

Article

## Aboveground Deadwood Deposition Supports Development of Soil Yeasts

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**Abstract:** Unicellular saprobic fungi (yeasts) inhabit soils worldwide. Although yeast species typically occupy defined areas on the biome scale, their distribution patterns within a single type of vegetation, such as forests, are more complex. In order to understand factors that shape soil yeast communities, soils collected underneath decaying wood logs and under forest litter were analyzed. We isolated and identified molecularly a total of 25 yeast species, including three new species. Occurrence and distribution of yeasts isolated from these soils provide new insights into ecology and niche specialization of several soil-borne species. Although abundance of typical soil yeast species varied among experimental plots, the analysis of species abundance and community composition revealed a strong influence of wood log deposition and leakage of organic carbon. Unlike soils underneath logs, yeast communities in adjacent areas harbored a considerable number of transient (phylloplane-related) yeasts reaching 30% of the total yeast quantity. We showed that distinguishing autochthonous community members and species transient in soils is essential to estimate appropriate effects of environmental factors on soil fungi. Furthermore, a better understanding of species niches is crucial for analyses of culture-independent data, and may hint to the discovery of unifying patterns of microbial species distribution.

**Keywords:** yeasts; soil; wood decomposition; forest; fungi; *Cryptococcus podzolicus*

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## 1. Introduction

Fungi are heterotrophic organisms supporting key processes in terrestrial ecosystems. Due to their wide range of enzymatic capabilities, they are involved in many element cycles, thus providing important ecosystem services, such as nutrient mobilization and turnover, but also the breakdown of persistent organic compounds, including toxic molecules [1]. Fungi are essential for forest ecosystems as they are the only organisms capable of substantial lignin decay (see, for example [2]). Thereby, fungi provide nutrition to a large variety of above- and belowground heterotrophic organisms. The role of fungi in the biological processes in soils cannot be overestimated as fungi have been estimated to contribute 60%–80% of the total microbial respiration and account for the bulk of soil biomass [3,4]. Further estimations suggest fungal biodiversity in the range of 1.5–5.1 million species, from which only *ca.* 100,000 have been described so far [5]. Especially complex habitats, such as soil, may harbor large numbers of undescribed fungi, estimated to outnumber plants by at least 6:1 [5].

In principle, fungi living in soils can be divided into two functional groups: filamentous multicellular fungi, and predominantly unicellular yeasts. Both comprise several unrelated lineages of various orders of both Asco- and Basidiomycota [6–8]. Unlike the typical saccharolytic phenotype often attributed to yeasts, soil-related species are able to utilize a wide spectrum of carbon sources, including products of the enzymatic hydrolysis of lignocellulosic plant materials such as simple aromatic compounds, hemicelluloses and organic acids [9–14]. This fact indicates that soil yeasts could play a crucial role in the decomposition of plant material and dissipation of nutrients within the soil (see, for example, [7]).

In forests, organic carbon acting as nutrient source for soil-borne microbes originates mainly from plants in form of exudates or dead organic matter, like litter or entire wood logs [15]. Wood and litter deposits are a major pool of organic carbon and are an essential element for forest biodiversity, providing resources or habitats for wide groups of organisms [2,15–18]. A recent meta-analysis showed negative effects of forest management on different taxonomical groups [19]. One explanation for this effect is that natural disturbances of vegetation and soil cover in unmanaged forests provide a multitude of diverse habitats of different scales and properties. In contrast, managed forests are characterized by low spatial heterogeneity and complexity that also affects biodiversity parameters [20]. For instance, the presence of large wood logs of different decay stages is an essential factor supporting higher diversity of substrate dependent taxa such as bryophytes, lichens and wood-inhabiting fungi [18,19]. Similar trends towards alteration of original community by forest management may be more common in nature and observed for other saprobic microorganisms that do not depend directly on the substrate.

Numerous studies aimed at yeast diversity assessments report diverse and often very dissimilar yeast assemblages from various forest soils [8,21–26]. The ultimate importance of the environment for development of soil yeasts has been long considered and variability in abiotic parameters explains to some extent observed differences between yeast communities [7,21,27,28]. However, soil yeast communities may vary extremely on a rather small spatial scale, between different forest types or

different forest management intensities. For example, recent studies showed that long-term alteration of the native broadleaf vegetation (beech forests) within three UNESCO Biosphere Reserves in Germany substantially changed soil yeast communities [8,26]. Interestingly, yeast communities analyzed in these studies reflected forest properties even though their diversity and biomass patterns did not depend on basic soil properties such as pH, clay content, total nitrogen, C/N ratio, nitrate, ammonium and plant-available phosphorus concentrations [29]. Although these most common abiotic parameters did not explain the effects of forest management on soil yeasts, abundance patterns of saprotrophic fungi as a whole were significantly related to these soil properties. Besides the environment, nutrition is another important factor for microbial community development. Because the regular presence of large wood logs is typical for unmanaged forests, soil yeasts may rely not on belowground resources alone, but also respond to the activity of wood-inhabiting fungi as they make aboveground carbon stocks available for soil microbes.

The main objective of the present study is to assess the impact of aboveground deadwood deposition on soil yeast communities in three managed forests using cultivation techniques with the subsequent identification of isolated cultures based on the ITS rDNA barcode [30,31]. To achieve this, we analyze yeast communities inhabiting soils underneath wood logs and apart from wood logs as control. Specifically, we test the hypothesis that true soil yeasts rather than transient species will respond to organic carbon sources leaching from decaying wood logs. We identify true soil yeast species and transient yeast species entering the soil profile with the leaf material in order to differentiate the influence of deadwood deposition. We apply incidence- and abundance-based community similarity indices to test whether environmental conditions formed underneath decaying wood logs are likely to select towards a few well-adapted species.

## 2. Methods

### 2.1. Sampling and Isolation of Yeasts

Sampling was performed in November 2009 in three spruce forests of the biosphere area of Schwäbische Alb (Swabian Jura, Baden-Württemberg) of the German Biodiversity Exploratories framework [32]. Additional details on these biotopes are given by Fischer *et al.* [33]. Soils were collected underneath logs and from adjacent areas, approximately one meter apart, placed in sterile plastic bags and transferred to the laboratory for analysis. We analyzed soils underneath logs larger than 20 cm in diameter. Samples were taken at 0–10 cm soil depth. Leaf litter was removed before sampling in areas adjacent to wood logs. Characteristics of studied logs and basic abiotic soil parameters (5 cm depth) are provided in Table 1. Soil acidity (pH) was measured in 0.01 M CaCl<sub>2</sub> suspensions, according to the guidelines for soil description [34]. For each sampled wood log, stem length, stem diameter, remaining stem mass, as well as the type of rot (brown or white), were estimated (Table 1). Remaining mass of stem was estimated as proportion of decaying stem mass (volume \* density) to the average wood weight of beech and spruce. In order to estimate stem mass, we measured the deadwood density by taking samples from the logs. The type of rot was determined based on fungal fruiting bodies growing on stems and by examining wood samples. Detailed description of methods to measure other abiotic soil properties and the respective results are given in Alvarez [35].

