Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh. I. Leaf sheaths

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Direct observation of fungal succession and community development on leaf sheaths of *Phragmites australis* have been studied over a period of 19 months in a brackish tidal marsh of the river Scheldt (The Netherlands). Seventy-seven taxa were identified: 33 ascomycetes (43%); 31 coelomycetes (40%); 9 hyphomycetes (12%) and 4 basidiomycetes (5%). Four microhabitats were screened, the top, middle and basal communities along the vertical axis of standing reed shoots and a community in the litter layer. Fungal community structure analyzed by multivariate analysis showed that all microhabitats are characterised by different mycota. Detrended correspondence analysis (DCA) of leaf sheath samples suggests the importance of a spatial separation (microhabitat) in explaining species variation between samples. Within each of those microhabitats DCA indicated a specific temporal pattern (succession). Fungal succession (community development) was described by a sequence of three phases in fungal sporulation. For the different microhabitats and all successional stages indicator species were assigned. The importance of screening host plants *in situ* and the use of indicator species analysis for fungal communities is addressed. The effect of seasonality on fungal succession is discussed.

Key words: ascomycetes, coelomycetes, common reed, detrended correspondence analysis, fungal community, hyphomycetes, indicator species analysis, vertical distribution.

Introduction

Bacteria have been considered to be the main contributors to macrophyte decomposition (Mason, 1976; Benner *et al*., 1986). However, there is an increased awareness of the importance of fungi in nutrient cycling in reed dominated wetlands (Newell, 1996; Komínková *et al*., 2000; Findlay *et al*., 2002; Gessner and Van Ryckegem, 2003). A high fungal diversity is known from *Phragmites australis* from both tropical (Poon and Hyde, 1998a; Wong and Hyde, 2001) and temperate (Apinis *et al*., 1972; Taligoola *et al*., 1972; Wirsel *et al*., 2001; Luo *et al*., 2004; Van Ryckegem and Verbeken, 2005)

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regions and a paper reviewing this diversity is in preparation (Van Ryckegem, unpubl. data). Hawksworth (2001) stressed the need for long term succession studies of fungi on hosts in order to obtain a better understanding of fungal diversity. Determining key species and their dynamics during the decay of a resource (*sensu* Swift, 1976) is an important step in recognizing the several successional phases during plant breakdown. Furthermore, a contrasting species composition between sample units might offer clues for the complex decay pattern mediated by fungi associated with a single host. Monitoring fungal succession by direct observation requires careful interpretation (Fryar, 2002; Jones and Hyde, 2002). Fungal succession is based on appearance of sporulating structures and could be attributed to mycelial replacement of one species by another i.e. real succession (Rayner and Todd, 1979; Fryar, 2002) or sporulation due to events such as nutrient depletion, species specific life cycle differences, competition effects, or other interactions between species or the biotic and abiotic environment (e.g. Frankland, 1992, 1998; Gessner *et al*., 1997).

Within the Scheldt estuary site specific conditions (mainly salinity and substrate quality differ) have been found to influence fungal community composition (Van Ryckegem and Verbeken, 2005). Site specificity has been noted by Apinis *et al*. (1972) and Poon and Hyde (1998b). Spatial and temporal factors have also been shown to influence fungal community development within one site (Apinis *et al*., 1972, 1975; Poon and Hyde, 1998b).

This paper records the succession of sporulating fungi on standing leaf sheaths of *P. australis* and those in the litter layer, during plant's growth and decomposition. The fungal community is characterised by multivariate analysis and indicator species for each of the recognised clusters.

Materials and methods

The study was situated in a brackish reed stand of the Scheldt estuary near Doel (51° 21′ N, 4° 14′ E). The tidal marsh is located 53.9 km inland. It is situated between the Dutch-Belgian border and the 'Verdronken Land van Saeftinghe' (The Netherlands), the largest tidal nature reserve along the Scheldt. The vegetation in our study area consists of a monotypic reed stand. The tidal exchange water floods the marsh with a frequency of 15.2% of the high tides with a flooding height of 17 cm on average and is characterised by a seasonally shifting salinity of 2455 ± 1510 mg $Cl^- l^{-1}$ (average ± STDEV, N = 19 in the year 2002, data from the Flemish Environment Agency) (see Van Ryckegem and Verbeken (2005) for additional environmental and reed characteristics).

The sampling comprised two cohorts of fungal succession of equal duration but with a year interval. This was to check for between year variations. Two plots of 6 m^2 (3 × 2 m) were therefore fenced (1.4 m high and mesh 1 cm) in order to prevent input of reed with unknown age in the litter layer. At the start of each survey, the plots where mown and the material removed (in January 2000 for plot 1 and January 2001 for plot 2). The plots were adjacent and considered to share similar characteristics. Standing shoots were sampled as follows: monthly 10 shoots were cut at bottom level and divided, if possible, into 3 standardised, 30 cm long parts: basal part, middle part and upper part (excluding inflorescence). Each part consisted of both stem and leaf sheath (if still present). In July, the year after emergence, 70 standing culms in each plot were marked at the base with red flagged cable ties, to ensure the age of dead culms. In order to follow litter decay, sampling commenced in November (both cohort-years) when the culms start senescencing and gradually enter the litter layer. Culm pieces considered to represent middle sections were randomly collected from the marsh surface. To be sure of the age and residence time of samples in the litter layer, we enclosed fifteen 30 cm long culm pieces (stems + leaf sheaths) from the last growing season, in plastic litterbags with a 4 mm mesh, 35 × 20 cm. Culms, representing *ca*. the middle section of snapped shoots were collected from the litter layer during the last week of November 2000 (plot 1) or 2001 (plot 2). Both plots were sampled monthly starting from May 2000 (plot 1) or May 2001 (plot 2) during 19 months for leaf sheath monitoring. The mycota were followed during 17 and 18 months in standing position in the middle and basal regions respectively and 13 months in the upper zone. Fungal sporulation was monitored for 13 months in the litter layer. The screening of the samples resulted in a relative occurrence of the fungal taxa on a scale of 10 for the leaf sheaths in a standing position, while a relative occurrence on a scale of 15 for the litter culms was obtained. Before data analysis the latter scores were transformed to a scale of 10 as a matter of conformity. All field samples were placed in plastic bags and returned to the laboratory and stored at 4°C. Samples were screened for fungal presence within 2 weeks after collecting by means of a dissecting microscope (magnification × 180). Before screening, the samples were gently wet to make subepidermal fruit bodies more visible. All observations are based on the presence of mature fruiting structures (judged on maturity of spores or conidia) formed *in situ*. Each unique taxon was described and illustrated (Van Ryckegem, 2005; see also http://biology.ugent.be/reedfungi).

The following indices were calculated to assess the importance of fungal taxa in the subcommunities (see Tables 1, 2).

$$\% \text{ abundance of a taxon } X_m = \frac{\sum \textit{records of taxon } X_m}{\sum \textit{records of all taxa}_m} * 100$$

$$\% \text{ recurrence of a taxon } X_m = \frac{\sum \textit{records of taxon } X_m}{\sum \textit{records of taxon } X} * 100$$

$$\% \text{ occurrence of a taxon } X_{m,t} = \frac{\sum \textit{records of taxon } X_{m,t}}{\textit{number of plant parts investigated}} * 100$$

With *m* the microhabitat (top, middle and basal height of standing shoots or litter layer) where the taxa were collected and *t* the time (months).

Both plots were compared using the Jaccard coefficient as qualitative index $S_j = a/(a+b+c)$, where a = # of species shared between two series, b = # of species restricted to the first series, c = # of species restricted to the second series and the Bray-Curtis (Sørensen) coefficient as quantitave index $S_{bc} = 2s/(a+b)$, where s is the sum of shared abundances and a and b are the sums of abundances in individual sample units. Species diversity was calculated by Shannon's diversity index: $H' = -\sum_{i}^{S} p_i \ln p_i$,

where S = # of species in the community, $p_i = n_i/N$, n_i = number of records of species i, N = total number of records collected.

Fungal species composition on reed leaf sheaths was investigated using cluster analysis (CA) to identify groupings of samples based on similarity in fungal assemblages (whole dataset, raw data) using the Bray-Curtis distance measure and group average method.

Gradients in the dataset were reconstructed using the indirect detrended correspondence analysis (DCA) algorithms based on raw data and without down-weighting of rare taxa in the final ordination.

Indicator species analysis (ISA) was used to ascertain which species were responsible for the differences among the groups found in the cluster analysis (Dufrêne and Legendre, 1997). The indicator value (IV) for a taxon was determined by combining relative frequency and relative abundance in a given group. This value can range from 0 (no indication) to 100 (perfect indication, meaning the species was present in all samples in the group and was absent from all samples in other groups). Like Dufrêne and Legendre (1997) we arbitrarily chose a threshold level of 25% IV, and a Monte Carlo simulation (1000 runs) was used to determine significance ($P \leq 0.01$) of species IVs.

The dataset used for CA, DCA and ISA was simplified by excluding species which were only found once or twice during the study. Furthermore, all samples with only one or two records were removed from the dataset. Also after preliminary ordinations, two samples (201LS1, 217LS1) were removed from the dataset because of their aberrant behaviour. The first sample was a collection with a single dominant species, and the second had abnormal high species richness with multiple rare species. Such outliers mask potential information in ordination by compressing the other ordination output (e.g. Manly, 1994). The dataset matrix used for CA, DCA and ISA consisted out 103 samples and 53 taxa.

