

# Temporal variation of fungal diversity in a mosaic landscape in Germany

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**Abstract:** This study aims at characterizing the diversity and temporal changes of species richness and composition of fungi in an ecotone of a forest border and a meadow in the Taunus mountain range in Germany. All macroscopically visible, epigeous fungi and vascular plants were sampled monthly over three years, together with climatic variables like humidity and temperature that influence fungal diversity and composition as shown by previous studies. In this mosaic landscape, a total of 855 fungal species were collected and identified based on morphological features, the majority of which belonged to *Ascomycota* (51 %) and *Basidiomycota* (45 %). Records of fungal species and plant species (218) for this area yielded a fungus to plant species ratio of 4:1, with a plant species accumulation curve that reached saturation. The three years of monitoring, however, were not sufficient to reveal the total fungal species richness and estimation factors showed that a fungus to plant species ratio of 6:1 may be reached by further sampling efforts. The effect of climatic conditions on fungal species richness differed depending on the taxonomic and ecological group, with temporal patterns of occurrence of *Basidiomycota* and mycorrhizal fungi being strongly associated with temperature and humidity, whereas the other fungal groups were only weakly related to abiotic conditions. In conclusion, long-term, monthly surveys over several years yield a higher diversity of macroscopically visible fungi than standard samplings of fungi in autumn. The association of environmental variables with the occurrence of specific fungal guilds may help to improve estimators of fungal richness in temperate regions.

**Key words:** *Ascomycota*, *Basidiomycota*, Fungi, Seasonal trend decomposition, Species composition, Temporal variation.

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## INTRODUCTION

The magnitude of global fungal diversity is largely unknown and it is estimated that only around 2–6 % of the existing fungal richness has been formally described (Jones & Richards 2011). Since many years, Hawksworth's proposal of 1.5 million fungal species (Hawksworth 1991) is one of the most frequently cited estimates of global fungal richness. This estimation was based on the observation that multiple habitats harbour an average of six fungal species per plant species (the Hawksworth index). However, updated estimates assume a much higher number of fungal species, ranging from 3.5 up to 5.1 million species based on high-throughput sequencing methods (Blackwell 2011). In a most recent publication, a variety of estimation techniques suggest a range for the number of fungal species worldwide between 2.2 and 3.8 million (Hawksworth & Lücking 2017).

Monitoring campaigns are necessary to describe fungal species richness in particular habitats or areas and can be used to infer the magnitude of fungal diversity on a larger scale (Lodge & Cantrell 1995, Danielsen *et al.* 2005, Peay 2014). Furthermore, monitoring is important to check the consistency of the proposed estimation factors and relationships among organisms (e.g. between fungi and their plant hosts), which are necessary for estimating global fungal diversity (Schmit & Mueller 2007).

In this paper we focused on the diversity of macroscopically evident (i.e., visible with a hand lens) fungi at the border of a temperate forest and a meadow to include different habitats and detect a higher fungal diversity. Several inventories of fungi are

known for temperate regions, but most are based on fruiting bodies of groups predominantly belonging to *Basidiomycota* (e.g. Hawksworth 1991, Straatsma *et al.* 2001, Newton *et al.* 2003, Karasch 2005, Unterseher *et al.* 2012, Angelini *et al.* 2015). Our approach to record all macroscopically evident macro- and microfungi on a monthly basis has previously been performed only in the tropics in Panama (Piepenbring *et al.* 2012) and in Italy (Angelini *et al.* 2015). For Germany, current checklists exist for *Basidiomycota* and *Ascomycota* (except rust fungi, i.e., Pucciniomycotina; smut fungi, i.e. Ustilaginomycotina; and powdery mildews, i.e. Erysiphales; Dämmrich *et al.* 2016), for lichenized fungi (Wirth *et al.* 2011), and for slime moulds (*Myxomycetes*) (Schnittler *et al.* 2011). Although *Myxomycetes* and *Oomycota* do not belong to the kingdom of Fungi, they were considered in this study due to their lifestyle that is comparable to species of Fungi and the traditional consideration of this group within mycology. It is estimated that approximately 14 000 species of fungi are known for Germany (Thines 2016). The sampling area is located in the federal state of Hessen, for which 3 682 species of fungi are known (Deutsche Gesellschaft für Mykologie 2017). The natural park of the Taunus mountain range is the largest in the region and has been the focus of plant inventories (Jestaedt 2012), but no comparable in-depth inventories for fungi exist for this area.

An inventory of fungal species should consist of repeated samplings to increase the chances of detecting species that previously remained undetected (Schmit & Lodge 2005) and should encompass seasonal variation within fungal communities

(Cannon 1997, Halme & Kotiaho 2012). This temporal variation is probably related to several factors, especially environmental conditions (Krivtsov *et al.* 2003, Stankeviciene *et al.* 2008). Inventories of macroscopically evident fungi allow to identify species and to obtain species lists, including information concerning lifestyle, association with other organisms, and morphological features (Schmit & Lodge 2005). With these data, sporulation patterns can be detected (Tóth & Barta 2010) and related to possible ecological drivers, such as abiotic climatic factors.

We have aimed to compare variation of fungal richness data with that of environmental factors, i.e., humidity, temperature, and precipitation, in order to identify factors shaping the temporal patterns of fungal diversity in general and of specific taxonomic and ecological groups of fungi. By gaining information on how temporal variation of fungal diversity is determined by such factors, we can build models that might improve predictions of fungal diversity in other, non-sampled areas. Specifically, the objectives of this study are: (i) to record fungal species richness and relate it to plant species richness; (ii) to analyse temporal variation in species richness, for all fungi and for the two most important divisions, i.e., *Ascomycota* and *Basidiomycota*, with a particular focus on the effect of climatic factors; and (iii) to analyse temporal variation in species composition, in general and in selected taxonomic and ecological groups of fungi.

