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Assessment of yeast diversity in soils under different management regimes

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ABSTRACT

Human activities, land management and climate change all have great impact on soil biology, but our knowledge of biodiversity of soil organisms is still very limited. Therefore, we assessed responses of soil yeasts to land management, and analysed 57 soils showing different land use from three distinct localities. We isolated and identified molecularly a total of 40 yeasts including several new species. Overall, species composition of different localities was very heterogeneous and nearly half of the species were found in a single site only.

The analysis of species abundance and community composition revealed a strong long-term effect of forest replacement by grassland vegetation. Unlike forests, grasslands harbour predominantly ascomycetous yeasts and their proportion increases with management intensity. In forests, evenness of yeast communities followed the gradient of land management intensity and natural beech forests harboured the most unevenly structured community, thereby mirroring the evenness of plant communities.

Introduction

Central Europe represents an ancient cultivated landscape and every spot holds traces of former use (Ellenberg 1988). Naturally, most of this area would be dominated by beech (*Fagus sylvatica*) forests. Extensive pasturing, felling and burning over more than 2000 yr have resulted in widespread and lasting woodland destruction and conversion. Although today, the total forested area of Germany again covers about 30 %, unmanaged beech forests account for only about 0.5 % (BfN 2008). Due to disturbances in vegetation and soil cover unmanaged forests provide a multitude of diverse habitats of different scales and properties, whereas managed forests are characterised by low heterogeneity and complexity (e.g. Christensen & Emborg 1996). In agricultural areas, fertilizer application or grazing considerably modify the environment

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by changing floristic composition, productivity, decomposition rates and many soil properties. Thus, forestry and agriculture radically change nutrient cycles and biodiversity in the affected ecosystems. Although, the significance of these processes is well recognised, the underlying mechanisms of ecosystem responses to human impact remain largely unclear (Wardle *et al.* 2004).

Soil biota interact with aboveground ecosystem components and influence ecosystem diversity, structure and functioning (Wardle *et al.* 2004). Being a significant component of all terrestrial environments, fungi have considerable impact on fundamental soil processes, like decomposition, aggregation, nutrient release and nutrient storage (Christensen 1989). Yeasts, a systematically artificial group of fungi designated by presence of a unicellular stage in their life cycle are found in soils worldwide (Bab'eva & Chernov 1995; Vishniac 2006; Botha 2011). Although their relevance for soil functioning is not fully understood yet, yeasts influence soil aggregation (Bab'eva & Moawad 1973), contribute to nutrient cycles (Botha 2011) but also interact with vegetation (Cloete *et al.* 2009) and soil animals (Yurkov *et al.* 2008). Next to the exploration of new habitats, the application of molecular methods has tremendously increased our knowledge of yeast species diversity, including soil yeasts, over the last 50 yr (Kurtzman & Robnett 1998; Fonseca *et al.* 2000; Scorzetti *et al.* 2002). Because an important factor that determines the validity of studies in yeast ecology is the correct identification of species in the ecosystem, application of molecular tools in biodiversity surveys was necessitated (e.g. Kurtzman & Fell 2006).

Although soil-inhabiting yeasts have been studied for over a century (reviewed by Starkey & Henrici 1927; Botha 2011), still little is known about their diversity. Up to 130 species have been reported from soils worldwide. From these studies, evidence for strong association with soil-related substrata is lacking for many of the reported species (Bab'eva & Chernov 1995; Vishniac 2006; Botha 2011). Most of the studies were conducted in boreal or temperate soils and utilized a combination of morphological and physiological characters for species identification (e.g. di Menna 1965; Sláviková & Vadkertiová 2000, 2003; Maksimova & Chernov 2004). However, unlike DNA markers, these commonly used assimilation tests are often not able to distinguish between closely related species, as physiological variations naturally exist between individual strains (e.g. Scorzetti *et al.* 2002). While this does not render these earlier surveys invalid, it suggests that conclusions drawn from these studies need to be re-examined using more accurate species identification (Kurtzman & Fell 2006). This is especially true, because so far only two studies worldwide investigated soil yeast diversity using molecular identification tools (Wuczkowski & Prillinger 2004; Vishniac 2006). Therefore, our knowledge about yeasts living in soils is rather limited.

In the present study we analyse the yeast communities across a series of 57 forest and grassland plots using cultivation techniques with the subsequent identification of isolated cultures based on rDNA sequence data. The surveyed biotopes reflected a gradient of land management: from near-natural climax beech forests to planted clear-cut forests and further to grasslands of different land use intensities. The latter represent former forested areas, which were converted to agriculture a long time ago. The plots were located in three separate regions along a latitudinal transect in Germany (Fischer *et al.* 2010).

The main objective of the present study was to assess responses of the soil yeast community to human impact. To achieve this, we, first, analysed ecological preferences and distribution patterns of yeasts across the three different regions. Second, long-term effects of forest conversion to open vegetation were investigated by comparing forest and grassland biotopes. Third, the influence of management intensity on forest and grassland communities was assessed in three different management categories for each vegetation type. We used the hierarchical design of the German Biodiversity Exploratories to analyse abundance, diversity, composition and structure of yeast communities quantitatively.

Materials and methods

Study sites and sampling

Soil samples were collected from three different localities in Germany on intensively studied experimental plots (VIPs) of the German Biodiversity Exploratories framework (www.biodiversity-exploratories.de) between Apr. and May 2008. Study sites were the "Biosphärenreservat Schorfheide-Chorin" in Brandenburg (North-Eastern Germany), the "Nationalpark Hainich" and its surroundings in Thuringia (Central Germany) and the "Biospärengebiet Schwäbische Alb" in Baden-Württemberg (South-Western Germany). In total, 57 sites consisting of 30 forest and 27 grassland plots were studied. Surveyed sites were selected to represent equally a gradient of land use intensity both in forests and grasslands (Table S1; Fischer *et al.* 2010). The three management categories, intensively managed, managed and extensively managed, were defined according to the type, intensity of annual use and the history of land management. Fertilisation, mowing and grazing were among the most important factors for selecting grassland plots. Intensively managed grasslands were fertilized meadows with 2–3 cuts per year, managed areas were mown pastures (grazed by cattle and horses) with one annual cut, and extensively managed plots were mostly unfertilized pastures grazed by cattle (Hainich and Schorfheide-Chorin) or sheep (Schwäbische Alb). Forest plots were selected on the basis of the main tree stand and the forest management. Intensively managed forest plots were the age-class coniferous forests, Scots pine (*Pinus sylvestris*) or Norway spruce (*Picea abies*), planted on land originally covered by broadleaf tree species. The managed forest type consisted of deciduous planted forests, with the main tree species being European beech (*F. sylvatica*). Extensively managed areas were mature near-natural (mostly protected and unmanaged for at least 60 yr) beech stands sometimes mixed with European ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*). Details of the study sites and of the soil properties are provided by Fischer *et al.* (2010).