**Table 1.** Study sites and soil properties.

Sample	Plot ID	Wood Logs	pH	DOC (mg/L)	C <sub>org</sub> (%)	Total N (%)	Remaining mass of original stem (%)	Stem length (m)	Stem diam. (foot) (cm)	Brown rot fungi	White rot fungi
8270	AEW1	<i>Fagus sylvatica</i>	4.7	251.84	6.97	0.42	65.06	3.25	22.0	No	Yes
8271	AEW1	<i>Picea abies</i>	4.6	348.35	11.57	0.62	70.46	11.25	25.0	Yes	Yes
8273	AEW1	<i>Picea abies</i>	4.1	280.27	5.48	0.31	80.81	13.25	28.0	Yes	Yes
8820	AEW2	<i>Picea abies</i>	5.3	250.49	9.29	0.55	53.13	15.30	34.0	No	Yes
8822	AEW2	<i>Picea abies</i>	5.0	300.13	10.95	0.50	36.26	1.90	27.0	No	Yes
8279	AEW3	<i>Picea abies</i>	4.2	247.97	5.40	0.35	64.30	13.10	21.5	Yes	Yes
8280	AEW3	<i>Fagus sylvatica</i>	3.7	240.00	7.04	0.51	60.03	9.80	24.0	Yes	Yes
8281	AEW3	<i>Picea abies</i>	4.8	291.20	9.96	0.57	57.96	16.10	23.0	Yes	Yes
8282	AEW3	<i>Fagus sylvatica</i>	5.3	296.56	14.10	0.78	98.89	3.25	23.0	No	Yes
8270	AEW1		4.1	216.65	6.23	0.39	n.a.	n.a.	n.a.	n.a.	n.a.
8271	AEW1		3.7	302.65	10.40	0.53	n.a.	n.a.	n.a.	n.a.	n.a.
8273	AEW1		3.9	241.67	5.10	0.30	n.a.	n.a.	n.a.	n.a.	n.a.
8820	AEW2		5.2	240.4	8.45	0.50	n.a.	n.a.	n.a.	n.a.	n.a.
8822	AEW2		4.9	331.24	19.41	0.78	n.a.	n.a.	n.a.	n.a.	n.a.
8279	AEW3		4.5	198.00	6.10	0.40	n.a.	n.a.	n.a.	n.a.	n.a.
8280	AEW3		5.1	168.52	6.13	0.50	n.a.	n.a.	n.a.	n.a.	n.a.
8281	AEW3		5.5	260.43	8.01	0.52	n.a.	n.a.	n.a.	n.a.	n.a.
8282	AEW3		5.5	311.3	13.7	0.80	n.a.	n.a.	n.a.	n.a.	n.a.

Abbreviations used: DOC, dissolved organic carbon; C<sub>org</sub>, organic carbon; Total N, total nitrogen; n.a., not applicable.

Note: C<sub>org</sub> (%) and Total N (%) values are related to dry soil weight.

## 2.2. Isolation of Cultures

From each soil sample, 7 g was placed in 50 mL plastic tube, suspended (w/v) 1:5 in 35 mL of sterile 0.01% peptone-water solution and shaken on an orbital shaker at 200 rpm for 1 hour. Aliquots were taken from 1:5 suspension to produce two other step-wise dilutions, 1:10 and 1:20. All soil samples were analyzed in duplicates (two sub-samples) and each of the replicates was used to produce three dilutions (1:5, 1:10 and 1:20), each of which was plated in a duplicate again. An aliquot of 0.15 mL was plated on the surface of acidified glucose-yeast extract-peptone agar (GPYA) [8,26]. Plates were incubated at room temperature for 2–3 days and kept at lower temperatures (6–10 °C) to prevent fast development of molds. 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 morphological type per plate were retained as a pure culture.

## 2.3. Identification of the Strains

PCR-fingerprinting with microsatellite-specific oligonucleotides were used to group pure cultures. Strains showing identical electrophoretic profiles were considered as conspecific clones and only 1–2 representatives of them were chosen for further identification by sequencing of rDNA regions. Details on oligonucleotide primer sequences and PCR conditions are given by Yurkov *et al.* [8].

Yeast cultures were identified using nucleotide sequences of the D1/D2 domains of the large subunit rDNA (LSU) and the internal transcribed spacer region (or rDNA ITS). A combination of these two genetic markers provides the highest probability of correct identification of asco- and basidiomycetous fungi [31]. Protocols describing DNA extraction, amplification, purification and sequencing are given by Yurkov *et al.* [8]. For species identification, the nucleotide sequences were compared with sequences deposited in the NCBI [36] and MycoID [37] databases, respectively. Nucleotide sequences were deposited in GenBank under the accession numbers given in Table 2. Pairwise sequence comparisons were derived from alignments obtained with the MAFFT algorithm [38].

## 2.4. Statistical Analysis

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 (incidence) 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 (CFU).

A total of 72 sub-samples were included in the analysis, 36 sub-samples collected underneath wood logs and 36 sub-samples from adjacent areas. Statistical evaluations were performed with Statistica 8–9 (StatSoft Inc., United States). Quantity values were  $\text{Log}_{10}$  transformed for the analyses. Normality of distribution was tested for absolute abundance values. Significant effects were confirmed with T-test. Differences in soil abiotic factors underneath logs and apart from them were assessed using the Wilcoxon signed-rank test (also referred to as Wilcoxon *T*-test) and the paired T-test. Effects were considered to be statistically significant at the level  $p < 0.05$ .

Beta-diversity was estimated using incidence- and abundance-based Jaccard similarity indices [39]. Similarity matrices were obtained using EstimateS 8.0 software [40]. Similarity matrices were analyzed with Multidimensional Scaling technique implemented in the Statistica software. The number of dimensions (axes) properly describing the observed similarity between samples was estimated on the basis of a scree plot, containing G-Star stress values.

Transient species were defined based on known ecological preferences (see Section 3) and from the analysis of Species Abundance Distribution (SAD) in the form of Rank-Abundance Diagram, or RAD [41]. Species evenness and productivity-evenness relationship in analyzed yeast communities were assessed on the basis of the distribution of species ranks in a community, RAD plots [41].

### 3. Results

Statistical analysis suggested that total yeast counts obtained from 1:10 and 1:20 soil-to-water suspensions were not significantly different (ANOVA:  $F = 2.37$ ;  $p = 0.13$ ), whereas the total quantity observed with 1:5 dilution was significantly lower (ANOVA:  $F = 6.92$ ;  $p = 0.002$ ). The number of yeast species isolated from 1:5 dilution was also lower than from two other dilutions. Specifically, molds developing on these plates made quantification and purification of yeast colonies difficult. Based on these results, total yeast quantity and species richness data obtained with 1:5 dilution were finally excluded from statistical analyses. Basic abiotic soil parameters such as pH, and amounts of organic carbon and nitrogen (Table 1) did not depend significantly on the presence of logs (Wilcoxon  $T$ -test and paired  $T$ -test; data not shown). However, dissolved organic carbon was significantly higher (paired  $T$ -test,  $p = 0.04$ ) under logs than in adjacent areas (mean values 278.53 and 252.32 mg/L, respectively).

#### 3.1. Yeast Species Inventory

A total of 25 species were found, 13 underneath logs and 18 species adjacent to logs (Table 2). Three yeasts, TSN-67, TSN-36, and TSN-5, showed low similarity to any known species and may thus represent novel taxa (Table 2). Analysis of the large subunit (D1/D2 domains) suggested the placement of the isolate TSN-67 in the Cystofilobasidiales (Tremellomycetes, Agaricomycotina). The closest match among currently recognized species was obtained with *Phaffia rhodozyma* (*Xanthophyllomyces dendrorhous*), showing more than 50 substitutions in the D1/D2 domains of LSU rRNA. Isolate TSN-36 (=CBS 11767) showed no difference in LSU to the strain CBS 7170, previously misidentified as *Candida atmosphaerica* (AY574389). Phylogenetic analysis suggested the placement of this novel species near the *Candida tanzawaensis* clade [42]. The other novel ascomycete, represented by the isolate TSN-5 (=CBS 11766) was closely related to the *Candida chilensis*, showing 10 substitutions in LSU. Additionally, several isolates showed a few nucleotide substitutions to the type strains of the currently described species (Table 2).