## MATERIALS AND METHODS

### Study area and samplings

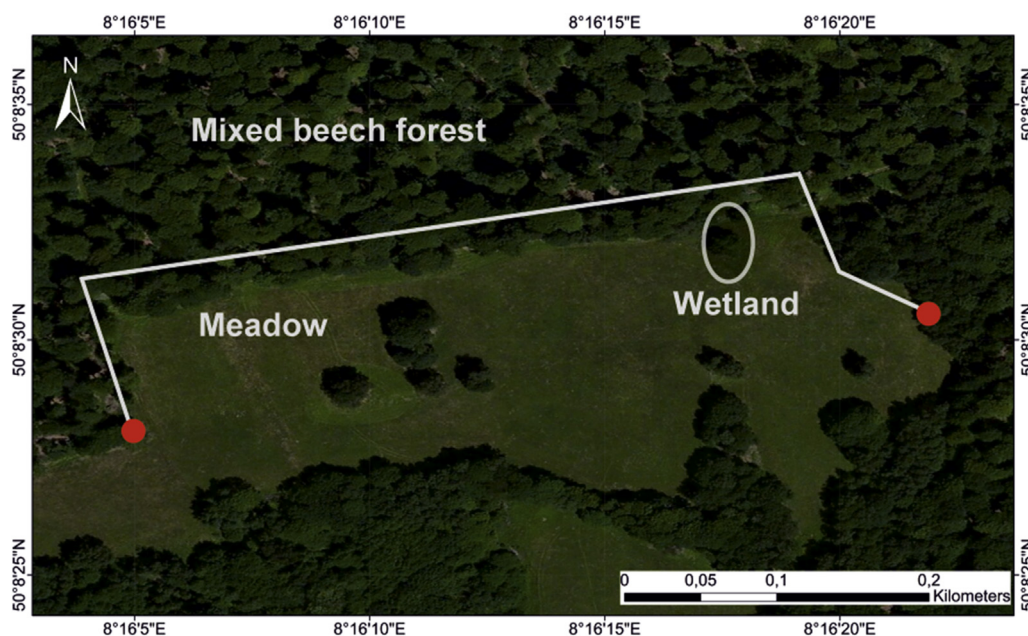
The study area is located on the southern slope of the Taunus mountain range (Vordertaunus), in the federal state of Hessen, central-western Germany (N 50° 08' 28.0", E 08° 16' 21.1", at approximately 400 m above sea level). The region is characterized by a mosaic landscape and comprises different types of habitat, such as broadleaved woodlands, mostly

composed of beech (*Fagus sylvatica*), and semi-managed meadows. It is integrated within the Natural Park "Troockenborn/Kellerskopf bei Rambach" of the Habitats Directive for Flora and Fauna (European Union 2012). The responsible Forestry Department in Wiesbaden was informed, a permit was not required.

A sampling transect was established along a 500-meter-long section of a footpath along a forest-meadow ecotone (Fig. 1), similar to the area investigated with the same sampling design by Piepenbring *et al.* (2012) in Panama. This mosaic landscape was chosen to cover a comprehensive and representative sample of the diversity of fungi living on different substrates. The vegetation was a mixed beech forest on one side of the path, and a semi-managed meadow that was mown twice a year by personnel of the Forestry Department on the other side. The sampling covered an area in a range of approximately 10 m at each side of the path, following a strip transect design (Hill *et al.* 2005).

Sampling was performed monthly for a period of three years, from May 2011 to April 2014. During each sampling, usually three persons recorded all visible fungi and vascular plants in the transect area for 2 h. All fungi discernible with the naked eye or with a hand lens on (fallen) plant material from the ground to two meters height were recorded (e.g., saprobionts on plant material, parasites on plants and insects, mycorrhizal fungi, lichens, as well as fungus-like organisms in Myxomycetes or Oomycota). Plants and fungi were collected for identification if necessary (see below).

Ambient temperature and relative humidity in the study area were continuously monitored with a Hygrochron data logger (model DS1923-F5, Fuchs Electronic, Weingarten, Germany). The device was placed on a tree branch at the border of the forest, a few centimetres above ground level. Recordings were programmed to take place daily at 12 a.m., 6 a.m., 12 p.m., and 6 p.m. Daily precipitation data were obtained from published records from the weather station Wiesbaden-Auringen (Deutscher Wetterdienst 2015), situated at 263 m above sea level, at 3.5 km distance from the study area.



**Fig. 1.** Sampling transect at Trockenborn in the Taunus mountain range. Starting and end points are marked by red circles. The map was produced with the software ArcGIS and ArcMap by Esri (2015). © OpenStreetMap (and) contributors, CC-BY-SA; Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community. Further editing of the map was made with Adobe Photoshop CS5.1.

## Identification of samples and recording of data

Field observations of fungi and vascular plants were recorded as incidence data once per sampling event. Samples from fungi and plants that could not be identified in the field were collected and brought to the laboratory for determination. For each fungal record, notes were taken on taxonomic classification, substrate or host, characteristic macromorphological features, cellular structures evident by light microscopy, and developmental stages. These data as well as photographs of fungi taken in the field and in the laboratory helped to assign species names or morpho-species concepts consistently over the years. Identifications and identification attempts for in total 2976 specimens of fungi (see results) were not supported by molecular sequence data because this was not possible within the scope of the present project.

The first approach to identify fungal specimens was based on the following literature for European fungi and its keys referring to macroscopical and microscopical features: Breitenbach & Kränzlin (1986-2005) for asco- and basidiomycetes, Knudsen & Vesterholt (2008) for basidiomycetes, Ellis & Ellis (1997) as well as Brandenburger (1985) for plant-parasitic microfungi, Gäumann (1959) as well as Vánky (2012) for rusts and smuts, respectively, and Wirth (1995a, b, c) for lichens. When necessary, specialised literature for particular groups (for example Seifert *et al.* 2011 for hyphomycetes) or monographic literature on specific groups was used. The fungus-host distribution database (USDA, <http://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm>) was used as orientation for the identification of plant parasites based on their host plant species. The nomenclature of fungi is based on Index Fungorum (Royal Botanic Gardens Kew 2008). Sexual and asexual developmental stages belonging to the same species were recorded only once per sampling event.

Fungal records were roughly assigned to ecological categories for statistical analyses. These categories consist of: (i) saprobionts, i.e., fungi growing on dead plant material, animals, or soil; (ii) plant parasites, present on living plant tissue and penetrating the host tissue; (iii) mycorrhizal fungi, recognized according to their morphology and taxonomy; (iv) lichens; and (v) fungi feeding on microorganisms, recognized according to their morphology and taxonomy. This classification was based on relevant literature (see above), *in situ* observations, and on the knowledge of researchers performing the samplings and/or identifications. Preserved fungal specimens were tagged with IPF-collection numbers and have been deposited in the Senckenberg Herbarium at Frankfurt (FR; Germany).

