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# Molecular diversity of fungal communities in agricultural soils from Lower Austria

**Sylvia Klaubauf · Erich Inselsbacher · Sophie Zechmeister-Boltenstern · Wolfgang Wanek · Richard Gottsberger · Joseph Strauss · Markus Gorfer**

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**Abstract** A culture-independent survey of fungal diversity in four arable soils and one grassland in Lower Austria was conducted by RFLP and sequence analysis of clone libraries of the partial ITS/LSU-region. All soils were dominated by the ascomycetous orders Sordariales, Hypocreales and Helotiales, taxa that are known from traditional cultivation approaches to occur in agricultural soils. The most abundant genus in the investigated soils was *Tetracladium*, a hyphomycete which has been described as occurring predominantly in aquatic habitats, but was also found in agricultural soils. Additionally, soil clone group I (SCGI), a subphylum at the base of the Ascomycota with so far no cultivated members, was identified at high frequency in the grassland soil but was below detection limit in the four arable fields. In addition to this striking difference, general fungal community parameters like richness, diversity and evenness were similar between cropland and grassland soils. The presented data provide a fungal community inventory of agricultural soils and reveal the most prominent species.

**Keywords** Agricultural soil · Fungal communities · Diversity · Soil clone group I · SCGI

S. Klaubauf · R. Gottsberger · J. Strauss · M. Gorfer (✉)
Department of Applied Genetics and Cell Biology,
Fungal Genetics and Genomics Unit,
Austrian Institute of Technology and BOKU University Vienna,
1190 Vienna, Austria
e-mail: markus.gorfer@boku.ac.at

E. Inselsbacher · W. Wanek
Department of Chemical Ecology and Ecosystem Research,
Faculty Center for Ecology, Faculty of Life Sciences,
University of Vienna,
1090 Vienna, Austria

E. Inselsbacher · S. Zechmeister-Boltenstern
Department of Forest Ecology and Soils, Unit of Soil Biology,
Federal Research and Training Centre for Forests,
Natural Hazards and Landscape (BFW),
1131 Vienna, Austria

*Present Address:*
S. Klaubauf
CBS-KNAW, Fungal Physiology,
Uppsalalaan 8,
NL-3584 CT Utrecht, The Netherlands

*Present Address:*
E. Inselsbacher
Department of Forest Ecology and Management, SLU,
SE-901 83 Umeå, Sweden

*Present Address:*
R. Gottsberger
AGES,
Spargelfeldstraße 191,
1220 Vienna, Austria

## Introduction

Fungi play a central role in most ecosystems and seem to dominate the microbial biomass in soil habitats (Joergensen and Wichern 2008), where they are important decomposers and occupy a notable position in the natural carbon, nitrogen and phosphorus cycles (Christensen 1989). Mycorrhizal and parasitic communities in different habitats are well characterised at the molecular level (Ryberg et al. 2009), and they directly affect plant community composition and productivity (Klironomos

2002; van der Heijden et al. 2008). In contrast, fungal species inventories from agricultural soils are so far mainly known from cultivation studies (Domsch and Gams 1970; Domsch et al. 1993; Hagn et al. 2003), while there are only few studies employing cultivation-independent techniques (de Castro et al. 2008; Lynch and Thorn 2006). A solid knowledge of the fungal community in agricultural soils provides the basis for functional studies about specific processes carried out by members of this group. The main contributions of the fungal community to functioning of the agroecosystem are soil stabilization and nutrient cycling (Stromberger 2005).

The presented study is part of a larger effort to elucidate the microbial processes in fertilizer nitrogen transformations. To gain a better insight into the role of fungi in the nutrient cycling processes in agricultural soils, we took an inventory of this important group, which we showed previously by quantitative real-time PCR to constitute a dominant microbial community in two agriculatural soils (Inselsbacher et al. 2010). These two soils are included in the present study.

The soils studied here derived from different locations in Lower Austria in the vicinity of Vienna. Four of the soils are used as agricultural fields, while one is a grassland. Several microbial parameters and nitrogen dynamics were investigated in previous studies (Inselsbacher et al. 2010; Inselsbacher et al. 2009). All five soils support higher nitrification rates than gross nitrogen mineralization rates leading to a rapid conversion of ammonium to nitrate. Accordingly, nitrate dominates over ammonium in the soil inorganic nitrogen pools (Inselsbacher et al. 2010; Inselsbacher et al. 2009). Following fertilization more inorganic nitrogen was translocated to the microbial biomass compared to plants at the short term, but after 2 days plants accumulated higher amounts of applied fertilizer nitrogen (Inselsbacher et al. 2010). Rapid uptake of inorganic nitrogen by microbes prevents losses due to leaching and denitrification (Jackson et al. 2008).

The aims of the presented work were (i) to identify the most prominent members of the fungal communities in agricultural soils, and (ii) to address the issue of fungal biodiversity in agroecosystems. Knowledge of community structure and composition will allow assessing the beneficial role of fungi in agriculture — besides their well established role as major phytopathogens. To this end the most prominent members of the fungal communities in four arable soils and one grassland in Lower Austria were identified by sequencing of cloned PCR products comprising the ITS- and partial LSU-region. The obtained dataset of fungal species present in the different agricultural soils provides the basis for future work on specific functions of fungi in agroecosystems.

## Materials and methods

### Field sites and soil sampling

Soils were collected from four different arable fields and one grassland in Lower Austria (Austria). The soils were selected to represent different bedrocks, soil textures, pH values, water, and humus contents. For a detailed description of the soils see Inselsbacher et al. (2009). Sampling site Riederberg (R) is a grassland for hay production, while sampling sites Maissau (M), Niederschleinz (N), Purkersdorf (P) and Tulln (T) are arable fields. Grassland soil R as well as arable field soil P were covered with vegetation (grasses and winter barley, resp.) at the time of sampling, while arable field soils M, N and T were bare. At each site five randomized samples of 5 kg each were taken from an area of 400 $m^2$ from the A horizon (0–10 cm depth) and mixed. Soils were sampled on April, 11th 2006 and immediately stored at 4°C until further analysis. Soils were homogenised, sieved (<2 mm) and kept at 4°C before processing.

