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Temporal patterns, species richness and spore size distribution of airborne fungi

Größenverteilung, Diversität und Jahresverlauf von Pilzsporen in der Luft

Bachelor thesis to obtain the degree Bachelor of Arts in biology

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1. Introduction

1.1 Fungal Spores – Aerosol Particles

Aerosols are suspensions of fine solid particles or liquid droplets in a gas which are crucial to the Earth's atmosphere and environment. Atmospheric aerosols affect precipitation and cloud formation as for example cloud condensation and ice nuclei, absorb solar and terrestrial radiation, as well as affect the abundance and distribution of atmospheric trace gases and therefore influence the water cycle and global climate. They originate from various sources including primary particle emission[1], volcanic eruptions, anthropogenic sources, soils or biological materials (Pöschl, 2005). Biological aerosols in ambient air offer a great variety of substances including bacteria, viruses, mircomycete spores, conidia and hyphal fragments as well as fragments of animals and plants. Primary biogenic aerosol (PBA) particles and components are directly emitted into the atmosphere. (Lugauskas *et al*., 2003).

PBA particles, such as fungal spores play an important role in the composition of all aerosol particles. Recent surveys have shown that fungal spores account in tropical rainforest air for up to 45% of coarse particle matter (2.5 – 10 µm) and up to 4-11% of fine particle matter ($PM_{2.5}$: < 2.5 µm) in urban and rural air (Womiloju, 2003). Annually countings have shown that fungal spores exceed pollen countings various times (Kurup *et al.*, 2002).
Next to their influence on the atmosphere, fungal spores can also cause or enhance human, animal and plant diseases. About 30% of fungi are living as parasites, most of them as plant parasites and are therefore crucial to agriculture. Fungal spore mycosis can have strong effects on human health, especially when spores are inhaled into the respirable fraction ($PM_{2.5}$) and then spread in the body (Campbell, 2002). Recently, considerable attention is paid on relevant fungal allergens. Major fungal allergic manifestations are asthma, rhinitis, allergic bronchopulmonary mycoses (Kurup, 2002). On the other hand, the climatic role of biogenic aerosols, such as fungal spores, remains undetermined and in order to understand their role and effects on the climate (e.g. as ice nuclei for precipitation) more profoundly, it is important to investigate in their detection, quantification and characterization. It is also very important to get a clearer picture of their composition, seasonal fluctuation, regional diversity and evolution (Georgakopoulos *et al.*, 2008)

[1] e.g. biomass burning

1.2 Fungal Diversity

Fungi are one of the most important organisms for the Earth's ecological system. Due to limited knowledge, current diversity estimates differ significantly for their global fungal species numbers and various estimations about total global fungal diversity have been suggested (Mueller & Schmit, 2007).

The most cited fungal diversity figure was hypothesized by Hawksworth (1991), who estimated 1.5 million species. The figure is based on the observed ratio between plant diversity and fungal diversity in countries where fungi have been studied sufficiently (Finland, Switzerland, UK). Assumed diversity was then calculated, given a ratio of 1:5 and compared to the total known number of plants worldwide (ca. 300 000 species) in order to estimate real diversities (Hawksworth, 1991). According to this estimate, only a fraction of about 5% of the total fungal species has been described and documented (Cannon, 1997).

Currently, there are 1000-1200 new fungal species discovered each year, which is a considerable number, but consequently it would take about 1000 years to describe the over 1 million missing species, if these estimates are correct (Mueller & Schmit, 2007).

Hawksworth (1991) also stated that richness of fungal species to plant species in tropical areas might be even higher, than in temperate areas. Other scientists, however, believe that fungi to plant ratios in tropical areas are lower than estimated, as are insect to plant relationships (insects in the tropical areas use a wider range of hosts compared to temperate areas). Since the tree diversity in tropical areas exceeds the one in temperate areas, individuals get less distributed, which affects their selective pressure and consequently their specialization (May 1991). The resolution of this issue depends on two critical points in order to be verified. Firstly, the ratio of fungal to plant species should not change when examined at broader spatial scales. Secondly, it should be constant, regardless of the diversity of the plant community. If either of these assumptions is false, the ratio estimate would be unreliable (Müller & Schmit, 2007). In order to broaden the knowledge about the missing species, investigations have to be made.

Species richness is a fundamental measurement of community and regional diversity and the simplest way to describe it (Gotelli & Colwell, 2001).

In order to make relevant assumptions about fungal species richness in given areas, various mathematical and statistical models have been established to describe their abundance and distribution (Unterseher *et al.*, 2008). Recently, there are many developments regarding the description of the diversity of mycological communities, including molecular tools that enhance

species identification, as well as different estimators, and computation applications for species richness estimation (Colwell, 2006; Unterseher *et al*, 2008).

Due to the fact that in fungal communities a considerable part of unexplored species is expected, these tools become more prominent for accessing species richness (Unterseher *et al*, 2008)

Additionally species groups like fungi, with a high number of undescribed species, often show sample sizes with considerably high numbers of singletons[2] (Mao & Colwell, 2005). The greater the number of singletons in a dataset, the more likely it is that there are many species not represented in the dataset. (Gotelli & Colwell, 2001).

The aim of this work is to test different suggested species richness estimators in order to get comprehensible estimates for the real species richness of the obtained data. Because estimated figures by mycologists often exceed the sampled species numbers by times, calculated numbers for the species data in this work are expected to be much higher than the actual fungal spore count showed. Furthermore, it is expected that the high number of singletons has significant impact on the calculation of fungal species richness.

1.3 Temporal patterns

Fungal variety depends on various factors, such as time of the day, meteorological parameters, seasonal variation, regional topography and the prevalent type of vegetation. Fungal spore abundances in the atmosphere are also influenced by anthropogenic sources including agricultural operations, such as watering, weeding, harvesting, and various other factors like vehicle movement or mechanical disturbances that are sufficient to deliberate particles (Oliveira *et al*, 2005; Lugauskas *et al*, 2003). Consequently the concentration, of fungal spores and other atmospheric aerosol particles are in general temporally and spatially highly variable.

Temporal patterns in abundances of airborne fungal spores may also have temporal affects on plant ecology and human health (Larsen, 1981). Various studies have shown that fungal peak times are mostly from early summer (June) until autumn (October) (Oliveira, 2005). Due to external factors like temperature humidity and precipitation fungal growth can be enhanced so that species show higher occurrences when external factors are fortunate for their development (Larsen, 1979). The beginning process of seasonal decay of vegetative matter may explain summer and autumn peaks. Lowest airborne fungal occurrences are mostly to be found in the winter season, which could be due to snow cover or low temperatures that prevent sporulation

[2] Species that only occur once during the whole sampling effort

(Li & Kendrick, 1995; Oliveira, 2005). According to Harvey (1972), warm and dry summers favor high spore concentrations. Other surveys have shown that airborne fungal appearance showed highest values in spring (Lugauskas, 2003). However, seasonal occurrences of fungal spores always correlate with the environmental circumstances of that area.

In many recent and past studies, the genus *Cladosporium*, which next to being a saprophytic species also represents a human pathogen, counted for the highest fungal species occurrences annually. For example cool summers decrease *Cladosporium* occurrences which has impact on humans having asthma (Hyde, 1972). Since temporal patterns of fungal occurrence differ from place to place, the impact on plants and humans differ likewise.
In this study, a principle component analysis (PCA) was used to show possible correlations between species and their annual patterns. Furthermore, the question was if there is a correlation between temporal fungal occurrence and their ecological adaptation.

1.4 Size distribution

Airborne fungal spores are primary biogenic aerosol (PBA) particles. They are ubiquitous to the Earth's atmosphere and thereby influence human health, the biosphere, the climate and atmospheric chemistry as well as physics. Fine aerosol particles, such as fungal spores, also have the potential to efficiently scatter and absorb solar radiation or function as ice nuclei for precipitation (Pöschl, 2005). Furthermore they play an important role for the propagation of biological organisms and can evoke human, animal and plant diseases (Pöschl, 2005).
Normally aerosol sizes range from 0.001 to 100 µm. PBA particles like fungal spores are usually collected in two different size categories fine particulate matter with a size $\leq$ 2.5 µm ($PM_{2.5}$) and total suspended particulates (TSP; >2.5 µm).
Especially fungal spores in the range of fine particulate matter ($PM_{2.5;<}$ 2,5 µm), are of medical importance since they can reach the human respiratory fraction. Particles with an aerodynamic diameter of below 10 µm can reach into the bronchiolar tree and thereby cause lung diseases (Riediker *et al*, 2000). To assess and minimize airborne fungal risks to human health, as well as their atmospheric behavior, it is important to develop a better understanding of the dynamics of these PBA particles in the air. (Kanaani *et al*, 2007).
Fine particles have long atmospheric residence times, typically on the order of weeks, and therefore they can be transported over long distances (Jaenicke, 1980). Rust fungi for example can be aerially dispersed over very long distances because their spores are comparatively robust against environmental damage (Brown & Hovmoller, 2002).

PBA particles, like fungal spores, include a wide range of organic matter with a large degree of variability in their physical and chemical characteristics such as size, shape, phase, composition, structure, volatility, hygroscopicity[3] and surface properties. Scientific investigation on PBA particles mainly concentrated on the detection and enumeration. Measurements of shape, optical and surface properties have been developed, however, there is no technique available that manages to acquire their physical complexity (Georgakopoulos *et al*, 2008).

Because of the importance of atmospheric particulate matter, such as fungal spores, many air particle size fractionators have been designed to collect size-fractioned samples (Solomon *et al.* 1983). High-volume virtual impactors are able to stream a high amount of airflow from an acceleration nozzle to a collection nozzle and thereby filter fine particles (< 2.5µm) and coarse particles (>2.5 µm).

As mentioned before, biogenic aerosol particles, like fungal spores, present high variability in their physical and chemical characteristics, which might influence their aerodynamic behavior, once they are sampled by virtual impactors (Solomon *et al*, 1983).

Consequently, this work tries to draw a relationship between airborne fungal spores that were sampled into the coarse and fine filters and their actual spore size. It will be studied, whether spore size or shape and ornamentation are influencing their sampling separation.

2. Materials & Methods

It has to be noted that the data of this work had been obtained by Fröhlich et al. (2009), who installed and acquired the filter samples, conducted the DNA isolation and later matched the data to create a list of species, that represents the source data for this work. In order to understand the origin of the analyzed data, the steps towards species characterization will be explained.

2.1 Filter Samples

Over a period of one year (March 2006 – April 2007) aerosol samples were collected by using dichotomous high-volume samplers (HVFS) on glass fiber filters (Solomon *et al.*, 1983). The sampling station was positioned on a mast at the top of the Max-Planck Institute for Chemistry in (MPIC) Mainz (130 meter above sea level, 5 m above the flat roof of the MPIC on top of the 3 story building) on the campus of the University of Mainz (49°59’31.36’’N 8°14’15.22’’E). The air

[3] Ability of a substance to absorb water molecules from the surrounding environment

masses investigated at the institute are characterized by a mix of urban and rural continental boundary layer air masses in central Europe. Against contamination, the filters were baked at 500°C over night. Loaded filters were stored at -80°C until DNA extraction (Fröhlich, 2009).

2.2 DNA Extraction, Quantification and Amplification

After sampling, DNA extraction, quantification, amplification, cloning and sequencing was applied by Fröhlich *et al* (2009). The samples were lysed and extracted by a commercial soil DNA extraction kit. The DNA was dissolved and measured. Polymerase Chain Reactions (PCR) were performed for Terminal Restriction Fragment Length Polymorphism (T-RFLP) and sequence analyses. For DNA sequence determination, amplification products were transferred into *Escherichia coli* and DNA sequences of single clones were determined. Finally, the DNA sequences were compared to the GenBank database of the National Center for Biotechnology Information (NCBI) (Fröhlich 2009).

2.3 Experimental Design

With the provided information on species names, the taxonomical data was generated by Fröhlich *et al* (2009). Sampling units of fine and coarse particles for each day were merged and the total amount of species calculated. 368 species were gathered, whereof 260 were singletons (ca. 70%) and 45 doubletons (ca. 12 %) (Table 1).

