Microbial Ecology

Fungi on Leaf Blades of *Phragmites australis* in a Brackish Tidal Marsh: Diversity, Succession, and Leaf Decomposition

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Abstract

Although fungi are known to colonize and decompose plant tissues in various environments, there is scanty information on fungal communities on wetland plants, their relation to microhabitat conditions, and their link to plant litter decomposition. We examined fungal diversity and succession on *Phragmites australis* leaves both attached to standing shoots and decaying in the litter layer of a brackish tidal marsh. Additionally, we followed changes in fungal biomass (ergosterol), leaf nitrogen dynamics, and litter mass loss on the sediment surface of the marsh. Thirty-five fungal taxa were recorded by direct observation of sporulation structures. Detrended correspondence analysis and cluster analysis revealed distinct communities of fungi sporulating in the three microhabitats examined (middle canopy, top canopy, and litter layer), and indicator species analysis identified a total of seven taxa characteristic of the identified subcommunities. High fungal biomass developed in decaying leaf blades attached to standing shoots, with a maximum ergosterol concentration of 548 ± 83 μg g^{-1} ash-free dry mass (AFDM; mean ± SD). When dead leaves were incorporated in the litter layer on the marsh surface, fungi experienced a sharp decline in biomass (to 191 ± 60 μg ergosterol g^{-1} AFDM) and in the number of sporulation structures. Following a lag phase, species not previously detected began to sporulate. Leaves placed in litter bags on the sediment surface lost 50% of their initial AFDM within 7 months ($k = -0.0035$ day^{-1}) and only 21% of the original AFDM was left after 11 months. Fungal biomass accounted for up to 34 ± 7% of the total N in dead leaf blades on standing shoots, but to only 10 ± 4% in the litter layer. These data suggest that fungi are instrumental in N retention and leaf mass loss during leaf senescence and early aerial decay. However, during decomposition on the marsh surface, the importance of living fungal mass appears to diminish, particularly in N retention, although a significant fraction of total detrital N may remain associated with dead hyphae.

Introduction

Emergent macrophytes are known for their high production potential. As a result, standing-dead shoot mass in marshes is often large and macrophytes play critical roles in carbon and nutrient dynamics of these ecosystems [46]. Emergent macrophytes can also be hotspots of biodiversity by providing shelter and food or substrate for a wide range of organisms [24, 67]. There is increasing evidence suggesting that fungi are the primary microbial decomposers colonizing the shoots of these plants (e.g., [38, 55]). This includes leaves of the common reed, *Phragmites australis* (Cav.) Trin. ex Steud. [18, 21, 39], a cosmopolitan grass that often forms extensive monospecific stands in freshwater and brackish wetlands under various water regimes. Fungi are well adapted to the diel wetting and drying cycles that characterize environmental conditions during standing-dead plant decay [36, 37, 39, 53]. Therefore, it is not surprising that fungal communities associated with *P. australis* [1, 69, 70, 75] and other wetland plants [7, 26, 32] are remarkably diverse.

Anatomical and chemical features of *P. australis* leaves change with shoot height, resulting in habitat conditions for fungi that vary with vertical position in the canopy. In upper, sun-exposed leaves, the number of stomata is greater [73] and the cuticle and epidermal cell walls are generally thicker [72]. Phytoliths, which may hamper fungal penetration of leaf surfaces [61], are more

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numerous in upper leaves (Deleebeeck, unpublished data, 2000). Furthermore, microclimatological conditions such as temperature and humidity vary with shoot height and may thus influence fungal colonization and growth in different canopy layers (e.g., [9, 39]). For example, the lower and middle portions of the canopy are more sheltered, resulting in less extreme variation in temperature and humidity between day and night [60]. Because both factors are important determinants of fungal activity in standing-dead shoots [39, 48], differences in temperature and humidity regimes in the canopy are likely to have consequences for fungal decomposers.

The natural decay pattern of *P. australis* leaves and other emergent macrophytes is characterized by an initial decay phase with leaves remaining attached to standing-dead shoots and subsequent decomposition occurring after leaves collapse to the litter layer on sediments or soils (e.g., [20, 21, 38, 51]). Initial decay of attached leaves proceeds sequentially from the shoot base to its tip, following the gradual senescence and death of leaves at different vertical canopy positions. This process may take several weeks to months [21, 50, 57]. It is during this period that leaves are colonized by fungi [1, 35, 47], concomitant with significant leaf mass loss (up to 28% for *P. australis*) [21] that is partly mediated by microbial decomposers [21, 39, 49]. Fungi may also influence the nutrient dynamics of standing-dead marsh plants, especially if fungal biomass accrual is high, and the C/N and C/P ratios are lower than those of decomposing plant tissues. When leaves eventually drop from the parent shoot, decomposition proceeds on the marsh surface (e.g., [20, 28, 58]). The radically different environmental conditions to which decomposers are exposed here may lead to important changes in fungal activity or species composition or both [65, 70]. Fungal biomass can dramatically decline at this stage, as observed in leaves of the rush, *Juncus effusus* [38], although this phenomenon may not be universal [33].