To study the relationship between the DCA-axes and a temporal factor we evaluated their correlation with the sample date by Kendall rank correlation (Legendre and Legendre, 1998). Differences in species composition found in the four microhabitats (subcommunities) were tested for significance using the multi-response permutation procedure (MRPP) a nonparametric method testing the hypothesis that there is no difference between the groups identified by a clustering method (Biondini *et al*., 1988). Euclidian distance and weighting of groups by $n.[sum(n)]^{-1}$ setup was used. Because we made multiple comparisons, *P*-values were adjusted using a Bonferroni correction (Rice, 1990). CA, MRPP, DCA and ISA were performed with PC-ORD version 4.26 (McCune and Mefford, 1999). For statistical tests we used the statistical package of S-plus (version 6.1 for Windows, professional edition, Insightful Corp. 2002).

Results

Sample size

Fig. 1 shows the species area curves on leaf sheaths in different microhabitats and in different successional stages. Preliminary screening of October 1999 leaf sheath collections, from the middle part of standing moribund culms, was used to set the sample size at ten for standing culms. This large sample replica is sufficient to control for variability caused by seasonality, succession and unknown differences between microhabitats. The sample size is shown to be large enough. All curves reach a platform before ten replica sheaths were screened (Fig. 1). Repeating the procedure for the other plant parts, a similar or lower sample size is suggested.

In the litter layer the sample replica was determined by screening culms of varied age and time of decay (October 1999 (not shown)). The results of the mixed litter layer sample showed a curve that levelled off at twelve leaf sheaths. Because of the high level of uncertainty of age distribution in the October 1999

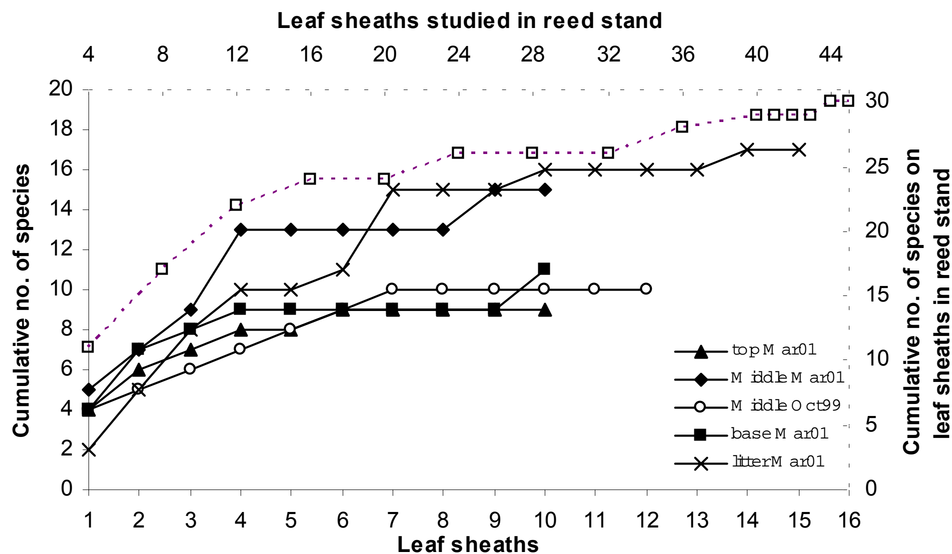


Fig. 1. Cumulative number of species forming sporulation structures on leaf sheaths. Primary axes, with data in full line, show results from the October 1999 sampling ─ used to set the number of replica samples at ten for standing reeds ─ compared with March 2001 samples with the highest species richness on leaf sheaths found during the study. Secondary axes, with data in dashed line, plot the cumulative species number in the reed stand on leaf sheaths if one collection is considered to consist out of a leaf sheaths collected at three heights in the canopy and one in the litter layer.

sample and the unknown seasonal and successional variability, the experimental sample size was set at fifteen litter layer sample units. This sample size proved to be large enough if plotted for leaf sheaths sampled in the litter layer at the time of highest species richness during decomposition (Fig. 1).

In total 122 leaf sheath samples were screened for fungal presence (see Table 3), rescaled to a total of 1220 individual leaf sheath sections screened (each month and in each subcommunity ten leaf sheaths) to calculate fungal occurrence.

For analysis, the two data sets were pooled, this seemed justified because of the high similarity between the two plots based on species composition (Jaccard similarity: 69%) and comparing species records between the two plots (Bray-Curtis similarity: 79%). Both plots had the same distribution as tested with a Mann-Whitney U test (P = 0.2064). Furthermore, the results of the cluster analysis showed that 81% of all leaf sheath samples compared at one year intervals, clustered in the same group identified (see Fig. 3).

Table 1. Taxa list and frequency distribution of all taxa found on leaf sheaths of *Phragmites australis* during growth and decomposition. Data for the two successive series pooled. % abundance (% ab.)[1] is the proportion of records of a taxon on the total number of records of all taxa. Taxa acronyms code for taxa names in Figs. 3 and 7. The first letter of the acronym codes for the pseudo-systematic position of a taxon: A = ascomycetes; B = basidiomycetes; C = coelomycetes; H = hyphomycetes.

Taxa	Acronyms	# rec.	% ab.[1]
Phoma sp. III	CPHOMAC	381	15.1
Septoriella sp(p).	CSEPTOR	281	11.2
Hendersonia culmiseda Sacc.	CHENCUL	169	6.7
Stagonospora vexata Sacc. sensu Diedicke	CSTAVEX	163	6.5
Massarina arundinacea (Sowerby: Fr.) Leuchtm.	AMASARU	118	4.7
Stictis sp.	ASTICTI	115	4.6
Phaeosphaeria sp. II	APHASP2	101	4.0
Phialophorophoma sp.	CPHIALO	100	4.0
Coelomycete sp. I	CCOELI	81	3.2
Phaeosphaeria culmorum (Auersw.) Leuchtm.	APHACUL	71	2.8
Mycosphaerella lineolata (Roberge ex Desm.) J. Schröt.	AMYCLIN	69	2.7
Didymella glacialis Rehm	ADIDGLA	69	2.7
Hyphomycete sp. III	HHYPSP3	62	2.5
Phaeosphaeria pontiformis (Fuckel) Leuchtm.	APHAPON	56	2.2
Phaeosphaeria sp. III	APHASP3	56	2.2
Phomatospora berkeleyi Sacc.	APHOBER	55	2.2
Myrothecium cinctum (Corda) Sacc.	HMYRCIN	52	2.1
Lophodermium arundinaceum (Schrad.) Chevall.	ALOPHAR	48	1.9
Phaeosphaeria eustoma (Fuckel) L. Holm *s.l.*	APHAEUS	38	1.5
Halosphaeria hamata (Höhnk) Kohlm.	AHALHAM	37	1.5
Ascochyta cf. *arundinariae* Tassi	CASCARU	30	1.2
Camarosporium sp.	CCAMASP	30	1.2
Neottiosporina australiensis B. Sutton & Alcorn	CNETAUS	23	< 1
Phoma sp. IIa	CPHOMBA	22	
Phoma sp. II	CPHOMAB	21	
Hendersonia sp. I	CHENDER	17	
Discostroma sp.	ADISCOS	15	
Asco sp. *Dothideales* incertae sedis II	AASCII	13	
Coelomycete sp. III	CCOELIII	13	
Hyphomycete sp. VI	HHYPSP6	13	
Pleospora abscondita Sacc. & Roum.	APLEABS	13	
Puccinia magnusiana Körn.	URPUCMA	13	
Pseudoseptoria donacis (Pass.) B. Sutton	CPSEDON	12	
Stagonospora elegans (Berk.) Sacc. & Traverso	CSTAELE	12	
Coelomycete sp. II	CCOELII	11	
Cladosporium sp(p).	HCLADOS	10	
Pleospora vagans Niessl	APLEVAG	10	
Tremella spicifera Van Ryck., Van de Put & P. Roberts	BTRESPI	10	
Morenoina phragmitidis J.P. Ellis	AMORPHR	9	

Table 1. continued. Taxa list and frequency distribution of all taxa found on leaf sheaths of *Phragmites australis* during growth and decomposition.

Taxa	Acronyms	# rec.	% ab.[1]
Phaeoseptoria sp.	CPHAEOS	8	
Didymella sp.	ADIDYME	7	
Hendersonia aff. *culmiseda* Sacc.	CHENCFC	7	
Microsphaeropsis sp. I	CMICSP1	7	
Septoriella phragmitis Oudem.	CSEPPHR	5	
Hendersonia sp. II	CHENDII	4	
Mollisia cf. *palustris* (Roberge ex Desm.) P. Karst.	AMOLPAL	4	
Stagonospora sp. II	CSTAINB	4	
Ascochyta cf. *leptospora* (Trail) Hara	CASCLEP	4	
Ascochyta sp. I	CASCOCI	3	
Cytoplacosphaeria rimosa (Oudem.) Petrak *s.l.*	CCYTRIM	3	
Periconia minutissima Corda	HPERMIN	3	
Phoma sp. IV	CPHOMAD	3	
Sporobolomyces sp.	BSPOROB	3	
Arthrinium phaeospermum (Corda) M.B. Ellis		2	
Asco sp. *Dothideales* incertae sedis I		2	
Gibberella zeae (Schwein.) Petch		2	
Nectria graminicola Berk. & Broome		2	
Paraphaeosphaeria michotii (Westend.) O.E. Erikss.		2	
Phaeosphaeria luctuosa (Niessl) Otani & Mikawa		2	
Puccinia phragmitis (Schumach.) Körn.		2	
Stagonospora incertae sedis III		2	
Alternaria alternata (Fr.) Keissl.		1	
Apiospora montagnei Sacc.		1	
Aposphaeria sp.		1	
Botryosphaeria festucae (Lib.) Arx & E. Müll.		1	
Camarosporium feurichii Henn.		1	
Fusarium sp. III		1	
Lewia infectoria (Fuckel) M.E. Barr & E.G. Simmons		1	
Massarina fluviatilis Aptroot & Van Ryck.		1	
Massarina sp. III		1	
Mollisia hydrophila (P. Karst.) Sacc.		1	
Mollisia retincola (Rabenh.) P. Karst.		1	
Periconia digitata (Cooke) Sacc.		1	
Phomatospora sp. IV		1	
Schizothecium hispidulum (Speg.) Lundq.		1	
Septoria sp.		1	
Stagonospora incertae sedis I		1	

[1] another index, the % occurrence of a taxon is not presented. This measure, which indicates the proportion of all leaf sheaths colonized by a specific taxon, was about double of the presented percentages for abundance.