The whole data set was organized to display the occurrence times of species in respect to different variables (months, seasons, spore sizes and ecology). Chronological division into months and seasons was conducted in order to test differences and correlations between temporal patterns of fungal spores. By distinguishing the spore sizes, patterns between distributions were observed. For further comparison the ecology of important fungi was checked on Mycobank. For many species there was no distinguished NCBI record which made it difficult to identify some specimens down to the species. However, for most of the species the data was sufficient so that they could be compared evenly. The ecological variables included plant pathogen, human pathogen and saprophytic.

(Ecological information and spore sizes were found on the fungal database Mycobank : http://www.mycobank.org/)

Table 1. Shows the occurrences of total species observed, Ascomycota (AMC), Basidiomycota (BMC), Fungi incertae sedis (FIS) and other not defined species. Singletons (ST) accounted for about 70%. Species that occurred more often than four times (>4) and species that occurred more often than ten times (>10). The percentages orientate on the values in their columns. Coarse and fine spore occurrence percentages orientate on the total value of individuals.

	Species	%	Individuals	%	Coarse	% of total	Fine	% of total
Total	368	100	956	100	519	54	437	45
AMC	124	34	370	39	188	19	183	19
BMC	238	65	579	60	325	34	253	26
FIS	4	1	2	0	4	1	1	0
Other	2	1	5	1	2	0	0	0
ST	260	71	260	27	114		146	
>4	31	8	496	42	331		175	
>10	16	4	386	32	194		115	

2.4 Coarse and Fine spores

$PM_{2.5}$ (<2.5 μm) and PM_{10} (≤ 10 μm) were often simultaneously collected into fine and coarse filter systems at the same sampling unit. The occurrence of species in both filters was calculated for further spore size comparison. Species with high occurrence in both filters and such that show large differences between the two sizes (coarse & fine) were looked at and possible explanations provided.

2.5 Statistical analysis

The statistical analyses were performed using Microsoft Office Excel 2007 for Windows Vista, Species richness estimator EstimateS Version 8.2 (Colwell, 2009) and SPSS for Windows Version 16 (SPSS Inc, 2007).

2.5.1 Species Richness

Sample based data was used for calculating the species richness throughout the year with EstimateS Version 8.2 (Colwell, 2009). The species richness was calculated and compared with 5 different estimators. As estimators, the incidence-based coverage estimator (ICE) (Chao *et al.*, 2000), first-order Jackknife richness estimator Jackknife 1 (Burnham & Overton, 1979), Chao2

richness estimator (Chao, 1987), Bootstrap richness estimator (Smith & Belle, 1984) and Michaelis-Menten richness estimator (Raaijmakers, 1987) were chosen. The different estimators were compared to their projection of species richness and their Standard Deviation (SD). The mentioned estimators have been successfully applied previously to the species estimation of fungal diversity (Unterseher *et al*., 2008). Graphs of the results were created and compared.

2.5.2 Principle Component Analysis

Data of the obtained species was merged into the temporal sections. A principle component analysis (PCA) was carried out on 368 species using the statistical analytic program SPSS (Version 16, SPSS Inc., 2007). The analysis included seasonal and monthly variations of species during the year (total sampling effort). Species counted for the dependant variables and the temporal occurrence for independent variables. For comparison, a PCA was performed for different groups (All data, >4 and >10) in respect to their time of occurrence. The results were summarized and compared by using Total Variance levels, distinguishing components, Scatter- and Scree-plots and Component Coefficient Matrix (CCM). With comparison of the different Eigenvalues (EV) of the Total Variance Explained, the significance of species and timeframe was established. Possible correlations between species were concluded and visually displayed. The CCM showed the coefficients of species towards different components that can explain and determine the distribution of the other species. The dataset was reduced due to values of the components displayed in CCM and relevant species projected onto a graph. Annual pattern graphs were created for better comparison of statistical similarities in CCM of the species that explain the componential behavior of most of the data.

3. Results

3.1 Species Richness Estimation

The species richness estimation was successfully applied and results generated. The real number of observations was 368 species and the highest possible estimation (including the SD) was calculated by the Chao2 estimator (~ 1288 species). The whole species richness calculation data output can be seen in the Appendix A.

3.1.1 The Estimators

The number of observed species was 368 for all data (Table 1). Strong similarities of estimated values could be observed between incident-based coverage estimator (ICE), and Chao2 richness estimator, as well as between the first-order Jackknife estimator (Jack1) and the Michaelis-Menten estimator (MM). The Bootstrap richness estimator showed the lowest estimated values but the nearest towards the actual data set. The standard Deviations (SD) varied strongly between the estimators (Table 2)

3.1.2 Species richness

The estimated species richness for the data was 1178.05 (± 27.17 SD) for ICE, 1136.18 (± 152.51 SD) for Chao2 , 621.81 (± 20.74 SD) for Jack1, 469.34 (± 4.56 SD) Bootstrap and 684.13 for MM. ICE, Chao2, Jack1, Bootstrap and MM estimator curves show different progressions towards the observed species (comp. Fig. 1). Each sampling unit represents 7 days of sampling approximately. After a total comparison, the various estimations were compared with the Sobs.

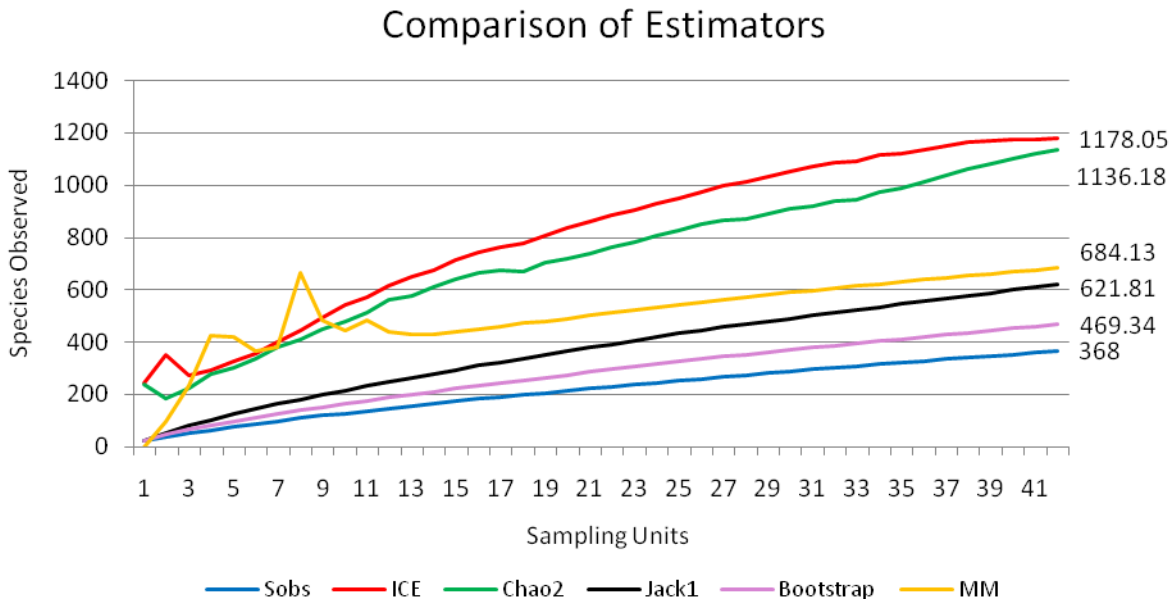


Figure 1 . Comparative view of the five different estimators and the observed species (Sobs). The lowest score (368), the actual species observed (Sobs), secondly the Bootstrap method calculated score of 469.34 species richness, Jackknife species estimator (Jack1) with an estimated value of 621.81 species, Chao2 richness estimator and incident-based coverage estimator (ICE) calculated much higher values of 1136.18 and 1178.05, respectively.

Figure 1 demonstrates that the graphs show similarities between the estimators. The ICE and Chao2 estimations are starting at higher values (both at 242.08) than estimations of Jack1, Bootstrap and MM (20.68, 20.68 & 0). After 50% of the sampling effort (21 SU) ICE already reaches (761.4) the maximum values of Jack1, Bootstrap and MM. Chao2 after 23 SU (703.08).

Table 2. Estimated species richness (ESR), Standard Deviation (SD)

Estimator	ESR	SD
Total	368	
ICE	1178.05	29.38
Chao 2	1136.18	152.51
MM	684.13	
Bootsrap	469.34	4.56
Jack 1	621.81	20.74

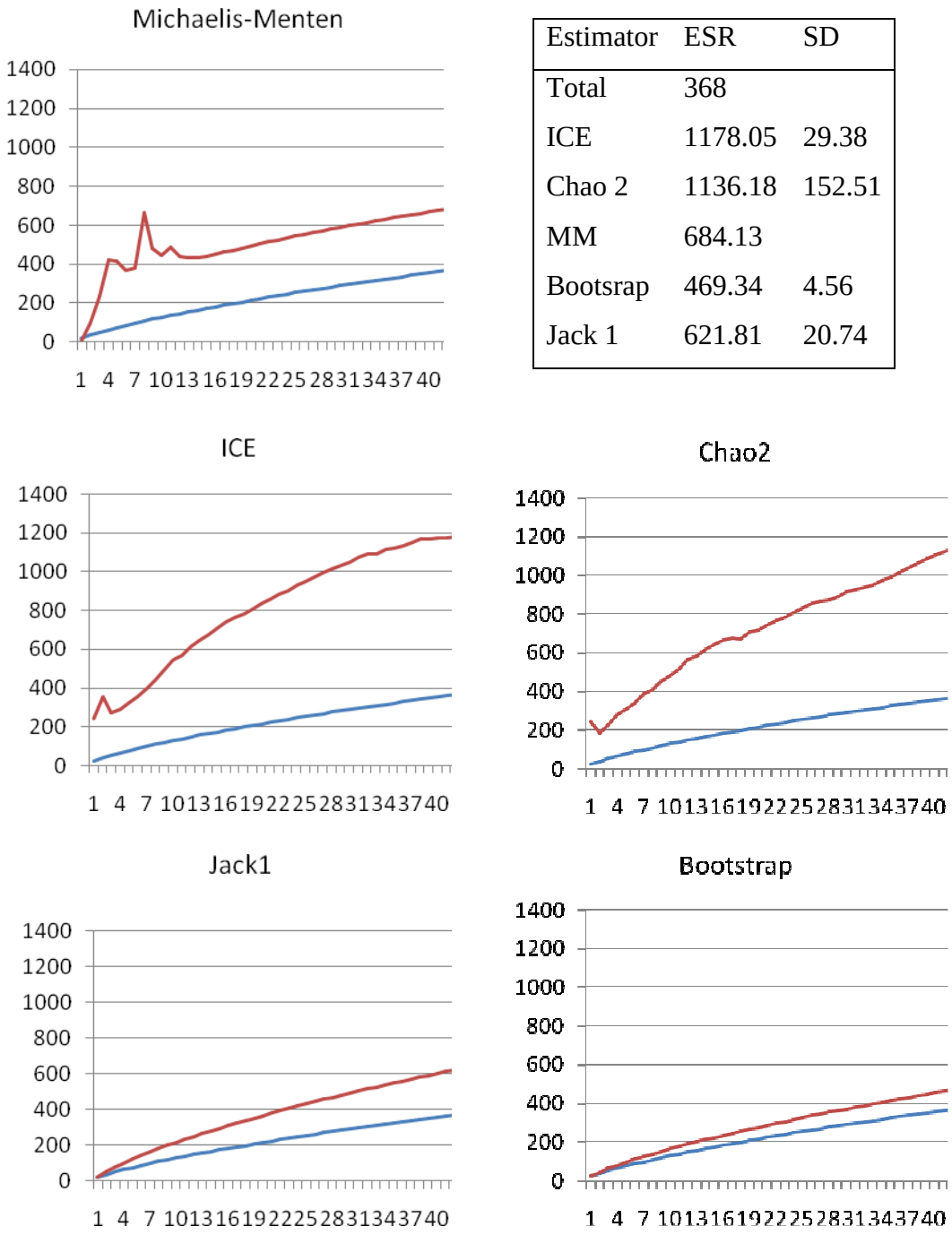


Figure 2. Show the various estimators (red) against the species observed (blue). Table 2 shows their values at the end of calculation and the standard deviations.