The following six yeasts were common in soils collected underneath logs and in adjacent areas: *Kazachstania piceae*, *Schizoblastosporion starkeyi-henricii* (Saccharomycetetales, Saccharomycetes), *Cryptococcus terricola* (Filobasidiales, Tremellomycetes), *Rhodotorula colostri* (Sporidiobolales, Microbotryomycetes), *Trichosporon dulcitum* and *Trichosporon porosum* (Trichosporonales, Tremellomycetes). *Cryptococcus podzolicus* (Tremellales, Tremellomycetes) was frequent in soils underneath logs, but was absent in soils of adjacent areas. Additionally, several single isolates of

pigmented yeasts, *Aureobasidium pullulans* (Dothideales, Dothideomycetes), *Cr. tephrensensis* (Tremellales, Tremellomycetes), *R. colostri* and *Sporobolomyces ruberrimus* (Sporidiobolales, Microbotryomycetes) were found in soils collected underneath logs. In contrast, soils collected adjacent to logs were characterized by regular occurrence of yeasts, which were reported as inhabitants of plant surface [43–47]: *Cr. filicatus*, *Cr. stepposus*, *Cr. victoriae* (Tremellales, Tremellomycetes), *Cystofilobasidium capitatum*, *Cy. macerans* (Cystofilobasidiales, Tremellomycetes), *R. colostri*, *R. fujiisanensis* (Microbotryomycetes) and *Sp. ruberrimus*. However, none of these species was observed in each of three studied plots.

**Table 2.** Yeast species inventory in analyzed soils.

Identification results	Taxon example		GenBank		
	Strain ID	Collection ID	ITS	LSU	Closest match *
<i>Aureobasidium pullulans</i>	TSN-43	-	FR716139	-	0/0 FJ150906
<i>Candida cretensis</i>	TSN-5	CBS 11766	-	FN824503	10/0 EU011654
<i>C. zeylanoides</i>	TSN-95	-	HF558653	FR716579	2/0 AY498861
<i>Candida</i> sp. CBS 7170	TSN-36	CBS 11767	-	FN824506	0/0 AY574389
<i>Candida</i> sp. CBS 11766	TSN-95-1	CBS 11778	-	FN908211	1/0 AY497688
<i>Cryptococcus aerius</i>	TSN-77	CBS 11764	JN942234	FN824504	2/2 AF181524
	TSN-82	CBS 12340	JN942257	FR716581	1/0 AF181544
<i>Cr. filicatus</i>	TSN-98	MUCL 52894	FN908210	FN908210	0/0 EU433984
<i>Cr. musci</i>	TSN-91	CBS 11765	FN824491	FN824491	0/0 AB126586
<i>Cr. podzolicus</i>	TSN-16	CBS 12385	HF558650	HF558650	1/0 AF075481
	TSN-41	CBS 12341	HF558651	HF558651	1/0 AF075481
	TSN-23	CBS 12388	HF558652	HF558652	2/0 AF075481
<i>Cr. ramirezgomezianus</i>	TSN-95-14	-	HF558654	HF558654	0/0 AB126584
<i>Cr. stepposus</i>	TSN-64	CBS 11763	FN824490	FN824490	0/0 DQ222456
<i>Cr. tephrensensis</i>	TSN-4	-	-	FR716587	0/0 CBS 8934
<i>Cr. terricola</i>	TSN-101	-	HF558655	HF558655	0/0 AF181520
<i>Cr. victoriae</i>	TSN-53	CBS 12045	HF558648	FR716585	4/0 AF363647
	TSN-84	-	HF558649	FR716586	0/0 AF363647
<i>Cystofilobasidium capitatum</i>	TSN-59	-	HF558659	FR716589	0/0 AF406889
<i>Cy. macerans</i>	TSN-58	-	HF558660	FR716588	1/0 AF075477
<i>Kazachstania piceae</i>	TSN-26	-	HF558661	FR716594	2/0 CBS 7738
<i>Phaffia</i> sp. CBS 11768	TSN-67	CBS 11768	HF558647	HF558647	56/3 AF189871
<i>Rhodotorula colostri</i>	TSN-60	-	HF558663	FR716590	0/0 AY372177
<i>R. fujiisanensis</i>	TSN-63	-	HF558662	FR716591	0/0 AF189928
<i>Schizoblastosporion starkeyi-henricii</i>	TSN-87	-	HF558658	FR716592	0/0 U40089
<i>Sporobolomyces ruberrimus</i>	TSN-25	-	HF558664	FR716593	1/0 AF070442
<i>Sp. tsugae</i>	TSN-37	CBS 11762	-	FN868152	0/0 AF189998
<i>Trichosporon dulcitum</i>	TSN-17	-	HF558657	FR716595	0/0 AF075517
<i>T. porosum</i>	TSN-24	-	HF558656	FR716596	0/0 AF189833
<i>Wickerhamomyces anomalus</i>	TSN-95-4	MUCL 52882	-	FN868150	3/1 EU057562

Abbreviations used: CBS, Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands); MUCL, Mycothèque de l'Université catholique de Louvain (Louvain-la-Neuve, Belgium).

\* the nearest match among currently recognized species; number of substitutions/gaps is based on an alignment (MAFFT algorithm).

## 3.2. Yeast Alpha- and Beta-Diversity

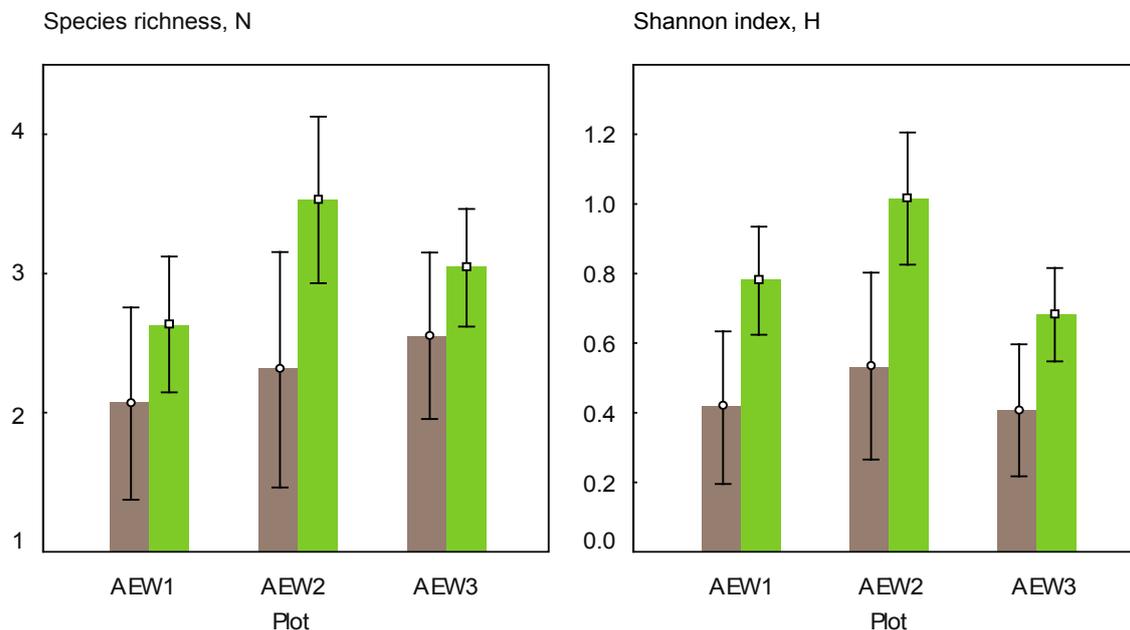
Total species richness estimated per plot varied from 5 to 9 yeasts in soils underneath logs and from 6 to 11 in soils in adjacent areas (Figure 1). Average species richness values per sample were slightly higher in soils collected in adjacent areas (2.5–3.5 species) than underneath logs (2.0–2.5 species). Also, Shannon index values were significantly higher in soils adjacent to logs (ANOVA:  $F = 22.72$ ;  $p < 0.001$ ). Communities' composition was strongly influenced by spatial factors, which resulted in observed dissimilarities between experimental plots. Three to four species were common for soil communities underneath logs, but the similarity between soil samples taken apart from the logs was always lower (Table 3). Specifically, five species were shared between AEW2 and AEW3 plots and one species only (*Cr. terricola*) was common for all three plots. Overall, *Cr. terricola* was the only species found in all soil samples underneath logs and in adjacent areas.