Given the significance of fungi on wetland plants and the current scarcity of information, this study aimed at elucidating fungal community structure on leaf blades during decomposition of leaves both naturally attached to standing shoots (living and dead) and in the litter layer of a brackish tidal marsh. We hypothesized that the fungal community establishing in the plant canopy undergoes major changes when leaves fall to the marsh surface. In addition, we tested whether the relatively small differences in habitat conditions at different layers in the plant canopy are important enough to give rise to distinct patterns of fungal sporulation and their changes over time. These aims were achieved by periodically screening leaves in the litter layer and at two canopy heights for fungal sporulation structures. Finally, we hypothesized that fungi play a major role in the mass loss and nitrogen dynamics of decaying leaf blades in the litter layer. This last question was explored by determining the magnitude and dynamics of fungal biomass in relation to litter decomposition and by estimating the N content in litter that can be accounted for by fungal biomass.

Materials and Methods

Study Site. The study was carried out in a brackish tidal marsh of the Scheldt Estuary located 53.9 km inland near Doel, The Netherlands (51°21′N, 4°14′E) (Table 1). Vegetation of the 50-ha marsh mainly consists of monospecific stands of *P. australis* with fringes of *Scirpus maritimus* L. near the river. The study site was about 50 m from the landward margin and 60 m from the river (Table 1). Growth of *Phragmites* in the marsh starts at the end of April and ends in August [62]. As shoots grow taller and form new leaves at the tip, leaves lower in the canopy start to senesce and to be dropped in late June or early July. However, most leaves do not senesce until September; they typically fall 4–6 weeks later, and some of the uppermost leaves remain attached to shoots even during winter ([62]; G. van Ryckegem, personal observations).

Field Procedures and Sample Processing. Seven different sets of samples were collected during the study

Table 1. Selected characteristics of the investigated tidal marsh

Variable	*Mean ± SD*	*N*
Tidal marsh		
Flooding frequency (%)[a]	15	
Estimated average flood height (cm)[b]	17	
Sedimentation rate (mm year^{-1})[c]	34	
Water chemistry[d]		
pH	7.59 ± 0.23	11
Cl^- (mg L^{-1})	2455 ± 1510	19
NH_4^+–N (mg L^{-1})	0.46 ± 0.35	21
NO_2^-–N (mg L^{-1})	0.06 ± 0.03	21
NO_3^-–N (mg L^{-1})	4.9 ± 0.65	21
Total P (mg L^{-1})	0.73 ± 039	21
Reed stand		
Shoot height (cm)	196 ± 21	20
Stem diameter at first internode (mm)[b]	4.0 ± 0.2	60
Density of living shoots (m^{-2})[e]	191 ± 65	6
Aboveground biomass (g m^{-2})[e]	927 ± 293	6
Leaf biomass (g m^{-2})[e]	320 ± 131	6

[a]Flooding frequency is the relative number of times high tides are higher than the marsh surface and are thus supposed to flood the marsh (Meganck, unpublished data, 1998).
[b]From (Meganck, unpublished data, 1998).
[c]Calculated as 157 × (flooding frequency) + 10; $r^2 = 0.52$, $P < 0.001$ [69].
[d]Annual average in 2002 (Flemish Environment Agency, Belgium; site code 154100, http://www.vmm.be).
[e]Estimated at end of the growing season (September 10, 1997) by harvesting all aboveground living reed matter in six 0.25-m^2 quadrats; biomass ≈ annual aboveground net production (Meganck, unpublished data, 1998).

period. This included four sets of leaf samples from the plant canopy: one set each from the middle and top canopy in each of two successive years (Cohort 2000 and Cohort 2001). Two additional sets of samples were collected from the litter layer of the marsh, corresponding to leaves from the two cohorts after leaves had dropped to the ground. These samples from the canopy and litter layer were used for fungal community analyses. In addition, dead leaves collected from standing shoot of the 2001 cohort were enclosed in litter bags to determine fungal biomass, litter mass loss, and nitrogen concentrations of leaves decomposing at the marsh surface (see below). These litter bag samples constituted the seventh sample set. Each of the seven sample sets consisted of multiple individual samples taken over time.

Ten leaf blades were collected from plant shoots every 4 weeks from May 2000 to July 2001 (Cohort 2000), and from May 2001 to July 2002 (Cohort 2001) until no more leaves remained attached to shoots. Collection of leaves from the litter layer started in the last week of November for both the 2000 and 2001 cohort. All samples were taken from fenced plots (3 × 2 m, 1.4-m-tall wire netting, 1-cm mesh) to prevent input of leaves of unknown age and origin. In addition, 10 leaf blades collected from the litter layer were enclosed in each of 12 litter bags (35 × 20 cm, 4-mm mesh) in November of both 2000 and 2001. Later, the bags were periodically retrieved over 9 months. All collected samples were placed in clean plastic bags and kept in the laboratory at 4°C for up to 2 weeks before they were inspected for fungal sporulation structures.