The other three estimators show proximities towards the Sobs values.In this case the Bootstrap estimator shows the strongest similarities in estimations towards the Sobs with 469.34 and 368, respectively. At the beginning of the MM estimation there are three different peaks (424.61, 663.95, 484.66), where the second shows a similar value to the final estimation (684.13). After

those peaks the values stabilize and nearly run parallel to Sobs. Interestingly, the ICE estimator (1178.05), indicates a slight flattening at the end of the sampling with stabilizing values. Chao2, however, shows no sign of converging at its maximum (1136.18). Table 2 lists the different Estimation maxima and also shows their SD.

3.2 Principle Component Analysis

The data was divided into different sections. The temporal succession was taken as the source of comparison and divided into species occurrences in respect to months and seasons. All species (all), species with more than four occurrences (>4) and species with more than ten occurrences (>10) were analyzed due to these ratings.

3.2.1 Total Variance and Eigenvalues

The PCA for the species (all, > 4 and > 10) calculated 11 (12 – 1) components for months and 3 (4 – 1) components for seasons, respectively, to reach a Total Variance (TV) of 100 %. The initial Eigenvalues (EV) of the components represent the number of species that describe the variance of the Components. The sum of Eigenvalues explains the number of species observed for all data (368), the species with more than 4 occurrences (31) and more than 10 occurrences (16)(comp. Table 1). For comparison, the Cumulative percentage generated by the different components was looked at C1 and C2 which represent the highest Eigenvalues of all components. Cumulative EV were marked at 75 % which represents ¾ of the dataset (Table 3-8).

In the total variance analysis of months, the data set for all species showed Eigenvalues (EV) of 61.997 for C1 and 51.074 for C2, from a total of 368. Cumulatively, they explain 31 % (numbers rounded) of the total variance (Table 3). After 7 components the values reached cumulative EV of 78% (numbers rounded). Species with > 4 occurrences showed EV of 7.298 for C1 and 7.229 for C2, from a total of 31 and explain 47% of the total variance with five components needed to reach cumulative EV of 77% (Table 4). Species with >10 occurrences showed EV of about 5 for C1 and 4 for C2, from a total of 16 and explain 55% of the total variance. After 4 components the values reached cumulative EV of 78% (Table 5).

In the TV analysis of seasons, the data set for all species showed EV of 160 for C1 and 118 for C2, from a total of 368 which explains cumulative EV of 76% (Table 6). Species with > 4 occurrences showed EV of 13 for C1 and 11 for C2, from a total of 31, which explains cumulative EV of 79% (Table 7). Species with > 10 occurrences showed EV of 8.5 for C1 and 5.5 for C2, from a total of 16, which explains cumulative EV of 88% (Table 8). It is to be noted that the

percentages of cumulative Eigenvalues always increase when less species are observed, which is due to the dataset being reduced and since less data is observed, less information is needed to explain the whole data. The Scree Plots (SP) visualize different EV of components (months 11-1, seasons 4-1) and the number of factors observed (all:368, >4:31, >10:16). They shows the fraction of total variance in the data as explained or represented by each PC. To have a better visualization of differences between individual components, the SP give an idea where the most important components cease and the least important start, as well as where the significant points of separation are located.

Table 3. Eigenvalues & Screeplot of all species regarding their occurrences during all months.

Months & all species			
	Initial Eigenvalues		
Comp.	Total	% of Variance	Cumulative %
1	61.997	16.847	16.847
2	51.074	13.879	30.726
3	44.435	12.075	42.801
4	37.814	10.276	53.076
5	34.138	9.277	62.353
6	30.002	8.153	70.505
7	28.508	7.747	78.252

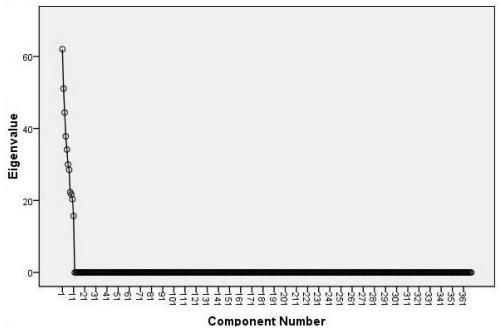


Table 4. Eigenvalues & Screeplot of species with more than 4 occurrences during the months.

Months & species > 4			
		Initial Eigenvalues	
Comp.	Total	% of Variance	Cumulative %
1	7.298	23.541	23.541
2	7.229	23.318	46.860
3	3.855	12.435	59.295
4	3.028	9.767	69.062
5	2.454	7.917	76.979

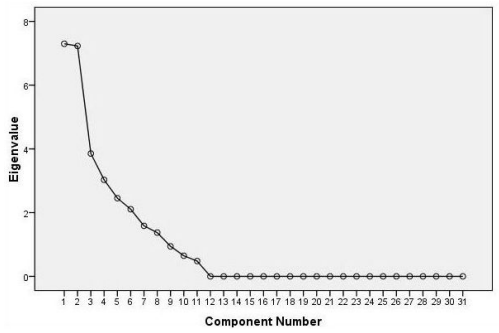


Table 5. Eigenvalues & Screeplot of species with more than 10 occurrences during the months.

Seasons & all species			
		Initial Eigenvalues	
Comp.	Total	% of Variance	Cumulative %
1	160.309	43.562	43.562
2	118.264	32.137	75.699
3	89.427	24.301	100

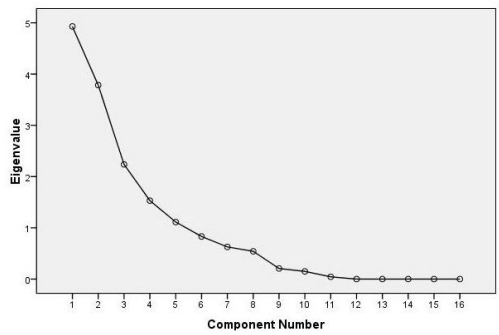


Table 6. Eigenvalues & Screeplot of all species regarding their occurrences during the seasons.

Months & species > 10			
	Initial Eigenvalues		
Comp.	Total	% of Variance	Cumulative %
1	4.929	30.808	30.808
2	3.784	23.651	54.459
3	2.238	13.985	68.444
4	1.531	9.57	78.015

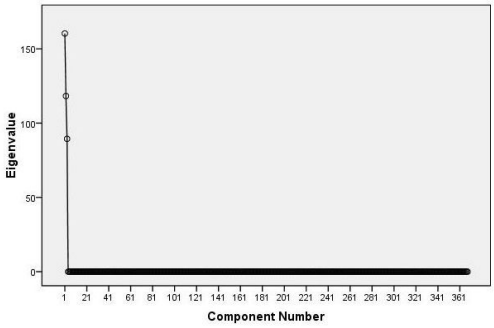


Table 7. Eigenvalues & Screeplot of species with more than 4 occurrences during the seasons.

Seasons & species > 4			
		Initial Eigenvalues	
Com.	Total	% of Variance	Cumulative %
1	12.904	41.624	41.624
2	11.442	36.911	78.536
3	6.654	21.464	100

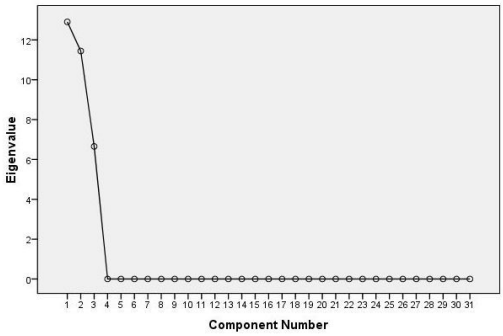
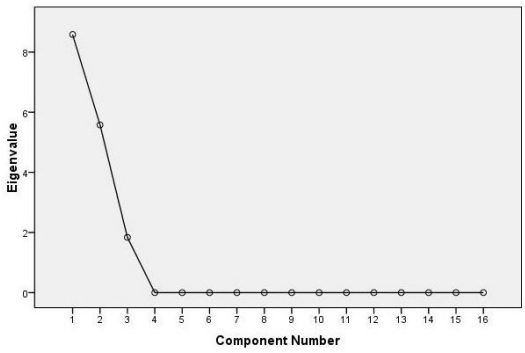


Table 8. Eigenvalues & Screeplot of species with more than 10 occurrences during the seasons.

Seaons & species > 10			
		Initial Eigenvalues	
Comp.	Total	% of Variance	Cumulative %
1	8.584	53.649	53.649
2	5.578	34.865	88.514
3	1.838	11.486	100



3.2.2 Coefficient Matrix and Species

Component Coefficient Matrix (CCM) and Component Plots (CP) results showed the possible correlations of variables (var.), which is also interesting for an ecological analysis since the data is merged and better accessible. All the 16 species were identified at least to genus level so that they provided enough information for a further ecological comparison (Table 10). The results were compared by their values in the CCM (Table 9). The five highest values for each timeframe (months, seasons) were observed and graphically visualized in order to display the most representative species of the principle component. The CP graphs showed the representation of variables (species) in relation to C1 and C2. The nearer they stand towards each other, the more they show similarities in their behavior towards C1 and C2. Possible clusters indicate correlations between the variables. Ecology aspects (plant pathogen, saprophytic or human pathogen) were added for further ecological comparison of the species (comp. fig. 8 and 9).

3.2.3 Representative Component Coefficients

CCM for the analysis of monthly and seasonal occurrences of all spores (including singletons) were ignored because singletons represented ¾ of the data and showed nearly the same CCM values as the species with more occurrences, which leads to an inadequate comparison of species regarding their occurrence and ecology.

As an example of comparison, the CCM of months and seasons for spores with more than 10 occurrences were compared to see differences between the values regarding monthly and seasonal occurrences (Table 9). Both showed highest EV for C1 & C2 for the time frames and a reasonable dataset for the species. The 5 highest values of C1 of both timeframes (months, seasons) were observed, because they explain most thoroughly the distribution of the data. Furthermore they were displayed in an EXCEL graph in order to see patterns between the species(comp. Fig. 3). It is to be noted that the comparison solely originated from the componential comparison and the graphs always show the annual pattern, which includes all the information provided by the data. For the observation of months, cumulative values of the first four components (30.8%, 23.7%, 12,4%, 9,8%) are needed to represent 78% of the EV (Table 4). 16 variables showed over 10 occurrences within the year. The variables occurrences emerge gradually in the table (Table 9). For example var001 stands for *Cladosporium herbarum* (41 occurrences).

Table 9. Shows the Component Coefficient Matrixes for species with more than 10 occurrences for months (left) and seasons (right) and the components needed to explain cumulatively more than 75% of the total variance. Species with high values are marked (green) when they are used for the analysis of their occurrence. Whole CCM tables can be found in Appendix B.

Months	**Component**				Seasons	**Component**	
>10	1 (30.8%)	2 (23.7%)	3 (12,4%)	4 (9.8%)	> 10	1 (53.7%)	2 (34.9%)
var001	0.214	0.030	0.032	-0.015	**var001**	0.164	-0.059
var002	0.254	-0.041	-0.144	-0.098	**var002**	0.166	-0.039
var003	0.020	0.212	0.267	0.240	**var003**	0.133	0.066
var004	0.043	0.183	-0.009	-0.071	**var004**	0.086	0.073
var005	0.168	-0.046	-0.133	0.224	**var005**	0.204	0.020
var006	0.009	0.043	0.248	0.128	**var006**	0.080	-0.160
var007	0.208	-0.042	-0.054	-0.172	**var007**	0.057	-0.090
var008	0.179	-0.125	0.143	0.041	**var008**	0.004	-0.198
var009	-0.139	0.275	0.240	0.003	**var009**	0.108	0.138
var010	-0.040	0.321	0.044	0.165	**var010**	0.063	-0.003
var011	-0.031	0.129	-0.216	-0.075	**var011**	0.081	0.193
var012	-0.090	0.180	0.080	0.530	**var012**	0.018	0.087
var013	0.012	0.040	0.012	0.359	**var013**	-0.043	-0.142
var014	-0.037	-0.092	0.055	-0.002	**var014**	-0.058	0.158
var015	-0.064	-0.011	0.390	-0.096	**var015**	-0.061	-0.033
var016	0.108	0.291	-0.159	0.071	**var016**	0.234	0.073

Table 10. Shows the variable numbers for PCA analysis matched with actual species names, the species ecology (Plant pathogen=PP, Human pathogen=HP and saprophytic=Sap) and occurrence during the seasons (Spring=Spr, Summer=Sum, Autumn=Aut, Winter=Win). The data on *Glonium pusillum* was incomplete but is understood to be a saprophytic species. Data on ecology was obtained on Mycobank (www.mycobank.org).