**Table 3.** Relative abundance of yeasts in analyzed soils.

Yeast species	underneath wood logs			in adjacent areas		
	AEW1	AEW2	AEW3	AEW1	AEW2	AEW3
<i>Aureobasidium pullulans</i>	-	-	0.003	-	-	-
<i>Candida cretensis</i>	-	-	-	s.i.	-	-
<i>C. zeylanoides</i>	-	-	-	s.i.	-	-
<i>Candida</i> sp. CBS 7170	-	-	0.013	-	-	-
<i>Candida</i> sp. CBS 11766	0.167	-	-	-	-	-
<i>Cryptococcus aerius</i>	-	-	-	>0.001	0.439	0.007
<i>Cr. filicatus</i>	-	-	-	0.096	-	-
<i>Cr. musci</i>	-	-	-	0.258	-	-
<i>Cr. podzolicus</i>	0.449	0.590	0.363	-	-	-
<i>Cr. ramirezgomezianus</i>	-	-	-	s.i.	-	-
<i>Cr. stepposus</i>	-	-	-	0.096	-	-
<i>Cr. tephrensis</i>	0.123	-	-	-	-	-
<i>Cr. terricola</i>	0.252	0.387	0.176	0.381	0.367	0.573
<i>Cr. victoriae</i>	-	-	-	-	0.116	0.109
<i>Cystofilobasidium capitatum</i>	-	-	-	-	-	0.017
<i>Cy. macerans</i>	-	-	-	-	-	s.i.
<i>Kazachstania piceae</i>	>0.001	>0.001	0.197	>0.001	>0.001	0.066
<i>Phaffia</i> sp. CBS 11768	-	-	-	-	-	0.148
<i>Rhodotorula colostri</i>	0.003	-	-	-	0.002	0.022
<i>R. fujisanensis</i>	-	-	-	-	-	0.039
<i>Schizoblastosporion starkeyi-henricii</i>	0.006	-	-	0.167	-	-
<i>Sporobolomyces ruberrimus</i>	-	-	0.002	-	-	-
<i>Sp. tsugae</i>	-	-	0.016	-	-	-
<i>Trichosporon dulciturum</i>	-	0.010	0.220	-	0.076	0.017
<i>T. porosum</i>	-	0.012	0.010	0.002	-	-
<i>Wickerhamomyces anomalus</i>	-	-	-	s.i.	-	-

Abbreviations used: s.i., single isolate; -, not observed.

**Figure 1.** Yeast diversity in soils underneath wood logs (brown) and of adjacent areas (green), species richness (N) and Shannon index (H). Whiskers correspond to a confidential interval and middle points to a mean.

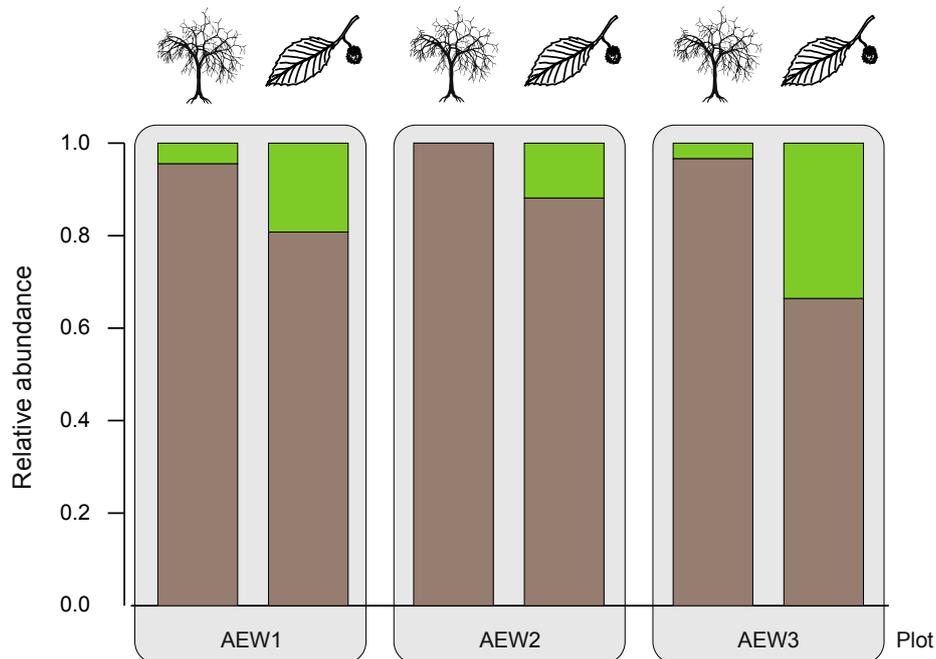


Up to 130 species have been reported from soils worldwide but evidence for strong association with soil-related substrata is still lacking for many of these species [7,28,48]. Unlike a few true soil-borne species (autochthonous soil yeasts) exclusively found in soils, transient yeast species are not restricted to this habitat but enter soil profile passively (e.g. with decaying leaves, fruits or mushrooms) or are transmitted to soils by animals (see, for example, [47,49]). Soil yeast communities underneath logs consisted to great extent of soil-borne yeasts and a few transient (phylloplane-related) species. Specifically, yeasts *A. pullulans*, *Cr. tephrensii*, *R. colostri* and *Sp. ruberrimus* were found in low numbers in a single replicate (Table 3). In total, relative abundance of these latter species did not exceed 4% (Table 4 and Figure 2) underneath logs. In contrast, communities of adjacent areas contained up to 30% (relative abundance) of phylloplane-related yeasts, which, therefore, contributed substantially to the total yeast quantity.