Additional litter bags were prepared in October 2001 to determine fungal biomass, litter mass loss, and litter nutrient dynamics. Fully brown leaves were collected from standing shoots at middle height, about 1 m above ground. Only leaves that were easy to detach from shoots were taken. Samples were returned to the laboratory and used to fill 38 litter bags with 5.0 g fresh mass. The next morning, litter bags were placed in the marsh in a mown plot, with all litter removed. The bags were positioned flat on the ground and secured with pegs. Ten bags were immediately returned to the laboratory, where the leaves were dried for 72 h at 40°C to determine initial dry mass (4.70 ± 0.09 g; mean ± SD). Subsequent sets of two bags were retrieved at monthly intervals over 12 months until leaves were almost completely decomposed. In the laboratory, leaves were thoroughly rinsed with distilled water to remove adhering clay and invertebrates. One leaf or a few fragments from each litter bag were blotted dry, freeze-dried, weighed, ground (2-mm mesh screen), and preserved in methanol–KOH (8 g L^{-1}) [23]. These samples were used to determine fungal biomass within 1 month after collection. The remaining leaves were dried at 40°C for 72 h to determine the mass remaining and carbon and nitrogen contents.

Fungal Identification and Estimation of Abundance. Leaf samples for fungal identification were maintained at 4°C and screened within 2 weeks under a dissecting microscope (180×) for mature sporulation structures. This involved preparing squashed slides of encountered sporulation structures (e.g., ascoma). Fungi were classified into four broad taxonomic groups (Ascomycetes, Basidiomycetes, Coelomycetes, and Hyphomycetes) [31]. They were microscopically identified by using the procedures described by Barnett and Hunter [2], Dennis [12], Ellis [14, 15], Ellis and Ellis [16], Sutton [64], and the extensive original taxonomic literature. Detailed descriptions of diagnostic features and illustrations of all identified taxa are available at http://intramar.ugent.be/nemys/fungi/web/Phragmiticolous%20fungi.asp. A taxon was scored as present on a leaf when at least one sporulation structure was found. Because there were 10 leaves per sample, this resulted in relative frequencies of occurrence of fungal taxa per sample ranging from 0% to 100% at 10% intervals.

Fungal Biomass. Fungal biomass in leaf material was determined from ergosterol concentrations using solid-phase extraction and high-performance liquid chromatography (HPLC) [22, 23]. The chromatographic systems consisted of a Kratos Analytical Instruments Spectroflow 400 with a Spectroflow 757 UV detector. The system was run isocratically with 100% methanol at a flow rate of 1.5 mL min^{-1} and an RP18 LiChrosphere column [23]. Column temperature was maintained at 30°C. Ergosterol was detected by UV absorbance at 282 nm. Peak area was quantified with an Applied Biosystems Model 610A data analysis system version 1.2.2. Conversion factors for calculating fungal biomass and fungal N content in litter were 5.8 mg ergosterol per g fungal dry mass [18], and 65 mg N per g fungal dry mass, as determined for fungal strains isolated from *P. australis* [18].

Carbon, Nitrogen, and Ash Content. The leaf material remaining from litter bags in the decomposition experiment was ground to pass a 2-mm mesh, and 5–8 mg was used to determine carbon and nitrogen content with a Carlo Erba NA-1500 elemental analyzer [11]. Nitrogen concentration in decomposing litter was corrected for the associated sediment, which had a lower nitrogen concentration than the leaf material (average N = 0.5%; Flemish Environment Agency, http://www.vmm.be) [68], based on an assumed 10% ash content of plant origin, as found in leaf blades attached to shoots [3, 30]. Ash content was determined by combustion of 250-mg ground subsamples for 4 h at 550°C [8].

Data Analysis. Litter mass loss data were fitted to the simple exponential model $m_t = m_o \cdot e^{-kt}$, where m_t is litter ash-free dry mass (AFDM) remaining after time t,

m_o is the original AFDM, and k is the decay coefficient [5]. Nonlinear regression analysis was used on untransformed data to estimate parameters (e.g., [8]) using the default settings in SYSTAT, version 10.2. Pearson correlation analysis was used to relate fungal biomass data with relative occurrence of sporulation records and with nitrogen concentrations during decomposition.

The frequency of occurrence (%) of a taxon was calculated as follows: $X_{m,t} = \frac{\sum records\ of\ taxon\ X_{m,t}}{number\ of\ leaves\ examined\ at\ t} \times 100$, where the subscript letter m represents the microhabitat (i.e., top or middle height of standing shoots or litter layer) from which a taxon was collected and t is time (months). Calculation of diversity and similarity indices [34] were performed with PC-ORD version 4.26 [44]. Shannon's diversity index was calculated as $H' = -\sum_i^S p_i \ln p_i$, where S is the number of taxa in the community, and $p_i = n_i/N$ with (n_i = number of records of taxon i and N = total number of records). Similarities between communities were assessed with Jaccard's index: $S_j = a/(a + b + c)$, where a is the number of taxa shared between two groups, b is the number of taxa restricted to the first group, and c is the number of taxa restricted to the second group. Sørensen's (Bray–Curtis') quantitative index was used for comparisons based on species' relative occurrences: $S_s = 2w/(x + y)$, where w is the sum of shared abundances and $x + y$ is the sum of abundances in the individual groups.