Variable	**Species**	**Ecology**	**Spr.**	**Sum.**	**Aut.**	**Win.**	**Total**
var001	*Cladosporium herbarum*	Sap & HP	14	10	10	7	41
var002	*Heterobasidion annosum*	PP	11	7	7	5	30
var003	*Thanatephorus cucumeris*	PP	12	5	5	7	24
var004	*Trametes versicolor*	Sap	11	4	3	7	25
var005	*Botryotinia fuckeliana*	PP	6	5	9	4	24

var006	*Peniophora incarnata*	Sap	7	7	6	2	22
var007	*Stereum hirsutum*	Sap	6	5	4	4	19
var008	*Glonium pusillum*	n/a (Sap)	5	7	5	1	18
var009	*Itersonilia perplexans*	PP	8	1	2	6	17
var010	*Vuilleminia comedens*	Sap	5	3	2	3	13
var011	*Eurotium amstelodami*	HP	5	0	2	6	13
var012	*Phlebia radiata*	Sap	0	2	6	4	12
var013	*Penicillium expansum*	HP	1	5	5	1	12
var014	*Penicillium brevicompactum*	PP & HP	3	2	2	5	12
var015	*Ascochyta sp.*	PP	4	3	1	3	11
var016	*Alternaria tenuissima*	PP	7	0	3	1	11
		Total	105	66	72	66	304

3.2.4 Species Observations

Component 1 in the observation for species with more than 10 occurrences for months explains an EV of 30.8 % in Total Variance (Table 5). The highest values were scored by *Cladosporium herbarum (0.214), Heterobasidion annosum* (0.254), *Botryotinia fuckeliana (0.168), Stereum hirsutum (0.208) and Glonium pusillum (0.179).* The table below shows the annual pattern of the species in comparison (for seasonal also comp. Table 10)

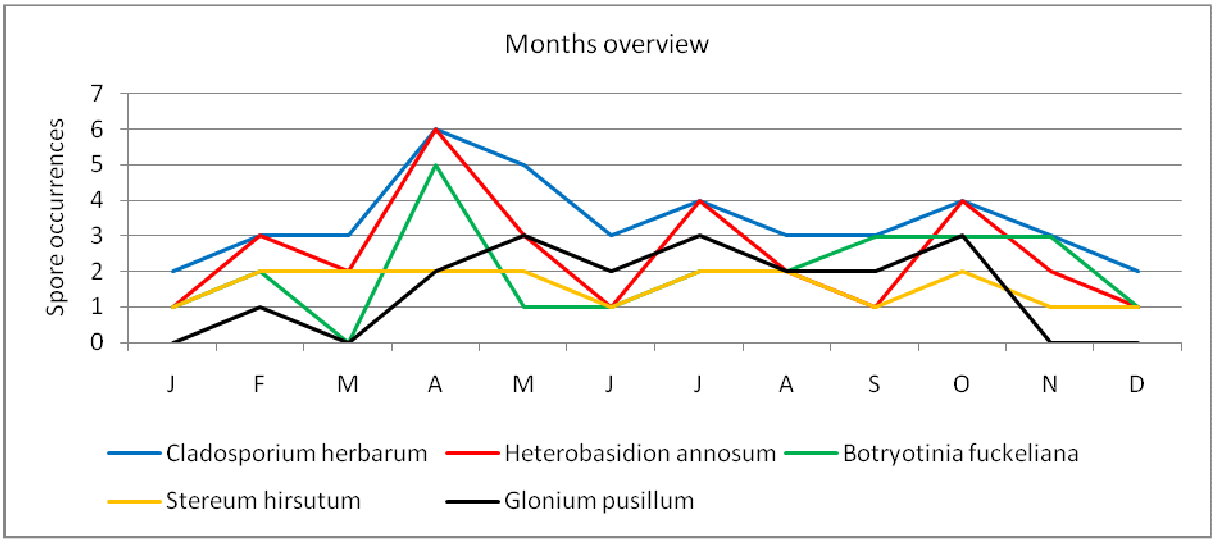


Figure 3. Selected species by the five highest values of component 1 of the monthly CCM. *Cladosporium* (blue), *Heterobasidion* (red), *Botryotinia* (green), *Stereum* (orange), *Glonium* (black). All species except *Glonium pulsillum* show maximum values during the month of April (peak occurrences in order of the figure: 6, 6, 5, 2, and 2).

In the seasonal observation, component 1 for species with more than 10 occurrences explains 53.7% of the EV (Table 8). The compared species show their highest occurrences in spring with a strong increase from winter to spring. *Cladosporium herbarum* (0.164), *Heterobasidion annosum* (0.166), *Thanatephorus cucumeris* (0.133) and *Alternaria tenuissima* (0.234) and *Botryotinia fuckeliana* (0.240) show their highest values for C1. All except *Botryotinia fuckeliana* show their highest values during spring (14, 11, 12, and 6).

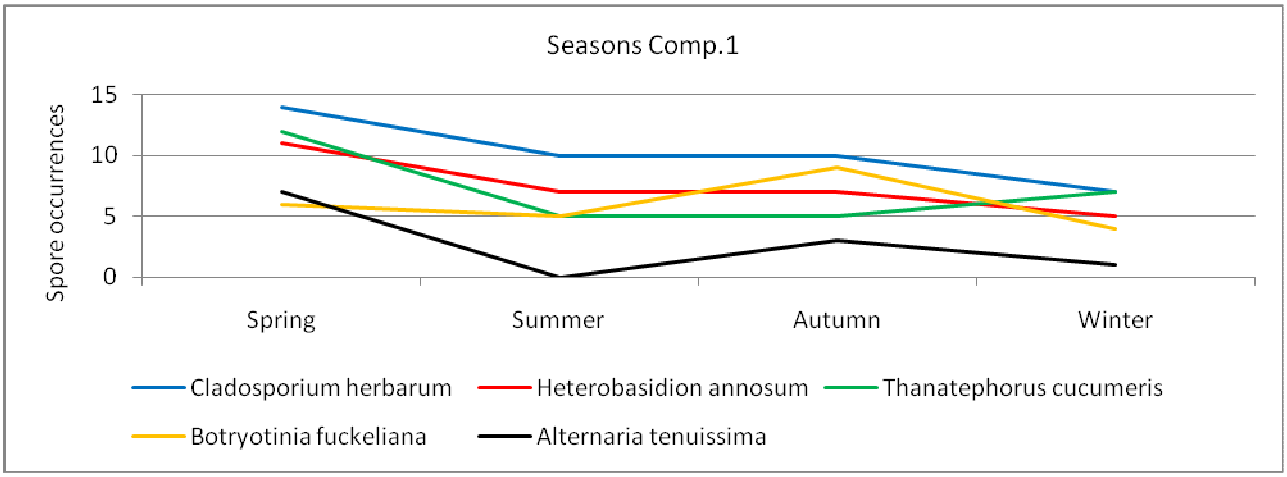


Figure 4. Selected species by the five highest values of component 1 of the seasonal CCM. *Cladosporium* (blue), *Heterobasidion* (red), *Thanatephorus* (green), *Botryotinia* (orange), *Alternaria* (black).

Because the second component for the seasonal observation explains an EV of 34.9%, it was observed in comparison to C1 in order to get to know more information about the data that cumulatively explains 88% of the Total Variance (Table8). *Itersonilia perplexans* (0.138), *Eurotium amstelodami (0.193)* and *Penicillium brevicompactum* (0.158) showed high values in that component. The overall trend in comparison to C1 is a clear increase in occurrences towards winter (6, 6, and 5). The main difference between C1 and 2 is, that in respect to the winter season, the highest values of C1 showed species with decreasing occurrences and C2 with increasing occurrences.

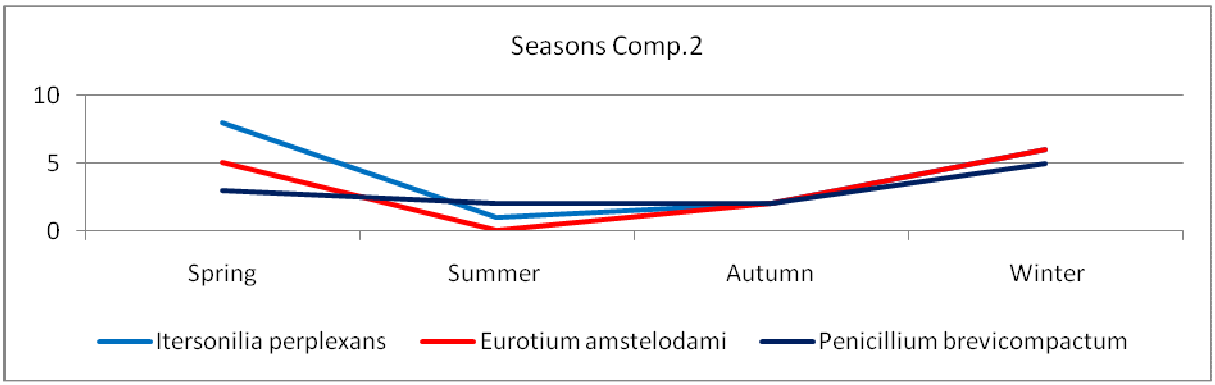


Figure 5. Selected species by the three highest values of component 2 of the seasonal CCM.

3.2.5 Component Plot Correlations

The comparison of negative and positive species patterns can be observed through the Component Plot (CP) that opposes C1 & 2 in a metrical graph (values are displayed in the rotated component matrix in Appendix C). CP for species >10 occurrences explains 88% of the Total Variance and possible patters have statistical significance (a CP for all species occurrences for months would explain 30% of the Total Variance and not be significant). Next to their annual occurrences, the species were compared due to their ecological factors (plant pathogen, human pathogen, saprophytic).

Figure 6 shows clusters of the variables (species) observed with near values (1, 2, 7 and 3, 4, 10, 16) and then displayed in an annual graph in order to see, whether they show a similar patterns. Figure 8 and 9 display the possible clusters (Cluster 1 and 2) of the species seasonal spore occurrence patterns. (var014=*Penicillium brevicompactum* and var011=*Eurotium amstelodami*). Figure 7 shows possible clusters of C1 & C2 in the CP for all species and seasons. However, even though the data explains 75% of the EV, it includes all 368 species with all 260 singletons which makes annual comparison of species difficult.

