 1996) and due to their ability to decompose complex macromolecules like lignin or chitin they are essential for making the nutrients like C, N, P, S available. Moreover the fungal mycelium plays an important role for the stabilisation of the soil because it binds soil aggregates and thus reduces erosion and helps to increase the waterholding capacity (KENNEDY and GEWIN, 1997). Soils also contain a lot of biotechnologically and pharmaceutically important fungi; penicillin and cyclosporin are two well known fungal products. The biological diversity in soil is closely related to abiotic and biotic factors (HUSTON, 1979; SHMIDA and WILSON, 1985; GRUBB, 1987), but soil moisture is generally assumed to be more important for

Zusammenfassung

Die Diversität der Mikropilz- und Hefeflora wurde im Boden und in der Streuschicht an sechs verschiedenen Stellen im Nationalpark Donauauen flußabwärts von Wien in einem periodisch überfluteten Gebiet bei Mannswörth (offene Au) und in einer trockeneren Region in der Nähe von Gr. Enzersdorf (gedämmte Au) untersucht, jeweils unter *Salix sp.* und *Populus sp.* Beständen. Zusätzlich wurden zwei landwirtschaftliche Standorte bei Gr. Enzersdorf untersucht, ein konventionell (außerhalb des Nationalparks) und ein ökologisch bewirtschafteter. An jedem Standort wurden Proben aus der Streuschicht, aus 0-5, 10-15 und 30-35 cm Tiefe entnommen. Die Mycelpilze wurden morphologisch auf Gattungsebene bestimmt, die Hefen wurden mittels Sequenzierung der D1-D2 Region der 26S-rDNA identifiziert. Es wurden 739 Mycelpilzstämme aus 35 anamorphen Gattungen und 129 Hefestämme aus 13 Gattungen isoliert.

Schlagworte: Mikropilze, Hefen, Diversität, Streuschicht, Boden.

Summary

The diversity of microfungi and yeasts in different soils in a river-floodplain landscape at the river Danube downstream of Vienna, Austria was analysed. Soil samples were taken from six different sites: under *Salix sp.*- and *Populus sp.* trees in a seasonally flooded and a not flooded forest and from agricultural fields with ecological and conventional farming. Material was taken from the litter and from three soil cores: 0-5, 10-15 and 30-35 cm. Fungi were identified morphologically to genus level, yeast strains by sequencing the D1-D2 region of the 26S-rDNA. The seasonally flooded and the not flooded forest showed no difference, but the diversity of microfungi was higher under *Populus* trees.

Key words: fungi, yeast, diversity, litter, soil.

microorganisms than temperature and pH-value (DONNELY et al., 1990).

A lot of studies were carried out world wide on the diversity of microfungi and yeasts in soil (BAATH, 1981; BABJEVA and CHERNOW, 1995; BABJEVA and RESHETOVA, 1998; BENKOVÁ, 1999; BUCKOVÁ et al., 2000; CABELLO and ARAMBARRI, 2002; KOK et al., 1984; PERSIANI et al., 1998; SAMPÒ et al., 1997; SLÁVIKOVÁ and VADKERTIOVÁ, 2000; VISHNIAC, 1996). However, from Austria there are only very few and relatively old data known in the literature (JANKE and HOLZER, 1929; SZILVINYI, 1941).

Thus, it was the aim of this study to acquire new data about fungi in soil, including both filamentous fungi and yeasts. To record data from a relatively undisturbed area, different sites in the "Nationalpark Donauauen" were chosen.

River-floodplain landscapes are dynamic areas with high biodiversity, especially for the complex downstream of Vienna along the river Danube a species-rich fauna and flora is recorded. This landscape is accepted as an ecosystem extremely worthy of protection, therefore it has been designated as a National Park ("Nationalpark Donauauen") (TOCKNER et al., 1998).

2. Materials and Methods

2.1 Sampling

Soil samples were collected underneath trees of *Salix sp.* and *Populus sp.* (both *Salicaceae*) both located in a seasonally flooded forest (next Mannswörth) and in a not flooded forest (next Gr. Enzersdorf) (Fig. 1, 2), and from two agricultural fields (ecological and conventional farming, also near Gr. Enzersdorf). All sites except the agricultural field with conventional farming are located in the "Nationalpark Donauauen" near Vienna.