Multivariate statistical analyses were run with PC-ORD version 4.26 [44]. Detrended correspondence analysis (DCA) was used to reconstruct the gradients and groupings of samples in the dataset. Default settings were used. Relative Euclidean correlation was calculated to express the proportion of variability explained by the axes [43, 44]. Cluster analysis was used to identify groupings of samples based on similarity in community composition of sporulating fungi. The Bray–Curtis distance measure and the group average linkage method were used in the cluster analysis, and the presented dendrogram was scaled by the percentage of remaining information [43, 44]. Clusters were tested for significance with the multiresponse permutation procedure, which tests the null hypothesis that there is no difference among identified groups [4]. Euclidean distance was used in this test, and groups were weighted by $n/\Sigma n$ as recommended by McCune and Mefford [44]. P values were Bonferroni-corrected [59]. Seven outliers (including three without fungal records) were omitted from these analyses, resulting in a total of 37 samples.

Indicator species analysis was used to discern which taxa were driving the differences among groups identified by cluster analysis [13]. The indicator value (IV) for a taxon was determined by combining relative frequency and relative abundance in a given group; it can range from 0 (no indication) to 100 (perfect indication, meaning the taxon was present in all samples in a given group and absent from all samples in all other groups). Following the procedure described by Dufrêne and Legendre [13], an IV threshold of 25% was chosen, and a Monte Carlo simulation (1000 runs) was used to determine the significance (set at $P \leq 0.01$) of species IV.

Results

Fungal Diversity and Succession. A total of 35 fungal taxa were recorded on living green and decaying leaf blades (Table 2). Fourteen taxa (40%) were Coelomycetes, 12 (34%) Ascomycetes, six (17%) Hyphomycetes, and only three (9%) were Basidiomycetes. Of all 513 fungal records, 45% (233) were Coelomycetes, 37% (189) Ascomycetes, 13% (65) Hyphomycetes, and only 5% (26) Basidiomycetes. The communities of fungi sporulating on leaf blades were highly similar in both years (Cohort 2000 vs Cohort 2001; Bray–Curtis similarity

Table 2. Fungal taxa recorded on living and decomposing leaf blades of *P. australis* attached to shoots and deposited in the litter layer of a brackish tidal marsh

Taxa	*No. of records*
Septoriella sp(p).	81
Phoma sp. III	70
Cladosporium sp(p).	49
Phomatospora berkeleyi Sacc.	49
Phaeosphaeria sp. III	48
Didymella glacialis Rehm	38
Hendersonia sp. I	19
Puccinia phragmitis (Schumach.) Körn	18
Stagonospora vexata Sacc.	17
Neottiosporina australiensis B. Sutton & Alcorn	14
Didymella sp	13
Phaeosphaeria sp. II	13
Pseudoseptoria donacis (Pass.) B. Sutton	11
Deightoniella roumegueri (Cavara) O.Constant.	10
Phaeosphaeria pontiformis (Fuckel) Leuchtm.	8
Halosphaeria hamata (Höhnk) Kohlm.	7
Puccinia magnusiana Körn	6
Coelomycete sp. I	5
Phaeosphaeria eustoma (Fuckel) L. Holm s.l.	5
Hendersonia culmiseda Sacc	4
Massarina arundinacea (Sowerby: Fr.) Leuchtm.	4
Microsphaeropsis sp. I	4
Arthrinium phaeospermum (Corda) M.B. Ellis	3
Ascochyta cf. *arundinariae* Tassi	2
Hendersonia aff. *culmiseda* Sacc.	2
Massarina fluviatilis Aptroot & Van Ryck.	2
Phoma sp. IIa	2
Sporobolomyces sp.	2
Alternaria alternata (Fr.) Keissl.	1
Anthostomella punctulata (Roberge) Sacc.	1
Coelomycete sp. II	1
Dictyosporium oblongum (Fuckel) S. Hughes	1
Periconia cf. *cookei* Mason & M.B. Ellis	1
Phaeosphaeria culmorum (Auersw.) Leuchtm.	1
Stagonospora incertae sedis III	1