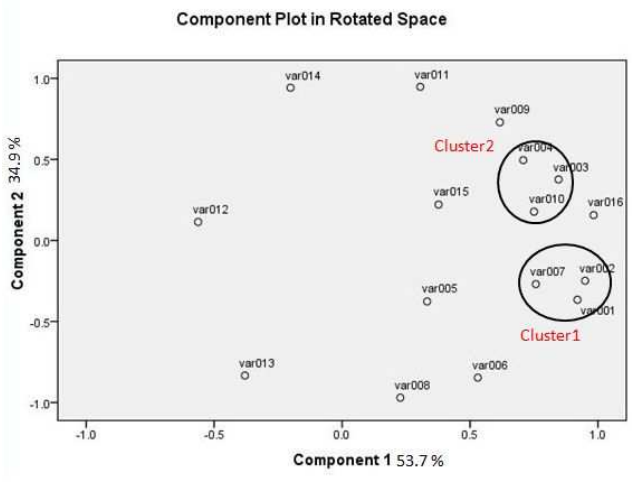


Figure 6. Component Plot for species > 10 occurrences and seasons. Component 1&2 account for 88% of EV.
The Clusters (1,2) indicate possible occurrence patterns for *Cladosprium herbarum* (var001), *Heterobasidion annosum (var002) and Stereum hirsutum (var007), as well as for Thanatephorus cucumeris (var003), Trametes versicolor (var004), Vuilleminia comedens (var010).*

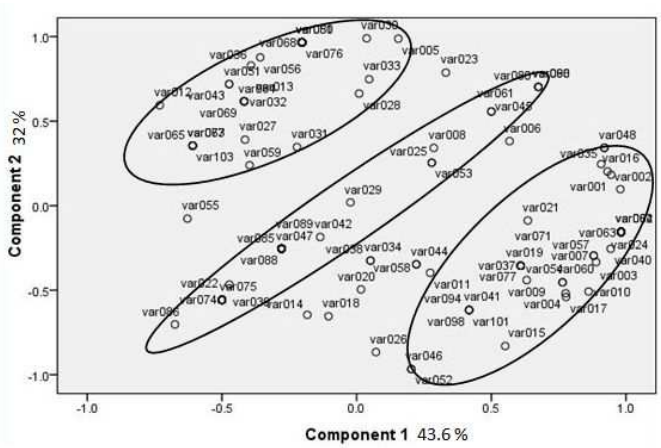


Figure 7. Component Plot for all species and seasons. Comp. 1&2 account for 75% of EV. 368 Species are observed with 260 singletons, representing 70% of the data. Cluster could be examined but due to the high number of singletons there is no real significant outcome, once the species are compared at an annual occurrence pattern.

Figure 8. First Cluster

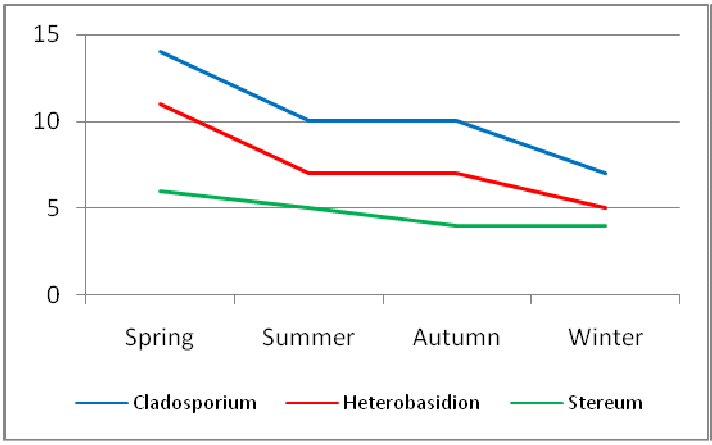


Figure 9. Second Cluster

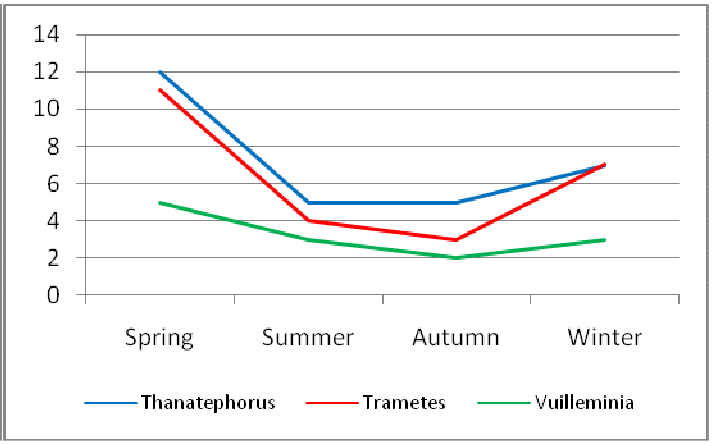


Figure 8 and 9. Show seasonal occurrences of *Cladosporium herbarum, Heterobasidion annosum, Stereum hirsutum & Thanatephorus cucumeris, Trametes versicolor, Vuilleminia comedens* as found in Cluster 1 & 2 from Figure 6.

Figure 8 represents the first cluster, which shows *Cladosporium herbarum* (var001) which is a saprobe on decaying plant material, *Heterobasidion annosum* (var002), which is a plant pathogen (butt rot and root rot) and stereum *hirsutum* (var007), which is a saprobe on deciduous wood, in their annual occurrences. They all have their highest values in spring and show a decrease of occurrence towards winter. *Cladosporium herbarum* and *Heterobasidion annosum* show a very similar occurrence pattern with a decrease towards summer, stable values towards autumn, a decrease towards winter and an increase towards spring. Figure 9 represents the second cluster, which shows *Thanatephorus cucumeris* (var003), which is a plant pathogen that can be found on *Solanum tuberosum*, *Trametes versicolor* (var004), which is a saprobe on dead and living deciduous trees, *Vuilleminia comedens* (var010) and which is a saprophytic species that is associated with a white rot. All species show a strong decrease in occurrence towards summer. *Thanatephorus*, *Trametes* and *Vuilleminia* show a further decrease or steady values towards autumn and an increase towards winter. All species show a strong increase towards spring.

3.3 Spore Size and Ornamentation

Species that were sampled into the PM2.5 (fine) section should show spore sizes of $\leq$ 2.5 µm. Everything above this size should go into the TSP sampler (Fröhlich, 2009). In order to prove the efficiency and sampling criteria of the high-volume filter sampler (HVFS), all species with more than four occurrences per year were compared and the ones that showed the highest differences between coarse and fine occurrence grouped. Many species also occurred in PM2.5 and TSP filters, respectively (Table 11).

In the coarse section the species with highest quotient towards their fine occurrences were *Glonium pusillum* (18:0), *Vuilleminia comedens* (13:0), *Blumeria graminis* (10:0), *Itersonilia perplexans* (16:1), *Alternaria tenuissima* (11:1), *Botryotinia fuckeliana* (20:8). All species showed spore sizes that were entirely in the range of TSP (comp. Table 11)

In fine section the species with highest quotient towards their coarse occurrences were *Penicillium expansum* (0:12), *Penicillium brevicompactum* (3:10), *Aspergillus conicus* (0:5), *Steccherinum fimbriatum* (1:4) and *Choiromyces venosus* (1:4). Only *Choiromyces venosus* showed very unusual sized spores for PM2.5 with a spore size of 10.0 x 15 µm (spikes 3.0-5.0 µm)

Species that showed high similarities between their occurrence in PM2.5 and TSP were *Cladosporium herbarum* (35:37), *Thanatephorus cucumeris* (22:21), *Eurotium amstelodami* (7:7), *Sistotrema brinkmanii* (3:3), *Peniophora incarnata* (3:3), *Botryobasidium subcoronatum* (5:4), *Trametes versicolor* (16:19), *Phlebia radiata* (6:7) and *Heterobasidion annosum* (12:26). Due to their minimal possible spore sizes only *Sistotrema, Botryobasidium, Trametes* and *Phlebia* could have reached the PM2.5 filter. All others showed minimal spore sizes over 2.5 µm.

Table 11. Spore sizes and ornamentation of the species with highest quotient towards coarse occurrences (fine), towards fine occurrences (coarse) and most similar occurrences in both filters (both). Fine filter PM2.5 (≤ 2.5 µm), coarse filter (TSP all >2.5 µm). The column Occ. (Occurrences) shows their individual distribution with coarse occurrences first and fine occurrences second (e.g. *Glonium* 18 coarse, 0 fine). Data on spore sizes was obtained through Mycobank (www.mycobank.org).

Coarse

Matches from Data	Occ.	Spore ornamentation	Sporesize
Glonium pusillum	18:0	ellipsoid, upper cell often larger	(9-) 11 - 12 (-13) x 4 - 5 µm
Vuilleminia comedens	13:0	suballantoid or cylindrical, smooth, blunt apiculus	13-16 x 4-5 µm
Blumeria graminis	10:0	in chains ellipsoid to lemon-shaped	(20-)24-35(-45) x (8-) 12-16 (-20) µm
Itersonilia perplexans	16:1	ellipsoidal, (sub)globose, ovoidal or pyriform	13.0 - 20.0 x 10.0 - 14.0 µm
Alternaria tenuissima	8:2	straight or curved, obclavate, smooth, slightly verrucous	22-95 x 8-19 µm
Botryotinia fuckeliana	20:8	obovoid to ellipsoid, smooth	8-16 x 6-12 µm

Fine

Matches from Data	Occ.	Spore ornamentation	Sporesize
Penicillium expansum	0:12	smooth, ellipsoidal	3-3.5 x 2.5-3 µm
Penicillium brevicompactum	3:10	ellipsoidal, finely roughened	2.5-3.5 x 2.0-2.5 µm
Aspergillus conicus	0:5	variable, elliptical	4.0-4.5(6.0) x 3.0-3.5(4.0) µm
Steccherinum fimbriatum	1:4	ellipsoid, smooth	3.25-4 x (2-)2.25-2.5 µm.
Choiromyces venosus	1:4	ellipsoid, spiky	15.0 x 20.0 µm (Spikes 3.0 - 5.0 µm)

Both

Matches from Data	Occ.	Spore ornamentation	Sporesize
Cladosporium herbarum	35:37	ellipsoidal to cylindrical, rounded ends, distinctly verrucous	5.5-13.0 x 3.8-6.0 µm.
Thanatephorus cucumeris	22:21	ellipsoid, smooth, prominently apiculate	8-12 x 5--6 µm & 6-14 x 4-8 µm but varying in size
Eurotium amstelodami	7:7	spinulose, subglobose to ellipsoid, ends flattened, rough	variable, 4.7-5.0 x 3.6-3.8 µm
Sistotrema brinkmannii	3:3	ellipsoid, variable	2-2,5-(3) x 4-4,5-(5) µm
Peniophora incarnata	3:3	smooth, cylindrical, slightly depressed	(7-)8-12 x (3-)3.5-5 µm
Trametes versicolor	16:19	cylindrical, slightly curved to allantoid, smooth	4.5-6.5 x 1.5-2.5 µm
Phlebia radiata	6:7	cylindrical narrowly oblond, ad axially flattened, smooth	(4-)4.5-5.5 x 1.5-2 µm.
Heterobasidion annosum	12:26	subglobose, asperulate, ellipsoid	4-6.5 x 3.5-4.5 µm

4. Discussion

"I don't believe it is usually possible to estimate the number of species [...] but only an appropriate lower bound to that number. This is because there is nearly always a good chance that there are a very large number of extremely rare species." (Good, 1953).

4.1 Species Richness

Despite the fact that fungi are important organisms in the world, because they have diverse roles in ecosystems and also can affect human health significantly, little is known about their real species numbers and estimates for fungal diversity differ significantly (Müller & Schmit, 2007). Fungi often lack characteristics for reliable identification and often one individual produces numerous fruiting bodies, which leads to ambiguous results in species occurrences (Unterseher *et al.*, 2008).

In this survey, 368 different taxa were identified during a period of 1 year. These specimens were afterwards matched by means of sequence comparison with the GenBank database. Many of them could only be assigned a genus name, as nucleotide differences were not high enough to assign single species. The genus with the highest occurrences was *Cladosporium*, which was also shown by other evaluations of airborne fungal species richness (Magyar *et al.*, 2009; Lugauskas *et al.*, 2003; Larsen, 1979). From the total number of species observed (368) 70% were singletons (260) which have a strong impact on the results of species richness estimation (comp. Table 1). In recent microbial surveys species richness revealed results, where singletons and doubletons can account

for about 90 % of the data (Hughes *et al.* 2000). Each singleton represents a unique species and thereby has a strong impact on the species richness. The more rare species appear, the greater will be the probability of existence of species that are not evaluated in the data set (Colwell *et al.*, 2001). Consequently, the percentage of singletons (70% for all data) leads to the strong assumption that a considerably high species number was not obtained by the sampling and is still missing. Corresponding to that, estimated curves of the incident-based coverage estimator (ICE) and Chao2 richness estimator, had a strongly linear increase in estimated figures (comp. Figure 1).