Sampling was performed in the corners and the centre of 20 m × 20 m plots using a motor driven soil column cylinder. Subsequently, for each plot, topsoil samples obtained from the five cores were then pooled and homogenized. Coarse

woody debris, roots and stones (>5 mm) were removed in the field. Samples were stored at 4 °C and transferred to the laboratory for analyses.

Isolation of cultures

Soil samples were placed in 50 ml plastic tubes, suspended in sterile water (w/v) 1:5, 1:10 and 1:20, and shaken on an orbital shaker at 200 rpm for 1 hr. Soil from one plot was analysed in five replicates (sub-samples) and each of the replicates was plated in triplicate. An aliquot of 0.15 ml was plated on the surface of solid media. Acidified glucose–yeast extract–peptone agar (GPYA) was used for cultivation experiments. Plates were incubated at room temperature for 2–3 d and kept at lower temperatures (6–10 °C) to prevent fast development of moulds. Plates were examined after 7, 14 and 21 d of incubation. Colonies were differentiated into macro-morphological types using dissection microscopy, counted and 1–2 representatives of each colony type per plate were transferred into pure culture.

Identification of cultures

DNA was isolated from 3–4 d old cultures using a technique described by Hoffman & Winston (1987), with the modification of cell lysate centrifugation at room temperature for 15 min, at 20,000*g*. DNA was precipitated with ethanol and then dissolved in 50 μl TE buffer containing RNAse (10 μg ml^{-1}). PCR-fingerprinting with minisatellite-specific oligonucleotides derived from the core sequence of bacteriophage M13 with the sequence provided by Sampaio *et al.* (2001) or microsatellite-specific oligonucleotides $(GTG)_5$, $(ATG)_5$ and $(GAC)_5$ as single PCR primer (Gadanho & Sampaio 2002) were used to group pure cultures. Strains showing identical electrophoretic profiles were considered as conspecific and only 1–2 representatives of them were chosen for further identification by sequencing of rDNA regions. DNA fragments were amplified by PCR using the primers ITS1f and NL4 (Gardes & Bruns 1993; O'Donnell 1993). Initial denaturation was performed at 96 °C for 2 min, followed by 35 cycles of 20 s at 96 °C, 50 s at 52 °C and 1.5 min at 72 °C, respectively. A final extension step of 7 min at 72 °C was conducted.

PCR products were purified with the my-Budget Double Pure kit (Bio-Budget Tech., Germany) and sequenced on an ABI3130xl sequencer using the same primers as for PCR amplification. Chromatograms were checked and corrected with Sequencher 4.8 – 4.10 (Gene Codes Corp., USA). For species identification the obtained nucleotide sequences were compared with sequences deposited in the NCBI (www.ncbi.nih.gov) and CBS (www.cbs.knaw.nl) databases, respectively.

Statistical data analyses

For each sub-sample, yeast quantity and community structure were determined. Yeast quantity was calculated as colony forming units (CFU) per gram of soil at natural humidity. Frequency of occurrence was calculated as the number of samples, where a species was observed, as a proportion of the total number of samples. Relative abundance was calculated as proportion of a particular species in the sample and is based on colony counts. Probability of dominance was calculated as the number of samples, where a species showed the highest abundance, relative to the total number of samples, where this species was observed. Species evenness was assessed using Pielou index (Pielou 1966) and Rank-Abundance Distribution (RAD) (McGill *et al.* 2007).

Out of 285 soil sub-samples, a total of 245 were included in the final analysis and 40 sub-samples were excluded from the analysis because they yielded no yeast cultures either due to low fungal quantity in a particular replicate or due to fast development of moulds, which made isolation and appropriate quantification of yeasts difficult.

Statistical evaluations were performed with Statistica 8-9 (StatSoft Inc., USA). Quantity values were Lg_{10}-transformed for the analysis. Statistical analyses were performed on the three hierarchical levels of factors: region (three Exploratories), type of vegetation (forests, grasslands) and land use intensity (intensively managed, managed or extensively managed). Normality of distribution was tested for the discussed variables. Effects were considered to be statistically significant at the level $p \leq 0.05$. Significant effects were additionally confirmed with Chi-square test.

Results

Quantity

All analysed soils yielded yeast. Yeast quantity ranged from hundreds of cells to 1.6×10^6 CFU per gram of soil. On average, the quantity of yeasts in grasslands was slightly higher than in forest biotopes (Table 1 and Fig S1). Yeasts were more abundant in grasslands than in forests at Hainich Exploratorium, but quantities did not differ significantly at the other studied regions. These differences were mainly due to higher yeast quantity in extensively managed grasslands than in natural forests, 3.6 and 3.0 Lg_{10} CFU g^{-1}, respectively (Table 1). Total yeast counts did not differ significantly ($p > 0.05$) in managed plots between the three Exploratories (Table 1). On average, no significant ($p > 0.05$) effects of land management on total yeast quantity in forest soils was observed (Table 1). In grasslands, yeast quantity decreased significantly ($p \leq 0.05$) in relation to land management intensity at the study sites of Schorfheide-Chorin and Schwäbische Alb but not at Hainich (Table 1 and Fig S2).

Diversity

A total of 40 yeast taxa were isolated and identified during this study. They belong to three lineages of Fungi, Saccharomycotina (15 species), Agaricomycotina (19 species) and Pucciniomycotyna (six species). According to the genetic distances and the physiological profiles, seven yeast taxa represented potential new species. Out of them, four novel species have been recently described as *Barnettozyma vustinii*, *Clavispora reshetovae*, *Holtermanniella takashimae* and *Leucosporidium drummii* (Yurkov *et al.* 2009a, b, 2011).

Although, observed species richness varied from 1 to 10 species per plot, average species richness values ranged between 2 and 3 species per sub-sample for a single region, vegetation type and land management category (Fig 1 and

Table 1 – Species list and relative abundance of soil yeasts isolated from a gradient of land use intensity. Total quantity, species richness, diversity and community evenness indices are provided below