### DNA extraction and PCR

DNA was extracted in triplicate from each soil (1 g fresh weight per extraction) using the Ultra Clean Soil DNA Isolation Kit (MoBio) according to the manufacturer's instructions and further purified with the QIAquick PCR Purification Kit (Qiagen). Fungal ITS-region and partial LSU were amplified with ITS1F (Gardes and Bruns 1993), which is specific for fungi, and the universal eukaryotic primer TW13 (Taylor and Bruns 1999). The resulting PCR products ranged from 1.1 to 1.8 kb in size. The LSU region serves for higher order identification of fungi without homologous ITS reference sequences in public databases.

PCRs contained GoTaq Green Master Mix (Promega), 1 μM of each primer, 0.5 mg/ml BSA and 0.5 μl soil DNA in a total volume of 20 μl. PCRs were run in triplicate on a T3 Thermocycler (Biometra). The following thermocycling program was used: 95°C for 2′30″ (1 cycle); 94°C for 30″–54°C for 30″–72°C for 1′30″ (30 cycles); and 72°C for 5′ (1 cycle). The nine replicate PCR products for each soil (three DNAs for each soil times three replicas for each DNA) were pooled before ligation to minimize effects from spatial heterogeneity and variability during PCR amplification (Schwarzenbach et al. 2007). For each soil a clone library (96 independent clones each) of ITS/LSU-PCR-products was constructed in plasmid pTZ57R/T (Fermentas) according to manufacturer's instructions. Insert PCR products (ITS1F/TW13) from individual clones were directly subjected to RFLP analyses. The reaction was performed with the restriction endonuclease *Bsu*RI (Fermentas, isoschizomere of *Hae*III) for 2 h at 37°C and the fragments were separated

on a 3% high resolution agarose gel. Initially up to 4 randomly selected clones that produced an identical pattern were sequenced (Big Dye Terminator v3.1, Cycle Sequencing Kit, ABI) using the primers ITS1F, ITS3 (White et al. 1990) and TW13. Sequencing reactions were purified over Sephadex-G50 in microtiterplates and separated on a DNA sequencer (ABI 3100 genetic analyzer, Pop69, BDv3.1) at the Department of Applied Genetics und Cell Biology, University of Natural Resources and Applied Life Sciences, Vienna (Austria). Where sequencing of more than one representative of one RFLP-pattern resulted in sequences with less than 97% identity in the ITS region or less than 99% identity in the LSU region (see cut-off values for species delineation below), all clones from the particular pattern were sequenced.

General molecular genetic manipulations were carried out according to Sambrook and Russell (2001).

## Sequence analysis

Forward and reverse sequence reads were assembled using the commercial software Vector NTI Advance™ 10 for Windows, version 10.3.0. Mended contig sequences were checked for chimeras by Bellerophon (Huber et al. 2004) and submitted to a nucleotide BLAST Search (Altschul et al. 1990). BLAST searches were performed separately with parts of the sequence corresponding to the ITS and partial LSU region, respectively. ITS- and LSU-taxonomies were compared for consistency to detect chimeras left undetected by Bellerophon. Reference hits from BLAST searches were scrutinised concerning their reliability (e.g. sequences from strains from collections like CBS were preferably taken as reliable references). In cases in which sequences could not be identified to a certain taxonomic level, the lowest common affiliation of reliable reference sequences was taken. Cut-off for distinct species was set to 97% for the ITS region (Hughes et al. 2009) and 99% for the LSU region, unless BLAST results for two closely related sequences gave distinct hits to well characterised strains. Chimeric sequences were excluded from further analyses.

Sequences are deposited at GenBank under accession numbers GU055518–GU055547 (soil M), GU055548–GU055606 (soil N), GU055607–GU055649 (soil P), GU055650–GU055710 (soil R) and GU055711–GU055747 (soil T).

## Statistical analysis

The data from each clone library were used for the calculation of estimates of species richness and diversity with EstimateS (Version 8.2.0, R. K. Colwell, http://purl.oclc.org/estimates). In addition to chimeric sequences, one sequence of eukaryotic but non-fungal origin (NG_R_F10, Acc. Nr. GU055695) from soil R was also removed prior to data analysis to obtain estimates of fungal richness and diversity. Richness estimators available in EstimateS 8.2.0 were compared to each other and gave comparable results for each of the five different soils. Only results for the Chao2 richness estimator (Chao 1987) are shown in Table 1.

**Table 1** Fungal richness and diversity indices for agricultural and grassland soils

| Soil | Management | Library[b] | Clones[c] | Sobs[d] | Chao2 ± SD[e] | % Cov.[f] | Shann.[g] | Simp.[h] |
|---|---|---|---|---|---|---|---|---|
| Maissau | Arable field | ITS/LSU | 96 | 19 | 20.4±3.1 | 92.8 | 2.33 | 7.37 |
| Niederschleinz | Arable field | ITS/LSU | 92 | 34 | 51.3±12.0 | 66.3 | 3.27 | 28.09 |
| Purkersdorf | Arable field | ITS/LSU | 94 | 32 | 44.9±9.5 | 71.3 | 3.18 | 23.76 |
| Riederberg | Grassland | ITS/LSU | 92 | 31 | 41.4±7.1 | 77.3 | 2.84 | 10.76 |
| Tulln | Arable field | ITS/LSU | 89 | 24 | 32.9±8.0 | 72.9 | 2.84 | 15.48 |
| Sourhope (UK)[a] | Grassland | SSU | 53 | 18 | 47.8±22.4 | 37.7 | 1.93 | 3.62 |
| Sourhope (UK)[a] | Grassland | ITS | 45 | 22 | 51.3±20.5 | 42.9 | 2.53 | 7.50 |
| Cristalina (BRA)[a] | Arable field (Soy) | SSU | 104 | 22 | 30.9±7.6 | 71.2 | 1.87 | 2.87 |

[a] Data for the soils "Sourhope" from the Sourhope Research Station in Scotland, UK (Anderson et al. 2003) and "Cristalina" from the district Cristalina in Goiás, Brazil (de Castro et al. 2008) were taken from the respective publications

[b] Library indicates on which region from rRNA-encoding cluster profiling of the fungal community was done

[c] Clones: number of analysed clones for each soil;

[d] Sobs: number of observed species in the clone libraries;

[e] Chao2 ± SD: Estimated species richness ± standard deviation for the sampling site based on the Chao2 richness estimator (Chao 1987) implemented in EstimateS 8.2;