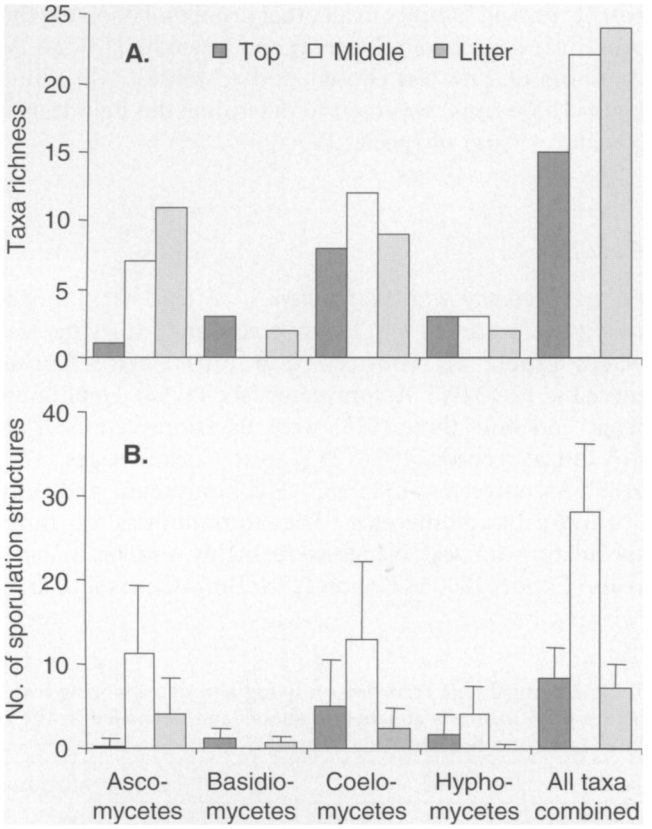

Figure 1. Fungal species richness and average number of sporulation structures of different fungal taxa on *P. australis* leaf blades in three microhabitats of a brackish tidal marsh. Error bars indicate mean ± 1 SD.

of 76.1%), as was the total number of records (268 vs 245), taxa richness (29 vs 26), and Shannon diversity (2.72 vs 2.74). Only the frequencies of two parasitic species differed: *Puccinia phragmitis*, a rust, was only found in the Cohort 2000 (18 records) and *Deightoniella roumegueri*, a dematiaceous hyphomycete, was only scored in the Cohort 2001 (9 records).

The three microhabitats examined (top canopy, middle canopy, and litter layer) differed in species richness and the number of sporulation structures of different taxonomic groups (Fig. 1). The lowest number of taxa and sporulation structures was recorded in the top canopy, and a significantly higher number of sporulation structures was observed in the middle canopy, compared to both the top canopy or litter layer (ANOVA, $F_{2,37} = 12.4$, $P < 0.01$, Tukey HSD pairwise comparisons of middle vs top canopy and middle canopy vs litter layer with $P < 0.01$ and $P < 0.001$, respectively). Sexual stages of Ascomycetes were virtually restricted to the middle canopy, whereas the asexual sporulation structures of Coelomycetes dominated the fungal community sporulating on leaf blades in the top canopy. Basidiomycetes and Hyphomycetes did not show a clear pattern.

DCA revealed different compositions of sporulating species in the three microhabitats, which were consistent between years (Fig. 2). All but one canopy samples were placed on the left along axis 1, whereas nearly all samples from the litter layer clustered on the right. In addition, axis 2 separated samples from the top and middle canopy. Cluster analysis indicated a total of four subcommunities (Fig. 3): a top and middle canopy community on leaves attached to standing shoots and an early and late successional community in the litter layer. Samples clustering in the branch designated early litter (EL) samples in Fig. 3 were typically less than 3 months in the litter layer and ranked between the middle canopy (MC) and late litter (LL) samples. Pairwise comparisons by multiresponse permutation procedure showed that the four subcommunities were all significantly different from one another ($P < 0.05$ after Bonferroni correction). Indicator species analysis identified five taxa as indicative of the community on middle-canopy leaves and one taxon each

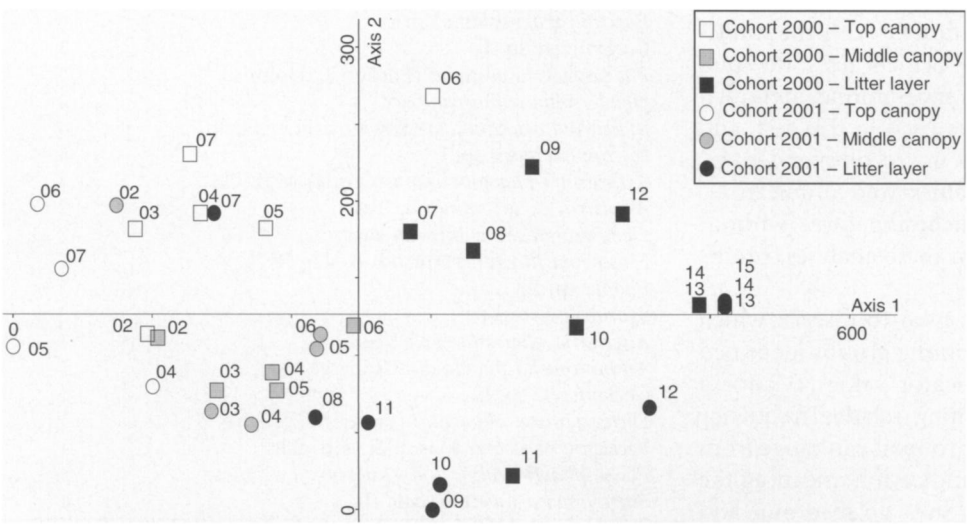

Figure 2. Projection of communities of sporulating fungi on leaf blades of *P. australis* in the canopy and litter layer of a brackish tidal marsh. Detrended correspondence analysis with eigenvalue: axis 1 = 0.77, axis 2 = 0.34; length of gradient: axis 1 = 5.10, axis 2 = 2.69. The first two axes account for 55% of the total variability in the data set. Numbers indicate collection months (1–15) with May = 1.