Species and region of isolation[a]		Forests			Grasslands		
		Intensively managed	Managed	Extensively managed	Intensively managed	Managed	Extensively managed
Barnettozyma californica		–	–	–	>0.01 (A)	–	0.11 (S)
B. pratensis		–	0.01 (A)	0.07 (H)	–	–	–
B. vustinii	H	–	>0.01	–	–	–	–
	S	–	–	–	0.68	0.19	0.33
Candida kruisii		–	0.08 (H)	–	–	–	–
C. sake		–	–	–	–	–	0.03 (A)
C. vartiovaarae	A	–	–	–	0.06	0.37	–
	H	0.06	–	0.07	0.38	0.18	0.09
Clavispora reshetovae		–	–	–	0.01 (A)	–	0.02 (H)
Cryptococcus adeliensis		–	–	–	–	–	0.12 (S)
Cr. aerius	A	–	–	–	0.04	–	0.76
	S	–	–	–	–	0.01	–
Cr. gastricus	A	–	–	–	–	0.02	–
	H	0.06	–	>0.01 (H)	0.08	–	–
	S	–	–	–	0.02	0.04	–
Cr. laurentii		–	–	–	–	0.01 (S)	–
Cr. podzolicus	A	–	0.02	–	–	–	–
	S	0.53	0.27	0.09	–	–	–
Cr. ramirezgomezianus	A	0.06 (A)	–	–	–	–	–
Cr. tephrensis	S	0.03	–	–	–	0.04	–
Cr. terreus	A	0.31	0.02	0.23	–	–	>0.01
	S	–	–	–	–	0.23	0.08
Cr. terricola	A	0.11	0.45	0.04	–	–	–
	H	–	0.09	–	–	–	–
	S	0.39	0.73	0.90	–	0.05	–
Cr. victoriae	S	0.02	–	–	–	0.04	0.01
Cystofilobasidium macerans		0.01 (H)	–	–	–	–	>0.01 (S)
Debaryomyces hansenii	H	0.03	0.10	0.05	–	–	–
Guehomyces pullulans	A	–	–	–	0.02	–	>0.01
	H	–	0.08	0.11	–	–	–
	S	–	–	–	–	0.03	0.01
Holtermanniella festucosa	S	–	–	–	–	–	0.01
H. takashimae	S	–	–	–	–	0.07	0.01
H. wattica	A	0.12	–	–	–	0.01	–
	H	0.03	–	–	–	–	–
	S	–	–	–	–	–	>0.01
Kazachstania piceae	A	–	0.06	0.07	–	–	–
	H	0.35	0.28	0.25	–	–	–
K. servazzii		–	–	–	0.21 (H)	–	–
Leucosporidium drummii		–	–	–	0.02 (S)	–	–
L. golubevii		–	–	–	–	–	0.01 (S)
Lindnera saturnus	S	–	–	–	0.25	0.02	0.16
Li. misumaiensis		–	0.11 (H)	–	–	–	–
Rhodotorula glutinis	S	–	–	–	–	0.20	0.01
Rhodotorula sp. AY167		–	–	–	>0.01 (H)	–	>0.01 (A)
Rhodotorula sp. AY214		0.03 (H)	–	–	–	–	–
Rhodotorula sp. AY211		0.03 (H)	–	–	–	–	–
Rhodosporidium babjevae		–	–	–	–	–	0.02 (S)
Schizoblastosporion	A	0.08	0.02	–	–	–	–
starkeyi-henricii	S	0.02	–	–	–	0.04	–
Schwanniomyces castellii	A	–	–	–	0.66	0.01	–
	H	0.01	–	–	0.33	0.66	0.86
	S	–	–	–	–	–	0.02
Sc. occidentalis		–	–	–	–	0.06 (A)	–
Trichosporon dulcitum	A	0.13	0.16	0.51	0.09	0.51	0.10
	H	0.20	0.11	0.45	–	0.03	0.03
	S	–	–	0.01	0.03	0.01	0.09
T. multisporum	A	–	–	–	0.11	–	0.06
	H	–	–	–	–	0.06	–
	S	–	–	–	–	>0.01	–

(continued on next page)

Table 1 – (continued)

Species and region of isolation[a]		Forests			Grasslands		
		Intensively managed	Managed	Extensively managed	Intensively managed	Managed	Extensively managed
T. porosum	A	0.19	0.27	0.15	0.01	0.03	0.05
	H	0.19	0.13	0.01	–	0.07	–
Species richness, N	A	7	8	5	9	7	7
	H	11	8	8	4	5	4
	S	5	2	3	5	15	16
Quantity, Lg CFU/g	A	3.4	2.8	3.0	3.1	3.2	3.5
	H	2.4	2.8	2.7	3.3	3.0	3.4
	S	3.1	3.5	3.4	3.0	3.5	4.0
Shannon index, H′	A	0.91	0.67	0.65	0.76	0.90	0.74
	H	0.54	0.71	0.32	0.17	0.65	0.29
	S	0.19	0.26	0.09	0.30	0.76	0.60
Pielou index, J′	A	0.76	0.59	0.66	0.69	0.88	0.66
	H	0.45	0.57	0.35	0.21	0.64	0.39
	S	0.24	0.38	0.10	0.32	0.72	0.48
Relative abundance of ascomycetous yeasts	A	0.08	0.09	0.07	0.08	0.42	0.03
	H	0.55	0.58	0.41	0.59	0.18	0.11
	S	0.02	>0.01	>0.01	0.92	0.23	0.59

a Region of isolation: A, Schwäbische Alb; H, Hainich; S, Schorfheide-Chorin.

Table 1). Without any regard to land use intensity, grasslands appeared to be slightly more species rich than forests at Schorfheide-Chorin (Table 1). In general, grassland plots showed decreased species richness in intensively managed biotopes in comparison to extensively managed areas. In contrast, natural forests harboured a lower number of yeasts than managed forests (Fig 1).

Community composition

The most frequent yeast species isolated was *Trichosporon dulcitum*, which was found in 57 % of forest and 54 % of grassland sub-samples (Fig 2). However, community composition differed considerably between vegetation types and regions. Basidiomycetes were more prominent in forest biotopes, e.g. *Cryptococcus terricola* and *Trichosporon porosum* were observed in 57 % and 46 % of analysed sub-samples, respectively. The most frequent ascomycetes in forest sub-samples were *Kazachstania piceae* (43 %) and *Candida vartiovaarae* (18 %). Frequency of occurrence of other species did not exceed 10 % (Fig 2). Grasslands harboured predominantly ascomycetous yeasts especially *Schwanniomyces castellii* (43 %) and *C. vartiovaarae* (32 %). The most frequent basidiomycetes in sub-samples of this habitat were *Cryptococcus aerius* (15 %), *Trichosporon multisporum* (11 %) and *T. porosum* (10 %) (Fig 2).