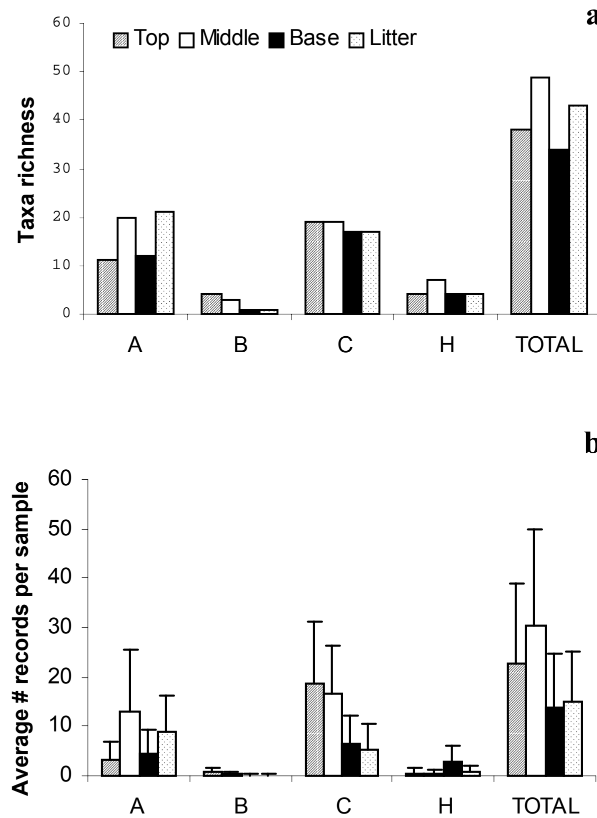


Fig. 2a. Taxa richness and distribution of the major (pseudo)systematic groups on leaf sheaths of *Phragmites australis* in the different microhabitats investigated. **b.** Average number of records per sample occasion in the different microhabitats investigated (error bar = STDEV). The same column coding for both panels. A = ascomycetes; B = basidiomycetes; C = coelomycetes; H = hyphomycetes.

Fungal diversity

During growth and decomposition of common reed 2516 records were made belonging to 77 fungal taxa. In general many taxa were rare, i.e. encountered only once or twice (Table 1). The screened leaf sheaths comprised 33 ascomycetes (43%), 31 coelomycetes (40%), nine hyphomycetes (12%) and only four basidiomycetes (5%). Coelomycetes and ascomycetes accounted for 93% of all records demonstrating the low presence of hyphomycetes and basidiomycetes (Figs. 2a,b). Three genera account for 56% of all fungal records on *P. australis* leaf sheaths. These include *Stagonospora s.l.* (11 taxa), *Phoma* (4 taxa) and *Phaeosphaeria* (6 taxa). *Stagonospora s.l.* was divided into several smaller genera [*Stagonospora s.s.* (5 taxa) (*sensu* Sutton, 1980), *Septoriella* spp. (2 taxa) (*sensu* Sutton, 1980; Nag Raj, 1993) and *Hendersonia* (4 taxa)

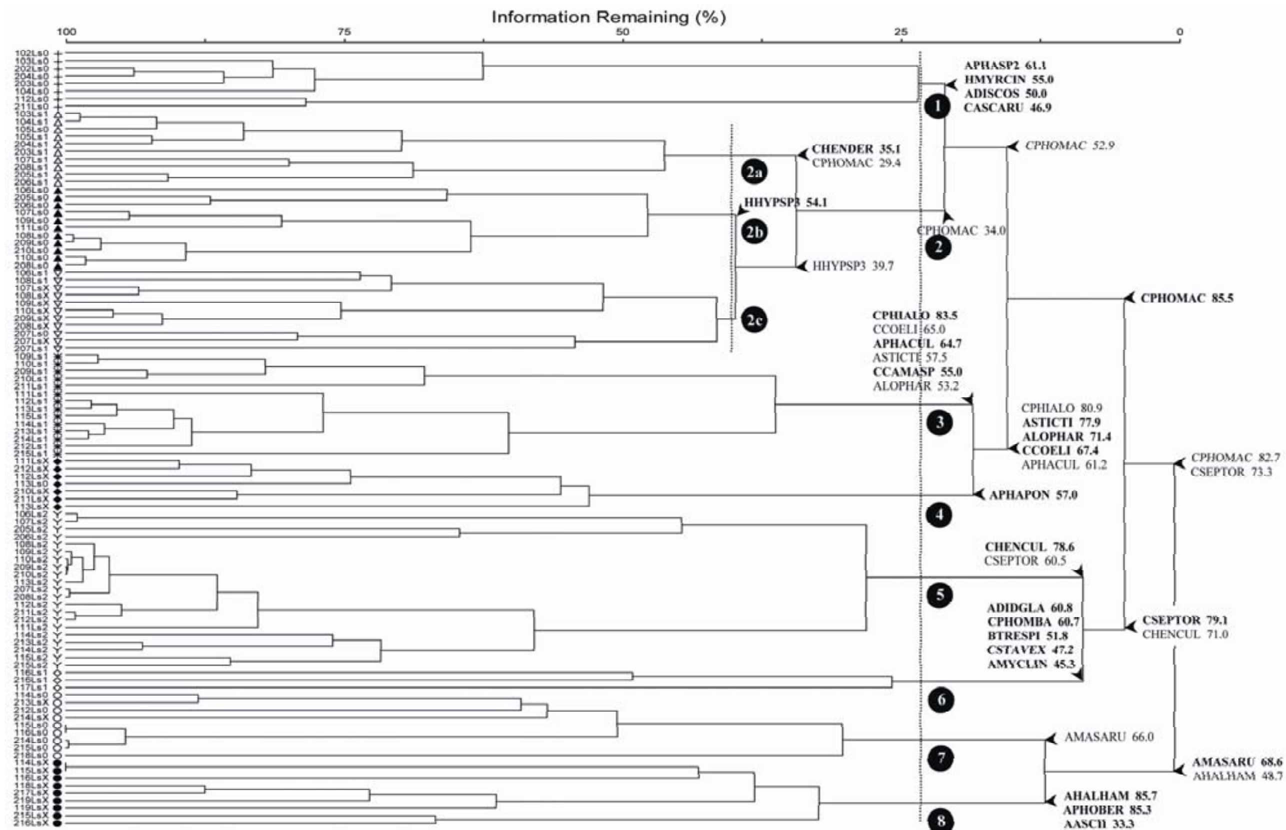


Fig. 3. Dendrogram from cluster analysis of the pooled data from plot 1 and 2 with 53 taxa and 103 samples included. Sample code exists of four character states. The first figure indicates the plot number being 1 or 2; the second number stands for month of collection (between 1-19), see Table 3; the letter code 'ls' stands for leaf sheath sample and the last figure indicates the microhabitat (0 = base, 1 = middle, 2 = top and X = litter layer) the samples were taken. Indicator species with indicator values are shown for the groups generated by cluster analysis. All species with indicator value > 25 and Monte-Carlo *P*-values < 0.01 are included. The maximum indicator values for a species are printed in bold. Acronyms abbreviations in Table 1. Dendrogram is scaled with the percentage information remaining in the branches.

(invalid name, see Wakefield, 1939, but for practical cause used to indicate *Stagonospora* spp. *s.l.* with brown conidia without obvious slime appendage and solitary conidiomata].

Of the four microhabitats (subcommunities) the middle canopy leaf sheath showed the highest taxa richness (49 taxa), while the lower and upper canopy leaf sheaths had 34 and 38 taxa respectively. The number of taxa found on the decomposing leaf sheaths in the litter layer was 43. A comparison of the distinct fungal groups within different microhabitats with an equal sample intensity (Fig. 2b) revealed a significant difference in ascomycete occurrence between middle and top shoot sections (Wilcoxon rank-sum test $P = 0.0063$; with α set at 0.0083, Bonferroni corrected), and between middle and basal leaf sheaths (Wilcoxon rank-sum test $P = 0.0221$, but not significant if Bonferroni corrected). Relative occurrence of coelomycetes, as measured by sporulation frequency, appeared to be substantially higher (although not significant) in the middle and upper canopy compared to the basal canopy and litter layer leaf sheaths (Fig. 2b).