[f] % Cov.: Estimated coverage of the libraries based on observed and estimated species richness;

[g] Shann.: Shannon Diversity Index

[h] Simp.: Simpson Diversity Index

For comparison, richness and diversity indices were calculated from published sequence datasets from a natural grassland at the Sourhope Research Station, Scotland (Anderson et al. 2003) and from a soybean plantation in Cristalina, Brazil (de Castro et al. 2008). Sourhope Research Station: Libraries A and B comprising overlapping 18S rRNA fragments were cured from non-fungal and chimeric sequences and richness and diversity was estimated from the combined A and B dataset as described above. The cut-off for operational taxonomic units was set to 99%. Similarly, species richness and diversity was calculated from Sourhope Research Station ITS library D. The cut-off was also set to 99%, since there was no difference in predicted species richness and diversity between cut-off values of 95–99%. Soybean plantation Cristalina: The published dataset did not contain chimeric or non-fungal sequences. The cut-off for further analyses was set to 99%.

UniFrac was used to compare the phylogenetic structures of the fungal communities from soils M, N, P, R and T (Lozupone et al. 2006). To this end sequences were aligned with the ClustalW algorithm in MEGA4 (Tamura et al. 2007), and a neighbor-joining tree was calculated from the aligned partial LSU sequences. The ITS-region was excluded, since it cannot be unambiguously aligned over such a broad phylogenetic distance. Sequences from an unknown eukaryote (NG_R_F10, Acc. Nr. GU055695) and from a fungus of uncertain affiliation (NG_R_F02, Acc Nr. GU055690) from site R were used as outgroups and excluded from further analyses. Data were weighted for abundance and normalized for branch length for calculating the UniFrac metric of the distance between each pair of soil samples (Lozupone et al. 2006).

## Results

Soil characteristics of the five soils used in the present study are given in Inselsbacher et al. (2009). All soil parameters are within the range for typical arable land as used for cultivation of barley in this area. Fungal communities were analysed by direct amplification of fungal ITS/partial LSU regions with primer pair ITS1F and TW13. Cloned PCR products from each soil were grouped by RFLP and up to four representatives from each RFLP type were sequenced. By this approach even closely related sequences (e.g. four different *Tetracladium* species from soil P with a maximum sequence difference of 3.7%) could be dissected. While the ITS region provides excellent resolution down to the species level, the partial LSU region provides good resolution at higher taxonomic levels when sufficiently identified ITS reference data in public databases are missing (Urban et al. 2008).

By this combined approach of RFLP typing and sequencing a total of 116 ribotypes were detected in the five soils. One sequence from soil R was of non-fungal, unknown eukaryotic origin. From the 115 fungal ribotypes, 42 could be classified to the species level, an additional 24 at least to the genus level, while the remaining 49 fungal sequences could only be classified to the family or higher taxonomic level.

Richness ranged from 19 to 34 for detected and from 20.5 to 51.3 for estimated species numbers (Chao2; Chao 1987) per sampling site. Coverage of the libraries ranged from 66.3 to 92.8% of estimated species numbers (see Table 1). As in a few cases sequencing of more than one representative clone from the same RFLP pattern resulted in closely related but dissimilar sequences, the species numbers given here most likely slightly underestimate the true fungal diversity in the investigated soils.

UniFrac analysis could not detect significant differences between the phylogenetic structures of the fungal communities from the herein studied soils. Bonferroni corrected P-values for pairwise comparisons were all above or equal to 0.1. The calculated environmental distances were between 0.43 and 0.60. No clustering of spatially close locations could be found (the distance between sampling sites M and N, P and R respectively R and T is less then 10 km).

All five soils are dominated by Ascomycota, which are represented by 77.7 to 88.2% of the clones in the respective libraries, followed by Basidiomycota, which are represented by 7.5 to 21.3% of the clones in the respective libraries (Fig. 1). Other phyla (Chytridiomycota, Blastocladiomycota as well as Mucoromycotina) were only detected occasionally and at low frequencies. No sequences belonging to the Glomeromycota were found. At all taxonomic levels from phylum to species soil M showed the lowest observed richness (see Fig. 1 and Table 2). Similarly, predicted species richness, several diversity indices (Magurran 2004) and evenness were lowest for soil M (see Table 1). The dominant species in soil M — a species related to *Trichocladium asperum* — was represented by nearly 30% of all analysed clones (see Table 2).

The most abundant orders for all soils were the Sordariales, Hypocreales and Helotiales, although Helotiales could not be detected in soil M. Additionally, the ascomycetous soil clone group I (SCGI; Porter et al. 2008) was found at a relatively high abundance in the grassland soil R, represented by 18.3% of all clones from the library, but was absent from the four libraries from arable soils. SCGI could be detected at a similar level in a published dataset from a study analysing fungal communities in a natural grassland: 17.5% of clones from the SSU library (A and B combined, and after removal of non-fungal and chimeric sequences) belonged to SCGI (Anderson et al. 2003).