Although, the total number of ascomycetous yeast species in the two different vegetation types was nearly equal (grasslands 10 species; forests 9 species), the community composition of the two habitats showed differences. While *Barnettozyma pratensis*, *Candida kruisii*, *Debaryomyces hansenii*, *K. piceae* and *Lindnera misumaiensis* were isolated from forest biotopes only, *Barnettozyma californica*, *B. vustinii*, *Cl reshetovae*, *Lindnera saturnus* and *Schwanniomyces occidentalis* occurred exclusively in grasslands (Fig 2, Table 1, Fig S3). *C. vartiovaarae* was isolated both from

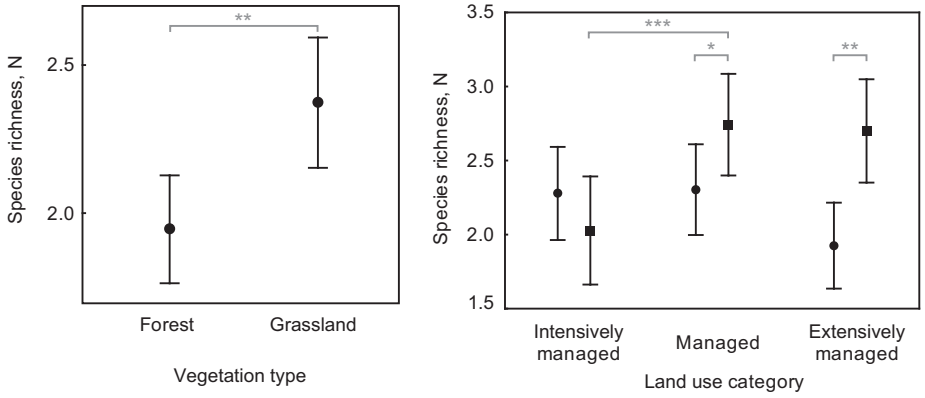

Fig 1 – Species richness in the sub-samples depending on vegetation type (left) and land use intensity (right) in forests (circle) and grasslands (square). Bars are confidence interval and middle points the respective mean. Significant differences are indicated by $^{*}p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$.

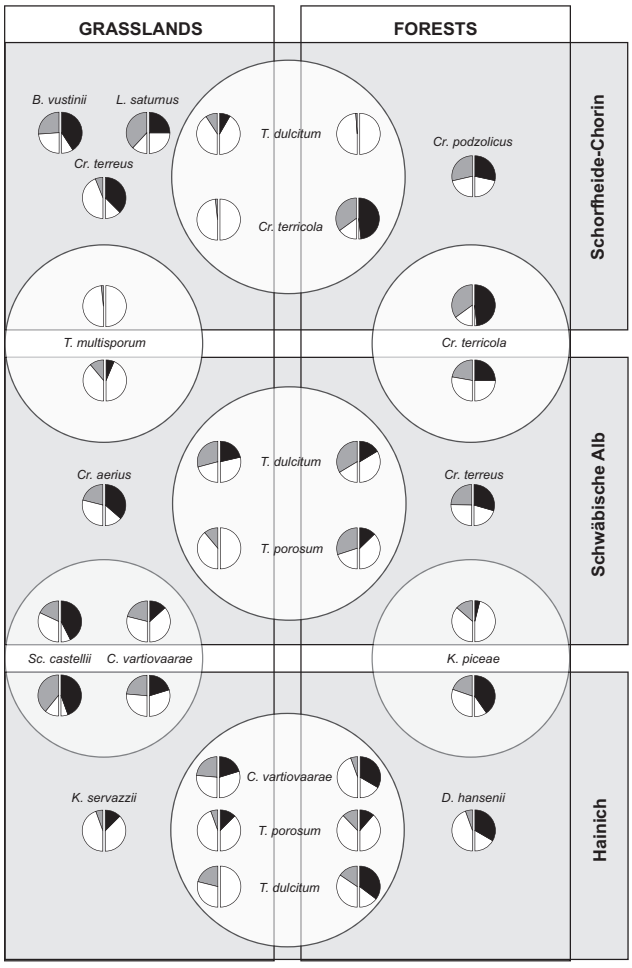

Fig 2 – Frequency and dominance of yeasts in forest and grassland soils at three localities as well as the species shared between biotopes and regions. Diagrams display frequency of occurrence (grey) and probability of dominance (black) of yeast species. Plot charts are scaled to 200 %, so each of the half-circles comprises 100 %.

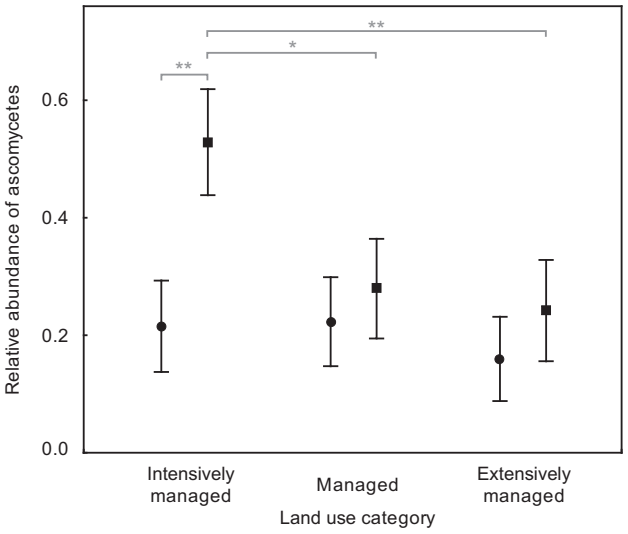

Fig 3 – Relative abundance of ascomycetous yeasts in forest (circle) and grassland (square) soils in relation to land use intensity. Bars are confidence interval and middle points the respective mean. Significant differences are indicated by $^{*}p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$.

forest and grassland soils at Hainich Exploratorium, but was found only in grassland at Schwäbische Alb (Fig 2).

Several yeasts displayed a strong association with a certain sampling region (Fig 2 and Table 1). In forests, the distribution of *Cryptococcus terreus* was restricted to the region Schwäbische Alb, *Cryptococcus gastricus* to Hainich and *Cryptococcus podzolicus* to Schorfheide-Chorin. *T. porosum* and *K. piceae* were obtained from soils of both Schwäbische Alb and Hainich. Ascomycetous yeasts were more numerous at Hainich, where all nine species were observed. Forest soils of Schorfheide-Chorin harboured a single ascomycete, *Schizoblastosporion starkeyi-henricii*, and Schwäbische Alb two additional species, *K. piceae* and *B. pratensis*. In grasslands, the distribution of *Sc. occidentalis* and *Cr. aerius* was restricted to the region Schwäbische Alb, while *B. vustinii* and *L. saturnus* were especially frequent at Schorfheide-Chorin. Yeasts *T. porosum*, *Sc. castellii* and *C. vartiovaarae* were isolated from soils of both Schwäbische Alb and Hainich (Fig 2 and Table 1).

Community structure

The abundance of species varied significantly depending on vegetation type and land management. Basidiomycetous yeasts, e.g. *Cr. podzolicus*, *Cr. terricola*, *Cryptococcus ramirezgomezianus*, *Holtermanniella wattica*, *T. dulcitum* and *T. porosum* were more prominent in forests than in grasslands (Fig S3). In contrast, relative abundance of ascomycetous yeasts was significantly higher in grasslands (Fig 3, Table 1, Fig S3). For example, *Sc. castellii* contributed 50–100 % of total yeast counts in grasslands, while in forests its abundance did not exceed 2 %. Similarly, *B. vustinii* was more abundant in grasslands than in forests, 25–100 % *vs.* 0–1 %, respectively (Table 1 and Fig S3). *K. piceae* and *D. hansenii* were the only two ascomycetes which were significantly more abundant in forest biotopes (Table 1 and Fig S3).