The *Salix*-location near Mannswörth is relatively young with sandy sediments, loosely bedded and well ventilated. The typical tree is *Salix alba*, additional species are *Populus x canescens*, *P. nigra* and *P. alba.* The shrub layer is dominated by *Humulus lupulus*, dominant in the herbal layer is *Urtica dioica* (indicator for very high nitrogen content), additional species are *Rubus caesius* and *Phalaris arundinacea.*

The *Populus*-location arose by siltation of old watercourses, with high amounts of clay and silt, it is poorly ventilated with spots caused by rust and reduction. *Populus x canescens* is the typical tree in this habitat, followed by

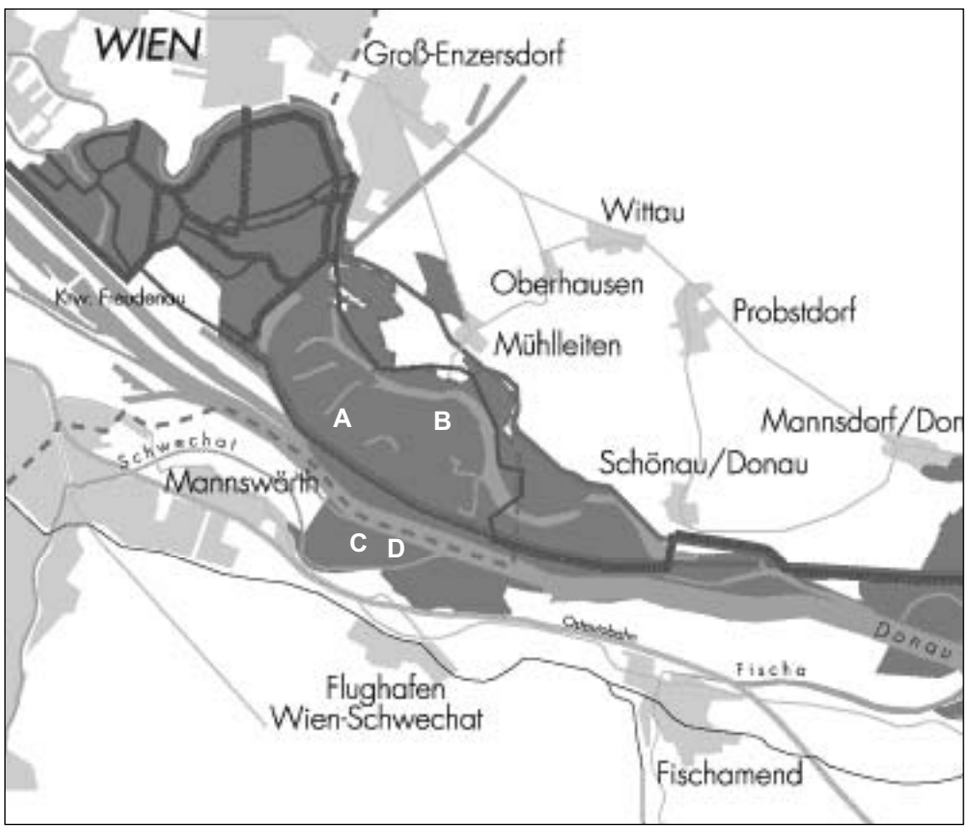

Figure 1: Map showing the regions of the river-floodplain landscape ("Nationalpark Donauauen") at the river Danube downstream of Vienna where the sampling points were situated
A ... *Salix*-, B ... *Populus*-location, both in the not-flooded forest and C ... *Populus*-, D ... *Salix*-location in the saisonally flooded forest (map from www.donauauen.at).

Abbildung 1: Lage der Probenahmestellen im Nationalpark Donauauen östlich von Wien
A ... *Salix*-, B ... *Populus*-Wald, beide im nicht überfluteten Wald; C ... *Populus*-, D ... *Salix*-Wald im saisonal überfluteten Auwald (Plan von www.donauauen.at).

Figure 2:
Pictures of the different sampling points in the forest
A ... *Salix*-, B ... *Populus*-locations near Gr. Enzersdorf, C ... *Salix*-, D ... *Populus*-location near Mannswörth.