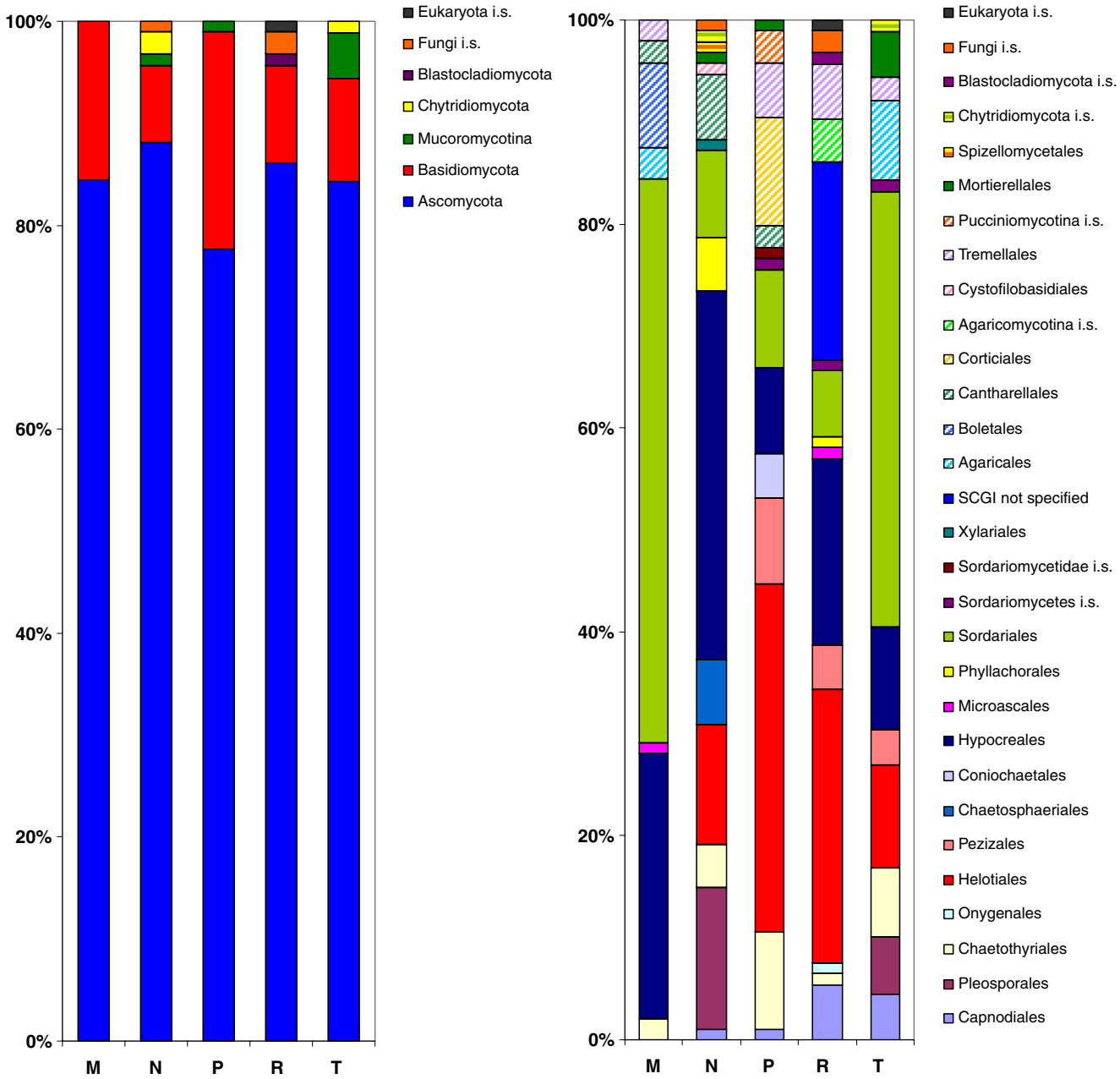


**Fig. 1** Relative abundance of fungal groups in arable and grassland soils. Relative abundances at the phylum (or where appropriate alternative taxonomic ranks; left part) and ordinal (right part) level of clones from libraries from arable soils Maissau (M), Niederschleinz (N), Purkersdorf (P) and Tulln (T) and grassland soil Riederberg (R)

The most abundant genus was *Tetracladium*, which could be found at all sites, except in soil M. *T. maxilliforme* was the most abundant species in the grassland soil R, represented by 22.6% of clones from the library. Another important group found in all soil samples are potentially phytopathogenic fungi, e.g. from the genera *Fusarium* and *Nectria*. From the 116 species detected in the five soil samples, 17 species could be detected in two soils, and four species could even be detected in three soils (co-occurring species are indicated in Table 2). No obvious patterns of soil clustering by common species could be observed.

## Discussion

While there is a plenitude of data available on fungal communities in different natural soil habitats (Anderson et al. 2003; Buee et al. 2009; Curlevski et al. 2010; Fierer et al.