The contribution of several species to yeast communities varied with land management (Fig 4). Relative abundance of *Cr. terricola* and *T. dulcitum* increased in unmanaged forests, while *Cr. podzolicus* and *Cr. terreus* displayed contrasting trends. In grasslands, relative abundance of ascomycetous yeasts significantly increased along with the intensification of land use. While contribution of *Cr. aerius* and *Sc. castellii* to the yeast communities decreased from intensively managed pastures to extensively managed meadows, distribution of *B. vustinii* displayed the contrasting trend (Fig 4 and Table 1).

Discussion

Yeast diversity

The diversity of unculturable soil microorganisms, mainly prokaryotes, is estimated to be extremely high (e.g. Curtis & Sloan 2005). This is also true for fungi even though molecular surveys of fungi have received much less attention

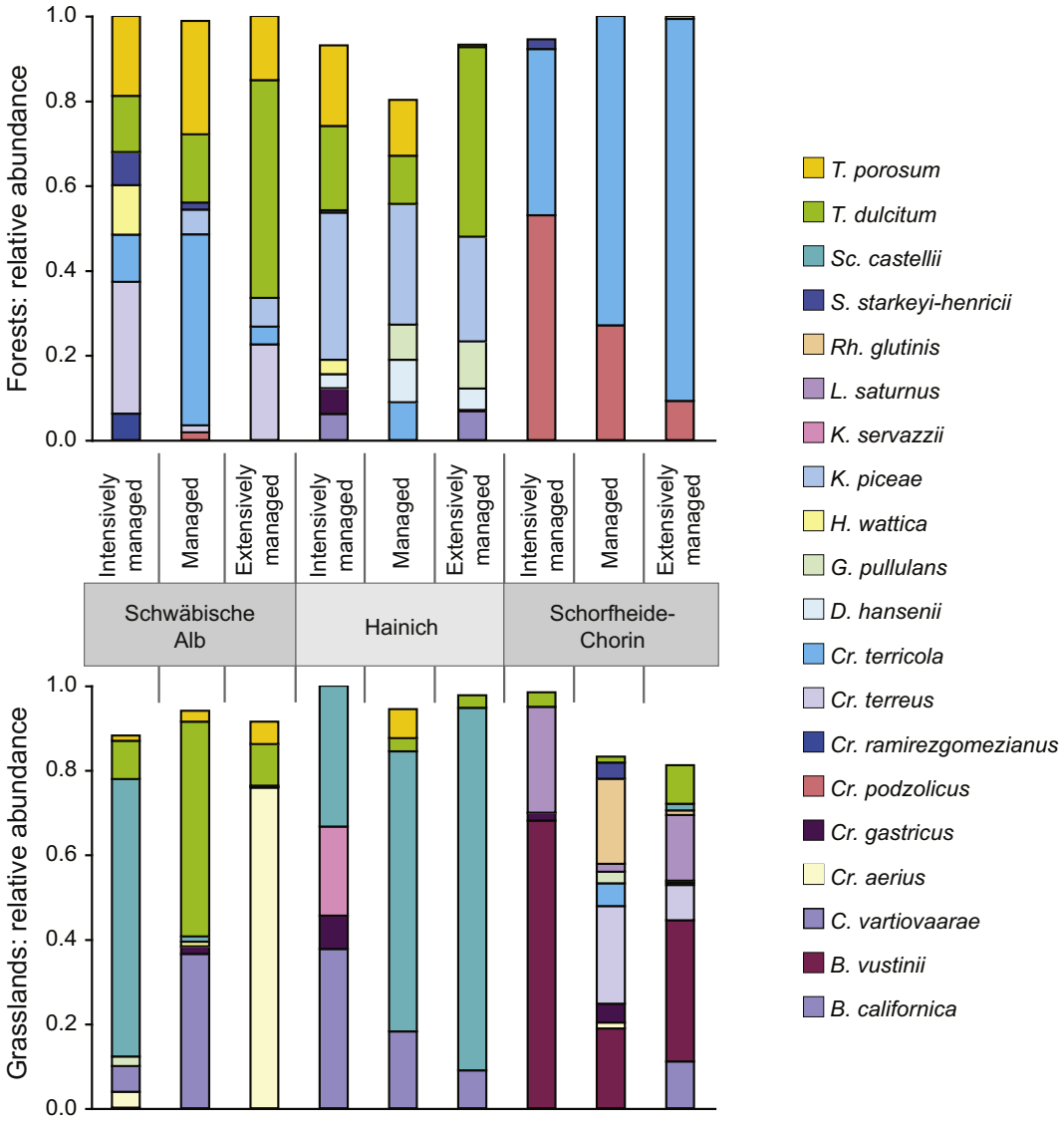

Fig 4 – Relative abundance of yeasts dominating in forest (above) and grassland (below) soils.

(Schmidt *et al.* 2008). To date yeasts in soils were mainly studied using culturing approaches and there are only a few reports of environmental sequences of fungi belonging to yeast lineages (Renker *et al.* 2004; Lynch & Thorn 2006; Buée *et al.* 2009). Lynch & Thorn (2006), using a cloning approach with subsequent Sanger sequencing, analysed basidiomycetes in arable soils and detected yeasts, which were already reported as soil inhabitants, *Cr. podzolicus*, *Cr. terreus*, *Cr. terricola*, *T. dulcitum* and *Guehomyces pullulans*. Similarly, 454-pyrosequencing of six forest soils showed a large number of sequence reads of the yeasts *Cr. podzolicus* and *Cr. terricola* (Buée *et al.* 2009). All these widespread pedobionts were also isolated and cultivated during the present study. Renker *et al.* (2004) identified several new lineages of Tremellomycetes, related to the genera *Cryptococcus* and *Dioszegia*, during the analysis of arbuscular-mycorrhizal roots and spores, suggesting the relevance of unculturable fungi. In our opinion, the new lineages observed by Renker *et al.* (2004) do not necessarily support the presence of great unculturable diversity in soils but exemplify that soils have not been sufficiently studied so far. The diversity assessment performed in the present work additionally illustrates that there is a large sampling bias currently existing in soil mycology. Despite the fact that the present study was aimed at analysing the structure of yeast communities of different biotope types and not at the isolation of new species, and used culture techniques, 15 % of the isolated species are new to science.