Spatial and temporal characterization of the subcommunities

Cluster analysis (Fig. 3) of the samples results in seven interpretable groups and one small rest group. The groups are mainly separated by spatial and temporal characteristics and correspond well to the natural subcommunity units sampled. Group 1 – a cluster of mainly basal leaf sheath samples with an initial fungal colonization on living sheaths. Group 2 – a large cluster of 3 smaller subcommunities comprising all initial species assemblages except for the samples from the top canopy. Group 2a are samples from the middle canopy showing initial fungal colonization on living, moribund and newly dead leaf sheaths. Group 2a is significantly different from Group 2b (Bonferroni corrected, MRPP, $P < 0.001$). The latter clusters all samples from moribund or dead leaf sheaths from the basal canopy. Group 2c predominantly consists of samples that had recently fallen into the litter layer. Group 3 – clusters all samples from the middle canopy height in a later phase of fungal community development. Group 4 – is a small cluster of transitional samples between the early and the late successional samples in the litter layer (Group 8). Group 5 – is a tight cluster of top canopy leaf sheaths with subclusters showing a temporal gradient. Group 6 – is a rest group of middle leaf sheaths. Group (7+8) – is the outgroup of the cluster dendrogram and illustrates the distinct species assemblages on leaf sheaths influenced by tidal exchange water in a last phase of decay. Group 7 – clusters basal leaf sheaths in a final phase of decay together with some litter samples showing comparable species composition. Group 8 – clusters leaf sheath samples in the litter layer in a final phase of decay.

DCA (Fig. 4) resulted in two interpretable axes with both axes having a high eigenvalue (axis 1: 0.69; axis 2: 0.42) and thus a high explanatory power. Axis 3 with an eigenvalue of 0.22 explained little of the observed variation in our leaf sheath dataset. The high eigenvalues and a large length of gradient (axis 1: 5.962; axis 2: 3.615) indicate a distinct β-diversity along both axes. An after-the-fact evaluation of the variation explained by the axes by relative Euclidean correlation (McCune and Mefford, 1999) estimated that 38% of the variation was explained by axis 1 and 14% by axis 2. DCA sample clusters (Fig. 4) are concurrent with the groups delimited by cluster analysis. A strong spatial component in fungal community structuring is illustrated in Fig. 4 showing the delimitation of the different microhabitats sampled in a reed stand. Axis 1 represents the spatial component, separating top, middle and (basal + litter) samples from each other. The latter two microhabitats show more resemblance to each other than the aerial reed parts, however along the second axis these basal samples are largely separated from the litter samples with the exception of the basal leaf sheath samples in the final phase of decay. Those leaf sheaths have almost identical fungal assemblages to those in the litter layer. Axis 2 shows a strong negative correlation with the sampling date (Kendall rank), representing a clear successional gradient in the fungal community development in the canopy (Fig. 6). Although axis 1 shows a weaker successional trend, as indicated by the lower correlation results, it appears to be associated with a temporal aspect for the litter layer samples (Fig. 6). In Fig. 7 a biplot of samples and taxa shows the taxa scores along axes 1 and 2. All of the sample scores represent a reciprocal calculation with species scores and are plotted as the centroid of the species present in the samples. This means that species plotted near samples show affinity to these samples. Species plotted at the margins of the ordination are generally rare species (McCune and Grace, 2002).

Indicator species analysis (ISA) reveals contrasting performance of several species in sample groups generated by CA. Those indicator taxa are plotted on the CA-diagram (Fig. 3). ISA can generate indicators at several levels of the community hierarchy. At the highest level (i.e. all samples) of the cluster dendrogram, group (7+8) is the first to be separated from the rest of the leaf sheath samples. Indicator species for this group are *Massarina arundinacea* and *Halosphaeria hamata*. Within group (7+8) *M. arundinacea* is more a generalist compared to *H. hamata* as the former reaches the highest IV for the whole group; while *H. hamata* is more a specialist as it reaches highest IV in the litter layer (group 8). These litter samples in a final phase of decay are further characterised by the presence of *Phomatospora berkeleyi* and Asco sp. *Dothideales* incertae sedis II. The best indicator for group 7, basal leaf sheaths

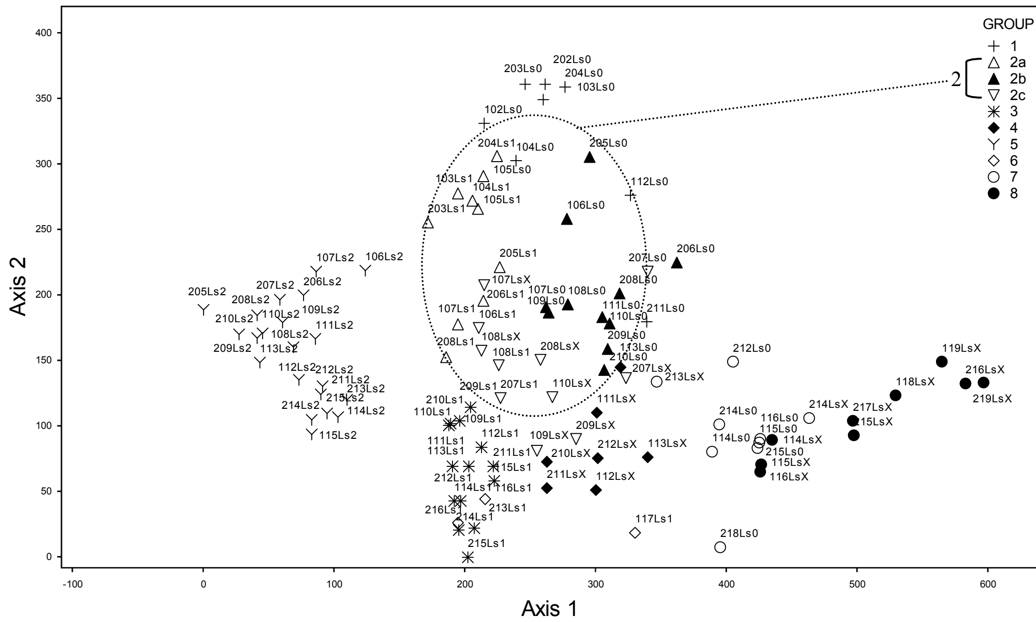


Fig. 4. First two ordination axes of a DCA based on species occurrences on leaf sheaths of *Phragmites australis* with 53 taxa (raw data) and 103 samples included. Sample grouping symbols and acronyms are the same as in the cluster analysis (Fig. 3). Axes are scaled in SD units (× 100).

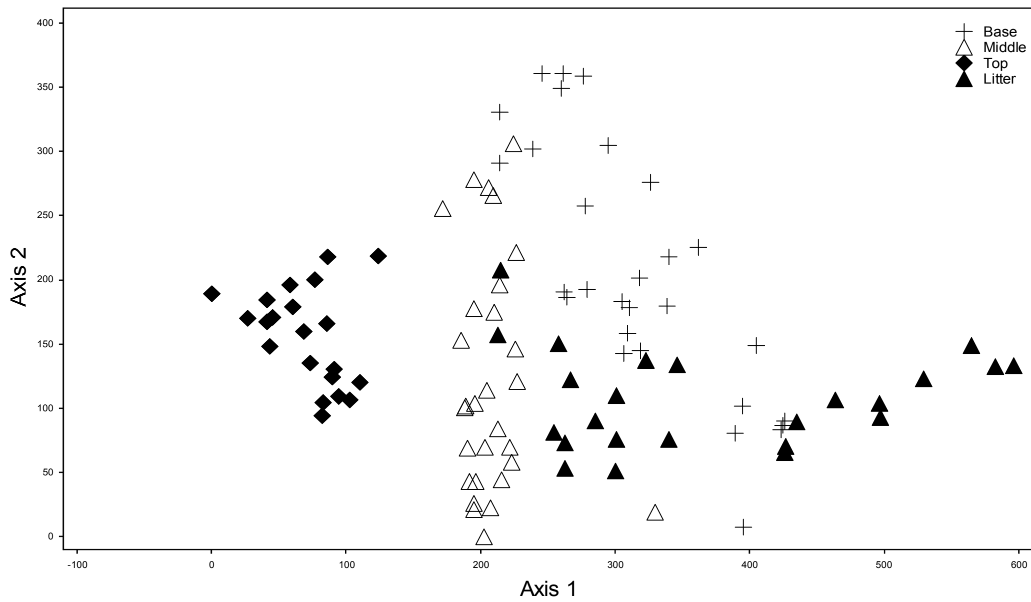


Fig. 5. DCA plot identical to Fig. 4 with the leaf sheath samples from *Phragmites australis* coded by the subcommunity they originated from. Axes are scaled in SD units (× 100).

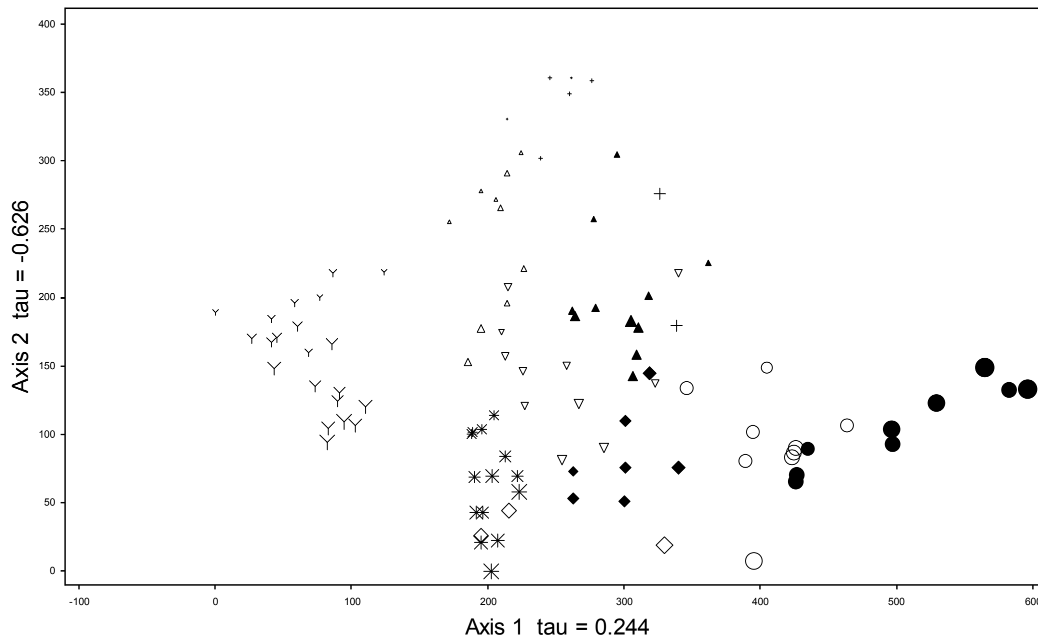


Fig. 6. DCA plot identical to Fig. 4 of leaf sheath samples from *Phragmites australis* showing the temporal correlation of the first two ordination axes with Kendall rank correlation (tau) indicated. Smallest symbols indicate earliest samples in the successional community development. Axes are scaled in SD units (× 100). Symbols are coded similar to Fig. 4.