Abbildung 2:
Bilder der verschiedenen Probenahmestellen im Auwald
A ... *Salix*-, B ... *Populus*-Standorte bei Gr. Enzersdorf, C ... *Salix*-, D ... *Populus*-Standorte nahe Mannswörth.

Ulmus laevis and *Salix x rubens*. In the shrub layer *Populus x canescens* and *Acer negundo* are the dominant species. The herbal layer is dominated by *Rubus caesius* and *Phalaris arundinacea* additional species are *Carex acuta* and *C. riparia* (both indicators for a high moisture content, ELLENBERG et al., 1992).

The locations next Gr. Enzersdorf represent a further level of siltation, with a lower groundwater table and therefore lower level of water supply. Calcaric fluvisols are typical for these locations. Typical for the *Populus*-location is *Populus x canescens*, additional species are *P. alba* and *Fraxinus excelsior*. In the shrub layer the most frequent species are *Juglans regia* and *Cornus sanguinea*. Dominant in the herbal layer are *Rubus caesius*, *Aegopodium podagraria* and *Salvia glutinosa*, additional species are *Physalis alkekengi*, *Parietaria officinalis* and *Solidago gigantea*. Mediate to high levels of nitrogen are indicated by *J. regia, S. glutinosa, F. excelsior, P. alkekengi, P. officinalis, R. caesius, A. podagraria and S. gigantea.* Mediate to high levels of moisture are indicated by *Populus x canescens* and *P. alba.*

Dominant in tree layer of the *Salix*-location are *Salix alba* and *Ulmus laevis*; followed by *Crataegus monogyna* (indicating drier site conditions). Dominant in shrub layer is *Clematis vitalba*; the additional species is *Cornus sanguinea.* Dominant in herbal layer is *Parietaria officinalis*; additional species are *Rubus caesius*, *Stachys sylvatica, Aegopodium podagraria, Brachypodium sylvaticum, Arctium tomentosum* (indicator for high nitrogen content) and *Viola cf. suavis*. *Salix alba* and *Ulmus laevis* may indicate a higher moisture content compared with the poplar stand [Identification of species according to ADLER et al. (1994)].

The pH-value was slightly basic in all cases, ranging from 7,5 to 8,0 (soil data from EBERL, unpublished).

The sampling dates were: November 1999, April 2000 and May 2001.

Material was taken from the litter, from 0–5 cm, 10–15 cm (both in the top soil level A) and from 30–35 cm depth. Several grams of soil were picked up from three different spots at each horizon and the three samples were mixed well.

2.2 Isolation of fungi

The litter samples were shaken with ¼-strength Ringer solution (MERCK, Darmstadt, Germany) and plated out. To remove conidia from the soil particles, the soil samples were washed through four sieves with different pore size, the smallest one of 0,06 mm mesh size. Therein, the small particles were restrained and were plated on agar.

Three different media were used: (1) 2 % malt extract agar (20 g malt extract, 1,0 g peptone, 20 g glucose, 20 g agar in 1 l water), (2) corn meal-agar (DIFCO, Detroit, USA) and (3) dichloran rose bengal agar (MERCK). The first two media were supplemented with ampicillin, streptomycin and chloramphenicol, each 50 mg/l. The plates were incubated at room temperature. Arising cultures were picked up and purified by one to several transfers onto malt extract agar. Yeast cultures were purified on potato-dextrose-agar.

2.3 Identification and conservation of fungal strains

Hyphomycetes were identified light-microscopically based on their morphology up to the genus level (ARX, 1981; BARNETT and HUNTER, 1999; DOMSCH et al., 1993; ELLIS, 1993; KIFFER and MORELET, 2000).