**Table 2** Species list of fungi from arable and grassland soils in Lower Austria

| Soil[a] | Clone[b] | Acc.No.[c] | Identification[d] | Order | Phy.[e] | RA[f] | CO[g] |
|---|---|---|---|---|---|---|---|
| M | NG_M_A03 | GU055520 | *Trichocladium asperum* related | Sordariales | A | 29,2 | |
| M | NG_M_A01 | GU055518 | *Myrothecium* sp. M_A01 | Hypocreales | A | 14,6 | |
| M | NG_M_A06 | GU055523 | *Cercophora costaricensis* | Sordariales | A | 13,5 | |
| M | NG_M_B07 | GU055525 | *Scleroderma bovista* | Boletales | B | 8,3 | |
| M | NG_M_A04 | GU055521 | *Hapsidospora irregularis* | Hypocreales | A | 5,2 | |
| M | NG_M_D07 | GU055530 | *Podospora dimorpha* | Sordariales | A | 4,2 | |
| M | NG_M_C04 | GU055528 | *Cercophora coprophila/terricola* | Sordariales | A | 3,1 | |
| M | NG_M_H03 | GU055544 | *Fusarium merismoides var. merism.* | Hypocreales | A | 3,1 | N, R |
| M | NG_M_D12 | GU055532 | *Hebeloma pallidoluctuosum* | Agaricales | B | 3,1 | |
| M | NG_M_C08 | GU055529 | Lasiosphaeriaceae M_G03 | Sordariales | A | 3,1 | |
| M | NG_M_G01 | GU055537 | *Cyphellophora laciniata* | Chaetothyriales | A | 2,1 | N |
| M | NG_M_H01 | GU055543 | *Minimedusa polyspora* | Cantharellales | B | 2,1 | N, P |
| M | NG_M_G11 | GU055542 | *Paecilomyces carneus* | Hypocreales | A | 2,1 | |
| M | NG_M_G04 | GU055539 | *Cryptococcus terricola* | Tremellales | B | 1,0 | P |
| M | NG_M_E04 | GU055534 | Hypocreales M_E04 | Hypocreales | A | 1,0 | |
| M | NG_M_D10 | GU055531 | Lasiosphaeriaceae M_D10 | Sordariales | A | 1,0 | R |
| M | NG_M_H07 | GU055546 | *Periconia macrospinosa* | Microascales | A | 1,0 | R |
| M | NG_M_A02 | GU055519 | *Thielavia hyalocarpa* related | Sordariales | A | 1,0 | |
| M | NG_M_E08 | GU055535 | *Trichosporon dulcitum* | Tremellales | B | 1,0 | |
| N | NG_N_A02 | GU055548 | *Fusarium merismoides var. merism.* | Hypocreales | A | 8,7 | M, R |
| N | NG_N_A06 | GU055552 | *Pyrenophora tritici-repentis* | Pleosporales | A | 7,6 | |
| N | NG_N_A09 | GU055554 | *Stachybotrys chartarum* | Hypocreales | A | 7,6 | |
| N | NG_N_A03 | GU055549 | Chaetomiaceae N_A03 | Chaetosphaeriales | A | 6,5 | |
| N | NG_N_A04 | GU055550 | Hypocreales N_A04 | Hypocreales | A | 5,4 | |
| N | NG_N_E02 | GU055577 | *Verticillium nigrescens* | Phyllachorales | A | 5,4 | |
| N | NG_N_B06 | GU055559 | *Botryotinia fuckeliana* | Helotiales | A | 4,3 | |
| N | NG_N_E10 | GU055583 | *Cyphellophora laciniata* | Chaetothyriales | A | 4,3 | M |
| N | NG_N_B09 | GU055561 | *Fusarium incarnatum* | Hypocreales | A | 4,3 | |
| N | NG_N_E07 | GU055581 | *Tetracladium maxilliforme* | Helotiales | A | 4,3 | P, R |
| N | NG_N_C08 | GU055568 | *Thanatephorus cucumeris* | Cantharellales | B | 4,3 | |
| N | NG_N_A08 | GU055553 | *Acremonium strictum* | Hypocreales | A | 3,3 | |
| N | NG_N_B01 | GU055557 | Pleosporales N_B01 | Pleosporales | A | 3,3 | |
| N | NG_N_B08 | GU055560 | Sordariales N_B08 | Sordariales | A | 3,3 | |
| N | NG_N_E04 | GU055579 | *Fusarium solani* | Hypocreales | A | 2,2 | R |
| N | NG_N_E01 | GU055576 | Lasiosphaeriaceae N_E01 | Sordariales | A | 2,2 | |
| N | NG_N_A12 | GU055556 | *Minimedusa polyspora* | Cantharellales | B | 2,2 | M, P |
| N | NG_N_D07 | GU055573 | *Nectria mauritiicola* | Hypocreales | A | 2,2 | P |
| N | NG_N_E06 | GU055580 | Pleosporales N_E06 | Pleosporales | A | 2,2 | |
| N | NG_N_E09 | GU055582 | *Chaetomium globosum* related | Sordariales | A | 1,1 | |
| N | NG_N_B12 | GU055562 | *Acremonium strictum* related | Hypocreales | A | 1,1 | |
| N | NG_N_G10 | GU055599 | *Alternaria* sp. N_G10 | Pleosporales | A | 1,1 | |
| N | NG_N_C01 | GU055563 | Chytridiomycota N_C01 | Chytridiomycota *i.s.* [h] | C | 1,1 | |
| N | NG_N_G11 | GU055600 | *Cladosporium herbarum* complex | Capnodiales | A | 1,1 | R, T |
| N | NG_N_C04 | GU055565 | Fungus N_C04 | Fungi *i.s.* | F | 1,1 | |
| N | NG_N_H08 | GU055604 | *Guehomyces pullulans* | Cystofilobasidiales | B | 1,1 | |
| N | NG_N_D09 | GU055575 | *Hypocrea lixii* related | Hypocreales | A | 1,1 | |
| N | NG_N_H02 | GU055603 | Hypocreales N_H02 | Hypocreales | A | 1,1 | |
| N | NG_N_G12 | GU055601 | Lasiosphaeriaceae N_G12 | Sordariales | A | 1,1 | P |
| N | NG_N_F01 | GU055586 | *Monographella nivalis* | Xylariales | A | 1,1 | |
| N | NG_N_C12 | GU055570 | *Mortierella alpina* | Mortierellales | M | 1,1 | |
| N | NG_N_F11 | GU055593 | Spizellomycetales N_F11 | Spizellomycetales | C | 1,1 | |
| N | NG_N_G09 | GU055598 | *Tetracladium* sp. N_G09 | Helotiales | A | 1,1 | |
| N | NG_N_E12 | GU055585 | *Tetracladium* sp. P_E08 | Helotiales | A | 1,1 | P |