Plant material was mainly determined based on Seybold (2011) and Jäger *et al.* (2013). The nomenclature of plants relies on Floraweb (Bundesamt für Naturschutz 2013).

A complete list of all fungal and plant species recorded during sampling events and their associated metadata is provided in Table S1. These data were imported into the database Diversity Workbench (Workbench contributors 2013), in cooperation with personnel and editors of this program from the Bavarian Natural History Collections (Munich).

## Analysis of the data

Statistical analyses were carried out with the program R version 3.1.3 (R Core Team 2015) including the package “stats” and

several specific R packages, especially “vegan” version 2.2-1 (Oksanen 2011), “Hmisc” version 3.16-0 (Harrell & Dupont 2014), and “gplots” version 2.17.0 (Warnes *et al.* 2015).

The analyses of fungal and plant occurrence were based on incidence data per sampling unit. For these analyses, only records identified to species and morphospecies level (with identification up to genus in the latter) were used. Observed and total estimated species richness of fungi and plants were calculated with EstimateS version 9.1.0 (Colwell 2013), using rarefaction curves with 1000 permutations, and the estimators Chao 2, Jackknife 1, Jackknife 2, and Bootstrap (Colwell & Coddington 1994). As the species accumulation curve for fungi did not reach saturation at the end of sampling, data were extrapolated until doubling the number of sampling events, following procedures proposed in the EstimateS manual (Colwell 2013).

The influence of seasons and other factors on fungal richness was investigated by a seasonal-trend decomposition process (*stf* function of “stats”) based on locally weighted regression (loess), a specific method for time series data (Cleveland *et al.* 1990) useful for visualizing patterns within the data (Kennedy *et al.* 2015). In order to test whether fungal species richness changed across months, the Kruskal-Wallis rank sum test was applied (Kruskal & Wallis 1952). Time series data per year were categorized by the sampling season, i.e., into fruiting season (May to November, comprising the period sampled most often in other monitoring studies) and non-fruiting season (December to April).

Data on temperature, humidity, and precipitation were analysed as potential drivers for seasonal changes of fungal richness and species composition. Average values of temperature and humidity eight days prior to each sampling were used for the analyses, because these showed the highest correlation with fungal species richness. Humidity and temperature were strongly correlated with each other (Spearman correlation:  $\rho = -0.71$ ,  $P < 0.001$ ). For total precipitation, average values from the four days before each sampling were used because they showed the highest correlation with fungal species richness. Data on humidity and precipitation were moderately correlated ( $\rho = 0.33$ ,  $P = 0.05$ ). Due to the correlations between the environmental variables, separate generalized linear models (GLMs) with a poisson error distribution were used to model the changes in species richness based on each explanatory variable separately. A quasi-poisson model was chosen because the dispersion parameter of the models was larger than two.

For analyses of species composition, ecological distances based on shared/unshared species among sampling events were calculated with the Sørensen dissimilarity index (Sørensen 1948). Prior to this, singletons (records that occurred in a single sampling event) were removed from the dataset (Ji *et al.* 2013). The ecological distances calculated and their correlation with temporal and ecological variables were visualized with Non-Metric-Multidimensional Scaling (NMDS). The NMDS species scores were grouped either by their taxonomic affiliation, using the most frequent orders within *Ascomycota* and *Basidiomycota*, or by their ecological category. The effect of the different abiotic factors (temperature, humidity, and precipitation) on species composition was investigated using a permutational analysis of variance (PERMANOVA; Anderson 2001) with 5000 permutations. This analysis is based on distance matrices that describe variation in species composition and evaluates the association of ecological variables (such as abiotic factors) with differences in species composition.



## RESULTS

### Species richness of fungi and plants

During the entire survey, 2976 records of fungi were obtained. These records were assigned to 855 species including 741 fully identified species (79 % of all records) and 114 morphospecies identified to genus level (12 % of all records). 80 records of fungi (9 %) could only be identified at higher taxonomic levels due to the absence of suitable characteristics for identification, with 28 records identified up to division level, and six up to order level. The remaining 46 morphospecies could not be assigned to any taxonomic category. A total of 3264 plant records were obtained, which were assigned to 218 species of plants.

The accumulation of fungal and plant species over the period of the survey followed distinctive patterns (Fig. 2A). Almost the entire plant richness in the sampling area was uncovered after 15–20 sampling events, as shown by a plateau reached by the plant species accumulation curve (Fig. 2A, grey lines). Saturation of fungal richness was not reached at the end of the survey, with the accumulation curve still showing a steady increase in the number of species with additional sampling effort (Fig. 2A, black lines). After three years of monthly sampling, a fungus:plant species ratio of 4:1 was obtained, which increased to 5:1 upon a rarefied extrapolation estimated for additional 36 months of sampling. Fungal richness showed no saturation even after data extrapolation (Fig. 2A, dashed black lines). The total estimated plant richness ranged from 225 to 234 (Chao 2 and Jackknife 1, respectively). Estimations of the total number of fungal species varied between 1032 (bootstrap), 1427 (Chao 2), and 1526 (Jackknife 2), yielding a fungus:plant ratio of 7:1 in the most species-rich scenario.

The distribution of fungal occurrence was uneven (Fig. 2B, black points), with 423 fungal species (49 %) found only once (singletons) and 152 species (18 %) found twice (doubletons). No fungal species was found in all sampling events. The occurrence of plants was more equally distributed, with only 16 plant species (7 %) found once and 17 species (8 %) twice (Fig. 2B, grey points). Twelve plant species (6 %) were found in all sampling events.