A correct identification of yeasts is not possible with morphological or physiological methods, therefore they were identified by sequencing the D1–D2 region of the 26S rDNA. DNA was extracted by using the hexadecyltrimethylammoniumbromide (CTAB) method as described in MESSNER et al. (1994). Amplification was performed in 150 µl volumes containing 10 mM KCl, 20 mM Tris-HCl (pH 8,8), 10 mM $(NH_4)_2SO_4$, 0,1 % Triton-X, 4,5 mM $MgSO_4$, 0,2 mM of each deoxynucleotide triphosphate, 2,5U Taq DNA polymerase (GENECRAFT, Münster, Germany) and 150 ng of each primer. Primers NL1 (GCATATCAATAAGCGGAGGAAAAG) and NL4 (GGTCCGTGTTTCAAGACGG) were used. The mixture was overlaid with 50 µl of light mineral oil. Amplification was performed as follows (Trio-Thermoblock TB1 Thermocycler, BIOMETRA, Göttingen, Germany): 35 cycles of: denaturation at 98 °C for 15 sec, annealing at 59 °C for 60 sec, extension at 72 °C for 120 sec, and a final extension period of 10 min at 72 °C. The PCR Products were purified using the Quiaquick PCR purification kit (QUIAGEN, Hilden, Germany).

Sequencing reactions were performed by a company (IBL, Vienna, Austria) using the dideoxynucleotide method, analysing on an automated DNA sequencer ALF-express (Amersham Biosciences Inc., Piscataway, USA). Primers were the same as used for amplification. Sequence assembly was done using the Seqman program (Dnastar Inc., Madison, USA). The sequences were then compared

with the database of the National Centre for Biotechnology Information, Bethesda, Md. (BLAST-search, http://www.ncbi.nlm.nih.gov/BLAST/). Strains showing at least 99 % sequence similarity with a maximum of three nucleotide differences are conspecific or sister species (FONSECA et al., 2000).

The isolated strains were conserved in the ACBR culture collection for further investigation.

3. Results and Discussion

739 fungal strains were isolated from the soil samples; based on their morphology these could clearly be assigned to 35 anamorph genera of *Ascomycetes* (table 1). Some strains were members of the artificial group of "*Coelomycetes*", few sterile mycelia and at each location some *Zygomycetes* were observed. Although the total number of fungal strains was

Table 1: Isolated genera of fungi and their distribution on the different sampling points. L ... litter, 0 ... 0–5 cm, 1 ... 10–15 cm, 3 ... 30–35 cm depth.
Tabelle 1: Isolierte Pilzgattungen und deren Verteilung auf die verschiedenen Probenahmestellen. L ... Streuschicht, 0 ... 0–5 cm, 1 ... 10–15 cm, 3 ... 30–35 cm Tiefe.

	Gr. Enzersdorf																Mannswörth							
	Salix				*Populus*				eco. field				conv. field				*Salix*				*Populus*			
horizon	L	0	1	3	L	0	1	3	L	0	1	3	L	0	1	3	L	0	1	3	L	0	1	3
Acremonium	3	5	5	3	0	18	5	4	4	13	6	1	4	13	6	13	1	3	1	1	1	7	6	6
Alternaria	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1
Aphanocladium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Aspergillus	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Beauveria	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0
Botryotrichum	0	0	1	0	0	0	1	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	1
Chrysosporium	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	1
Cladosporium	9	0	0	1	2	1	1	0	0	3	0	1	5	1	1	2	5	0	0	0	0	3	1	0
Cylindrocarpon	0	4	2	5	0	4	4	4	0	1	0	0	0	0	0	0	0	2	0	2	1	4	4	8
Dictyosporium	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Doratomyces	0	0	1	3	0	0	3	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	2
Fusarium	0	0	1	0	0	1	0	0	1	4	5	2	0	4	0	1	1	0	2	1	0	0	2	0
Gliocladium	0	1	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	2	1	2	0	1
Gonytrichum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Hormiactis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Humicola	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Idriella	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2	0	1	0	0
Mammaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Myrothecium	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	1
Nectria	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Paecilomyces	0	3	2	3	0	3	2	6	0	1	1	3	0	3	2	1	0	0	0	3	0	3	2	2
Penicillium	6	11	4	1	6	17	2	0	2	2	4	3	0	2	3	6	2	6	2	3	2	16	12	9
Periconia	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phialophora	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1
Scopulariopsis	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
Seimatosporium	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sporothrix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Stachybotris	0	0	0	0	0	0	2	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
Tricellula	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichoderma	0	4	5	0	0	2	1	0	0	2	0	1	0	0	0	0	1	3	4	5	1	3	3	2
Trichophyton	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
Trichosporiella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Verticillium	0	0	0	0	0	0	2	0	0	1	0	2	0	0	1	0	0	0	0	0	0	1	2	0
Volutella	0	1	2	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Wardomyces	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coelomycetes	4	3	2	0	1	4	2	0	0	5	4	1	5	9	4	4	0	9	7	0	3	6	6	7
sterile	0	4	8	7	0	8	13	7	1	6	3	1	3	1	1	6	0	3	5	4	1	4	12	8
unidentified	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	1	0	0
total	24	38	36	27	9	62	41	28	8	41	26	20	18	36	22	40	10	27	23	27	11	59	54	52

alike in the seasonally flooded forest (263) and in the not flooded forest (265), the diversity was higher under *Populus sp.* trees, especially in the flooded forest.