**Table 2** (continued)

| Soil[a] | Clone[b] | Acc.No.[c] | Identification[d] | Order | Phy.[e] | RA[f] | CO[g] |
|---|---|---|---|---|---|---|---|
| P | NG_P_B05 | GU055621 | *Corticium* related P_B05 | Corticiales | B | 10,6 | |
| P | NG_P_A12 | GU055616 | *Exophiala* sp. RSEM07_18 | Chaetothyriales | A | 9,6 | |
| P | NG_P_D08 | GU055634 | *Tetracladium* sp. P_D08 | Helotiales | A | 8,5 | |
| P | NG_P_A04 | GU055610 | *Cryptococcus terricola* | Tremellales | B | 5,3 | M |
| P | NG_P_C08 | GU055628 | Helotiales P_C08 | Helotiales | A | 5,3 | T |
| P | NG_P_A07 | GU055613 | *Schizothecium vesticola* | Sordariales | A | 5,3 | T |
| P | NG_P_E09 | GU055641 | *Tetracladium* sp. P_E09 | Helotiales | A | 5,3 | T |
| P | NG_P_B01 | GU055617 | *Byssonectria* sp. P_B01 | Pezizales | A | 4,3 | |
| P | NG_P_A11 | GU055615 | Coniochaetaceae P_A11 | Coniochaetales | A | 4,3 | |
| P | NG_P_F03 | GU055642 | *Kotlabaea* sp. P_F03 | Pezizales | A | 4,3 | R |
| P | NG_P_C02 | GU055626 | *Nectria mauritiicola* | Hypocreales | A | 3,2 | N |
| P | NG_P_A02 | GU055608 | Pucciniomycotina P_A02 | Pucciniomycotina *i.s.* | B | 3,2 | |
| P | NG_P_C09 | GU055629 | *Tetracladium furcatum* | Helotiales | A | 3,2 | R |
| P | NG_P_B03 | GU055619 | *Tetracladium maxilliforme* | Helotiales | A | 3,2 | N, R |
| P | NG_P_C01 | GU055625 | Chaetomiaceae P_C01 | Sordariales | A | 2,1 | |
| P | NG_P_D07 | GU055633 | Helotiales P_D07 | Helotiales | A | 2,1 | |
| P | NG_P_E05 | GU055637 | *Leptodontidium orchidicola* | Helotiales | A | 2,1 | |
| P | NG_P_B06 | GU055622 | *Minimedusa polyspora* | Cantharellales | B | 2,1 | M, N |
| P | NG_P_B04 | GU055620 | *Neonectria radicicola* | Hypocreales | A | 2,1 | R |
| P | NG_P_H08 | GU055649 | *Arthrinium phaeospermum* | Sordariomycetidae *i.s.* | A | 1,1 | |
| P | NG_P_H06 | GU055647 | Bionectriaceae P_H06 | Hypocreales | A | 1,1 | |
| P | NG_P_E02 | GU055635 | *Chaetomium* sp. P_E02 | Sordariales | A | 1,1 | |
| P | NG_P_B10 | GU055623 | *Chalara* sp. P_B10 | Helotiales | A | 1,1 | |
| P | NG_P_E03 | GU055636 | *Fusarium* sp. P_E03 | Hypocreales | A | 1,1 | |
| P | NG_P_B11 | GU055624 | Helotiales P_B11 | Helotiales | A | 1,1 | |
| P | NG_P_D03 | GU055632 | Helotiales P_D03 | Helotiales | A | 1,1 | |
| P | NG_P_C03 | GU055627 | Lasiosphaeriaceae N_G12 | Sordariales | A | 1,1 | N |
| P | NG_P_B02 | GU055618 | Mortierellaceae P_B02 | Mortierellales | M | 1,1 | |
| P | NG_P_G05 | GU055644 | *Ramularia* sp. P_G05 | Capnodiales | A | 1,1 | |
| P | NG_P_E06 | GU055638 | Sordariomycetes P_E06 | Sordariomycetes *i.s.* | A | 1,1 | |
| P | NG_P_E08 | GU055640 | *Tetracladium* sp. P_E08 | Helotiales | A | 1,1 | N |
| P | NG_P_H07 | GU055648 | *Trichoderma spirale* | Hypocreales | A | 1,1 | |
| R | NG_R_B12 | GU055661 | *Tetracladium maxilliforme* | Helotiales | A | 22,6 | N, P |
| R | NG_R_H09 | GU055707 | SCGI R_H09 | SCGI *i.s.* | A | 18,3 | |
| R | NG_R_E08 | GU055685 | *Cladosporium herbarum* complex | Capnodiales | A | 5,4 | N, T |
| R | NG_R_C06 | GU055666 | *Cryptococcus aerius* | Tremellales | B | 4,3 | T |
| R | NG_R_E09 | GU055686 | *Fusarium oxysporum* | Hypocreales | A | 4,3 | T |
| R | NG_R_B03 | GU055656 | Hypocreales R_B03 | Hypocreales | A | 4,3 | |
| R | NG_R_D03 | GU055673 | Lasiosphaeriaceae M_D10 | Sordariales | A | 4,3 | M |
| R | NG_R_D10 | GU055679 | Agaricomycotina R_E03 | Agaricomycotina *i.s.* | B | 2,2 | |
| R | NG_R_F02 | GU055690 | Fungus R_F02 | Fungi *i.s.* | F | 2,2 | |
| R | NG_R_G12 | GU055703 | *Fusarium* sp. R_G12 | Hypocreales | A | 2,2 | |
| R | NG_R_B09 | GU055660 | *Kotlabaea* sp. P_F03 | Pezizales | A | 2,2 | P |
| R | NG_R_D04 | GU055674 | Lasiosphaeriaceae R_D04 | Sordariales | A | 2,2 | |
| R | NG_R_F04 | GU055692 | *Neonectria radicicola* | Hypocreales | A | 2,2 | P |
| R | NG_R_B08 | GU055659 | Pyronemataceae R_B08 | Pezizales | A | 2,2 | |
| R | NG_R_C09 | GU055668 | *Tetracladium furcatum* | Helotiales | A | 2,2 | P |
| R | NG_R_D12 | GU055681 | *Tetracladium* sp. R_D12 | Helotiales | A | 2,2 | |
| R | NG_R_B04 | GU055657 | Agaricomycotina R_B04 | Agaricomycotina *i.s.* | B | 1,1 | |
| R | NG_R_D01 | GU055671 | Agaricomycotina R_D01 | Agaricomycotina *i.s.* | B | 1,1 | |
| R | NG_R_C01 | GU055662 | *Auxarthron umbrinum* | Onygenales | A | 1,1 | |
| R | NG_R_D09 | GU055678 | Blastocladiomycota R_D09 | Blastocladiomycota *i.s.* | Bc | 1,1 | |
| R | NG_R_D02 | GU055672 | *Cryptococcus tephrensis* | Tremellales | B | 1,1 | |