Records of fungi and fungus-like organisms were assigned to five major groups, namely *Ascomycota*, *Basidiomycota*, *Myxomycetes*, *Oomycota*, and zygomycetous fungi (Mucorales). *Ascomycota* (436 species) and *Basidiomycota* (389 species) were the most frequently represented divisions. *Myxomycetes* were represented by 25 species, *Oomycota* by three, and zygomycetous fungi by two species. The proportion of fungus-like organisms, i.e., *Myxomycetes* and *Oomycota*, in this study was small and their removal would not affect the general results. Overall, 66 orders and 454 genera of fungi were identified. The ecological groups, to which fungal records were most frequently assigned, were saprobionts (591 species, 69%), parasites (169 species, 20%), and mycorrhizal fungi (41 species, 5%). Lichenized fungi (29 species, 3%) and fungus-like organisms feeding on microorganisms (25 species, 3%) represented smaller parts. The most frequently recorded fungal species were *Fomes fomentarius* (*Basidiomycota*; in 33 sampling events), *Schizophyllum commune* (*Basidiomycota*; in 32 sampling events), and *Hypoxylon fragiforme* (*Ascomycota*; in 32 sampling events). For this and further information on frequently recorded species see Table 1. A complete list with all taxonomic classifications is provided in supplementary Table S1.

### Temporal variation of fungal species richness

In total, 394 species (46 %) were recorded only during the fruiting season (May to November), 182 species (21 %) only during the non-fruiting season (December to April), and 279 species (33 %) in both periods. Examples of species only occurring in winter/beginning of spring are *Ciboria amentacea* (*Ascomycota*), which was recorded only in February and March of all sampling years, and *Mycena tintinnabulum* (*Basidiomycota*), which was recorded four times between January and March over the three years. Species with durable fruiting bodies like bracket fungi and lichens were detected all year round, independent of season.

The raw data on species richness per sampling event were analysed by seasonal trend decomposition to unravel seasonal components and general trends in the number of records (Fig. 3). A general increasing trend with a lag of one year was evident in the data, which was confirmed through decomposition into

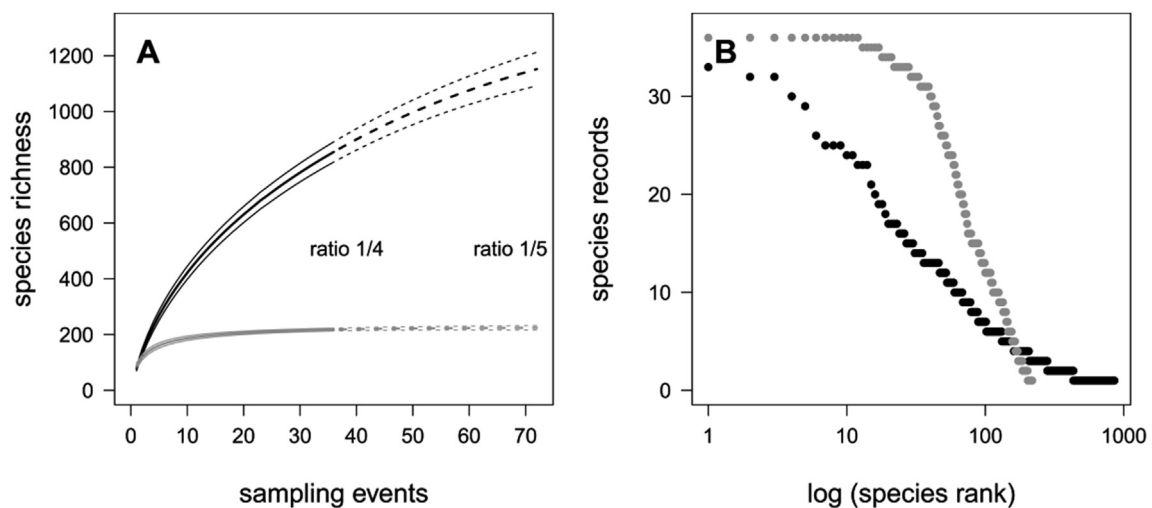


Fig. 2. Patterns of fungal (black) and plant (grey) species richness recorded during this study. A. Rarefaction curves of species accumulation with sampling effort. The lines show the number of species recorded (36 sampling events, continuous lines) and estimated (for sampling events 37–72, dashed lines) with 95 % confidence intervals, calculated with Chao 2 unbiased estimator. B. Rank-frequency plot showing the number of records per fungal (black points) and plant (grey points) species, ranked by decreasing frequency (log scale).

**Table 1.** Systematic overview of fungi recorded for at least ten sampling events in the context of the present investigation.