Of 40 species isolated in this study, 11 had been reported to be associated with soil substrata, i.e. the basidiomycetous *Cr. aerius*, *Cryptococcus laurentii*, *Cr. terreus*, *Cr. terricola*, *Cr. podzolicus*, *G.* (*Trichosporon*) *pullulans* and the ascomycetous *B.* (*Williopsis*) *californica*, *B. pratensis*, *Sc.* (*Debaryomyces*) *occidentalis*, *L.* (*Williopsis*) *saturnus*, *S. starkeyi-henricii* (Bab'eva & Chernov 1995; Lachance & Starmer 1998; Vishniac 2006; Botha 2011). In accordance with other studies (e.g. Slávíková & Vadkertiová 2000; Golubtsova *et al.* 2007), our study also yielded a small

number of pigmented yeasts, i.e. *Cryptococcus tephrensis*, *Cryptococcus victoriae*, *Rhodotorula glutinis* and *Rhodosporidium babjevae*. Although these yeasts are found regularly in soils, they are typically associated with the phylloplane and enter the soil profile with plant material (Fonseca & Inacio 2006).

The ecology of most other species isolated in our survey was previously unknown and evidence for association with soil for the yeasts mentioned below is provided here for the first time. *B. vustinii* was dominant in grasslands of Schorfheide-Chorin (Fig 2) but the distribution range of this species is obviously larger. According to sequence data, deposited in GenBank (acc. numbers AB525764–67), it was recently found in Japan along with *B. californica* and *B. pratensis*, and this species might be a widespread but so far overlooked pedobiont.

K. piceae was frequently found in beech forests at Schwäbische Alb and in all forest types at Hainich (Fig 2). This species was described based on isolates obtained from the rhizosphere of spruce but its association with bulk soil was unknown. The most frequent and the most abundant ascomycete isolated from forests, *K. piceae*, was previously found in Austria (Wuczkowski & Prillinger 2004) suggesting that it might inhabit various forest soils of Central Europe.

C. vartiovaarae, which was described from a soil in Finland, was observed regularly at Schwäbische Alb and Hainich. The high frequency of occurrence along with the high abundance of this species suggests soil being its primary habitat (Fig 2 and Fig S3). Phylogenetically, *C. vartiovaarae* is related to the well-known pedobiont *L. saturnus* species complex (Kurtzman *et al.* 2008) and *Candida mengyuniae* (Chen *et al.* 2009), representing an eco-clade, which is associated with soils.

Sc. occidentalis was detected in our study at low frequency and abundance (Table 1) that does not support its association with analysed soils even though it was recognised as a pedobiont (e.g. Lachance & Starmer 1998). We found *Sc. castellii*, of the same monophyletic group, to be very prominent in soils especially of grasslands at Hainich and Schwäbische Alb (Fig 2 and Table 1). Together with *Schwanniomyces polymorphus*, *Schwanniomyces pseudopolymorphus*, *Schwanniomyces yamadae* and *Schwanniomyces vanrijiae*, *Sc. occidentalis* is characterised by very low divergence of rRNA gene sequences (Martorell *et al.* 2005). Thus, a proper differentiation of this species complex requires multi-gene analyses, as performed in the present study. Importantly, the other closely related (and cryptic) species comprising the above-mentioned complex were never observed.

Arthroconidia-forming yeasts morphologically assigned to the genus *Trichosporon* inhabit various soils, and *T. pullulans* is among the most frequently reported soil species (Bab'eva & Chernov 1995; Sláviková & Vadkertiová 2000; Maksimova & Chernov 2004). We occasionally found *G. pullulans* in all three Exploratories and its abundance never exceeded 25 % per subsample (Table 1). In contrast, the true members of the genus *Trichosporon*, *T. dulcitum* and *T. porosum* were more frequent and abundant (Fig 2, Table 1, Fig S3). *T. dulcitum* was the only common species in both forests and grasslands, without any regard to region or land management type (Figs 2 and 4 and Table 1). Although *T. dulcitum* has been known for nearly 90 yr, its ecological preferences remained obscure. Several isolations of this yeast from soils were reported (Wuczkowski & Prillinger 2004; CBS database). Additionally, *T. dulcitum* was detected using a culture-independent approach from an arable field in MI, USA (Lynch & Thorn 2006). Interpreting these reports in the light of our findings, *T. dulcitum* should be considered as a widespread pedobiont.

Distribution of yeasts

Several earlier studies reported that yeasts are unevenly distributed in soils (Bab'eva & Chernov 1995; Sláviková & Vadkertiová 2000; Maksimova & Chernov 2004; Botha 2011). However, the uneven character of distribution often refers to quite distinct parameters of the yeast community simultaneously (quantity, diversity or structure) and often the claimed uneven distribution might be simply the result of non-representative sampling. Using a large-scale isolation approach and differentiating between quantity, diversity and community structure, we discuss the distribution patterns of yeasts based on counts of individual species on regional, biotope and community level. We found yeast quantities ranging from hundreds to millions of cells per gram of soil, even within a single locality. This is in agreement with many previous studies and was expected (e.g. Sláviková & Vadkertiová 2000; Maksimova & Chernov 2004). The uneven distribution of microbial biomass, including yeast cells, most likely reflects environmental heterogeneity, which is typical for belowground biota (e.g. Frey 2007).

Several studies report that yeast communities of different biotopes are often characterised by a limited number of shared species while some taxa often seem to be restricted to a single sampling site (e.g. Sláviková & Vadkertiová 2000; Maksimova & Chernov 2004; Vishniac 2006). This was also found in a study on yeast distribution along a large latitudinal gradient (approx. from 77°S to 64°N) in which nearly 40 % of species were found in a single locality only (Vishniac 2006). This might be a sampling bias closely associated with the analysis of remote biotopes, i.e. the strong heterogeneity of environmental parameters and vegetation type between study sites. Alternatively, soil communities might be highly endemic and many different ecological niches could be observed within the same natural zone and vegetation. Our results also showed that yeast communities of the same type of habitat, e.g. beech forests, were extremely dissimilar independent of the soil type. For example, of ten yeasts isolated from unmanaged beech forests at Schwäbische Alb and Hainich, only three were common: *T. dulcitum*, *T. porosum* and *K. piceae*. The similarity between other forest and grassland sites was even lower (Fig 2 and Table 1).