in a final phase of decay, was *M. arundinacea*. Although *P. berkeleyi* is a good indicator for leaf sheaths (and leaf blades, unpublished data) in late stage of decay in the litter layer, it was also observed higher up in the canopy on recently senescent sheaths (Table 2; unpublished data). General, eurytopic indicators for all canopy samples and litter layer leaf sheaths recently fallen on the sediment are *Phoma* sp. III and *Septoriella* sp(p). *Phoma* sp. III is mainly indicative for the basal and middle canopy leaf sheaths during the early colonization, while *Septoriella* sp(p). is a typical coloniser of the upper moribund and dead leaf sheaths of common reed. The latter was however, occasionally found in the middle canopy (group 2a and 6), and in particular it had a high % occurrence on green middle canopy leaf sheaths (Table 2). The third level in cluster hierarchy showed highest IV for *Didymella glacialis*, *Phoma* sp. IIa, *Tremella spicifera*, *Stagonospora vexata* and *Mycosphaerella lineolata* (group 6). *Hendersonia culmiseda* is a specific indicator for the entire subcommunity of top canopy leaf sheath (group 5) (Table 4). Group (3+4) clustering the middle leaf sheaths in a second phase of fungal colonization and some leaf sheath litter layer samples are characterised by *Stictis* sp., *Lophodermium arundinaceum* and Coelomycete sp. I. On a lower level group 3,

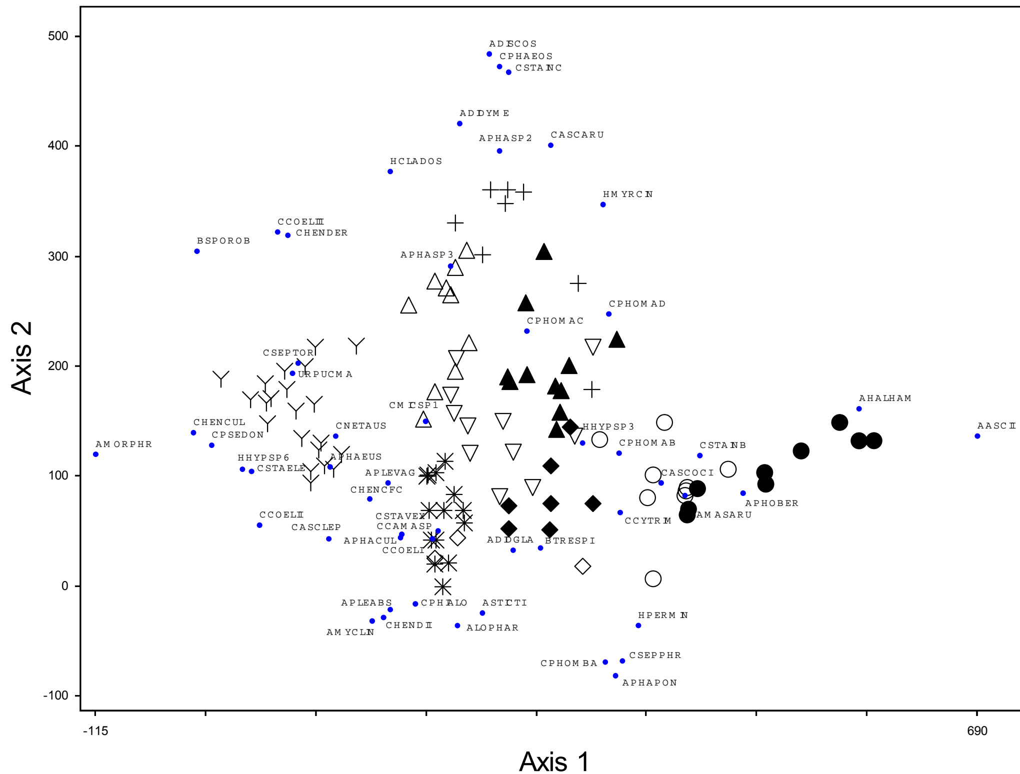


Fig. 7. First two ordination axes of a DCA biplot showing *Phragmites australis* leaf sheath samples (103) (Figs. 3, 4) and the species distribution (53 taxa). Acronyms as in Table 1. Symbols are coded similar to Fig. 4.

clustering solely the middle leaf sheaths, is characterised more specifically by *Phialophorophoma* sp., *Phaeosphaeria culmorum* and *Camarosporium* sp. Group 4 is specifically characterised by *Phaeosphaeria pontiformis* although it has a number of species in common with group 3 but in a lower frequency. Group 1, containing living green and moribund leaf sheath at the basal culm part, is characterised by *Phaeosphaeria* sp. II, *Myrothecium cinctum, Discostroma* sp. and *Ascochyta* cf. *arundinariae*. Group 2 and its subgroups show few good indicator taxa. In general group 2 and 2a are well characterised by *Phoma* sp. III with group 2a further typified by *Hendersonia* sp. Within group (2b+2c) Hyphomycete sp. III is the most typical taxon, with highest indicative power for group 2b. Initial litter layer samples (group 2c) showed no good indicator taxa.

Discussion

The chosen replica of the sample unit was evaluated by plotting the species area curves for the month with highest species richness during the entire

Table 2. Sequence in fungal sporulation on leaf sheaths of *Phragmites australis* during growth and decomposition in four different microhabitats. Only taxa with more than 10 records (39 taxa) during the entire study are shown with their number of records, percentages abundance (calculation based on all taxa) and percentage recurrence for each of the different microhabitats. The percentages of frequency of occurrence of the taxa in each month sampled starting from May (see Table 3) and for each microhabitat sampled. Data from the two plots are pooled, 20 leaf sheaths screened per sampling time per microhabitat. Microhabitats without taxa records were omitted. 'Gr' codes for the pseudo-systematic position of a taxon: A = ascomycetes; B = basidiomycetes; C = coelomycetes; H = hyphomycetes.

Gr	**SPECIES**	**Micro-habitat**	**∑ records**	**% ab.**	**% rec.**	**% occurrence (months)**																		
						M	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**
A	*Discostroma* sp.	base	15	3	100	10	15	45	5															
H	*Cladosporium* sp(p).	top	1	0	10					5														
		middle	7	1	70	10	5	10									5	5						
		base	2	0	20		10																	
C	*Septoriella* sp(p).	top	173	29	62					30	65	75	80	95	100	100	85	90	70	75				
		middle	62	6	22	5	10	55	35	0	25	25	15	10	25	15	15	15	10	5	35	10		
		base	29	6	10		20	25	25	20	5	15	5	20	0	5	0	5						
		litter	17	4	6							15	20	5	20	15	10							
C	*Phoma* sp. II	middle	7	1	33	5																30		
		base	12	3	57							5	0	25	0	25	0	5						
		litter	2	1	10												5	0	0	5				
A	*Phaeosphaeria* sp. II	middle	32	3	32			25	40	35	5						2	3	3	3				
		base	64	14	64		90	80	60	35	10	5				15	15	5	5					
		litter	4	1	4											15	0	5						
C	*Phoma* sp. III	top	15	3	4						10	15	15	10	5	5	10	5						
		middle	176	17	46			60	90	100	75	80	70	75	85	85	70	45	15	25	5			
		base	132	27	35		40	55	50	65	80	60	75	70	65	55	20	20	5					
		litter	58	15	15							60	60	65	45	40	10	10						
H	*Myrothecium cinctum*	base	45	10	87		10	45	30	15	15					30	50	30						
		litter	7	2	13										5	10	20							
C	*Ascochyta* cf. *arundinariae*	top	2	0	7														10					
		middle	1	0	3																5			
		base	25	5	87		5	45	45	10	15			5	0	5								
		litter	1	0	3													5						
A	*Phaeosphaeria* sp. III	top	5	1	9						5					20								
		middle	41	4	73			45	45	25	5			5		5	15	25	20	5	5	5		
		base	5	1	9			5	10	5	0	5												
		litter	5	1	9								5				10	10						

Table 2 continued. Sequence in fungal sporulation on leaf sheaths of *Phragmites australis* during growth and decomposition in four different microhabitats.