All estimators reached the actual sample size after at least 50% of the sampling effort, and ICE, Chao2 and MM performed the fastest and most reliable estimations for the required species richness, after just 11,11 and 10 days, respectively, which also matched with other surveys (Unterseher *et al.*, 2008). However, the MM estimator calculated a final value of 684 which, if the high over all fungal diversity is considered, may not be satisfactory. ICE and Chao2 are the most promising calculators with total values of 1178 and 1136. The great advantage of the Chao2 species richness estimators in respect to fungal studies is that it is not bound to specific information on the number of individuals per sample. Chao2 is an incident based estimator that relies on the number of unique units and duplicates and not on the number of singletons and doubletons (Unterseher *et al.*, 2008). In our case the singletons account for 70% of the overall data, which might have a significant impact on the outcome.

Although a large number of fungal species were sampled in the survey, the steep slope and lack of an asymptote (regarding Chao2, Jack1, MM and Bootstrap) in the species accumulation curve (Graph 1) suggests that the sampling of the airborne fungal communities is far from complete. The ICE estimator shows a slight asymptote which also might be a temporal flattening. Colwell predicts that an ideal estimator would reach its own asymptote much sooner than the sample based rarefaction. (Colwell, 2001).

The asymptotic species richness is of crucial importance because it gives trends for certain patterns of species richness, especially in exhaustively rich communities or taxa, such as fungi (Cannon *et al.*, 1998). However, under the current estimates of fungal species richness research (Hawksworth, 2001; Cannon, 1997; Arnold 2000), the asymptote for the ICE estimator could show a wrong trend, since it approaches finite richness and species richness numbers are expected to be much higher.

The most promising estimation of total fungal species richness was postulated at a number of 1.5 million species worldwide (Hawksworth, 1991). Thus the estimate suggests that only around 5% of the species are basically described (Hawksworth, 1991). There are many skeptics that criticize

this estimate due to low rates of new species descriptions from tropical areas (May, 1991; Fröhlich *et al.*, 1999) but also others that suggest that 1.5 x 10^6 underestimates fungal diversity. Little-explored guilds (such as endophytes), could solemnly account for about 1 million species (Arnold *et al.*, 2000). In respect to that ratio it would be interesting to know the prevalent plant to fungi rate, and observe whether it fits with the one Hawksworth (1991) calculated and based his estimate on. In order to get more distinguished information about fungal diversity, Cannon suggests a rapid assessment of fungal diversity. He suggests that either fungal diversity in litter or endophyte diversity should be analyzed in order to get an approximation of the range of target organisms (Cannon, 1997).

As was described by Cannon (1997) the asymptote generated by the ICE estimator, could show a trend towards total diversity. However, in respect to the total diversity postulated and small numbers of species already discovered, the Chao2 estimator generates most promising values, with steadily increasing species richness. Due to the high number of singletons it is reasonable that the trend will continue with a larger sampling effort. Consequently, the Chao2 species richness estimator presents the most sensible results for the estimation of fungal species richness, which was also found by Unterseher *et al* (2008).

4.2 Temporal Patterns

Species peaks in this study occurred in spring (comp. Fig. 10) and thereby show different patterns to most other surveys performed on airborne fungal diversity where summer and autumn account for the highest diversity (Larsen, 1979; Li & Kendrick 1995; Oliveira *et al*, 2003). The number of species with more than 10 occurrences showed a high rate in winter which usually is considered as the season with lowest airborne fungal counts (Lugauskas, 2003). However, despite that irregularity, seasonal patterns between fungal occurrences could be determined by reducing the data to the conclusive speciesthat are relevant for comparison.

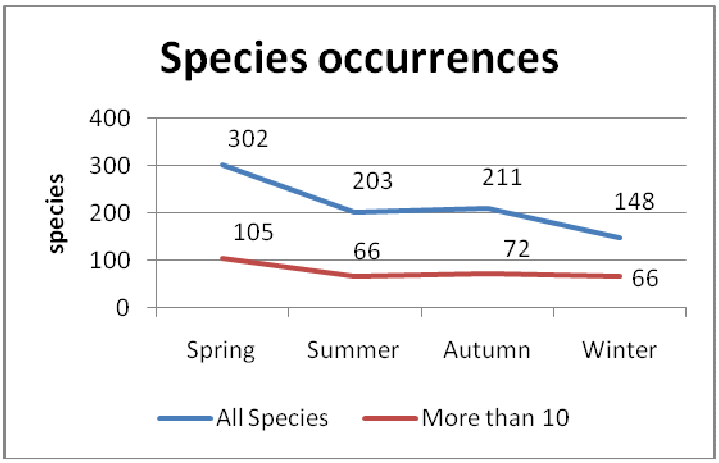


Figure 10. Shows comparison of seasonal species occurrences for all species sampled (blue) in comparison to the species that occurred more than 10 times (red).

4.2.1 Monthly Occurrences

The results of the principle component analysis for all species, more than 4 and 10 occurrences (all, >4, >10) were carried out for months and seasons and aimed to distinguish between important species and their temporal patterns. The Eigenvalues (EV) (Tables 3-8) gave the information to choose the most representative constellation. In the case of monthly occurrence (12) observation, 7 components (all species), 5 components (>4) and 4 components (>10) are needed in order to explain at least 75% of the data. This already shows the success of the dimension reduction provided by the PCA which states that a small set of components is needed to explain most of the variance of the whole data. It could lead to the assumption that regarding the months there are timeframes that show stronger correlations between fungal occurrence and others that are less important for the overall pattern. However, these components also always include the huge set of singletons which show similar componential coefficient values as the species that occurred more often than 10 times. This shows that there may be relationships between singletons and species with more occurrences. But since their occurrence is only of minor temporal importance, it is not recommendable to study this relationship for the huge set of singletons provided by the data. The ecological validity of the monthly componential analysis should also not be overestimated, since C1 and C2 only explain 30% to 55% of the data (comp. Table 3-5). However, as a mean of comparison two scenarios (monthly and seasonal occurrence with more than 10 species occurrences) were compared (Table9). In order to get knowledge about the monthly variances of the species towards their annual patterns, the five highest variable values (species) of CCF in Component1 (30% EV) were compared. *Cladosporium herbarum* ,*Heterobasidion annosum* , *Botryotinia fuckeliana* , *Stereum hirsutum* and *Glonium pusillum* are most likely to explain the highest possible annual patterns of fungal dispersal regarding their monthly occurrences. *Cladosporium* showed the highest occurrence rate of all species (41 times on 42 sampling units), which matches with findings worldwide (except the arctic), where *Cladosporium* is the dominant genus (Larsen, 1979; Li & Kendrick, 1995; Marshall, 1997; Kurup, 2002; Lugauskas, 2003; Oliveira, 2005). All fungi except *Glonium* showed highest values in the month of April (comp. Figure 3). This leads to the assumption that the month of April could have the greatest impact on airborne fungi dispersal. However, studies show that different months are the main source of fungal growth and dispersal, and the period from June to October was most favorable for fungal occurrence and was higher than in April (Li & Kendrick, 1995). This can be due to different environmental factors (Viswanath, 2002) that influence fungal abundance and their peak times, so that different fluctuations between regions exist (Marshall, 1997; Oliveira, 2005; Li & Kendrick 1995). Summer and autumn peaks may be explained by the beginning process of seasonal decay

of vegetable matter. Lowest airborne fungal occurrences are mostly found to be in the winter season, which can be due to snow cover or low temperatures that prevent sporulation (Li & Kendrick, 1995; Oliveira, 2005). Because the species were picked due to their high values in the calculation of C1, which only explains 30% of the EV, the temporal pattern might be significant but an overall ecological pattern would be too vague for the collection of species.

4.2.2 Seasonal Occurrences

The seasonal analysis, however, showed more promising results for a thorough explanation of the total dataset. In all calculations (all, >4, >10), two components were needed to explain 75% of the total data set (comp. Table 6-8). The higher the cumulative EV of C1 and C2, the bigger is the data that is explained by it. In this case 88% of EV is explained with C1 and C2, which makes this observation the most interesting. Species with the highest values for C1 (explaining 53% EV) of the seasonal with more than 10 occurrences CCF were *Cladosporium herbarum*, *Heterobasidion annosum*, *Thanatephorus cucumeris*, *Alternaria tenuissima* and *Botryotinia fuckeliana*. All expect *Botryotinia* showed their highest occurrences in spring (Figure 4), which lets assume that spring could be the season with highest impact on all airborne fungi occurrences in this survey. In the overall comparison (including singletons), spring also was the season of highest airborne spore counts. This matches with the CCM results regarding months, where many of the highest values showed monthly patterns with peaks in April (comp. Figure 3). However, other surveys mostly found out that their seasonal peak in airborne fungal spores could be counted in summer and autumn after having measured an annual circle. (Lugauskas *et al.*, 2003; Oliviera *et al.*, 2003; Li & Kendrick 1995; Larsen, 1979).

For component 2, (35% EV) of the seasonal CCF the species with highest values (above 0.1) were *Itersonilia perplexans, Eurotium amstelodami*, *Penicillium brevicompactum.* Due to their statistical values they explain the behavior of fungal spores towards C2 most thoroughly. All showed similar patterns with a rise towards winter which lets assume, that many species with >10 occurrences show a similar seasonal patterns with a rise in winter, which is different to many surveys dealing on airborne fungal seasonal patterns (Marshall, 1997; Oliveira, 2005; Li & Kendrick 1995), that found lowest values of fungal occurrences during winter. Since the ground is covered by snow and the outdoor fungal is expected to be low (Pasanen *et al.*, 1990; Larsen, 1979). In the overall score of the survey (Figure 10) winter showed lowest values, but still they were near the values of summer and autumn. Most surveys on seasonal fungal distribution found that summer and autumn were accounting for highest fungal spore dispersal (Stepalska, 1999; Oliveira, 2005).

However, other studies (Oliviera *et al,* 2005; Stepalska *et al,* 1999; Larsen, 1979) often used different measurement techniques that calculated fungal spore concentration and abundance throughout the year, whereas this study used a different quantification and identification approach. Since the other surveys were rather concerned with fungal biomass, the total diversity was not taken into account, which could be a major difference to this survey. Another difference is the high number of singletons that could be characterized by this study and is often not taken into account by different studies in the field of fungal diversity.

Since tests were performed at a stationary filter on the Max-Planck-Institute, the source of indoor fungal spores in winter could also not be far. Indoor fungi account for higher percentages than outdoor spores during winter (Li & Kendrick, 1995), when outdoor plants are not present. A possible explanation could also be that the sampling site was located near the urban center of the city and surrounded by inhabited space. Buildings, roads, cars, people and other heat producing facilities can be the source of warmer urban temperatures than in their surrounding areas (Environmental Protection Agency, 2009), which then also has an effect on the vegetation. Rising temperatures can have an impact on fungal growth because some species have persistent sporocarps that sporolate as soon as temperatures are high enough (Deacon, 2006), which could be applied to some of the species that were compared in respect to their seasonal patterns and ecological adaptation. It should never be forgotten that another possibility why the values of this work differ to other studies, could be that it was a year with exceptional seasonal conditions for that favored fungal dispersal.

4.2.3 Ecological Patterns

Patterns in the occurring species ecology were tested by the CCF values of C1 and C2 for species >10 occurrences and seasons, as well as with the combination of those components in a Component Plot. (Figure 6). The components for the monthly observation explained small EV, so they were not analyzed for patterns of in-between species. For further understanding of this emphasis, the Component Plot (Figure 7) includes a large set of singletons which makes it difficult to analyze possible interactions between single species, since they only can occur at one specific time.

In the CCM values for C1 (explaining 53% of total EV) *Cladosorium herbarum* (saprophytic and pathogen to humans), *Heterobasidion annosum* (plant pathogen), *Thanatephorus cucumeris* (plant pathogen), *Botryotinia fuckeliana* (plant pathogen) and *Alternaria tenuissima* (plant pathogen) showed possible correlations in their values towards C1 (comp. Table 4). All of them except *Thanatephorus cucumeris* show a similar pattern with an increase towards spring, a decrease

towards stable values or an increase towards autumn and a decrease towards winter. Normally outdoor spore occurrences decrease towards winter, which was also shown during other surveys (Oliveira, 2005; Lugauskas 2003). Next to being a plant pathogen, it is reported that *Thanatephorus cucumeris* is able to live as a saprophytic species on wood (from Mycobank), which could explain why it is showing an increase in winter, where it would be possible to sporulate when temperatures temporally rise. Most of the species (mainly plant pathogen) show decrease towards winter, when plants that function as hosts are absent due to low temperatures. Consequently, these species show correlations between their temporal occurrence and ecological strategy.