Global distribution patterns in the microbial world are a focus of intensive scientific debate (e.g. Whitfield 2005; Taylor *et al.* 2006; Whitaker 2006) and the opinion that the distribution of microbes is determined solely by environmental factors and, therefore, no biogeography is plausible for organisms smaller than 1 mm (see Whitfield 2005) has dominated for a long time. However, several studies have provided evidence for different geographic ranges of pro- and eukaryotic microbes (Taylor *et al.* 2006; Whitaker 2006). For instance, although pedobionts, like *Cr. aerius*, *Cr. podzolicus*, *Cr. terreus* and *Cr. terricola*, were found in different regions of the world (Bab'eva & Chernov 1995; Vishniac 2006), our study suggests that their distribution areas are very fragmented. We have observed that despite relatively low species richness in a given locality, i.e. 19–25 yeasts (Table 1),

yeast communities are much more diverse on larger geographic scales (Fig 4). This contrasts broad ranges of soil microfungi, including other filamentous members of Pezizomycotina (Ascomycota), which are believed to have a cosmopolitan, i.e. continuous wide range distribution (e.g. Gams 2007). Thus, two different life forms of fungi seem to have distinct distribution patterns in terms of continuity in a given area and, therefore, exhibit contrasting ecological strategies: specialists with high level of fragmentation (yeast fungi) and generalists demonstrating cosmopolitan distribution (filamentous fungi).

Finally, in most cases a few species accounted for the majority of observed yeast colonies. On average, in forest biotopes the most frequent species (the first rank) accounted for 70 % of the total yeast counts, while the second and the third ranks accounted for 14 % and 4 %, respectively (Fig 5). Although species abundance distribution (SAD) was introduced into ecology more than 100 yr ago, our knowledge of the shape of SADs of microorganisms is still poor (McGill *et al.* 2007). Our analysis showed that soil yeasts produce a hollow-curve SAD similar to those of plant and animal communities. In particular, yeasts in soils form species-poor communities with uneven SADs, a pattern that is also common to climax communities such as boreal forests (McGill *et al.* 2007).

Spatial trends

A total of 57 forest and grassland plots, located in three different regions were analysed in the present survey. For a single region, each land management category was represented by three plots, which were treated as replicates. Analysis of three different regions simultaneously was assumed to be advantageous for statistical comparisons across taxa and for testing hypotheses regarding land use effects (Fischer *et al.* 2010). However, we found that yeast communities in these three Exploratories were too distinct to treat them as replicates in the analyses (Fig 2 and Table 1). The assignment of 20 yeast species, which accounted for 80–100 % of total abundance (Fig 4), clearly illustrates dissimilarities between regions. Yeasts shared between forest biotopes dominated in a single locality and were found as second and third rank in other regions (Fig 4 and Table 1). In contrast, species highly abundant in grasslands were extremely rare in other Exploratories. Additionally, the ordination of results of the principal component analysis of species abundance data displayed separation of forest and grassland communities along the first axis and showed regional differences in both vegetation types along the second axis (Fig 6). We should, however, note that due to a low similarity between the analysed communities the first two axes describe less than a quarter (19.6 %) of the total variance.

In addition to spatial dissimilarities on a regional scale, heterogeneity between individual plots also contributed to the observed variability. The number of unique species observed in a plot correlated positively with its species richness ($r = 0.94$; $p \leq 0.001$), which suggests that dissimilarities of yeast communities within a given location are mainly due to occurrence of rare species. This implies that the analysis of species rich communities requires a larger sampling in order to achieve reliable data (see Curtis & Sloan 2005).

Yeast responses to vegetation type

Because the impact of human activity on nature has increased during the last few centuries and, thus, is an important concern

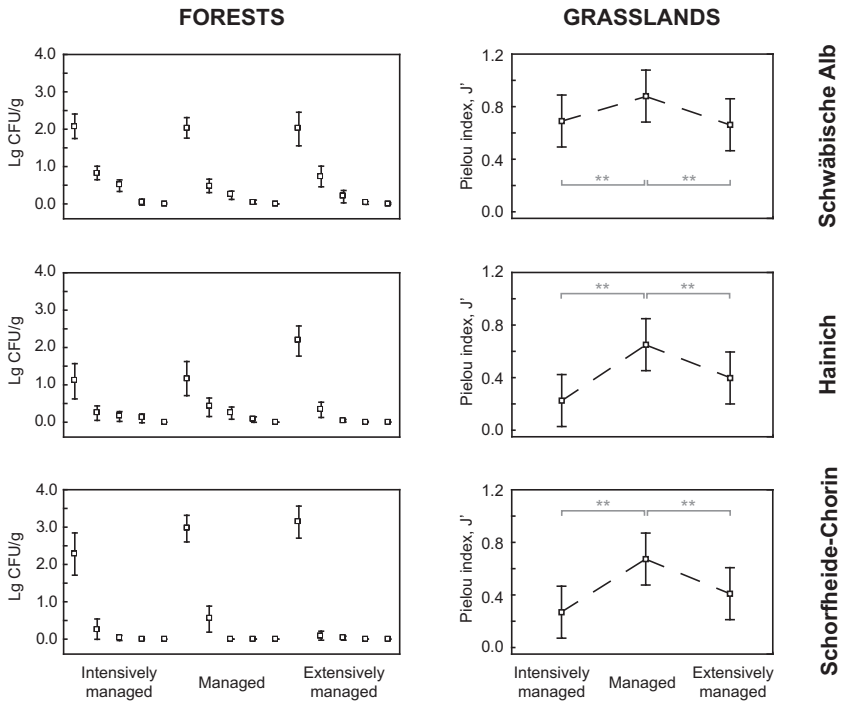

Fig 5 – Evenness of yeast communities (Pielou index, J') and Rank-Abundance diagrams displaying alteration of community evenness in forest and grassland soils in relation to land management at three localities. Bars are confidence interval and middle points the respective mean. Significant differences are indicated by $^{*}p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$.

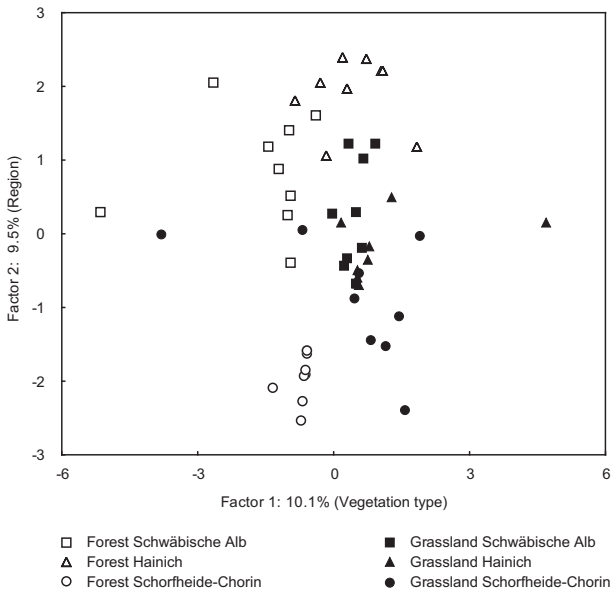

Fig 6 – Principal component analysis showing the grouping of soil samples in relation to vegetation type (Axis 1) and locality (Axis 2). Percent of the total variance described by the first two extracted factors are given on the axes.