Gr	SPECIES	Micro-	∑ records	%	%	% occurrence (months)																		
		habitat		ab.	rec.	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
C	*Hendersonia* sp. I	top	8	1	47					5	15									20				
		middle	8	1	47			10	5	20	5													
		base	1	0	6					5														
C	*Neottiosporina*	top	13	2	57								10	5	5	0	15	10	10	10				
	australiensis	middle	8	1	35						5	0	5	10	15	5								
		base	1	0	4			5																
		litter	1	0	4									5										
C	Coelomycete sp. III	top	7	1	54					5	5	10	5	10										
		middle	6	1	46				10	15	5													
A	*Phaeosphaeria eustoma*	top	19	3	50											15	5	45	25	5				
		middle	16	2	42	5						10					10	10	30	10	5			
		base	3	1	8			15																
A	*Didymella glacialis*	top	7	1	10						5					5	5	0	5	15				
		middle	53	5	78					25	20					10	5	50	40	50	50	15		
		base	1	0	1											5								
		litter	7	2	10											5	10	10	5	0	5			
C	*Hendersonia culmiseda*	top	148	25	88							25	80	85	95	85	100	100	95	75				
		middle	14	1	8					15	5	15	20	10	5									
		litter	7	2	4								10	10	10	0	5							
C	*Pseudoseptoria donacis*	top	12	2	100					5	5							30	20					
A	*Phaeaosphaeria*	middle	12	1	21					5	0	5				5	0	10	10	10	15			
	pontiformis	base	4	1	7													10	10					
		litter	40	10	71							5	10	45	30	35	50	15	0	10				
A	*Halosphaeria hamata*	base	6	1	17						20	5	5											
		litter	30	8	83												5	5	20	10	50	10	10	40
A	*Phaeosphaeria culmorum*	top	15	3	21						10	0	5	5	5	0	10	15	20	5				
		middle	47	4	66						5	10	5	25	15	40	30	45	45	15				
		litter	9	2	13									15	5	10	15							
A	*Massarina arundinacea*	top	4	1	3													20						
		middle	5	0	4							5				5			10		5			
		base	53	11	46						10				5	20	25	35	50	70	40	0	10	
		litter	53	14	46									10	20	50	55	45	35	40	5	5		

Table 2 continued. Sequence in fungal sporulation on leaf sheaths of *Phragmites australis* during growth and decomposition in four different microhabitats.

Gr	SPECIES	Micro-habitat	∑ records	% ab.	% rec.	% occurrence (months) M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
H	Hyphomycete sp. III	top	1	0	2													5						
		middle	3	0	5								5				5	5						
		base	49	11	79						5	30	40	50	45	60	15							
		litter	9	2	15								15	10	5	10	5							
B	*Puccinia magnusiana*	top	10	2	77						5	10	15	0	5	10	5							
		middle	3	0	23								10			5								
A	*Phomatospora berkeleyi*	middle	9	1	18						5	5	15									20		
		base	1	0	2							5												
		litter	41	11	80							5	5	5	0	5	10	30	15	45	35	25	10	15
A	*Stictis* sp.	middle	93	9	81						5	5	5	10	5	50	100	100	100	80	5			
		base	6	1	5											15	0	15						
		litter	16	4	14									5	0	25	40	10						
A	*Pleospora vagans*	top	1	0	10														5					
		middle	9	1	90						5	5			10	10			15					
C	*Phoma* sp. IIa	top	1	0	5								5											
		middle	12	1	55													5			30	25		
		base	4	1	18						5			5	10									
		litter	5	1	23							5	0	5	10	5								
C	*Stagonospora vexata*	top	61	10	37									10	0	45	60	45	75	70				
		middle	77	7	47							5	20	50	30	45	30	55	40	20	70	20		
		base	1	0	1													5						
		litter	24	6	15							5	10	25	15	30	15	10	5	5				
C	Coelomycete sp. I	top	14	2	18											15	5	15	35					
		middle	58	6	73							5	10	55	55	40	35	50	30	10				
		litter	8	2	10								5	5	5	15	5	5						
A	*Pleospora abscondita*	top	1	0	8															5				
		middle	12	1	92							5	5			10	15	10	10	5				
C	Coelomycete sp. II	top	8	1	73												10			30				
		middle	2	0	18														5	5				
		base	1	0	9							5												
C	*Camarosporium* sp.	top	2	0	7											5			5					
		middle	26	2	87								5	15	40	15	20	15	20					
		litter	2	1	7								5					5						
C	*Stagonospora elegans*	top	11	2	92								5			5	0	15	10	20				
		base	1	0	8									5										

Table 2 continued. Sequence in fungal sporulation on leaf sheaths of *Phragmites australis* during growth and decomposition in four different microhabitats.

Gr	SPECIES	Micro-habitat	∑ records	% ab.	% rec.	% occurrence (months) M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
C	*Phialophorophoma* sp.	top	4	1	4														10	10				
		middle	89	9	91									15	45	70	85	85	100	40	5			
		litter	5	1	5											10	10	5						
B	*Tremella spicifera*	top	2	0	20											5	5							
		middle	5	0	50									5	5						10	5		
		base	2	0	20											10								
		litter	1	0	10											5								
A	*Lophodermium arundinaceum*	middle	41	4	85										5	30	35	55	40	40				
		base	2	0	4													10						
		litter	5	1	10											10	15							
H	Hyphomycete sp. VI	top	9	2	69											15			30					
		middle	4	0	31											15			5					
A	*Mycosphaerella lineolata*	top	17	3	25														35	50				
		middle	51	5	74												5	50	80	40	80			
		litter	1	0	1													5						
A	Asco sp. *Dothideales* incertae sedis II	litter	13	3	100															20	30			15

Table 3. Sampling design and the different stages in fungal succession on leaf sheaths of *P. australis* recognised in our study. The complete dataset exists out two successional series with one year interval, with series 1 started in May 2000 and series 2 in May 2001.

Month	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov
Code number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Top					Phase I						Phase II				*1				
Middle					Phase I							Phase II					Phase III	*2	
Base			Phase I					Phase II							Phase III				
Litter layer								Phase X				Phase II				Phase III			*3

*[1] There are indications that phase III of fungal succession starts in month 15.
*[2] Lose observations on leaf sheath remains showed an ongoing – decreasing – sporulation till January the next year when the leaf sheaths were almost completely decomposed.
*[3] Probably a fourth phase of fungal succession, characterised by common soil fungi, is established.

study (Fig. 1). In each of the investigated microhabitats those curves reached a platform before the chosen replica size was reached. If we consider a pooled species area curve including all sample units collected in one month, we could argue that we over-sampled to have a general idea of the fungal community. This comment is however only valid where all microhabitats in a reed stand are included during sampling. This is illustrated by the dotted line in Fig. 1. This species area curve was compiled by recognizing a sample as consisting out of a litter layer sheath and 1 sheath from each of three heights monitored in the canopy. Such a collection is a representative sample of all different types of leaf sheaths available in a reed stand in March. No fresh shoots have emerged and leaf sheaths from the previous growing season have (almost) completely decomposed (pers. observ.). The 45 leaf sheaths screened in March are considered to give a representative picture of fungal species richness. The curve shows that after screening 16 leaf sheaths, four from each microhabitat, we had already found 80% of all fungal species sporulating on leaf sheaths in March. A replica size of four is considered as a minimum for describing fungal community development in a specific microhabitat. Thus, to have a fairly complete view on fungal richness and composition on a single host, it seems important to invest more effort in screening a high number of potential

Table 4. Indicator taxa with indicator value (IV) on *Phragmites australis* leaf sheaths identified for the four different subcommunities sampled: top, middle and basal height leaf sheaths along the vertical axis of standing shoots and litter layer leaf sheaths.

Top		**Middle**		**Base**		**Litter**	
Taxa	**IV**	**Taxa**	**IV**	**Taxa**	**IV**	**Taxa**	**IV**
Hendersonia culmiseda	77.2	*Phialophorophoma* sp.	45.8	Hyphomycete sp. III	28.9	*Phomatospora berkeleyi*	58.9
		Stictis sp.	43.8				
Septoriella sp(p).	69.0	*Didymella glacialis*	40.3	*Ascochyta* cf. *arundinariae*	28.9	*Phaeosphaeria pontiformis*	43.8
		Phoma sp. III	39.4				
		Phaeosphaeria culmorum	37.1	*Phaeosphaeria* sp. II	28.5	*Halosphaeria hamata*	42.9
		Phaeosphaeria sp. III	35.7			*Massarina arundinacea*	31.4
		Coelomycete sp. I	34.5				
		Camarosporium sp.	28.8				
		Lophodermium arundinaceum	28.8				

Table 5. Jaccard similarity between different microhabitats screened for fungal sporulation on leaf sheaths of *Phragmites australis*.

	Litter	**Base**	**Middle**	**Top**
Litter	100			
Base	46	100		
Middle	48	40	100	
Top	35	30	62	100

microhabitats and less on the number of sample units. In investigating fungal diversity on submerged wood samples it was found the asymptote was reached after examination of *ca*. 100 samples of bamboo and 70 wood sample, with 30 samples considered optimal for sampling the most common species (Cai *et al*., 2003).

The natural decay pattern of *P. australis* consists of an initial standing decomposition phase and a secondary phase of breakdown in the litter layer. The importance of studying plant decay in this natural sequence has been stressed by Newell (1993) and others (e.g. Gessner, 2000). The criticism by the above authors mainly originated from the practice of unnaturally clipping of plant parts and incubating them (often in litterbags) on the soil surface or hanging them in an aerial position. However, the natural time in standing position and the physical contact with the whole plant are important for plant resorption processes (Granéli, 1990; Gessner, 2001). Therefore, decomposition studies manipulating reeds in an unnatural way describe breakdown rates and nutrient patterns probably not existing in field conditions. Furthermore, this study demonstrates the importance of natural decay patterns with specific

fungal microhabitats along the vertical axis of a reed shoot, an aspect noticed before (Apinis *et al*., 1972, 1975; Poon and Hyde, 1998b; Van Ryckegem and Verbeken, 2005). Each of the four studied microhabitats seemed to develop a unique fungal species composition (MRPP overall $P < 0.0001$, all six comparisons of microhabitats were highly significant $P < 0.0001$ even if Bonferroni corrected). This implies a natural vertical taxa distribution and a different taxa composition in the litter layer (Table 5). Such a vertical zonation of fungal taxa seems to be a feature in various reed wetlands and not restricted to tidal systems (e.g. Apinis *et al*., 1972; Poon and Hyde, 1998b; Van Ryckegem and Verbeken, 2005).