**Table 4.** Quantity, diversity and relative abundance of soil-borne yeasts and transient species.

	underneath wood logs			in adjacent areas		
	AEW1	AEW2	AEW3	AEW1	AEW2	AEW3
Total yeast quantity, Log <sub>10</sub> (CFU/g)	4.985	5.368	4.985	5.401	5.261	5.045
Quantity of soil-borne yeasts, Log <sub>10</sub> (CFU/g)	4.968	5.386	4.972	5.309	5.206	4.865
Species richness	7	5	9	11	6	10
Number of soil-borne species	4	5	6	6	4	4
Number of transient species	3	0	3	5	2	6
Relative abundance of soil-borne species	0.96	1.00	0.97	0.81	0.88	0.66
Relative abundance of transient species	0.04	-	0.03	0.19	0.12	0.34

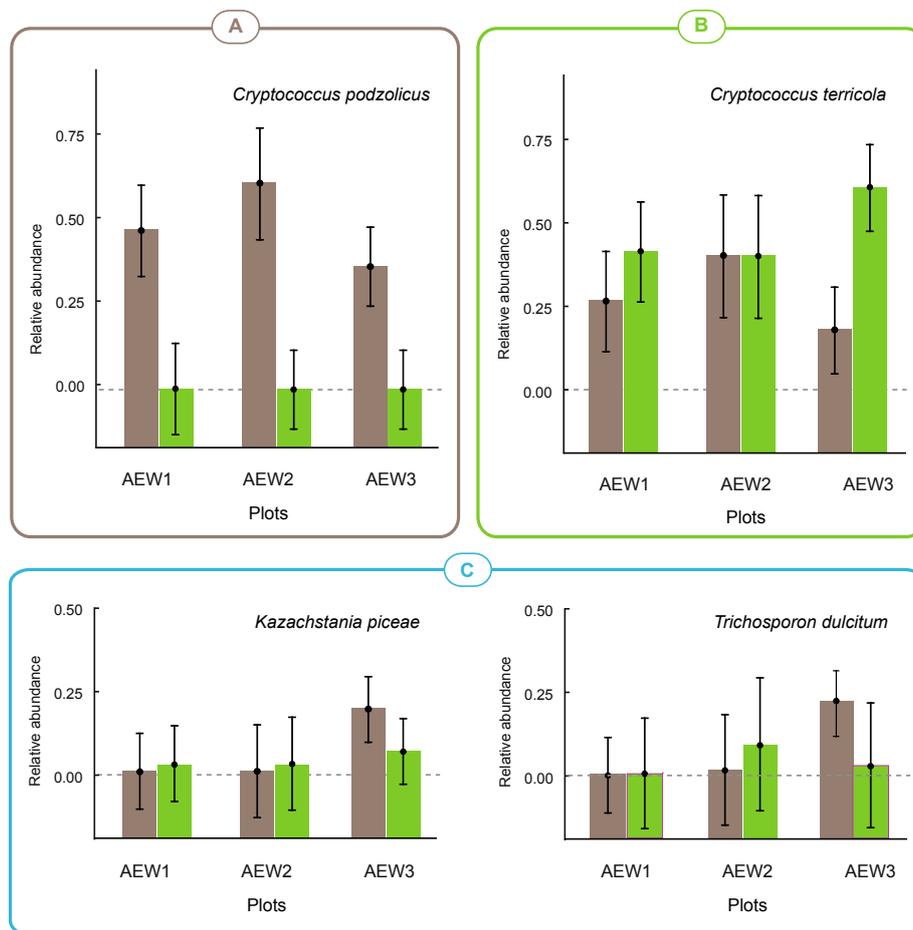
**Figure 2.** Relative abundance (average values) of soil-borne (brown) and transient (green) yeast species in soils underneath wood logs and in adjacent areas.



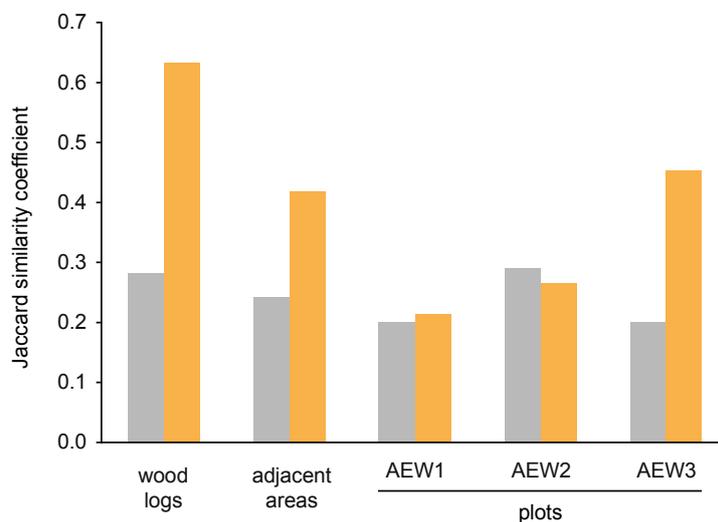
Analysis of communities' structure also revealed striking differences between soils influenced by decaying logs and ones of adjacent areas (Figure 3). For example, *Cr. podzolicus* was the most frequent and abundant species underneath logs, but it was absent in soils collected apart from logs (Table 3). In contrast, the relative abundance of *Cr. terricola* was slightly higher in soils apart from logs, where it was clearly dominant (ANOVA:  $F = 5.48$ ;  $p = 0.02$ ). In contrast, *K. piceae* and *T. porosum* were significantly more abundant in soil underneath logs (ANOVA:  $F = 5.17$ ;  $p = 0.02$  and  $F = 5.58$ ;  $p = 0.02$ , respectively). In contrast to species clearly reacting on the deposition of logs in all plots, yeasts *K. piceae* and *T. dulcitum* showed a plot-specific pattern being more abundant at the AEW3 plot than at AEW1 and AEW2 (Table 3 and Figure 3). Relative abundance *Cr. podzolicus* showed a contrasting trend declining at the AEW3 plot (Table 3 and Figure 3). In soils from adjacent areas, *Cr. musci* and *Schizoblastosporion starkeyi-henricii* were more abundant at the AEW1 plot (Table 3), whereas relative abundance of *K. piceae* and *Cr. terricola* were significantly higher at the AEW3 plot (Table 3 and Figure 3).

Analyses of communities' similarity using incidence- and abundance-based Jaccard similarity coefficient showed a low similarity level between all yeast communities (Figure 4). In all pair-wise comparisons, incidence-based Jaccard coefficient values did not exceed 0.30 (mean values 0.28 and 0.24 for soils under logs and from adjacent areas, respectively). Interestingly, similarity within a single plot, between soils collected underneath and apart from wood logs ranged from 0.20 to 0.29. Application of the abundance-based Jaccard coefficient showed that yeast communities in soils collected underneath logs were more similar in average than the ones of adjacent areas, 0.63 and 0.42, respectively (Figure 4). Additionally, the ordination of these results using the multidimensional scaling technique clearly displayed a separation between soil yeast communities underneath logs and of adjacent areas (Figure 5).

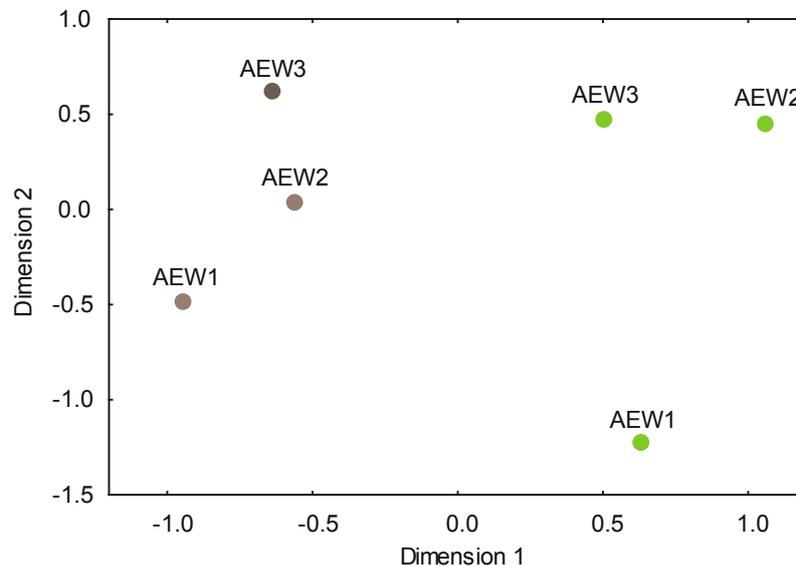
**Figure 3.** Relative abundance of dominating soil yeasts in soils collected underneath wood logs (brown) and in adjacent areas (green): **(A)** exemplifies species like *Cr. podzolicus* positively responding to log deposition, **(B)** exemplifies species like *Cr. terricola* negatively responding to log deposition, **(C)** exemplifies species like *K. piceae* and *T. dulcitum* showing plot-specific pattern. Whiskers correspond to a confidential interval and middle points to a mean.