**Table 2** (continued)

| Soil[a] | Clone[b] | Acc.No.[c] | Identification[d] | Order | Phy.[e] | RA[f] | CO[g] |
|---|---|---|---|---|---|---|---|
| R | NG_R_F10 | GU055695 | Eukaryote R_F10 | Eukaryota *i.s.* | E | 1,1 | |
| R | NG_R_D07 | GU055677 | *Exophiala* sp. RSEM07_18 | Chaetothyriales | A | 1,1 | T |
| R | NG_R_C12 | GU055670 | *Fusarium solani* | Hypocreales | A | 1,1 | N |
| R | NG_R_C10 | GU055669 | *Fusarium* sp. R_C10 | Hypocreales | A | 1,1 | |
| R | NG_R_E02 | GU055682 | *Fusarium merismoides var. merism.* | Hypocreales | A | 1,1 | M, N |
| R | NG_R_F11 | GU055696 | Hypocreales R_F11 | Hypocreales | A | 1,1 | |
| R | NG_R_H12 | GU055710 | *Nectria lugdunensis* | Hypocreales | A | 1,1 | |
| R | NG_R_B06 | GU055658 | *Periconia macrospinosa* | Microascales | A | 1,1 | M |
| R | NG_R_H11 | GU055709 | *Plectosphaerella* sp. R_H11 | Phyllachorales | A | 1,1 | |
| R | NG_R_G01 | GU055697 | SCGI R_G01 | SCGI *i.s.* | A | 1,1 | |
| R | NG_R_G03 | GU055699 | Sordariomycetes R_G03 | Sordariomycetes *i.s.* | A | 1,1 | |
| T | NG_T_B06 | GU055716 | Chaetomiaceae T_B06 | Sordariales | A | 16,9 | |
| T | NG_T_A04 | GU055713 | *Schizothecium vesticola* | Sordariales | A | 10,1 | P |
| T | NG_T_A01 | GU055711 | Lasiosphaeriaceae T_A01 | Sordariales | A | 9,0 | |
| T | NG_T_A06 | GU055714 | *Exophiala* sp. RSEM07_18 | Chaetothyriales | A | 6,7 | R |
| T | NG_T_H11 | GU055747 | *Fusarium oxysporum* | Hypocreales | A | 6,7 | R |
| T | NG_T_C10 | GU055724 | Helotiales T_C10 | Helotiales | A | 5,6 | |
| T | NG_T_B11 | GU055717 | Pleosporales T_B11 | Pleosporales | A | 5,6 | |
| T | NG_T_H09 | GU055745 | *Trichocladium asperum* | Sordariales | A | 5,6 | |
| T | NG_T_D07 | GU055729 | *Cladosporium herbarum* complex | Capnodiales | A | 4,5 | N, R |
| T | NG_T_C05 | GU055721 | *Coprinellus* sp. T_C05 | Agaricales | B | 4,5 | |
| T | NG_T_E09 | GU055733 | Mortierellales T_E09 | Mortierellales | M | 4,5 | |
| T | NG_T_E04 | GU055732 | Pyronemataceae T_E04 | Pezizales | A | 3,4 | |
| T | NG_T_F08 | GU055736 | *Cryptococcus aerius* | Tremellales | B | 2,2 | R |
| T | NG_T_C01 | GU055718 | *Nectria ramulariae* | Hypocreales | A | 2,2 | |
| T | NG_T_D03 | GU055727 | *Psathyrella* sp. T_D03 | Agaricales | B | 2,2 | |
| T | NG_T_A03 | GU055712 | *Apodus deciduus* | Sordariales | A | 1,1 | |
| T | NG_T_F11 | GU055737 | *Chytridiomycota* T_F11 | Chytridiomycota *i.s.* | C | 1,1 | |
| T | NG_T_H01 | GU055742 | Helotiales P_C08 | Helotiales | A | 1,1 | P |
| T | NG_T_D02 | GU055726 | Helotiales T_D02 | Helotiales | A | 1,1 | |
| T | NG_T_D06 | GU055728 | Helotiales T_D06 | Helotiales | A | 1,1 | |
| T | NG_T_D01 | GU055725 | Hypocreales T_D01 | Hypocreales | A | 1,1 | |
| T | NG_T_H06 | GU055743 | Sordariomycetes T_H06 | Sordariomycetes *i.s.* | A | 1,1 | |
| T | NG_T_C03 | GU055720 | Stephanosporaceae T_C03 | Agaricales | B | 1,1 | |
| T | NG_T_H10 | GU055746 | *Tetracladium* sp. P_E09 | Helotiales | A | 1,1 | P |

[a] M, Maissau; N, Niederschleinz; P, Purkersdorf; R, Riederberg; T, Tulln

[b] representative sequenced clone from library

[c] Acc.No., Accession number at GenBank

[d] Sequence identification based on separate BLAST searches of the ITS-region and the partial LSU-sequence; clone epithets are used to distinguish different species were identification to the species-level was not possible (e.g. Hypocreales M_E04 is different from Hypocreales N_A02)

[e] phylogenetic affiliation to a phylum (or other higher taxonomic ranks where appropriate); A, Ascomycota; B, Basidiomycota; Bc, Blastocladiomycota; C, Chytridiomycota; E, Eukaryota; F, Fungi; M, Mucoromycotina

[f] RA: relative abundance in percent of analysed clones per soil type based on RFLP and sequencing data

[g] CO: co-occurence of the same species in a second (and third) soil

[h] *i.s.*, *incertae sedis*

2007; Urich et al. 2008; Vandenkoornhuyse et al. 2002), much less is so far known about fungal communities in agricultural soil (de Castro et al. 2008; Domsch and Gams 1970; Lynch and Thorn 2006; Stromberger 2005). Molecular fingerprinting approaches like DGGE or T-RFLP allow rapid profiling of distinct communities and are especially useful for comparative analyses of numerous samples, but provide no information on species identities (Kennedy and Clipson

2003). Cloning and sequencing, on the other hand, is more labour-intensive but allows identification of the community members. Care must, however, be taken when using GenBank for species identification, since many sequences are incorrectly named (for a case study see e.g. Cai et al. 2009).

In this study we obtained by sequencing of ITS/partial LSU clones from four arable and one grassland soil a dataset of 115 fungal species, of which 96 were found in arable soils. This species inventory contains both, actively growing mycelium and dormant structures like spores (Anderson and Cairney 2004). The majority of fungal sequences belonged to the Ascomycota, which is not unusual for soil habitats lacking ectomycorrhizal host plants (Schadt et al. 2003) and is in good agreement with findings from a soy bean plantation site (de Castro et al. 2008) and from numerous studies using cultivation techniques to describe agricultural soil fungal communities (Domsch and Gams 1970). Dominance of Ascomycota is probably enhanced by relatively high nitrogen contents of all soils analysed herein (Nemergut et al. 2008). The grassland soil analysed by Anderson et al. (2003), however, was dominated by Basidiomycota (60% of the clones in the combined SSU library and 47% in the ITS library), while Basidiomycota were only the second most abundant group in all five soil samples from our study (7.5–21.3% of the analysed clones).

A similar distribution of sequences between fungal phyla was observed in a sandy lawn by a metatranscriptomic approach, which assessed abundance of soil RNAs by pyrosequencing (Urich et al. 2008). Since no PCR step is involved, this approach is unbiased by amplification. The main difference was the presence of ca. 20% sequences belonging to the Glomeromycota, which are completely absent from our datasets.