Species richness differed between the vertically recognised microhabitats. We observed lower species richness in the upper canopy. This corresponds to the results of Poon and Hyde (1998b). Although the latter authors did not state specifically the part of the plant screened (stem or leaf sheath), their low species richness in the upper canopy probably corresponds to the low number of taxa on upper leaf sheaths. This is deduced from the fact that leaf sheaths form the major fraction of plant material in the top canopy. However, the observed dominance of coelomycetes on top leaf sheaths in this study (Fig. 2b) was not recorded during the screening of tropical intertidal reeds (Poon and Hyde, 1998b). The observed highest species richness on the lower parts of standing culms by Poon and Hyde (1998b) must have been due to the higher species richness on basal stem parts compared to leaf sheaths.

It may be concluded from this section that the species rich fungal colonisers have a considerable impact on standing leaf sheath decay, which is dependant on the duration of leaf sheaths in a standing position and on the enzymatic capabilities of the microbial community.

The standing phase is abruptly ended for some culms when they fall into the litter layer (e.g. modelled by Soetaert *et al*., 2004). This is influenced by extreme weather conditions such as storms or extreme winds that snap the culms. The shift from standing decay to the litter layer results in an almost complete change in species composition. A set of characteristic taxa appears with both high % abundance and % recurrence in the litter layer: *Phaeosphaeria pontiformis*, *Halosphaeria hamata*, *Phomatospora berkeleyi*, *Massarina arundinacea* and *Phoma* sp. III (Table 2). The best indicators for the litter layer leaf sheath are late successional species (Table 4). Most of the above taxa are uncommon in the canopy with the exception of *Massarina arundinacea* and *Phoma* sp. III, which are also common in the basal canopy indicating the resemblance of basal leaf sheath mycota to the litter layer mycota. This is not surprising due to the proximity and similar environmental conditions in both these microhabitats characterised by periodical flooding by brackish water.

Several species such as *Halosphaeria hamata* and *Massarina arundinacea* are well adapted to sporulate in these saline conditions. *Halosphaeria hamata* was found to be adapted to brackish conditions both in the field and in culture experiments (Van Ryckegem and Verbeken, 2005). *Massarina arundinacea* is a eurytopic species, sporulating vigorously on *P. australis* litter from fresh water till mesohaline conditions and a culture experiment revealed tolerance for salinity (Van Ryckegem and Verbeken, 2005). The overall low frequency of fungi sporulating in the litter layer (Fig. 2b) is mainly due to the lower sporulation of coelomycetes compared to ascomycetes in the litter layer (Figs. 2b, 8b). This aspect has also been noticed in other reed habitats (Van Ryckegem and Verbeken, 2005).

Successional sequence

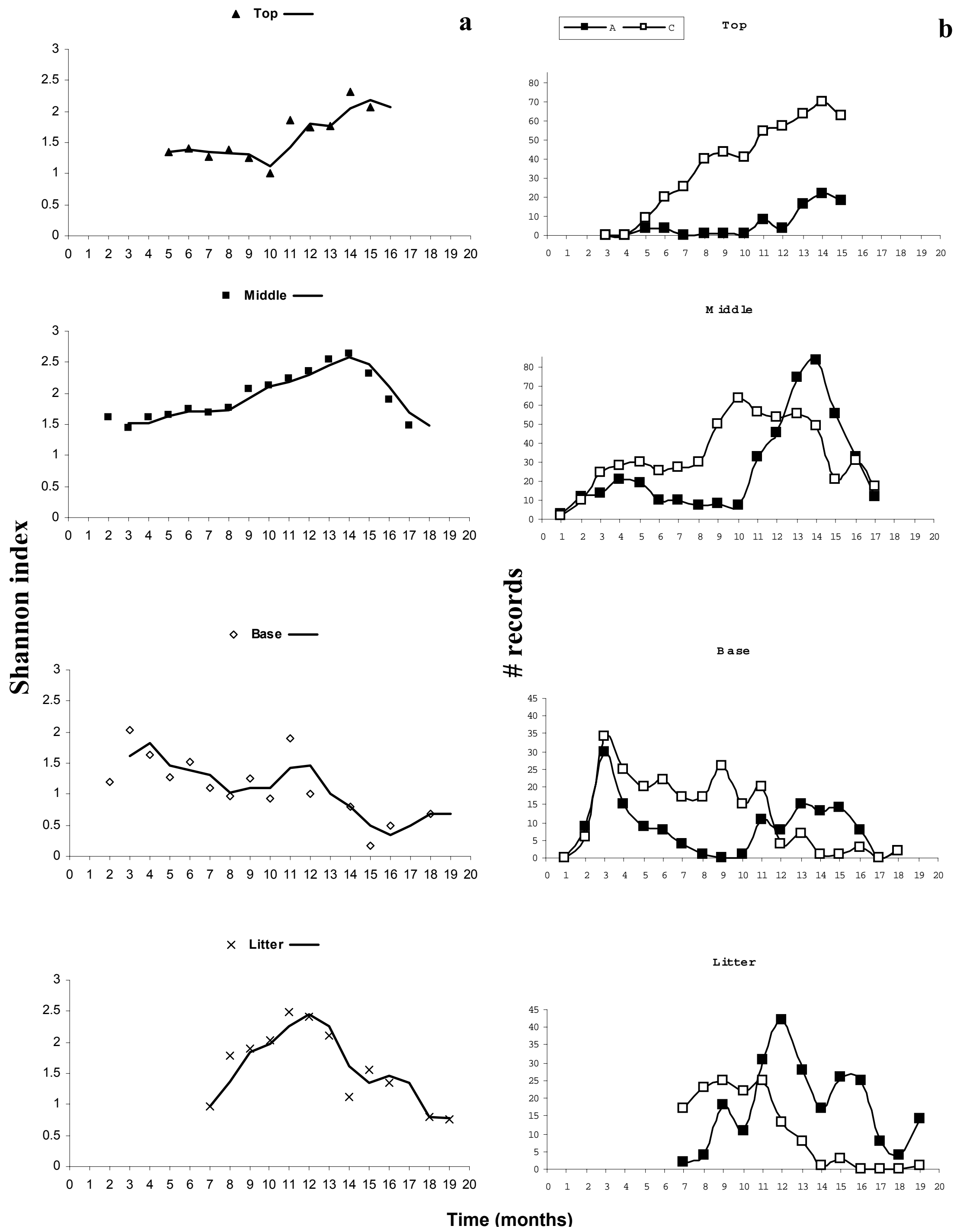
The successional sequence of fungal sporulating structures at different stages of heterotrophic succession has been observed by several authors and summarised in Frankland (1998) and Hyde and Jones (2002 and references herein). Several successional stages can also be discerned in fungal colonization of *P. australis* leaf sheaths. Canopy subcommunities (basal, middle and top leaf sheaths) show their own unique pattern comprising three different stages in fungal succession (Figs. 3, 5, 8a, b and summarised in Table 3). This three-step-succession of fungal community development is comparable to the observations by Apinis *et al*. (1972) and the general patterns described by Dix and Webster (1995) and Cooke and Rayner (1984). We recognise an initial pioneer community (Phase I), which is relatively open for fungal invasion and usually characterised by low species diversity, with few stress tolerant or ruderal species having a high abundance. The second stage (Phase II) is a more closed, mature community with high diversity of more combative species. The third phase (Phase III) is characterised as an impoverished community dominated by few species with low sporulation frequency typified as stress tolerant and/or highly combative taxa.

In particular, during Phase I, *P. australis* leaf sheaths are characterised by weak pathogens, biotrophic species and opportunistic saprotrophs found on living, moribund or initially dead leaf sheaths. Some taxa, such as *Sporobolomyces* sp. could be considered as phyllosphere fungi, but this group of fungi are not easily observed through direct observation on the leaf surface. More phyllosphere fungi would have been found if leaf sheaths were incubated in moist conditions or other techniques were used as presented in Apinis *et al*. (1972). Some other initial colonisers seemed to be confined to damaged or necrotic parts, or the thin margins of leaf sheaths where browning occurs

quickly. These fungi are *Alternaria alternata* and *Cladosporium* sp(p). (also on leaf blades; unpubl. data). These species are regarded to be opportunistic, non host specific saprotrophes found on nearly all plant species (Hudson, 1968, 1971). Although *Alternaria* and *Cladosporium* have also been found to be endophytic genera on *P. australis* (Wirsel *et al*., 2001), and may be host specific endophytes growing biotrophically. Other species were able to form sporulatation structures on the green, living tissues e.g. *Phaeosphaeria* sp. II, *Septoriella* sp(p). and *Phoma* sp. III. *Phaeosphaeria* spp., were shown to be systemic endophytes of *P. australis* that are also dispersed by seed (Ernst *et al*., 2003) and characterised by *Stagonospora* like anamorphs (Leuchtmann, 1984). Our *Septoriella* sp(p). and *Hendersonia* spp. show morphological affinities to these anamorphs. *Septoriella* sp(p). showed a disjunct appearance in space and time and this raises the suspicion that several species are lumped in this taxon with highly variable conidia, in size and septation. The *Septoriella* sp(p). initially colonizing the basal and middle leaf sheaths is probably different from the taxon dominant on the dead upper leaf sheaths (Table 2). Phase I is species poor for top and middle leaf sheaths but appears to be the most diverse stage for the basal leaf sheaths (Fig. 8a). The latter is probably due to the fact that most basal leaf sheaths soon lose their assimilation function and die. Furthermore, the tough bud scales surrounding the young emerging reed cones were screened in this study as if they were leaf sheaths. These bud scales are dead within the first month after shoot emergence and available for fungal decay. The peak sporulation (Fig. 8b) during the first months is mainly on these bud scales, which are characterised by some typical mycota (group 1, Figs. 3, 4). Of those species, *Discostroma* sp. is most typical. The first phase of fungal community development in the litter layer (Phase X, Table 3) is not comparable with the initial pioneer community described above for standing shoots because it represents a turnover phase of fungi. Species originating from various heights in the canopy, not adapted to the physicochemical stress present in the litter layer, are replaced by more typical estuarine fungi.