**Figure 4.** Similarity of yeast communities revealed with classical, incidence based (grey) and abundance-based (orange) Jaccard coefficients.



**Figure 5.** Ordination using multidimensional scaling technique of pair-wise abundance-based Jaccard similarity coefficients. Brown symbols represent soil samples collected underneath wood logs and green symbols represent soils of adjacent areas.



### 3.3. Yeast Abundance

Total yeast quantity varied largely from  $2.4 \times 10^3$  to  $9.7 \times 10^5$  CFU/g or 3.38 to 5.99  $\text{Log}_{10}$  (CFU/g), respectively (Table 4 and Figure S1). Average quantity in soils collected in adjacent areas was slightly higher than in soils underneath logs, 5.24 and 5.10  $\text{Log}_{10}$  (CFU/g), respectively (ANOVA:  $F = 17.67$ ;  $p < 0.001$ ). However, when colony counts corresponding to phylloplane-related yeasts were not considered (Figure 4), quantity of soil-borne species appeared to be in the same range in both soils, 5.15 and 5.16  $\text{Log}_{10}$  (CFU/g), respectively (Table 4).

## 4. Discussion

In this study we assessed the impact of aboveground log deposition in forests on yeast communities using cultivation technique coupled with identification based on rDNA ITS sequencing. We analyzed total abundance, alpha- and beta-diversity indices of yeast communities in soils underneath logs and in adjacent areas. Most of the species isolated from soil in this study were basidiomycetous like in previous studies (see for review [7]) and only two out of eight ascomycetes were found repeatedly, *viz.* *Schizoblastosporion starkeyi-henricii* and *Kazachstania piceae*. While association of *S. starkeyi-henricii* with soil substrates is well supported [50-52], soil origin of *K. piceae* was only revealed recently [8]. However, both species display positive response to the presence of decaying wood even though relative abundance distribution of *K. piceae* shows a plot-specific trend (Figure 4). Among basidiomycetous yeasts isolated in our survey, *Cryptococcus aerius*, *Cr. musci*, *Cr. podzolicus*, *Cr. terricola*, *Trichosporon dulcitum* and *T. porosum* were reported as soil-inhabiting species (see, for example, [8,22-26]). A previous study, which was performed at the same forest sites, yielded seven yeast species [8], and five of them were also observed in the present work. Some of these yeasts were found under logs (*Cr. terricola*, *S. starkeyi-henricii*, *T. dulcitum* and *T. porosum*) or in adjacent areas (*Cr. ramirezgomezianus*, *Cr. terricola*). Surprisingly, during this study, we could not isolate

*Cr. terreus*, which was the dominant species in these soils during the spring sampling [8]. This observation supports previous reports highlighting the fact that soil yeast communities in spring and in autumn might differ considerably [26]. In the present survey, we also observed higher species richness in the end of the vegetative season. However, the question of whether or not diversity of soil yeast communities increases during the vegetative season should be addressed in additional studies with more expansive time points.

#### 4.1. Factors Influencing Distribution of Soil Yeasts

Temperature, annual precipitation, pH and carbon content are the most frequent soil parameters, traditionally provided to explain distribution of soil yeasts (see, for example, [7,21,28]). Two studies that analyzed yeast distribution along a large latitudinal gradient revealed a positive correlation between high rainfall values, low soil pH, and occurrence of *Cr. podzolicus* [27,28]. However, none of these parameters explain the association, in our study, of *Cr. podzolicus* with soils underneath decaying logs (Table 1 and Figure S2). This disagreement might be explained by the fact that both pH and rainfall do not impact directly environmental factors determining the ecological optimum of this yeast. According to the original description, *Cryptococcus podzolicus* (basionym *Candida podzolica*) is associated with soddy-podzolic soils or podzols (according to FAO classification) [53]. Formation of this type of soil relies both on high raw organic matter income and on washing water regime [54]. However, rainfall values do not necessarily correlate with the type of soil water regime, as the latter strongly depends on physical field properties such as soil structure, clay content, ground water table and parent rock. Similarly, soil acidity (often referred to as pH) is the result of two distinct processes: decomposition of organic matter, and parent rock weathering [54]. In podzols, decomposing plant material produces a large amount of organic acids and aromatic compounds that are readily utilized by soil microbes, including yeasts. In contrast, acid acrisols and ferralsols contain less labile carbon, and their highly weathered parent rock releases toxic metal ions such as aluminum and iron [54,55]. Even though all these acid soils may have pH in the same range, environmental conditions for development of soil microorganisms in such habitats are quite different [54–56].

Our results showed positive effects of decaying logs on abundance of *Cr. podzolicus*, thereby suggesting the importance of dissolved (leaching) carbon compounds rather than soil pH for development of this soil yeast. Therefore, the distribution range of *Cr. podzolicus* might be significantly larger and include different acid well-drained soils worldwide, like podzols, andosols, umbrisols, acrisols and ferrasols. Even though the soil seems to be the primary habitat for this species, its occurrence in other substrates with similar environmental properties is feasible. For example, *Cr. podzolicus* was isolated from *Sphagnum* moss and peat [57,58], habitats that are also characterized by high moisture, acidity, and raw organic matter content. Although in some cases the distribution range of *Cr. podzolicus* may correlate with high water content in a substrate (washing water regime in case of soils), temperature, pH, or rainfall data alone are unlikely to be able to explain the distribution of this soil yeast.

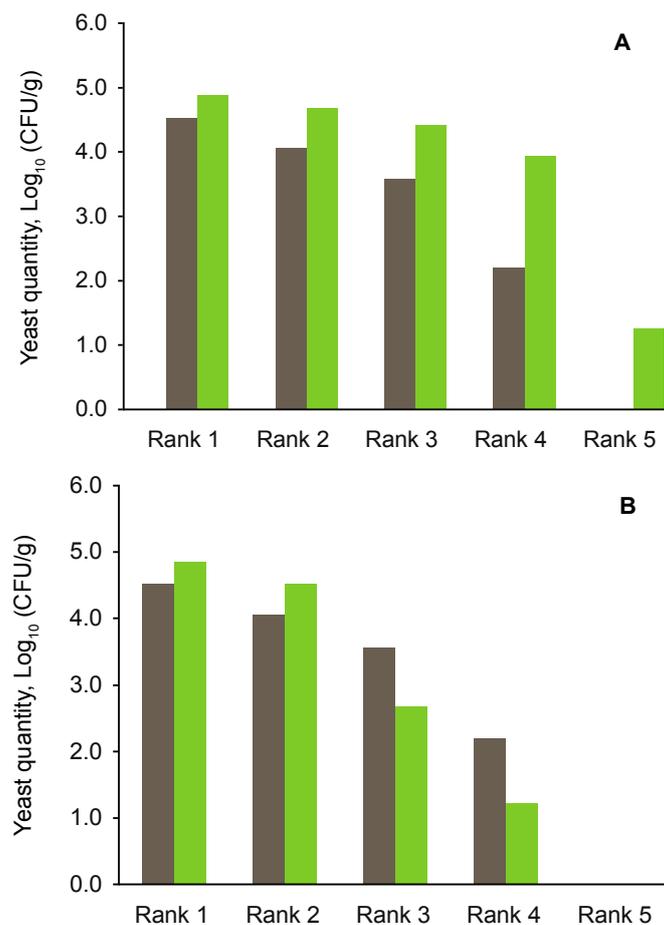
#### 4.2. Autochthonous and Transient Species in Soils