There are higher indoor spore counts during winter, because the circumstances (especially for human pathogen fungi) are better indoors for sporulation when temperatures outside are low (Pasanen *et al.*, 1990). In the CCM values of C2 (Explaining 35%of total EV) *Itersonilia perplexans* (plant pathogen), *Eurotium amstelodami* (human pathogen) and *Penicillium brevicompactum* (human pathogen) showed the highest values (comp. Table 9). Ecology and seasonal patterns match again in this case because they all show similar occurrence rates (high in spring, low and steady in summer and autumn, high in winter). *Itersonilia perplexans* is reported to be saprophytic on dead and decaying wood or roots (Legon & Heinricki, 2005) so that it could also occur in winter. The other two species are human pathogens and mostly occur in winter when indoor fungal counts are usually higher than outdoor (Viswanath *et al.*, 2002). As in other surveys, indoor pathogens like *Eurotium amstelodami* and *Penicillium brevicompactum* showed their seasonal maximum occurrences in winter (Li & Kendrick, 1995). Again this could be due to the fact that the high-volume sampler that collected the spores was always stationed near warm buildings (Max-Planck-Institute Mainz and urban area of Mainz), which could affect the spore occurrences. But even though indoor pathogens could show high occurrences during winter, there are many outdoor saprophytic species that show an increase towards winter. The species of Cluster 2 of Figure 9 illustrate annual patterns of *Thanatephorus cucumeris, Trametes versicolor* and *Vuilleminia comedens*, which all show a similar ecological strategy, being saprophytic, and similar temporal patterns (high in spring, low in summer, low in autumn, high in winter). Unexpectedly, they show an increase in winter (especially *Thanatephorus*) which is unusual for outdoor fungal spores (Li & Kendrick, 1995). However, since the location was located near the city of Mainz, temperatures in winter could be temporally high which could be in favor for saprophytic fungi sporulation. Consequently, possible ecological patterns between these species could be determined. The annual pattern of the species of the first Cluster (Figure 8) show *Cladosporium herbarum, Heterobasidion annosum* and *Stereum hirsutum*. *Cladosporium* shows an overall high occurrence during the year, since it is an omnipresent seasonal species with high occurrences

(Marshall, 1997), that has can live as a saprobe outdoors and as a human pathogen mold indoors. However, *Cladosporium* and *Heterobasidion* tend to decrease towards winter and *Stereum* shows stable occurrences. Due to their specific ecology, the mold *Cladosporium* and the plant pathogen *Heterobasidion* show a decline towards winter. *Cladosporium* needs herbaceous material to grow on, which is rare in winter, when temperatures are lower. The butt rot and root rot *Heterobasidion* could have difficulties to sporulate during winter when temperatures are low and the ground could be covered by snow. On the contrary, *Stereum* lives as a saprophytic fungi on deciduous wood and like other fungi with that ecological adaptation (e.g. *Tanatephorus, Trametes, Vuilleminia*), it has persistent fruit bodies that could sporulate quickly in winter once temperatures increase, which would explain the stable occurrences during that season.

4.3 Spore Size Distribution

In the coarse filter (Table 11), all spores observed were in the range (>2.5 µm) given by the high-volume filter sampler (HVFS) (Solomon *et al.*, 1983). Spore sizes ranged from a minimum of 8.0 to 6.0 µm (*Botryotinia fuckeliana*, 20 times coarse, 8 times fine – 20:8) to a maximum 35.0 to 16.0 µm (*Blumeria graminis*, 10:0). Nearly all of the species show normal spore sizes larger than 10 µm and consequently, if it comes to human health, these spores would not reach the bronchiolar tree in respect to human allergies (Riediker, 2000).

In the fine filter nearly all species show spore sizes that can be found in the range up to PM2.5 (Table11). But in this filter spores that are larger than $PM_{2.5}$ were also found. *Choiromyces venosus* (1:4) shows spore sizes way above that criterion (15.0 to 20.0 µm). Even if the spike length (3.0 to 5.0 µm in length) is substracted from the minimal possible spore size, spores would range above 10 µm in size. Since this size really represents one of the coarse sections, it should not occur in the fine filter. Another interesting species is *Aspergillus conicus* which only was found in the fine filter but showes spore sizes that ranged out of the fine particle filter region given by Solomon (1983). Spore sizes range from 4.0-6.0 x 3.0-4.0 µm. The ornamentation of *Aspergillus* is variable but mostly elliptical with a roughened surface.

Also, spores of other taxa could be found in the fine filter, that show a larger spore sizes than the criterion for the fine particle section $PM_{2.5}$ allowed (Fröhlich, 2009). *Cladosporium herbarum* (35 times in coarse filter: 37 times in fine filter) and *Heterobasidion annosum* (12:26) showed spore sizes ranging out of the $PM_{2.5}$ criterion that are characterized with an asperulate surface. *Eurotium amstelodami* (22:21) also showed larger spore sizes than $PM_{2.5}$ but shows a roughened surface. *Peniophora incarnata* (3:3), *Trametes versicolor* (16:19), and *Phlebia radiata* (6:7) all show

cylindrical spore shapes. Consequently, the ornamentation and shape of many of the coarse sized spores showed a pattern that might be crucial to their selection towards the fine filter.

Cylindrical shapes or asperulate surfaces could favor spore selection for the $PM_{2.5}$ since the sampler applies an air flow to the air mass and the fine spores follow the streamlines of the major airflow into the fine particle filter (Solomon, 1983). Particles that have cylindrical shapes or roughened ornamentations could be favored by that airflow because they give higher aerodynamic drag and are streamed into the fine filter. Consequently, the respective spores (e.g. *Peniophora, Heterobasidion, Eurotium*) can be found in the $PM_{2.5}$. For some species, like *Thanatephorus cucumeris* (22:21), a species with spore sizes much larger then $PM_{2.5}$ (8.0-12.0 x 5-6 µm), an ellipsoid shape and smooth ornamentation, it is hard to tell what made the species occur in the fine particle filter. Another explanation for the finding of coarse spores in the fine filter could be a wrong taxonomical assignment in GenBank. For instance there were several genetic sequences assigned to *Choiromyces venosus*.

Whatever is the cause of the discovery of coarse sized spores in the fine filter, it has profound effects. $PM_{2.5}$ particles are assumed to reach the human alveolar fraction, which enables them to cause different respirable diseases like asthma, rhinitis or pneumonitis (Viswanath *et al*., 2002). A false categorization based on findings of spores in the HVFS bears wrong interpretations for allergenic capabilities of fungi.

5. Conclusive Thoughts

The study shows promising results in the areas of fungal species richness, their possible temporal patterns and criteria of spore size that can favor or hinder spore selection to aerodynamic filters.

In the area of species richness, the study outcome matches with estimations of other surveys (Unterseher *et al*, 2008), that also came to the conclusion that incidence based estimators like ICE and Chao2 are most representative for fungal species richness estimation. Since singletons represented 70% of the dataset in this study, they have to be taken into account carefully. It was questioned in the beginning at what scale the species richness estimation exceeds the species samples and expected that the estimation is much higher than the actual spore count. The total sampling size was 368 species and the predicted species numbers for the two estimations were 1178 species for the ICE estimator and 1136 for the Chao2 estimator. The estimated species numbers are about 3 times higher than the amount of species sampled during the one year at the MPI Mainz. In respect to the overall estimated high fungal species diversity these values sound

most promising. The other estimators (MM, Jack1, Bootstrap) showed curves that approximated their values towards the observed species. Consequently, Chao2 and ICE species richness estimators can be recommended for the estimation of fungal diversity, where the representation of singletons is very important due to high numbers of rare sampling of most fungi.

Observed temporal patterns in this study showed peak diversity in spring and also high occurrences in winter, which differs from other surveys done on annual fungal species patterns (Marshall, 1997; Oliveira, 2005; Li & Kendrick 1995), which mostly studied fungal occurrences due to their seasonal concentration. The principle component analysis (PCA) showed that monthly comparison of species occurrences are not really relevant due to low Eigenvalues. However, in the calculation of all species and their monthly occurrences 5 components explain about 75% of Eigenvalue which is already a promising observation. It became clear that the huge amount of singletons influences the correlation comparisons once we look at different species and their annual patterns. The PCA showed that it is more meaningful to make this comparison on a seasonal scale where the first component explained 53% of Eigenvalues for species with more than 10 occurrences. The calculations highlighted similar temporal occurrence patterns of various genera (e.g. *Cladosporium, Heterobasidion* and *Stereum*, as well as *Thanatephorus, Trametes* and *Vuilleminia*), and also their ecological adaptation showed commonalities. Since fungal species diversity in most surveys is very high, PCA represents a useful tool to reduce the whole dataset to the most important data for further comparison. Consequently, in this study, PCA showed that there are important patterns that mainly can be seen in seasonal differences of fungal occurrences as well as for species with more occurrences (excluding singletons).

The study of fungal spore size sometimes showed strong imbalances between given filter sizes and spore occurrences in those filters. Spores that according to size criteria ($\geq$ 2.5 µm) would not be favored for fine selection can often be found in those filters. Spores of several species often occurred in both ‚fine' and ‚coarse' filters. Since the high-volume samplers are aerodynamic filters, not only fungal spore size is most important for selection into $PM_{2.5}$ or PM_{10} but selection can also be affected by spore ornamentation (verrucose, spiky, roughened surfaces) and shape (cylindrical). Since high-volume samplers are based on aerodynamic filters, ornamentation and shape can play a crucial role in the selection of species like *Cladosporium herbarum, Thanatephorus cucumeris, Eurotium amstelodami, Sistotrema brinkmannii, Peniophora incarnate, Trametes versicolor, Phlebia radiata, Heterobasidion annosum.* All of them show spores sized above $PM_{2.5}$ but have high occurrences in ‚fine' filters. Future aerodynamic samplers should be constructed by considering the heterogeneity of fungal spores in order to increase their accuracy. Eventually, it could already help to conduct test series with selected particles that present

heterogeneous ornamentations and thereby mirror variations natural particles. Since these filters are often used to obtain quantities of particles that can be found in nature, these aspects should also be taken into account. Furthermore, it is proven that $PM_{2.5}$ particles can reach the respiratory tract. The consistency of the human respiratory tract (with mucous surfaces until the alveolar tract) is different to the one of the HVFS, and spore sizes above 2.5 µm with smooth ornamentations could enter the alveoli as the results show. Spores with verrucouse to spiky surfaces could eventually get caught in the ciliated epithelium and therefore not penetrate the alveolar fraction.

6. Abstract English

Fungi are biogenic aerosols that affect precipitation and cloud formation, e.g. cloud condensation and ice nuclei, absorb solar and terrestrial radiation, as well as affect the abundance and distribution of atmospheric trace gases and therefore influence the water cycle and global climate. Next to their influence on the atmosphere, fungi can also cause or enhance human, animal and plant diseases. Diversity of fungal spores in the aerosol depends on various factors, such as time of the day, meteorological parameters, seasonal variation, regional topography and the prevalent type of vegetation. Because of limited knowledge, the total fungal diversity still remains unknown. Fungal spores nowadays are mostly collected in high-volume filter samplers and little is known about their species diversity and the relationship between aerodynamic filter sizes and actual spore sizes.

Based on weekly air samples from March 2006 until April 2007 at the Max-Planck Institute for Chemistry (MPIC) in Mainz, Germany a total of 368 fungal species were observed. 70 % of the species were only found once during the whole sampling time, 12 % were found twice and only about 4 % occurred more often than ten times.