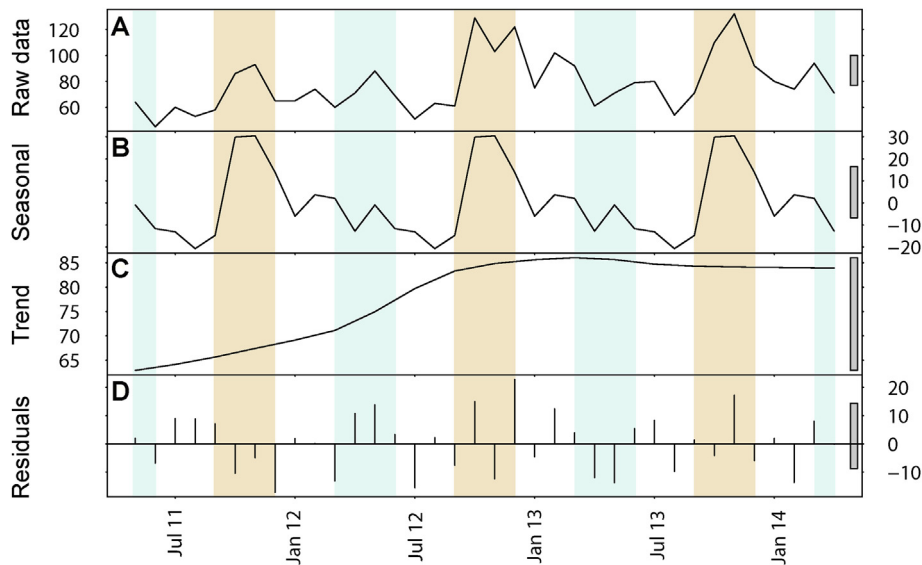
Division	Total	%	Order	Total	%	Genus	Total	%	Species	Records	Life style						
<i>Ascomycota</i>	436	51	<i>Helotiales</i>	87	20	<i>Lachnum</i>	6	7	<i>Lachnum impudicum</i>	10	saprobic						
						<i>Bisporella</i>	3	3	<i>Bisporella citrina</i>	12	saprobic						
						<i>Neodasyscypha</i>	1	1	<i>Neodasyscypha cerina</i>	16	saprobic						
			<i>Pleosporales</i>	64	15	<i>Lachnellula</i>	1	1	<i>Lachnellula occidentalis</i>	13	saprobic						
									<i>Epicoccum</i>	1	2	<i>Epicoccum nigrum</i>	13	saprobic			
									<i>Melanomma</i>	1	2	<i>Melanomma pulvis-pyrius</i>	13	saprobic			
						<i>Xylariales</i>	45	10	<i>Hypoxylon</i>	6	13	<i>Hypoxylon fragiforme</i>	32	saprobic			
									<i>Diatrype</i>	4	9	<i>Diatrype stigma</i>	21	saprobic			
									<i>Diatrype decorticata</i>	17	saprobic						
			<i>Hypocreales</i>	41	9	<i>Dialonectria</i>	1	2	<i>Dialonectria episphaeria</i>	19	saprobic						
									<i>Nectria</i>	4	10	<i>Nectria cinnabarina</i>	14	saprobic			
									<i>Cladosporium</i>	6	22	<i>Cladosporium herbarum</i>	19	saprobic			
						<i>Capnodiales</i>	27	6	<i>Mycosphaerella</i>	5	19	<i>Mycosphaerella punctiformis</i>	16	parasitic			
									<i>Ramularia</i>	4	15	<i>Ramularia digitalis-ambiguae</i>	17	parasitic			
						<i>Diaporthales</i>	18	4	<i>Gnomonia</i>	4	22	<i>Gnomonia setacea</i>	14	saprobic			
						<i>Chaetosphaeriales</i>	6	1	<i>Chaetosphaeria</i>	4	67	<i>Chaetosphaeria ovoidea</i>	13	saprobic			
						<i>Rhytismatales</i>	5	1	<i>Colpoma</i>	1	20	<i>Colpoma quercinum</i>	17	saprobic			
									<i>Propolis</i>	1	20	<i>Propolis farinosa</i>	12	saprobic			
						<i>Basidiomycota</i>	389	45	<i>Agaricales</i>	174	45	<i>Schizophyllum</i>	1	1	<i>Schizophyllum commune</i>	32	saprobic
												<i>Cylindrobasidium</i>	1	1	<i>Cylindrobasidium evolvens</i>	12	saprobic
			<i>Polyporales</i>	45	12				<i>Polyporus</i>	5	11	<i>Polyporus brumalis</i>	10	saprobic			
									<i>Trametes</i>	5	11	<i>Trametes versicolor</i>	29	saprobic			
									<i>Fomes</i>	1	2	<i>Fomes fomentarius</i>	33	parasitic			
									<i>Ganoderma</i>	1	2	<i>Ganoderma applanatum</i>	23	parasitic			
									<i>Bjerkandera</i>	1	2	<i>Bjerkandera adusta</i>	14	saprobic			
									<i>Piptoporus</i>	1	2	<i>Piptoporus betulinus</i>	13	saprobic			
									<i>Daedaleopsis</i>	1	2	<i>Daedaleopsis confragosa</i>	11	saprobic			
<i>Pucciniales</i>	43	11							<i>Puccinia</i>	21	49	<i>Puccinia coronata</i>	24	parasitic			
									<i>Puccinia annularis</i>	15	parasitic						
									<i>Puccinia obscura</i>	14	parasitic						
			<i>Puccinia hieracii</i>	11	parasitic												
<i>Phragmidium</i>	5	12	<i>Phragmidium violaceum</i>	25	parasitic												
			<i>Melampsora</i>	3	7				<i>Melampsora populnea</i>	24	parasitic						
			<i>Melampsora caprearum</i>	23	parasitic												
<i>Russulales</i>	39	10	<i>Stereum</i>	10	26				<i>Stereum hirsutum</i>	30	saprobic						
			<i>Peniophora</i>	6	15				<i>Peniophora quercina</i>	15	saprobic						
			<i>Peniophora incarnata</i>	13	saprobic												
<i>Hymenochaetales</i>	12	3	<i>Hericium</i>	1	3				<i>Hericium coralloides</i>	12	saprobic						
			<i>Fuscoporia</i>	2	17				<i>Fuscoporia ferruginosa</i>	13	parasitic						
			<i>Schizopora</i>	1	8				<i>Schizopora paradoxa</i>	20	saprobic						
<i>Auriculariales</i>	7	2	<i>Exidia</i>	4	57				<i>Exidia plana</i>	15	saprobic						
			<i>Exidia glandulosa</i>	13	saprobic												
<i>Dacrymycetales</i>	7	2	<i>Dacrymyces</i>	5	71				<i>Dacrymyces stillatus</i>	26	saprobic						

"Total" refers to the number of species recorded during the present investigation within the respective taxon and "%" is the percentage of the species number in the respective taxon within the taxon one level above (for example: 87 species of Helotiales were recorded and represent 20 % of the species recorded for *Ascomycota*).

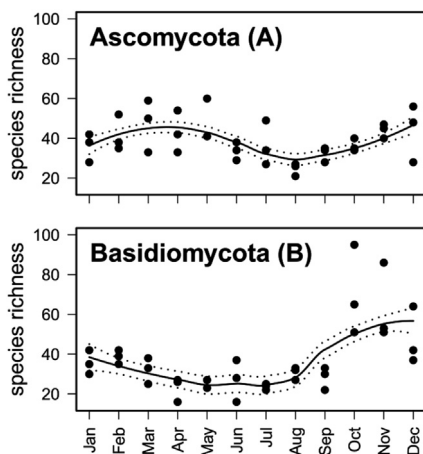
different components (Fig. 3A,C). An assessment of the temporal autocorrelation of fungal species richness over three sampling years reveals a pronounced seasonal variability of fungal species richness (Fig. 3B). Fungal richness differed significantly across months (Kruskal–Wallis:  $\chi^2 = 21$ ,  $df = 11$ ,  $P = 0.033$ ). Autumn and the beginning of winter were the periods showing the highest fungal richness, whereas the lowest values were found in August and September (Fig. 3).