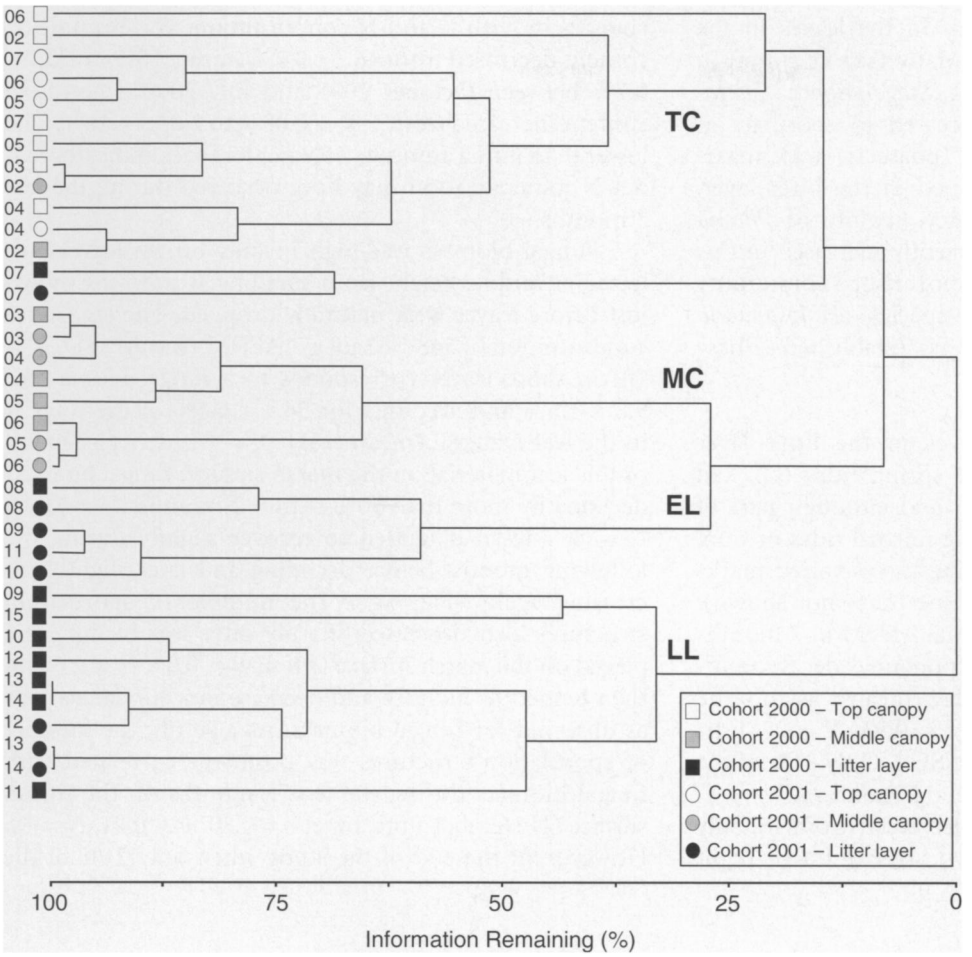

Figure 3. Cluster analysis of fungal communities in 37 leaf blade samples from a brackish tidal marsh dominated by the emergent macrophyte, *P. australis*. Bold letters TC (top canopy samples), MC (middle canopy samples), EL (early litter samples), and LL (late litter samples) designate the recognized major subcommunities of sporulating fungi. Leaf blades were screened for fungal taxa starting in May 2000 and 2001, respectively. Numbers indicate collection months (1–15) with May = 1.

of the top-canopy and late litter communities (Table 3). No specific indicators were found for the early litter samples.

The temporal sequence of fungal sporulation structures differed among microhabitats in regard to both composition and timing. In the litter layer, samples tended to be arranged in sequential order from left to right along axis 1 of DCA, and a similar trend was observed for samples from the middle canopy (Fig. 2). In contrast, no clear temporal pattern was apparent for the top canopy. Although not clear-cut, similar trends are also suggested by cluster analysis (Fig. 3). In the canopy, phase I was characterized by an initial pioneer community with low diversity, followed by a gradually developing mature community (phase II) with higher diversity, as indicated by positive correlations between collection date and Shannon diversity (middle canopy $r = 0.66$, $P = 0.04$, $N = 10$; top canopy: $r = 0.63$, $P = 0.054$, $N = 10$). Phase I in the middle canopy was characterized by the sporulation of *Septoriella*. The end of phase I in the middle canopy coincided with leaf senescence and the appearance of specific indicator taxa for phase II such as *Didymella glacialis*, *Microsphaeropsis* sp. I, and *Stagonospora vexata*. The successional sporulation phases in the top canopy are less well characterized by indicator taxa; *Septoriella* and *Cladosporium* were the most typical taxa of phase I, and the former remained dominant in phase II of succession after leaf senescence. In the litter layer, the initial stage was a transition phase characterized by a drop in taxa richness and the disappearance of sporula-