Surprisingly, the inventory of agricultural soil fungal taxa found by cultivation techniques (Domsch and Gams 1970) correlates well with the molecular data obtained from our cultivation-independent survey as there is e.g. the dominance of Ascomycota or frequent occurrence of fungi from the orders Sordariales, Hypocreales and Helotiales. Even at the genus and species level many fungi found in our study were already previously described to occur in agricultural soils, as is the case e.g. for the genus *Tetracladium* and for the potentially phytopathogenic genera *Fusarium* and *Nectria*. It should, however, be considered that 49 of the 115 fungal species in our study could not be classified below family level. This group of 49 species is probably composed of formally described fungal species for which no ITS or LSU reference sequences are deposited in GenBank and for another part harbours species not yet formally described. No attempts for a cultivation-dependent description of the soil fungal communities were undertaken in our study. The relatively good correlation between cultivation-dependent and -independent techniques for fungal communities in agricultural soils is not unprecedented for environments dominated by ascomycetes (Götz et al. 2006) but in striking difference to bacterial communities (Smit et al. 2001). Traditional soil bacterial genera known from cultivation techniques make up only 2.7 to 3.7% of the total community investigated by cultivation independent techniques (Janssen 2006).

*Tetracladium*, which was the most prominent genus found in the soils from our study, is mainly known to occur in aquatic ecosystems, where it is involved in leaf litter decay (Bärlocher 1992), or as plant endophyte (Selosse et al. 2008). Nevertheless, this genus has been found also in agricultural soils (Domsch and Gams 1970; Domsch et al. 1993), where it is most likely involved in plant debris degradation. A survey of insufficiently identified sequences from environmental samples in emerencia (Ryberg et al. 2009) revealed that *Tetracladium* actually commonly occurs in soil samples or associated with plant roots. In our study, *Tetracladium* was only absent from soil M, the soil with the lowest clay content (see Inselsbacher et al. 2009) and therefore lowest water holding capacity from all five soils. Similarly, relatively dry soil conditions and consequently good aeration resulted in highest nitrification activities and highest $NO_3^-$-N/$NH_4^+$-N ratios in soil M (Inselsbacher et al. 2009).

Predicted species richness (Chao2; Chao 1987) for the soils studied here ranged from 20.4 to 51.3, which is in a similar range as found in comparable studies (see Table 1), but substantially lower than fungal richness estimations from studies employing high throughput sequencing (Buee et al. 2009; Fierer et al. 2007). In addition, richness estimation is strongly dependent on the prediction model (Fierer et al. 2007). For these reasons predicted species richness allows direct comparison of datasets similar in size analysed by identical models, but gives little information about the actual number of species present in a sample.

Predicted species richness, diversity and the phylogenetic composition of fungal communities from arable soils did not differ from the grassland soil R (see Table 1), although soil R showed higher levels of microbial biomass and activity compared to the four arable soils (Inselsbacher et al. 2009). Likewise, vegetation cover at sampling time did, within the limits of our experimental resolution, not substantially influence richness, diversity and phylogenetic composition of soil fungi. This finding is in agreement with data reported by Waldrop et al. (2006) who showed that aboveground plant richness does not directly influence belowground fungal richness.

While there does not seem to be a difference in general parameters of fungal communities between arable and grassland soils, the most striking difference is the obvious absence of SCGI from arable soil, a group of fungi that could

be found at high frequencies in grassland soils (soil R and natural grassland field site at the Sourhope Research station (Anderson et al. 2003)). SCGI is an only recently detected subphylum at the base of the Ascomycota with thus far no cultivated members (Porter et al. 2008). Presence in grassland and absence in arable soil could be an indication that SCGI fungi directly depend on a continuous plant cover, which is in good agreement with the list published by Porter et al. (2008) summarising sites where SCGI fungi were found. Although site characteristics ranged from tundra to forest and from tropical to boreal, not a single arable site was included in this listing. SCGI fungi are frequently found directly associated with grass roots (Vandenkoornhuyse et al. 2002) or ectomycorrhizal root tips (Izzo et al. 2005; Menkis et al. 2005; Rosling et al. 2003; Urban et al. 2008), further pointing to an obligate-biotrophic lifestyle, which was already proposed by Porter et al. (2008). Such a direct dependence of the fungus on living plants could be the reason for the hitherto inability to cultivate SCGI fungi.

Fierer et al. (2007) suggested that diversity is independent of soil parameters but an intrinsic feature of microbe types, the fungal specific Simpson's diversity index being 134±39. This value is however far above the values found in our study (7.37–28.09), in Brazilian soy bean plantation soil (2.87; de Castro et al. 2008), Scottish grassland soil (3.62–7.50; Anderson et al. 2003) or soil with mixed grass-legume-shrub vegetation in Tennessee (2.56–41.67; Castro et al. 2010). Underestimation of diversity indices due to smaller sizes of libraries is unlikely to be the cause for this discrepancy, since predictions for the diversity indices of soils M, N, P, R and T stabilised after analysis of a maximum of 50 sequences. This is in good agreement with a comparative evaluation of diversity indices by Giavelli et al. (1986), who found that Simpson's diversity index is least sensitive to small sample size. While the diversity in our study is potentially underestimated due to the use of RFLP for clone selection, even lower diversity indices were found in published studies for grassland (Anderson et al. 2003) and arable (de Castro et al. 2008) soil by directly sequencing SSU libraries without preselection by RFLP (see Table 1), an approach adopted at larger scale by Fierer et al. (2007). Underestimation of diversity at the species level by analysing SSU libraries is expected since the phylogenetic resolution of the fungal SSU is commonly thought to be restricted to the genus or family level but not to be sufficient for species identification (Anderson and Cairney 2004; Seena et al. 2008). More comparative studies are needed to give a solid answer whether arable and grassland soils indeed sustain a lower fungal diversity compared to desert, prairie or rainforest soils, which are the ecosystems studied by Fierer et al. (2007).

Our study provides a fungal community inventory of agricultural soils and reveals the most prominent species. Considering, however, the known seasonal dynamics of soil fungal communities and the diversity of agricultural practices, further studies are needed to extend and corroborate the presented initial findings. At least at the regional scale some general conclusions can be drawn from this study, i.e. (i) different agricultural soils harbour common fungal taxa from the species to the phylum level; (ii) the fungal biodiversity of our four investigated arable soils was in a similar range as one investigated and one reference grassland soils, and (iii) SCGI fungi seem to be absent from agricultural soils. These findings will certainly facilitate future studies on the relationship between fungal community structure and function and how these fungal-specific functions influence microbial nutrient cycling and the soil food web. The culturability of the majority of agricultural soil fungi opens the possibility for laboratory culture experiments to study genetics and molecular physiology of a number of potentially important species and thus to better determine their role in agroecosystems.

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