Phase II, the mature community, develops along the vertical axis of the shoots and is dependant on the moment of leaf sheaths senescence. The most basal leaf sheaths are already dead a few months before the middle and top leaf sheaths. The retarded senescence of the top leaf sheaths did not detectably slow the establishment of a mature community as compared with the middle height

Fig. 8a. Shannon diversity indices for the different microhabitats – subcommunities – sampled on leaf sheaths of *Phragmites australis* during the experimental period. The line is a moving average fit with a period of two. **b.** sporulation records in de different microhabitats on leaf sheaths of *Phragmites australis* for the two dominant pseudo-systematic groups: A = ascomycetes; C = coelomycetes.


a
b
Top
Middle
Base
Litter
A
C
Shannon index
records
Time (months)

leaf sheaths. Considering the natural senescence pattern of leaf sheaths one would expect to see a gradual appearance of species first colonizing the initially dead middle leaf sheaths followed by colonization of senescent top leaf sheath. However, because of the dissimilarity between vertically separated fungal subcommunities (Table 5) and possibly the inappropriate time lag of one month between the subsequent collections, these temporal colonization patterns were observed for only a few species (e.g. *Phoma* sp. III, Table 2). Furthermore, colonization and fruiting patterns could also be inferred by interspecific competition more than by the lag in senescence. At the end of phase II fungal communities reach their peak diversity (Fig. 8a). This pattern is less clear for the basal subcommunity. Although, it appears that the mature subcommunity on basal leaf sheaths matures together with the litter layer assemblages (Fig. 8b). While all subcommunities show a peak in species richness and sporulation of asexual coelomycetes before the sexual ascomycetes, the top community is characterised by a common peak of sexual and asexual taxa richness and sporulation (Fig. 8b). Whether these asexual and sexual sporulation structures represent the sequential appearance of the different stages of the holomorphic fungus remains to be established for most of the observed taxa. Few corresponding ana- and teleomorphs are known.

Soon after maximal diversity the communities start to become impoverish (Fig. 8a) and <u>Phase III</u> in fungal succession commenced. This phase shows few stress tolerant species that persist to sporulate in the last stage of decay, characterised by increasing carbon and/or nutrient stress and a reduced resource size by fragmentation. The top leaf sheath community could not be sampled long enough to demonstrate clearly a third phase, although the last samples indicated an already lowered diversity. A final fourth phase in fungal succession is to be expected in the litter layer characterised by common soil fungi (e.g. Frankland, 1976), but these where not found sporulating during this study on the leaf sheath remains in a final state of decay.

Occurrence and indicator taxa

Several taxa recorded during this study appeared to sporulate accidentally on reed sheaths with only one or few records (Table 1). Besides being rare, these taxa could be common mycelial inhabitants or the recorded life stage (the sexual or asexual stage) is formed rarely on the leaf sheaths. Only four taxa had overall abundance higher than 5% (Table 1). How to evaluate species importance and whether to call taxa common or rare and where to draw the line for these terms is an ongoing point of discussion between different fungal diversity studies (Jones and Hyde, 2002). Based on sporulation data the most objective way to pinpoint a species as characteristic for a certain phase or

microhabitat during fungal community development is thought to be an indicator algorithm such as TWINSPAN (two-way indicator species analysis) (Hill, 1979) or ISA (indicator species analysis) (Dufrêne and Legendre, 1997). The latter technique is preferred in ecological studies with several underlying gradients (McCune and Grace, 2002) and was used in this study. The hierarchical output in Fig. 3 gives ecological information by contrasting the performance of taxa at different levels of the community (generalists-eurytopic versus specialists-stenotopic). Ideally, the best indicators should be abundant, present on all unit samples of a group and faithful to a group. Such a species would be easily detected during field sampling. Alternatively a less abundant species that is faithful to a group to the point that its mere presence in all group samples is also a reliable indicator with high IV. However, such a species will be more easily overlooked when the community is undersampled. The species shown to have a significant IV (Fig. 3) could have a relatively low IV. Those taxa are often confined to a single group but their presence remained undetected on several sample units (asymmetrical indicators *sensu* Dufrêne and Legendre, 1997). Such taxa should not be used alone to predict a subcommunity type or a temporal phase. Asymmetrical indicators are considered to be a common type of indicators for fungal communities because of the many rare, specialised taxa. Therefore, typifying fungal (sub)communities should be based on all indicators united in ‘core’ assemblages of taxa (Fig. 3).

The natural falling of whole shoots on the sediment explains the absence of indicative taxa for reeds initially in the litter layer and lowers the overall indicative power of some taxa typical in canopy communities. Some terrestrial taxa could persist with fruit bodies on the reed in the litter layer for a few months. Therefore, the IV of taxa characteristic for the standing subcommunities (Table 4), and more specific for group 2a, 2b and group 5, are considered to be minimum values (Fig. 3).

Seasonality

While most taxa showed sporulation during a specific, continuous period, few taxa showed a seasonal pattern observed as a sporulation stop during colder winter months. Most obvious examples of species presumably not adapted to sporulate under colder temperatures are *Phaeosphaeria* sp. II and III and *Myrothecium cinctum* (Table 2; see Fig. 9 for seasonal weather dynamics). However, those taxa probably stay cryptic as mycelia during winter or they could be pleomorphic. Although seasonality is mentioned before as an important effect on fungal succession (e.g. Swift, 1976; Zhou and Hyde, 2002) most studies seem to be unable to demonstrate regular seasonal patterns directly linked to climate (Hawker, 1966; Widden, 1981). For microfungi on specific

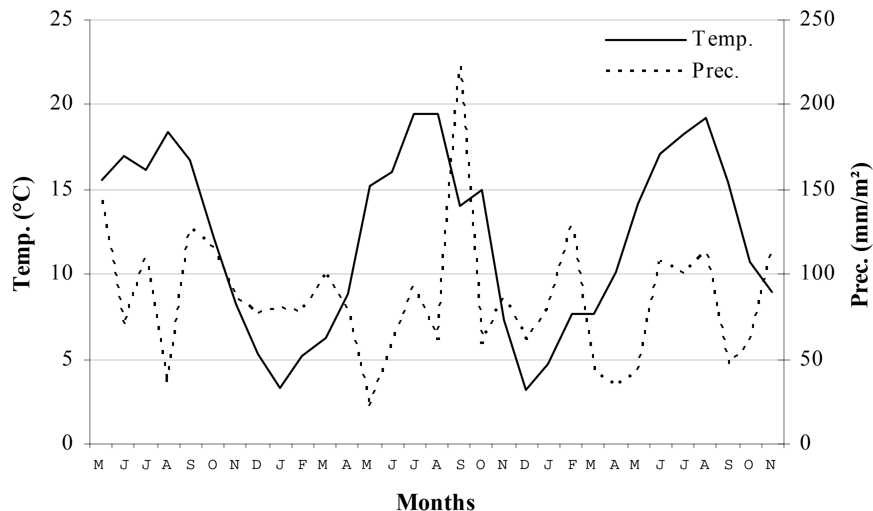


Fig. 9. Average daily air temperature and monthly precipitation during the study period. Both temperature and precipitation were measured in a nearby official weather station of the Royal Meteorological Institute of Belgium (Stabroek, Antwerp).

substrates, part of the difficulty is due to the close relationship between host physiology, life history and changing nutritional quality of the resource with seasons or time. Sporulation of taxa soon after the resource senescences is probably controlled by endogenous changes within the resource while sporulation on the dead resource is likely to be influenced more by environmental variables. Changes in resource quality could however have a substantial impact on fungal dynamics (Gessner *et al*., 1997; Unpublished data). During our study, sporulation of many taxa started already during winter, with maximal species richness recorded in March-April which meant that temperatures were still below 11°C and night frost was not uncommon. However, diversity increased till the start of the summer (Fig. 8A). It would seem that coelomycetes can bear lower temperatures (Fig. 8B) (see Anonymous, 1979). Perhaps their fruit body formation is triggered by low temperatures causing sporulation sooner in the season, while ascomycetes were mainly found later in the season, suggesting that their ascomata formation (i.e. fertilization) could be triggered by different factors such as increasing temperatures. The sequential occurrences of teleomorphs and anamorphs could be due to the fact that anamorphs are simpler and thus develop more rapidly (Müller, 1981). The adaptation of fungal phenology to the host's life history was demonstrated by comparing leaf blade and sheath communities. In our study site leaf blades had all but three of their 35 species in common with leaf

sheaths (unpublished data). However, all these species predominantly sporulated on moribund and senescent hanging leaves in autumn, serving as potential inoculum resource for the leaf sheaths. While the same species sporulate on the standing leaf sheaths in spring, closing the circle by inoculating the newly formed leaf blades and sheaths.

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