Table 3. Indicator values and Monte Carlo *P* values for species with indicator values >25 and Monte Carlo *P* values <0.01, for three of the four distinct subcommunities of sporulating fungi identified by cluster analysis (Fig. 3)

Indicator taxon	*Fungal subcommunity*	*Indicator value*	*P*
Didymella sp.	Middle height canopy	87.5	0.001
Phaeosphaeria sp. III	Middle height canopy	82.8	0.001
Cladosporium sp(p).	Middle height canopy	65.7	0.002
Neottiosporina australiensis	Middle height canopy	64.7	0.001
Didymella glacialis	Middle height canopy	55.3	0.005
Phomatospora berkeleyi	Late litter layer	94.5	0.001
Septoriella sp(p).	Top height canopy	48.3	0.008

No particular taxa were associated with the early litter layer community.

tion structures previously present on the leaves in the canopy (black arrows in Fig. 4). Many taxa common in the canopy, such as *Phaeosphaeria*, *Stagonospora*, *Cladosporium*, and *Septoriella* species, ceased to sporulate at this stage. A mature community (phase II) with maximum diversity was not well defined in the litter layer, although the sporulation frequency, mainly of *Phoma* sp. III, and the taxa richness transiently increased in this microhabitat (Fig. 4) before an impoverished community with few dominant sporulating species (*Halosphaeria* hamata and *Phomatospora berkeleyi*) established (Phase III).

Litterbag Experiment. Leaves in the litter layer were fully covered by mud after spring tides (G. Van Ryckegem, personal observation), and although part of the deposits was washed off during normal tides or rain, the ash content of the decomposing leaves varied markedly (9–51% of dry mass) over time (data not shown). Leaves lost about 50% of their initial AFDM in 7 months (Fig. 5A), corresponding to an exponential decay coefficient, k, of -0.0035 ± 0.0002 per day [mean ± asymptotic standard error (ASE); corrected $r^2 = 0.93$, $N = 27$, estimated $m_o = 96.8 \pm 2.4\%$ (mean ± ASE)]. Only 21% of the original leaf AFDM remained after 11 months.

The C/N ratio of leaf litter decreased from initially 24.8 to 16.6 in July 2002 (Fig. 5B), as a result of slight changes in both C and N concentration. For example, C content decreased from 46.2 ± 0.2% (mean ± SD) to 33.7 ± 0.7% between October 2001 and July 2002, whereas N content increased from 1.86 ± 0.08% to 2.03 ± 0.16%. The lower than initial amounts of N per leaf pack indicates that net N mineralization may have occurred during the last 3 months.

Fungal biomass was high in fully brown leaves collected at middle height from standing *P. australis* shoots just before leaves were naturally dropped. The ergosterol concentration of 548 ± 83 μg g^{-1} AFDM (mean ± SD, N = 10) in those leaves corresponds to a fungal biomass of 9.8 ± 1.6% and accounts for 34.4 ± 6.6% of the total N in the leaf material (mean ± SD, $N = 4$). After placement of this leaf material on the marsh surface, fungal biomass declined by more than 50% within 2 months ($P = 0.002$, $N = 4$, t test); it tended to recover slightly during the following months before declining and later slightly increasing again (Fig. 5C). The number of sporulation structures also decreased sharply after leaf blades were placed on the marsh surface (t test, $P = 0.04$, $N = 2$), and then tended to increase and decrease in a similar pattern as observed for fungal biomass. As a result, the number of sporulation structures was positively correlated with fungal biomass during the first 8 months on the marsh surface (November until June; $r = 0.80$, $P < 0.01$, $N = 8$). However, at the end of the study when only 21% of the