The overall values of species richness within the *Ascomycota* (Fig. 4A) were rather stable over the year ( $\chi^2 = 16.01$ ,  $df = 11$ ,

$P = 0.14$ ). The model of richness variation within this division along the year displayed only slight increases at the end of autumn (Nov, Dec) and at the end of winter (Feb). Conversely, species richness of *Basidiomycota* varied significantly over the year ( $\chi^2 = 27.53$ ,  $df = 11$ ,  $P = 0.004$ ), with a considerable increase at the beginning of autumn (Fig. 4B). However, different groups of fungi within this division followed distinct patterns, e.g., species richness in the *Agaricales* varied broadly over the year, while species richness in bracket fungi, i.e., *Polyporales* and other polyporoid fungi, *Pucciniales*, and lichens remained rather constant.



**Fig. 3.** Seasonal trend decomposition for fungal species richness recorded monthly during three years in the Taunus area. **A.** Original species richness data for each sampling event, including the sum of **B** seasonal variation, **C** trend, and **D** residuals. Scales are different for each panel; therefore, the relative variance of the components is displayed by grey bars on the right side of each panel. The brown background shows autumn months (Sep – Dec) and the blue background shows spring months (Mar – Jun).



**Fig. 4.** Changes in the numbers of species recorded for *Ascomycota* (A) and *Basidiomycota* (B) across months during the entire sampling period. The points show the recorded species richness per sampling event over the three years. The continuous lines represent the loess model of richness across months, and the dashed lines show the corresponding standard errors.

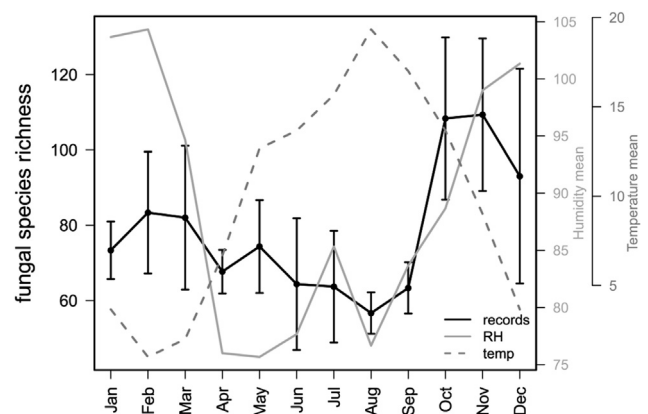
### Effects of climatic factors on fungal species richness

Values of fungal species richness, humidity, and temperature varied significantly during the year. Temperature and humidity showed rather opposing patterns, whereas changes in fungal species richness matched well with changes in humidity (Fig. 5). Fungal species richness negatively correlated with temperature (Estimate = -0.01, SE = 0.007, adj.  $R^2 = 0.11$ ,  $T = -1.9$ ,  $P = 0.06$ ; Fig. 6A), whereas a significant positive correlation was recorded between fungal species richness and humidity (Estimate = 0.01, SE = 0.003, adj.  $R^2 = 0.24$ ,  $T = 3.2$ ,  $P = 0.003$ ; Fig. 6B), as well as between fungal richness and precipitation (Estimate = 0.01, SE = 0.004, adj.  $R^2 = 0.18$ ,  $T = 2.7$ ,  $P = 0.01$ ). Changes in humidity and precipitation had a stronger impact onto fungal species richness in the division of *Basidiomycota* than onto species richness in *Ascomycota* that instead was affected by temperature (Table S2). Concerning different life styles, humidity

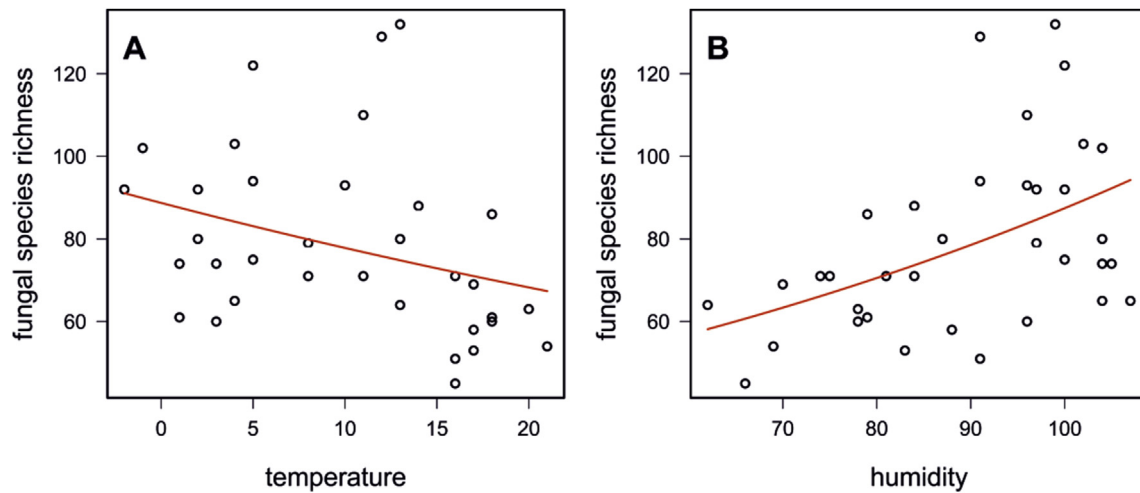
had a relatively strong impact onto fungal species richness of saprobionts and parasites, while mycorrhizal fungi were slightly affected only by temperature (Table S2). Species richness in the *Agaricales* increased with humidity and precipitation, whereas records of species belonging to other orders within the *Basidiomycota* and *Ascomycota* did not vary significantly with these factors (Table S3).

### Temporal variation in fungal species composition

The composition of fungal species during the survey was significantly affected by temporal (sampling year and month, Table S4) and climatic factors (temperature, precipitation, and humidity, Table S5). The assemblage of fungal species recorded in the first sampling year differed significantly from assemblages obtained in subsequent years, with the factor sampling year accounting for 7 % of the variation in species composition (Fig. 7A,  $F = 3.00$ ,  $R^2 = 0.07$ ,  $P = 0.0002$ ). The strongest effect on



**Fig. 5.** Temporal variation of fungal species richness across three years of sampling shown by averaged values of fungal species richness, relative humidity (RH), and temperature (temp) across months, during the entire sampling period. For RH and temp, average values of eight days prior to each sampling event were considered. Error bars indicate standard deviations.