For the estimation of fungal diversity various estimators like the incident-based estimator (ICE) and Chao2, as well as first-order Jackknife (Jack1), Boostrap and Michaelis-Menten (MM) estimators were compared. The results of the incidence-based species richness estimators showed significantly higher species numbers than the actual species occurrences. In the analysis of temporal species patterns, a principle component analysis (PCA) was performed to reduce the dataset (consisting of a high number of singletons) in order to find possible correlations between fungal species occurrences. The results showed temporal correlations between species appearances, as well as possible links in their ecological adaptations. For example *Thanatephorus cucumeris, Trametes versicolor* and *Vuilleminia comedens*, which all show a similar ecological

strategy, being saprophytic also were being found to show similar temporal patterns (high in spring, low in summer, low in autumn, high in winter. Furthermore, various species were analyzed due to spore size occurrence in fine and coarse filters. The results showed that fungal spores with spore sizes above 3 µm (sometimes much more than that) often occurred in fine filters that should select particulate matter of $\geq$ 2.5 µm and therefore aerodynamic behavior of complex ornamented fungal spores was questioned.

7. Zusammenfassung

Pilze sind biogene Aerosole, die den Niederschlag und die Wolkenbildung als Eiskernchen beinflussen können. Desweiteren können sie die Sonneneinstrahlung absorbieren und Erdabstrahlung reflektierten, sowie die Verteilung von atmosphärischen Spurengasen beeinflussen und haben somit einen großen Einfluss auf den Wasserkreislauf und das globale Klima. Neben ihrem Einluss auf die Atmosphäre können Pilzsporen menschlische, tierische und pflanzliche Krankheiten hervorrufen oder verstärken. Ihre Vielfalt in der Luft hängt von verschiedenen Faktoren, wie Tageszeit, meteorologische Parameter, jahreszeitliche Veränderungen, regionale Topography und der vorherrschenden Vegetation ab. Aufgrund limitierter Informationen bleibt die gesamte Pilzvielfalt bisher jedoch ungewiß. Pilzsporen werden heute meist in Filtersystemen mit hoher Durchflußmenge gesammelt, jedoch ist bisher wenig über die Artenzusammensetzung und Beziehung zwischen Sporengrößen und deren Filterselektion bekannt.

Basiert auf wöchentlichen Luftproben vom Max-Planck Institut für Chemie (MPIC) in Mainz, Deutschland wurden zwischen März 2007 bis April 2007 eine Gesamtanzahl von 368 Arten bestimmt. 70 % der Arten wurden nur einmal während der gesamten Untersuchungszeit gefunden, 12 % wurden zweimal gefunden und cicra 4 % traten mehr als 10 mal auf.

Um die Pilzvielfalt anhand der Daten zu messen wurden 5 verschiedene Kalkulatoren (ICE, Chao2, Jackknife, Bootstrap und Michaelis-Menten) verglichen. Die Resultate der Kalkulatoren, welche auf das Aufkommen spezialisiert sind, zeigten signifikant höhere Pilzvorkommen (bis zu dreimal mehr), als die Gesamtanzahl der gefundenen Pilzarten erbrachte. Die Analyse des jahreszeitlichen Auftretens der Pilze wurde mit einer Hauptkomponentenanalyse (PCA) durchgeführt, um den großen Datensatz (mit hoher Anzahl an Einzelvorkommen) zu reduzieren, damit letztendlich Korrellationen zwischen einzelnen Arten gefunden werden konnten. Zeitliche Korrelationen zwischen dem Auftreten der Arten, sowie mögliche Verbindungen zu deren ökologischer Anpassung wurden gefunden. Als ein Beispiel zeigten *Thanatephorus cucumeris*,

Trametes versicolor und *Vuilleminia comedens ein* ähnliches jahreszeitliches Auftreten, sowie gleiche ökologische Strategien (saprophytisch).

Desweiteren wurden ausgewählte Arten in Bezug auf ihre Sporengröße und aerodynamische Selektion auf feine und grobe Filter untersucht. Die Resultate zeigten, dass Pilzsporen mit Sporengrößen über 3 µm (teilweise weit darüber) oft in feinen Filtern vorkamen die nach der Vorgabe nur eine maximale Größe von 2.5 µm zulassen sollten. Dementsprechend wurde das aerodynamische Verhalt von komplex gestalteten Pilzsporen untersucht.

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Appendices

Appendix A

Shows the whole species richness calculation data for the various estimators and the observed species (Sobs). Incidence-based coverage estimator (ICE), first-order Jackknife richness estimator (Jack1), Chao2 richness estimator (Chao2), Bootstrap richness estimator (BS) and Michaelis-Menten richness estimator (MM).

Samples	Sobs	ICE	ICE SD	Chao2	Chao2 SD	Jack1	Jack1 SD	BS	BS SD	MM
1	20.68	242.08	167.14	242.08	93.25	20.68	0	20.68	6.9	0
2	35.72	352.27	262.73	186.13	110.14	51.45	4.07	43.59	8.58	99.27
3	50.12	272.92	95.73	223.19	96.97	78.08	6.18	62.77	10.21	235.23
4	62.5	289.8	91.63	278.1	112.31	100.48	7.76	78.98	11.53	424.61
5	75.1	325.93	92.62	305.08	104.94	122.84	8.85	95.38	12.68	418.98
6	86.1	353.63	90.72	335.8	106.13	141.98	10.22	109.51	13.03	368.5
7	96.9	398.25	95.92	382.62	115.73	161.15	11.51	123.51	14.47	379.62
8	107.58	443.16	101.18	411.42	115.19	179.77	12.2	137.28	15.94	663.95
9	117.84	492.31	100.26	452.84	122.26	197.8	13.16	150.53	15.11	483.6
10	127.26	541.29	102.93	479.35	122.91	214.42	13.53	162.72	15.62	445.97
11	136.64	570.48	99.69	513.53	126.99	230.82	14.1	174.81	15.63	484.66
12	146.72	615.89	114.86	562.23	136.95	248.65	14.54	187.88	16.74	440.42
13	155.7	646.74	107.64	579.93	134.39	264.05	14.9	199.38	15.37	431.06
14	164.1	674.05	112.9	613.9	139.89	278.46	15.15	210.13	15.89	431.75
15	172.64	710.18	115.53	642.37	141.79	293.34	15.64	221.12	15.35	438.33
16	182.12	741.84	125.48	666.01	141.63	309.66	16.32	233.31	14.81	449.8
17	190.06	761.4	118.19	676.36	137.73	323.12	16.61	243.43	15.47	461.1
18	197.54	778.66	103.48	671.82	129.52	335.66	16.9	252.97	16.34	472.26
19	205.84	807.8	101.62	703.08	133.41	349.95	17.13	263.6	16.47	481.1
20	214.08	834.57	94.21	719.35	132.23	364.07	17.38	274.19	15.04	490.75
21	222.82	859.87	88.02	740.06	132.42	378.95	17.75	285.38	14.47	504.1
22	230.62	881.78	102.73	762.74	133.63	392.26	17.97	295.35	14.66	514.55
23	237.92	902.52	100.11	781.91	134.42	404.58	18.2	304.65	13.61	523.68
24	245.98	928.68	91.46	807.61	136.7	418.46	18.6	315	12.76	533.9
25	254.24	949.42	87.33	828.16	137.14	432.28	18.75	325.48	11.97	544.14
26	261.3	973.38	90.13	851.72	139.28	444.34	19.03	334.49	12	553.79
27	268.9	994.89	93.12	864.85	138.15	456.99	19.2	344.14	12.58	562.95
28	275.72	1012.2	82.82	874.12	136.5	468.15	19.47	352.74	12.3	572.13
29	282.6	1031.94	80.71	891.54	137.26	479.51	19.55	361.41	12.23	581.23
30	289.14	1050.65	78.09	910.41	138.65	490.38	19.54	369.66	12.18	589.45
31	296.2	1070.12	72.91	922.91	137.8	502	19.65	378.57	12.18	597.86
32	303.18	1086.26	71.95	939.15	138.04	513.61	19.76	387.41	11.49	606.53
33	308.8	1091.75	71.07	946.15	137.13	522.31	19.89	394.32	11.09	614.27
34	316.48	1113.78	64.35	973.6	139.79	535.43	20.05	404.12	10.93	622.34
35	322.82	1121.24	56.85	989.98	140.62	545.84	20.2	412.09	10.54	630.26
36	329.76	1131.95	51.69	1013.26	142.66	557.51	20.29	420.88	9.54	638.3
37	336	1147.11	52.39	1037.69	145.44	568.21	20.34	428.85	9.21	645.97
38	342.28	1163.98	46.97	1061.63	147.94	578.98	20.42	436.86	7.75	653.42
39	348.64	1167.04	45.03	1083.43	150.05	589.42	20.46	444.83	6.87	660.91
40	355.3	1173.68	35.9	1103.19	151.23	600.75	20.56	453.31	5.9	668.59
41	361.66	1172.71	27.17	1122.59	152.61	611.26	20.58	461.31	4.56	676.35
42	368	1178.05		1136.18	152.51	621.81	20.74	469.34		684.13

Appendix B

1) Shows the Component Coefficient Matrix for all species with more than 10 occurrences in the monthly observation. The variables (001-016) can be matched with the species in Table 10 of this bachelor thesis

Component Coefficient Matrix Months

	Component				
	1	2	3	4	5
var001	.214	.030	.032	-.015	.014
var002	.254	-.041	-.144	-.098	.011
var003	.020	.212	.267	.240	.242
var004	.043	.183	-.009	-.071	.022
var005	.168	-.046	-.133	.224	.363
var006	.090	.043	.248	.128	-.139
var007	.208	-.042	-.054	-.172	-.184
var008	.179	-.125	.143	.041	.021
var009	-.139	.275	.240	.003	-.056
var010	-.040	.321	.044	.165	-.042
var011	-.031	.129	-.216	-.075	.103
var012	-.090	.180	.080	.530	.132
var013	.012	.040	.012	.359	-.062
var014	-.037	-.092	.055	-.002	.550
var015	-.064	-.011	.390	-.096	.066
var016	.108	.291	-.159	.071	-.251

2) Shows the Component Coefficient Matrix for all species with more than 10 occurrences in the seasonal observation. The variables (001-016) can be matched with the species in Table 10 of this bachelor thesis

Component Coefficient Matrix Seasons

	Component		
	1	2	3
var001	.164	-.059	-.057
var002	.166	-.039	-.053
var003	.133	.066	-.020
var004	.086	.073	.044
var005	.204	.020	-.381
var006	.080	-.160	.016
var007	.057	-.090	.151
var008	.004	-.198	.094
var009	.108	.138	-.050
var010	.063	-.003	.125
var011	.081	.193	-.106
var012	.018	.087	-.262
var013	-.043	-.142	-.022
var014	-.058	.158	.053
var015	-.061	-.033	.293
var016	.234	.073	-.218

Appendix C

1) Shows the values of the rotated component Matrix. These are the values that indicate the orientation of variables in the Component Plot of Figure 6. The species behind the variables can be found in Table 10. In the CP of Figure 6 only the first two components were used which display about 88% of cumulative Eigenvalue.

Rotated Component Matrix Seasons >10

	Component		
	1	2	3
var001	.920	-.366	.140
var002	.950	-.249	.190
var003	.846	.376	.378
var004	.708	.495	.504
var005	.332	-.377	-.865
var006	.530	-.846	.048
var007	.757	-.270	.595
var008	.228	-.970	.084
var009	.617	.729	.298
var010	.750	.177	.637
var011	.306	.948	.094
var012	-.563	.114	-.819
var013	-.380	-.834	-.401
var014	-.202	.942	.270
var015	.377	.221	.900
var016	.983	.156	-.094

Honesty Declaration

Hiermit erkläre ich, dass ich die heute eingereichte Bachelorarbeit selbstständig verfasst und keinen Anderen als die angegebenen Quellen und Hilfsmittel benutzt, sowie Zitate kenntlich gemacht habe. Bei der vorliegenden Bachelorarbeit handelt es sich um in Wort und Bild völlig übereinstimmende Exemplare.

Weiterhin erkläre ich, dass digitale Abbildungen nur die originalen Daten enthalten und in keinem Fall inhaltsverändernde Bildverarbeitung vorgenommen wurde.

Erstgutachter: Prof. Dr. Dominik Begerow

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Köln, den 11.10.2009

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Philipp Wagnitz