Many different ascomycetous and basidiomycetous yeasts were reported from soils but just a few of them were found exclusively in this substrate [7,8,28,51]. Other species, which could be also detected in soils, are entering soil profile with animal activity or annual leaf fall [47]. In particular, leaf fall may contribute substantially to the composition of soil yeast communities because phylloplane contains an abundant yeast population reaching millions of cells per gram [43]. In the present study, typical phylloplane-related yeasts were numerous and abundant in soils collected from adjacent areas that are exposed to the annual litter fall (Figure 2). These transient (or allochthonous) species substantially affected estimations of yeast diversity values, such as species richness and Shannon diversity index (Figure 1). In contrast, yeast communities underneath logs contained only three so far phylloplane-associated species, viz. *Aureobasidium pullulans*, *Cr. tephrensensis* and *Rhodotorula colostri*. Three more ascomycetous yeast species (retrieved as single isolates) could represent transient species, as well. They are closely related to yeasts that were previously isolated from decaying material. Specifically, strain TSN-95 was identified as *Candida cretensis*, which was originally isolated from a rotten polypore *Inonotus tamaricis* [59]. Another yeast (strain TSN-36) is prospectively conspecific to the strain *Candida sp.* CBS 7170, which was isolated from a fungal fruiting body of *Tyromyces ptychogaster* growing on a fallen spruce trunk (CBS database). Lastly, strain TSN-5 was closely related to *C. chilensis*, a species described from rotting *Nothofagus* wood in Chile [60]. All these yeasts were observed as single colonies during plating experiments that utilized 1:5 and 1:10 soil-to-water dilutions. Because these species were not detected from soil suspension with a lower soil-to-water ratio, we assume that these three ascomycetous yeasts are present in soils in very low quantities. They might originate from aboveground sources such as decaying logs and fungal fruiting bodies growing on fallen stems or trunks [8,13,42,59,60]. Overall, our results suggest that unlike phylloplane-related yeasts, yeasts inhabiting decaying logs provide minor contribution to soil communities.

While influence of transient species on communities' parameters in animal ecology has been considered [41], the division of microbes into resident and transient species is rare and has little application in microbial ecology. In the 1970s, extensive analysis of species abundance distribution patterns (SAD) along gradients by animal and plant ecologists resulted in revealing an empirical pattern of increasing community evenness with ecosystem productivity [41]. We found that the extrapolation of results based on species distribution patterns (abundance and incidence) in soils might be biased by the substantial presence of transient species (comp. Figure 2). This may also lead to incorrect conclusions regarding ecosystem properties. Both species richness and the Shannon diversity index suggested higher diversity in soils of adjacent areas in our study. Furthermore, we found that distribution of taxa (ranks) in Rank-Abundance diagram (RAD) was more even (flat) in soils collected apart from tree logs (Figure 6 A). This leads to a contradictory conclusion that without any regard to the soluble organic carbon leaching from decaying wood (Table 1), soils underneath logs are less productive and, therefore, harbor less diverse yeast communities. It is worth mentioning that yeasts entering soil with litter develop their biomass by consuming plant exudates and mainly retain their activity on fresh fallen leaves during early stages of succession [47,48,61]. These yeasts do not necessarily rely on the nutrients available in soil and, therefore, do not explain differences in

productivity between soils collected underneath logs and in adjacent areas. Remarkably, these yeast species were rarely detected at the same plots in soils collected in spring [8]. After removal of transient (phylloplane-related) species from the analysis, the structure of soil yeast community underneath logs displayed higher evenness than communities of adjacent areas (Figure 6 B). Therefore, our results show that external carbon originating from decaying logs supports higher productivity of soil-borne yeast species.

**Figure 6.** Rank-abundance diagram of yeast communities in soils underneath wood logs (brown) and in adjacent areas (green): (A) analysis includes all species and (B) soil-borne species only.



It has been repeatedly pointed out that proper identification of fungi using molecular markers is important for microbial biodiversity assessments. Our results additionally suggest that correct attribution of resident and transient species is crucial for microbial diversity assays, both culture-dependent and culture-independent ones. Importantly, detection of fungal taxa with culture-independent techniques does not necessarily require the presence of living cells and is often possible from resting stages, such as spores [62]. This may also bias estimations of sampling depth. It has been shown that completely surveyed communities usually follow lognormal distribution, whereas undersampled communities are better described with power law functions [41]. Recent analysis of fungal communities in the rhizosphere and on plant surfaces showed the presence of core and satellite species (molecular operational taxonomic units, MOTUs) groups [63]. While species from the core

group followed lognormal distribution, species from the satellite group were log-series. At the same time, a few studies have shown that the resident species of fish and beetles were lognormal, but the transient species were log-series [41]. We would like to emphasize that additional studies (also using culture-dependent approaches) are required to resolve the ecology of rare (or satellite) fungal species (and MOTUs) in order to find out whether or not they represent transient community members.

#### 4.3. Effects of Aboveground Deadwood Deposition

Yeast populations in soils collected from adjacent areas was strongly dominated by a single species, *Cr. terricola*, which contributed with 40%–60% to the total yeast abundance. In soils collected underneath logs, relative abundance of several soil-related yeasts, *Cr. podzolicus*, *T. dulcitum* and *T. porosum*, was higher than in soils from adjacent areas (Table 3 and Figure 3). These species are known to be able to sustain low pH and their occurrence in acid soils has been reported previously [10,21,28,48]. Besides the tolerance to soil acidification with soluble organic carbon substances, good development of these yeasts in soils underneath logs could be additionally explained by their assimilation profiles, *i.e.*, abilities to assimilate intermediates of wood decomposition such as complex polysaccharides, organic acids and aromatic compounds [9,11,13]. For example, strains of *Cr. podzolicus* and *T. porosum* isolated from decomposing wood were found to be the most active in degradation of plant-related carbohydrates [13]. In contrast, the members of the Cystofilobasidiales and Tremellales (Tremellomycetes), frequently detected in phylloplane and litter [43,47,48,61] either exhibited a reduced capacity to metabolize aromatic compounds, or did not utilize them at all [14].