In agricultural soil the number of species was around 20 % below the values of the forest with no significant difference between conventional and ecological farming. This agrees with former investigations showing that in general biodiversity is higher in forest soil, therefore they are characterised as "hot spots" in agricultural landscapes (HAGVAR, 1998).

The results of this study show that the highest number of fungal species is located in the upper core, followed mostly by the subjacent layer. This can be explained by the fact that the contact zone between the litter and the soil is the zone of highest remineralisation of the organic material. However, in the seasonally flooded forest, no significant difference in the total number of fungi was found between the three cores. The two agricultural soils show different results, while in the field with ecological farming the highest diversity could be observed in the upper layer (0–5 cm), in case of conventional farming most fungi were isolated from the deepest core (30–35 cm). This may be due to soil cultivation methods like ploughing, leading to a disturbance of the soil stratification.

The most frequently isolated genera are *Acremonium* and *Penicillium*, overall about 34 % of the fungi belong to these genera. Also often found are members of several sterile mycelia and diverse *"Coelomycetes"*, including *Phoma* and similar genera. The genera *Cladosporium*, *Cylindrocarpon*, *Fusarium*, *Paecilomyces* and *Trichoderma* were each represented with about 5 % of the total count. Rarely found genera are *Alternaria*, *Botryotrichum, Chrysosporium*, *Doratomyces*, *Gliocladium*, *Idriella*, *Myrothecium*, *Phialophora, Stachybotris*, *Verticillium* and *Volutella*, each with approximately 1 % part. Several other genera were found in all samples less than five times, these are: *Aphanocladium*, *Aspergillus, Beauveria*, *Dictyosporium*, *Gonytrichum*, *Hormiactis*, *Humicola*, *Mammaria*, *Nectria*, *Periconia*, *Scopulariopsis*, *Seimatosporium*, *Sporothrix*, *Tricellula*, *Trichophyton*, *Trichosporiella*, *Wardomyces*. *Zygomycetes* were found everywhere, namely some members of the genus *Mucor* and one representative of the genus *Coemansia*.

Acremonium, *Gliocladium*, *Penicillium*, *Volutella* and members of *Coelomycetes* have their maximum occurrence in the top soil level (0–5 cm), *Fusarium* and *Trichoderma* are additionally similar present in the subjacent layer (10–15 cm). Sterile mycelia and *Verticillium* also prefer this horizon, whereas *Doratomyces*, and *Paecilomyces* can mostly be found in the lowest core (30–35 cm). Two-thirds of the *Cladosporium* species are located in the litter. The other genera have no favoured residence.

Populus-habitats show more diversity than *Salix* forests, although members of the genera *Aphanocladium*, *Aspergillus*, *Gonytrichum*, *Trichosporiella* and *Wardomyces* were only found under *Salix*. *Trichoderma spp.* were found more frequently in the latter location. The genera *Dictyosporium*, *Hormiactis*, *Humicola*, *Mammaria*, *Phialophora*, *Seimatosporium*, *Sporothrix* were isolated only under *Populus*, whereas the genera *Beauveria*, *Stachybotris* and *Verticillium* were additionally found in agricultural soil.

Agricultural soils show some different results, e. g. *Penicillium* here prefers deeper soil layers. *Fusarium* species were found primarily on agricultural land, whereas *Cylindrocarpon* and *Trichoderma* could be isolated rarely there. The genera *Nectria*, *Periconia* and *Tricellula* appeared only in the field with ecological farming. The stratification in agricultural soils however, might be less significant than in untouched soils because they are ploughed in regular terms each year.