**Fig. 6.** Generalized linear models showing the relationships between climatic variables and fungal species richness during 36 sampling events in the study area. **A.** Effect of temperature. **B.** Effect of humidity.

species composition was due to the factor month ( $F = 1.44$ ,  $R^2 = 0.38$ ,  $P = 0.0002$ ), which explained nearly 40 % of the variance in species composition (Fig. 7A).

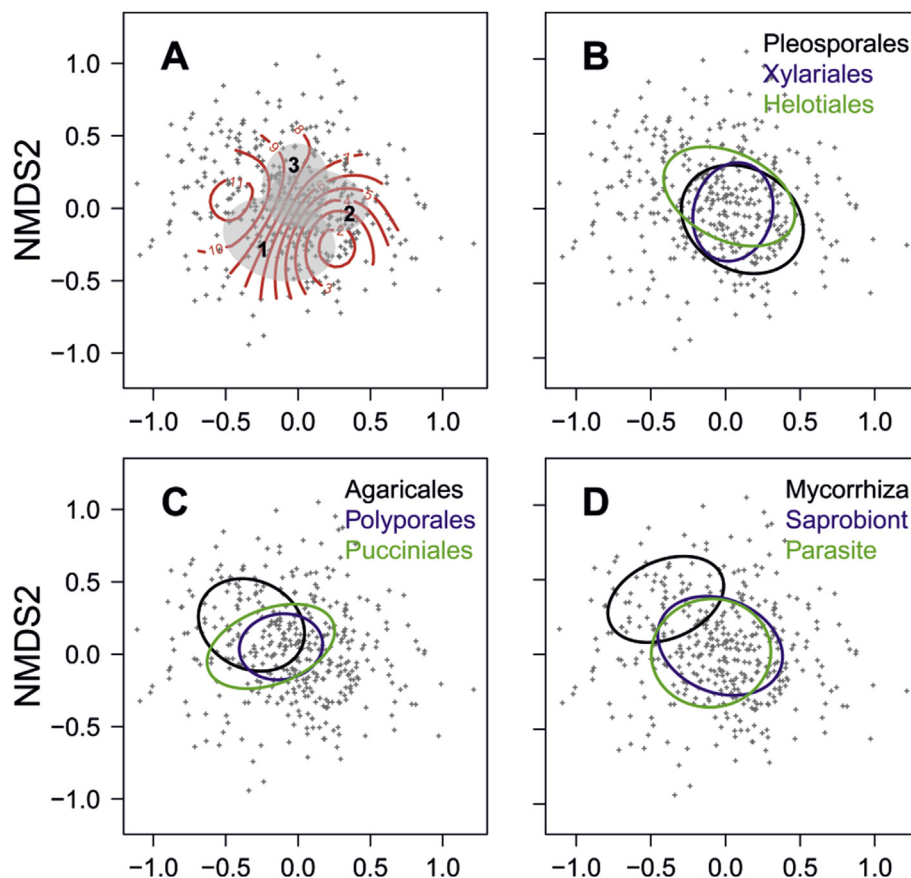
A partition of the temporal changes of species composition into different taxonomic and functional groups of records yielded different patterns (Fig. 7B–D). Fungal groups within the most frequent orders of *Ascomycota* (Fig. 7B) or with a saprotrophic and plant parasitic lifestyle (i.e., *Polyporales*, *Pucciniales*; Fig. 7C) did not show a temporal variation in their assemblages. In contrast, records that were either classified within the

basidiomycetous order *Agaricales* (Fig. 7C) or as mycorrhizal (Fig. 7D) had a preferential occurrence in autumn and winter.

## DISCUSSION

### Species richness of fungi and plants

In an area of approximately 1 ha, we documented fungal and plant species richness over a period of three years, resulting in



**Fig. 7.** Species composition of the study area for 36 sampling events displayed in a non-metric multidimensional scaling (final stress = 25 %) based on Sørensen dissimilarities. The dark grey crosses display the species scores along the first two axes. **A.** Dispersion ellipses for the sampling years numbered with 1, 2, and 3 are based on the standard deviations. The variable month (red lines) is fitted to a smooth surface using generalized additive model (GAM). Plots B–D show data for the three most frequent orders within the *Ascomycota* (B), the three most frequent orders of the *Basidiomycota* (C), and the three most frequent ecological groups (D).



lists including 855 species of fungi and 218 species of plants. Several previous inventories have revealed varying numbers of fungal species below or above the values reported here, ranging between 305 and 1 166 fungal species in areas located in Europe or North America (Zehfuß 1999, Straatsma et al. 2001, Krivtsov et al. 2003, Straatsma & Krisai-Greilhuber 2003, Karasch 2005, Ceska 2013, Angelini et al. 2015). The broad variation in fungal species richness reflects differences in the sampling methodologies and ecological features of the particular habitats. The use of the same methodology would facilitate the comparison of fungal richness obtained in different areas and projects (Cannon 1997, Dornelas et al. 2012). In our study, we included all visible fungi, comprising a large variety of taxonomic groups rather than focusing only on fungal fruiting bodies larger than a few centimetres, as done in the previously mentioned studies. The high fungal species number in our study, sampled in a relatively small sampling area and over three years, shows the importance of including different taxonomic groups in monitoring projects, in order to obtain a comprehensive species list, as proposed by Rudolf et al. (2013). Furthermore, the development of standardized sampling protocols is essential, because comparable assessments of biodiversity are important, among other things, to prioritize possible areas for conservation (Margules et al. 2002).

For the first year, a lag phase (Fig. 3C) indicates a number of recorded fungal species that is lower than during the second and the third year. This lag phase is probably due to a learning effect in the sampling team, that is common in similar studies (e.g., Piepenbring et al. 2012) and corresponds to the period of activities necessary for the researchers to get acquainted with the local species diversity. The overall increase in fungal richness over time was, however, independent of the seasonal variability in diversity patterns that was largely consistent across all sampling years.