of biodiversity management, we assessed responses of belowground yeast communities to intensification of land use. Not surprisingly it was found that quantity, diversity and proportion of ascomycetous yeasts differed most significantly between grassland and forest biotopes (Tables 1 and 2). Therefore, we assume that the conversion of forests into open vegetation types in historic times had the largest effect on soil yeast communities. This is in accordance with di Menna (1960), who found that replacement of the native broadleaf flora by pastures resulted mostly in an increased yeast quantity. The situation is different in arable soils from which Sláviková & Vadkertiová (2003) reported a nearly ten times lower yeast quantity compared to forests. In this context it is important that although the effect of the vegetation type on yeast quantity was significant in our study, it describes only 5 % of the total variability (Table 2). Therefore the transformation from one vegetation type into another might not be the sole factor to explain observed differences. The alteration of species diversity followed the same trend and species richness was significantly higher in grassland than in forest soils (Tables 1 and 2). The high relative abundance of ascomycetes was common and significant for the analysed grasslands and explained 19 % of the total variability in a given locality (Table 2). Therefore, the increased contribution of ascomycetous yeasts to the yeast community distinguishes grasslands much better than total quantity or species richness.

Yeast responses to land use intensity

The effects of land management on several parameters that influence soil yeast communities also varied between grasslands and forests (Table 2). Because community composition between different localities was often very dissimilar, we were not able to perform any plausible statistical analysis of species distribution across all studied plots. Nevertheless, two significant trends in the structure of communities reflecting land management were found. First, the proportion of ascomycetous yeasts increased with intensity of land use in grasslands (Fig 1). Second, land management considerably affected community evenness. Evenness was assessed by means of Pielou index and RAD as these approaches enable proper comparison of communities with a few common species (see McGill *et al.* 2007). In grasslands, communities' evenness increased along the land use gradient from meadows to pastures but then decreased again to mown pastures (Fig 5). The most even communities were observed in pastures at all Exploratories. In forests, community evenness decreased with increase in land management (Fig 5 and Fig S4).

Community evenness appears to be a universal marker to reflect the impact of land management on soil yeast communities. Forest conservation leads to pronounced dominance of a few highly specialized pedobionts. Abilities to decompose complex polysaccharides and some aromatic compounds by *T. dulcitum*, *T. porosum*, *Cr. podzolicus* and

Table 2 – Three-way analysis of variance (ANOVA) for yeast quantity, species richness and relative abundance of ascomycetes in studied soils

Variance source	Quantity, Lg CFU/g				Species richness, *N*			Proportion of ascomycetes		
	Degree of freedom	Sum of squares	Total variance, %	F-level and *p*-level	Sum of squares	Total variance, %	F-level and *p*-level	Sum of squares	Total variance, %	F-level and *p*-level
Total	244	262.5	100.0		426.3	100.0		30.5	100.0	
Exploratories, region (E)	2	14.6	5.5	9.0***	70.1	16.4	26.9***	3.0	9.9	23.5***
Vegetation type (V)	1	13.5	5.1	16.7***	11.3	2.7	8.7**	1.3	4.2	20.0***
Land-use category (C)	2	5.9	2.2	3.7*	5.2	1.2	2.0 ns	1.1	3.8	8.9***
E × V	2	19.2	7.3	11.9***	9.6	2.3	3.7*	5.9	19.2	45.4***
E × C	4	8.9	3.4	2.8*	9.4	2.2	1.8 ns	2.4	7.7	9.1***
V × C	2	0.1	0.0	0.04 ns	6.5	1.5	2.5 ns	0.7	2.4	5.7**
E × V × C	4	6.9	2.6	2.1 ns	2.3	0.5	0.4 ns	1.9	6.4	7.5***
Error	227	193.4	73.7		311.9	73.2		14.1	46.4	

Statistical significance: ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Cr. terreus, which were the most prominent in the surveyed forests, have been previously reported (Sampaio 1999; Middelhoven 2006) and their possible involvement in organic matter decomposition and dissipation has been considered (Botha 2011). Ecosystem disturbances, like clear-cut forestry and replacement of original vegetation cause niche fragmentation, which likely enables the development of species that are normally unable to compete successfully for the resources in a climax community. In turn, this should result in a more equal contribution of various species to the given community and, thus, increase species evenness.

The conversion of forests into open vegetation resulted in an increased proportion of ascomycetous yeasts. Nutritional differences in grassland soils, i.e. quality of soil organic matter, may have caused the observed effect. Unlike basidiomycetes, ascomycetes do not have the appropriate enzymatic machinery to break down complex compounds but display fast growth rates in the presence of mono-, di- and trisaccharides (e.g. Fonseca & Inacio 2006). Land management, grazing and fertilisation, change soil nutrient balance (e.g. Matlou & Haynes 2006) and, therefore, provide easy-to-use carbon sources for ascomycetous yeasts. Remarkably, extensively managed and therefore oligotrophic pastures at Schwäbische Alb contained yeast communities that resembled the ones of forest sites and displayed a high abundance of basidiomycetous yeasts. In contrast, fertilized grasslands contained mainly ascomycetes (Fig 4 and Table 1). Besides a greater specialisation, ascomycetous yeasts of the genera *Barnettozyma*, *Schwanniomyces* and *Lindnera* produce mycocines (proteins or glycoproteins), which might also hinder the growth of other species. The production of mycocines is thought to be advantageous for development in substrata with population density higher than 10^4 CFU g^{-1}, like phyllosphere (Golubev 2006) or grassland soils in the present study.

Conclusions

Human activities and land management have a great impact on the soil cover. This is the first study, which assessed the effects of different levels of land management intensity in forestry and agriculture on soil yeast diversity and community structures. We found that alteration of vegetation type affected both quantity and diversity of yeasts, thereby strongly influencing their distribution in soil. In contrast, land use intensity had only a low impact on yeast quantity and species richness but significantly changed composition and structure of soil yeast communities.

Two significant patterns of community alteration along land use intensity were revealed in this study. First, ascomycetous yeasts dominated in grasslands and their proportion in analysed soils increased from extensively to intensively managed grasslands. Second, the evenness of yeast communities followed the gradient of land management. Evenness increased with intensity of forest use and natural beech forests harboured a yeast community with the most uneven structure. In particular, species evenness curves in unmanaged forests were hollow-shaped and resembled the ones known for climax communities of higher plants. In contrast, the curves obtained for intensively managed biotopes were more flat and, thus, similar to juvenile or disturbed plant communities. These patterns have not been reported for microbial communities so far, but seem to support one of ecology's oldest and most universal laws.

Finally, the present study demonstrated that species composition differed considerably between different sampling regions even within the same natural zone. We observed that soil yeast communities were relatively species-poor and heterogeneous between regions, biotopes and management type. Determining if this is the result of endemism or range fragmentation should be the focus of future research.

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Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.funeco.2011.07.004.

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