All fungi recorded in this study were known as inhabitants of different soils, some are also found on decaying plant material, as for example *Doratomyces* and *Stachybotris*, some are also plant parasites like *Alternaria* (a few species are ubiquitous and found in soil), *Cylindrocarpon*, *Fusarium* (some species cause storage rots and are important toxin producers), *Idriella*, *Myrothecium* and *Verticillium*. Members of the genus *Cladosporium* are one of the most common air-borne fungi, they have been isolated from many sources (DOMSCH et al., 1993). One of the most frequent isolated genus in this study was *Penicillium*, which is widespread in different soil types mainly in the temperate climate. These fungi produce hundreds of different metabolites, which among other things give them the possibility to suppress or even stop the growth of their competitors (BENKOVÁ, 1999). In contrast to the previous genus, *Aspergillus* species prefer warm climates. This is the reason why only three representatives of this genus were found in this study.

In addition to the hyphomycetes 129 strains of yeasts were isolated, sequence data resulted in 36 different sequences. Most of them could be assigned to 13 genera, with exception of four strains (Tables 2, 3 and 4).

The most frequently represented genera were *Cryptococcus* (nearly half of the strains), *Aureobasidium*, *Sporobolomyces* and *Trichosporon*. More rarely isolated genera are: *Bulleromyces*, *Candida*, *Cystofilobasidium*, *Debaryomyces*, *Geotrichum*, *Kluyveromyces*, *Pichia*, *Rhodotorula* and *Williopsis*.

Table 2: Isolated yeasts and their distribution on the different sampling points. L ... litter, 0 ... 0–5 cm, 1 ... 10–15 cm, 3 ... 30–35 cm depth.
Tabelle 2: Isolierte Hefen und deren Verteilung auf die verschiedenen Probenahmestellen. L ... litter, 0 ... 0–5 cm, 1 ... 10–15 cm, 3 ... 30–35 cm depth.

	Gr. Enzersdorf																Mannswörth							
	Salix				*Populus*				eco. field				conv. field				*Salix*				*Populus*			
Horizon	L	0	1	3	L	0	1	3	L	0	1	3	L	0	1	3	L	0	1	3	L	0	1	3
Aureobasidium	6	0	0	0	2	0	0	0	2	1	0	0	1	0	0	0	3	0	0	1	4	0	0	0
Bulleromyces	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Candida	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
Cryptococcus	8	1	0	0	7	3	0	1	5	4	2	1	3	1	1	2	5	0	2	1	3	2	3	1
Cystofilobasidium	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
Debaryomyces	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Geotrichum	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Kluyveromyces	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Pichia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Rhodotorula	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Sporobolomyces	0	0	0	0	1	0	0	1	6	0	0	1	3	0	0	0	1	0	1	0	0	0	0	0
Trichosporon	1	1	3	0	0	1	2	0	0	0	1	0	0	0	1	0	0	2	0	0	0	0	0	0
Williopsis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1
unidentified	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	2	0
total	18	3	4	1	10	5	2	2	13	5	3	2	9	3	3	2	10	4	7	2	9	2	7	3

Table 3: Number of different yeast species on the basis of their 26S rDNA sequence
Tabelle 3: Anzahl der verschiedenen Hefearten aufgrund deren 26S rDNA Sequenz

	Yeast
Aureobasidium	1
Bulleromyces	1
Candida	2
Cryptococcus	13
Cystofilobasidium	1
Debaryomyces	1
Geotrichum	1
Kluyveromyces	1
Pichia	2
Rhodotorula	2
Sporobolomyces	2
Trichosporon	3
Williopsis	2
unidentified	4
total	36

The relationship between *Asco-* and *Basidiomycetes* was 39 to 61 percent. Former investigations have shown similar results, namely the yeasts in soil being mostly *Basidiomycetes*, with a high frequency of the genus *Cryptococcus* (SLÁVIKOVÁ and VADKERTIOVÁ, 2000; SPENCER and SPENCER, 1997). This predominance can be explained by the fact that due to their capsules they are capable to survive under disadvantageous conditions such as low moisture.

In contrast to the microfungi the diversity of yeasts is in agricultural soil similar as in the not flooded forest, the highest diversity could be observed in the seasonally flooded forest near Mannswörth. This agrees with literature data showing that the number of yeasts in soil depends heavily on the amount of available nutrients which is higher in the latter location (SPENCER and SPENCER, 1997).