A high number of singletons and new records in each additional sampling event even after three years of monthly sampling show that the macroscopically visible fungi of the study area remain undersampled. To estimate the total diversity, the estimator Chao 2 (Unterseher et al. 2008) suggests a fungus to plant ratio of approximately 6:1 for all macroscopically visible fungi. This result is consistent with the fungus to plant ratio of 6:1 proposed by Hawksworth (1991) based on inventory data, with the rationale that a higher diversity of available plant substrates influences fungal diversity (Rudolf et al. 2013). This ratio of 6:1 was shown to be very conservative because the most species-rich scenario revealed a higher ratio of 7:1 (Hawksworth & Lücking 2017). Molecular studies based on environmental sequencing tend to reveal higher fungal diversities than visual assessments (O'Brien et al. 2005), but these data are prone to methodological errors (Hawksworth & Lücking 2017). We conclude that a fungus to plant ratio of 6:1 is conservative and only applicable to visual monitoring assessments.

The proportion of species of *Ascomycota* recorded in our study area was larger (51 %) than that found in other monitoring activities of fungi, which reported mainly species of *Basidiomycota* (between 78 % and 97 %) and only between 3 % and 18 % species of *Ascomycota* (Krivtsov et al. 2003, Karasch 2005, Angelini et al. 2015, Kutszegi et al. 2015). The high percentage of *Ascomycota* in the present study shows that many inconspicuous species are overlooked in most monitoring campaigns (Mueller & Gerhardt 1994). Intensive sampling covering

several fully-sampled years and a wide range of fungal groups across different habitats yield proportions of *Ascomycota* similar to those found in molecular studies (e.g. between 46 % and 49 % in Tedersoo et al. 2014 and O'Brien et al. 2005). These numbers also reflect the actual proportion of known *Ascomycota* species respective to the total number of known fungal species (Kirk et al. 2008).

## Temporal variation of fungal species richness and composition

We detected a strong seasonal effect on total fungal species richness, which was associated with variation in humidity, precipitation, and temperature during the years. This effect of seasonality has also been shown in other studies, with a high species richness evident by fruiting bodies of macrofungi during late summer and autumn in regions with temperate climate (Watling 1995, Egli et al. 1997, Stankeviciene et al. 2008, Piepenbring et al. 2012). In tropical climate, a maximum of macrofungi is evident at the beginning of the rainy season (Piepenbring et al. 2015). Inventory activities all year round indicate that different fungi can be observed at different times of the year (Piepenbring et al. 2012). Therefore, sampling activities should not be restricted to mushroom fruiting seasons especially in temperate regions.

Recently, the importance of seasons for species richness has also been demonstrated by high-throughput sequencing for fungi in soil (He et al. 2017). According to these results, the impact of season on fungal species richness is stronger in temperate deciduous forest than in subtropical evergreen forests, probably due to the seasonal changes being more pronounced in temperate ecosystems.

By correlating changes of different ecological and taxonomic groups to climatic variables, we found distinct temporal patterns especially for *Basidiomycota* and for mycorrhizal fungi, which form a subset of *Basidiomycota* in our study. The fructification of macrofungi, which are mostly mycorrhizal or saprotrophic species of *Basidiomycota*, is correlated with precipitation and humidity (Baptista et al. 2010, Ceska 2013). Probably, this relationship is at least partly due to the relatively large fruiting bodies, which need rainfall for their development (Krivtsov et al. 2003). In contrast, species of *Ascomycota* are mostly small and live in microhabitats often protected from dehydration (Mueller & Gerhardt 1994), entailing a lower dependency on environmental conditions than for most species of *Basidiomycota*. Species of bracket fungi (*Polyporales* and other polyporoid fungi; *Basidiomycota*) and *Xylariales* (*Ascomycota*) as well as lichens produce persistent fruiting bodies, which are usually observable all year round. For these groups it would be interesting to document seasonal changes in sporulation that were not documented in the present investigation.

In our study, ectomycorrhizal fungi mostly belong to the order *Agaricales*. Due to their mutualistic symbiosis, these species dependent on nutrients of their associated plant partner, and probably receive more photosynthates once the latter interrupt their biomass production by late summer/early autumn (Egli et al. 1997). He et al. (2017), however, found that soil fungal species diversity was highest during summer (July) and declined later toward autumn and was relatively low in spring. Based on these results, the increase in fruiting body production in autumn may



not be due to more fungi growing in soil during this season, but to changes in their physiology in response to seasonal stimuli. For saprotrophic fungi, such as cultivated species of Agaricales, experiments showed that the reduction of temperature may be the trigger for the differentiation of primordia (Eastwood *et al.* 2013, Kang *et al.* 2013).

Plant parasitic rust fungi in the order *Pucciniales* (*Basidiomycota*) were found throughout the entire year, mostly as telia on dead leaves in winter, and represented by other spore stages in spring, summer, and autumn (Kolmer *et al.* 2009). Due to these changes of spore stages over the year and their ability to feed on living plant cells or to survive as resting cells in or on dead plant tissue, the presence of rust fungi was less dependent on climatic variables. Our findings show that different ecological groups of fungi adopt different strategies and that these groups have to be considered individually for the analysis of temporal changes of fungi.

The abiotic factors considered in this study, i.e., temperature, precipitation, and humidity, vary non-independently across seasons. Therefore, it is not possible to disentangle the individual effects of these collinear variables on fungal species richness. Further changes related to season are light availability and photoperiod, which, in combination with humidity, have been shown to induce sporulation in species of *Ascomycota* (e.g., Rossi *et al.* 2001, Ehlert *et al.* 2017). The temporal patterns are most likely the result of the combined effects of all these factors.

## CONCLUSION

A high fungal diversity can be detected by considering all macroscopically visible fungi, including small species of *Ascomycota* and plant parasites. Different fungal groups show different patterns of seasonality, inferring that the timing of sampling activities has to be taken into account. The results of our study contribute to the understanding of the changes in fungal diversity driven by temporal and environmental factors. Unravelling these patterns of variation can help to improve current models aimed to estimate local and global fungal richness, to estimate fungal species diversity for conservation purposes, as well as to predict the impact of climate change on biodiversity.

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## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.simyco.2018.01.001>.

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