Even though the concentration of dissolved organic carbon in topsoil under logs was higher than in adjacent areas (Table 1), we did not observe any pronounced correlation of this factor with yeast quantity, species richness, abundance of soil-related yeasts, and relative abundance of *Cr. podzolicus* (data not shown). Indeed, log deposition had little effect on total yeast quantity and species richness values (Table 4 and Figures 1, S1). However, it caused substantial alteration of yeast communities' structure and composition (Table 3 and Figures 2, 3). Although detailed analysis of environmental factors associated with wood decay was not in the scope of this study, our observations imply that selection towards well-adapted soil-borne species is driven by the same mechanisms in all analyzed plots. It has been repeatedly pointed out that yeasts in soils are distributed unevenly [7,8]. However, analysis of spatial variability using abundance-based Jaccard similarity coefficient showed that yeast communities formed underneath logs are more similar than the ones in adjacent areas (Figure 4). Additionally, ordination of this data displayed larger between-plot heterogeneity (distance) of yeast communities in soils collected apart from logs (Figure 5). This, in our opinion, suggests that aboveground deadwood deposition establishes selective (but stable) conditions in soils that promote growth of a few highly specialized soil-borne yeasts. Nevertheless, additional studies are required to find out which substances influenced soil yeasts and, therefore, are responsible for the observed effects. Besides altering belowground microbial communities with soluble organic carbon, large wood logs cover soil surface and thereby hamper the proliferation of plant-related yeasts in the soil profile.

## 5. Conclusions

Recent studies investigated effects of forest management on soil yeasts and suggested that yeasts depend on forest properties [8,26]. Although biomass and diversity of yeasts did not depend on soil abiotic parameters [29], substantial alteration of yeast communities' structure in relation to forest management has been revealed [8]. This is the first study, which tested the hypothesis that soil yeasts rely on forest properties such as substrate availability in a way similar to bryophytes or fungi [19]. Although aboveground deadwood deposition did not alter total quantity and species richness values, it changes the structure of soil yeast communities. While yeast populations in soils collected from adjacent areas were strongly dominated by a single species *Cryptococcus terricola*, several soil-borne yeasts successfully co-occurred in soils underneath wood logs. On average, decaying wood logs supported a more evenly structured yeast community, suggesting also higher productivity of this habitat. Additionally, soil yeast communities in adjacent areas harbored a considerable number of transient (usually phylloplane-related) yeasts reaching up to 30% of the total yeast quantity. The fact that transient yeast species are present in soils in autumn but not in spring additionally supports their origin from leaf litter. Our study highlights the necessity of distinguishing resident and transient species for correct conclusions regarding diversity and functional relationships within soil microbial communities.

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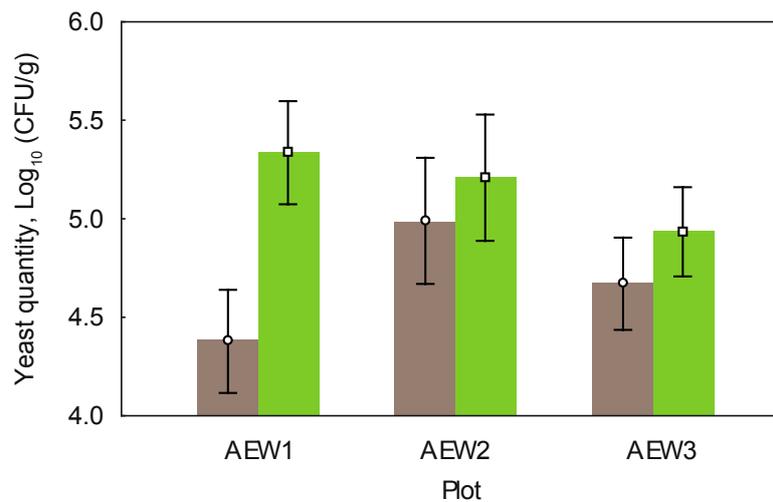
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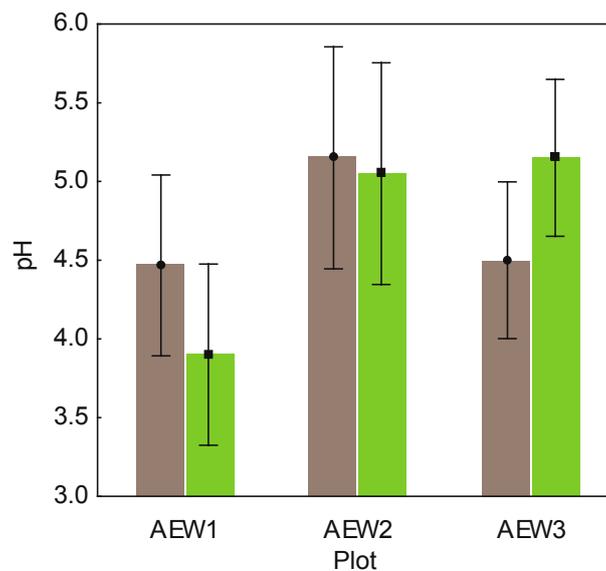
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### Supplementary

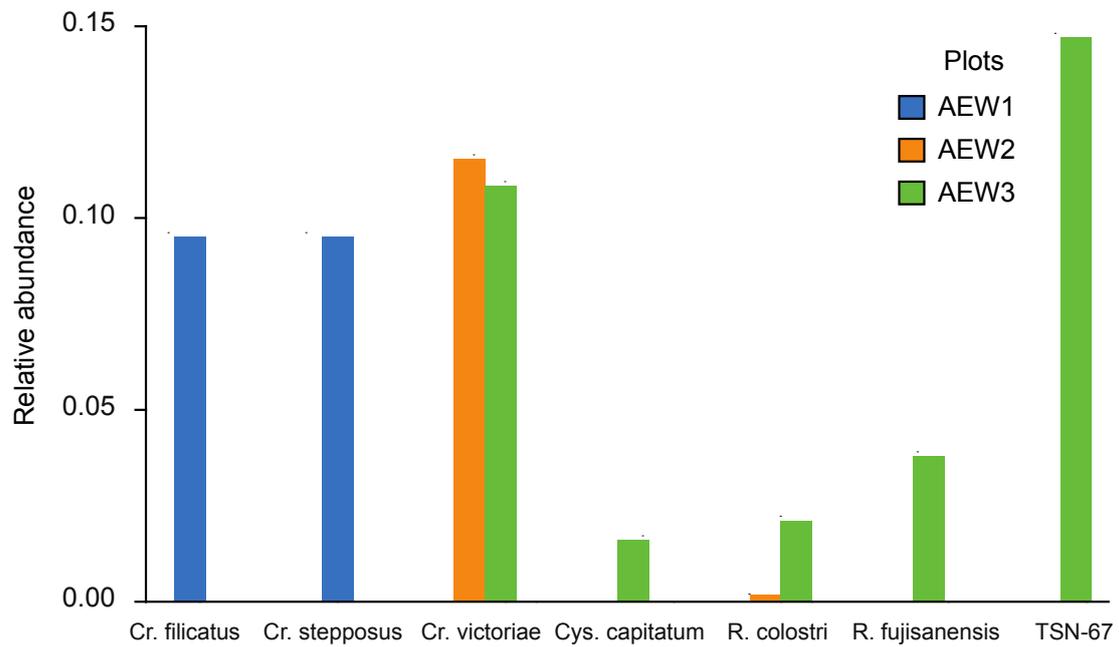
**Figure S1.** Yeast quantity,  $\text{Log}_{10}$  (CFU/g) in soils underneath Jwood logs (brown) and of adjacent areas (green). Whiskers correspond to a confidential interval and middle points to a mean.



**Figure S2.** Soil acidity ( $\text{pH CaCl}_2$ ) underneath wood logs (brown) and in adjacent areas (green). Whiskers correspond to a confidential interval and middle points to a mean.



**Figure S3.** Relative abundance (average values) of transient yeast species in soils of adjacent areas in plots AEW1 (blue), AEW2 (orange) and AEW3 (green).



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