Most of the yeasts could be isolated from the litter (at least 50 %), *Aureobasidium* and *Sporobolomyces* were almost exclusively found there. The quantity decreased with soil depth, one exception was the soil under *Populus* in the seasonally flooded forest, here the deepest soil layer showed more diversity than the upper ones. *Geotrichum* could be isolated only from soil under *Salix*.

The distribution of the yeasts can be explained by their ecology: Yeasts are residents of different habitats, they are also often found on the phylloplane, the leaf surfaces of different plants. Members of the genera *Aureobasidium*, *Cryptococcus*, *Rhodotorula*, *Sporobolomyces* and other *Basidiomycetes* live there (SPENCER and SPENCER, 1997). In the litter complex, between the epiphytous and the soil complex, saprobionts like *Trichosporon* are the most ubiquitous ones, but also inhabitants of the neighbouring habitats can exist or even dominate there (BABJEVA and CHERNOV, 1995).

A recent publication (BUCKOVÁ et al., 2000), dealing also with microfungi under *Salix* and *Populus* shows some different results in comparison with this study. The genera *Cylindrocarpon*, *Gliocladium* and *Stachybotris* occurred only in forests with *Populus*, whereas in this work the first two could be also isolated under *Salix*. Members of the genera *Fusarium*, *Geotrichum*, and *Scopulariopsis* were investigated

Table 4: List of different 26S rDNA sequences and their accession-numbers to the EMBL-databank
Tabelle 4: Liste der unterschiedlichen Sequenzen und deren Nummern in der Genbank

Genus	Strain	EMBL No.
Aureobasidium	HA1556	AJ507454
Bulleromyces	HB948	AJ508232
Candida	HB1041	AJ509856
	HA1403	AJ507662
Cryptococcus	HB1062	AJ507769
	HB980	AJ507767
	HB1043	AJ509857
	HB939	AJ510141
	HB949	AJ510142
	HA1546	AJ510143
	HB1042	AJ510145
	HB1052	AJ510146
	HB953	AJ510147
	HB946	AJ510201
	HB1048	AJ510202
	HA1558	AJ510144
	HB1061	AJ507805
Cystofilobasidium	HA1567	AJ508233
Debaryomyces	HA1560	AJ508234
Geotrichum	HA1393	AJ507661
Kluyveromyces	HA1400	AJ507455
Pichia	HA1553	AJ509854
	HA1559	AJ509855
Rhodotorula	HB919	AJ508235
	HB1068	AJ510198
Sporobolomyces	HB1050	AJ507768
	HB1056	AJ510199
Trichosporon	HB940	AJ507663
	HB1046	AJ507664
	HB942	AJ507665
Williopsis	HA1568	AJ510200
	HA1561	AJ507804
unidentified	HA1379	AJ511334
unidentified	HA1406	AJ511335
unidentified	HB1047	AJ511333
unidentified	HA1554	AJ511336

by BUCKOVÁ et al. only in the *Salix*-locations, we found *Fusarium* and *Scopulariopsis* also under *Populus*.

Identification of fungi on the species level is often problematic. For example within the genus *Trichoderma* it proved as difficult due to morphological similarities between the different species. Therefore the members of this genus were identified by molecular methods (WUCZKOWSKI et al., 2003).

For a reliable yeast identification on the species level the results obtained by sequencing the D1–D2 region of the 26S rRNA, especially for those which revealed not 100 % similarity, are object of further studies. They must be verified by microsatellite- and RAPD-PCR (random amplified polymorphic DNA polymerase chain reaction).

Acknowledgement

The project was financed by:
- Magistratsabteilung 49 – Forstamt und Landwirtschaftsbetrieb der Stadt Wien
- Leiter der Forstverwaltung Lobau, Nationalpark-Koordinator DI. Gottfried Haubenberger
- Amt der Niederösterreichischen Landesregierung
- Rektor der Universität für Bodenkultur Wien, Univ. Prof. DI. Dr. nat. techn. Dr. h. c. Leopold März

We would like to thank Anton Pajan and Peter Halwachs for their kind help with the sample collection.

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Eingelangt am 29. November 2002
Angenommen am 18. April 2003