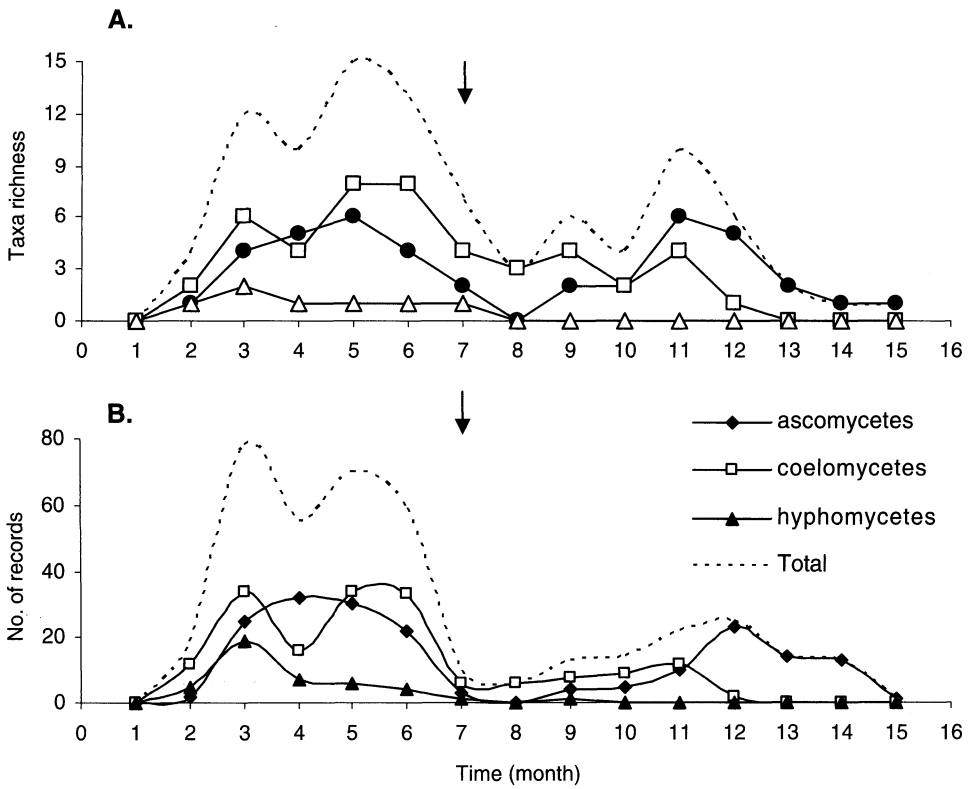

Figure 4. Fungal taxa richness (A) and relative occurrence of sporulation structures (B) during growth, senescence, and decay of *P. australis* leaf blades at middle canopy height and in the litter layer. Single records of taxa were excluded. *Black arrows* point to the first collection of leaf blades after 1 month of decomposition in the litter layer. Numbers along the *x-axis* indicate collection months (1–15) with May = 1. The legend applies to both panels.

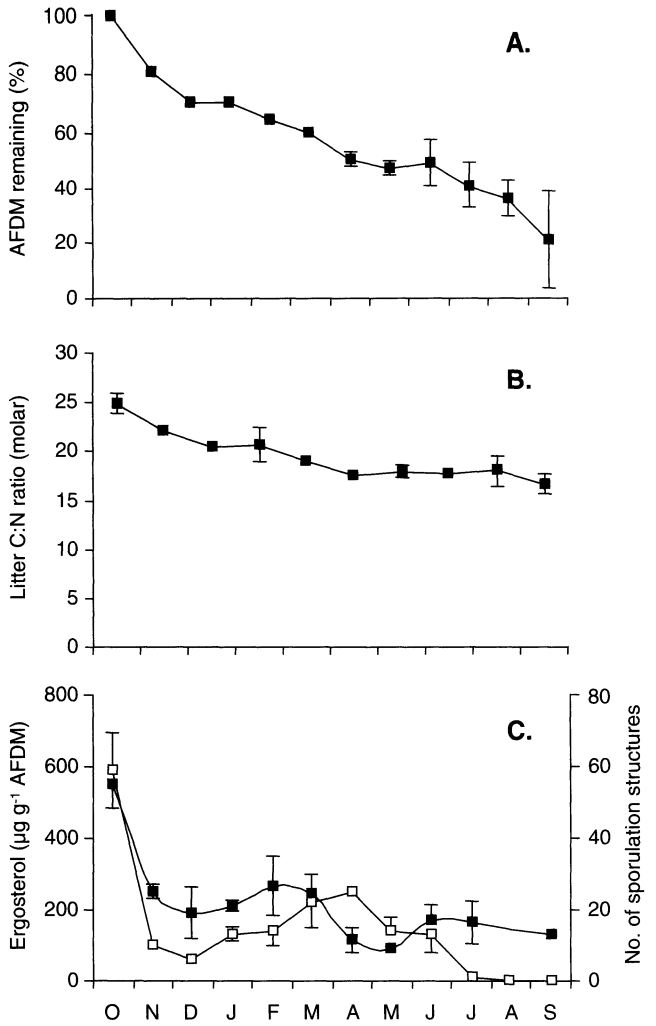

Figure 5. Mass loss (A), changes in the C/N ratio (B), and dynamics of fungal biomass as ergosterol content (■) and sporulation structures (□) (C) in leaf litter bags of *P. australis* placed on the sediment of a brackish tidal marsh. Error bars indicate ±1 SD with $N = 4$, except for June, July, and September, where $N = 3$ for ergosterol and $N = 2$ for sporulation structures.

initial AFDM remained, fungal biomass was still sizable (127 ± 8 µg ergosterol per g AFDM, $N = 3$), whereas sporulation structures could not be detected at this stage.

Discussion

Fungal Diversity and Community Composition on Leaf Blades. The number of fungal taxa we observed on leaf blades attached to standing *P. australis* shoots (29 taxa) is comparable with the richness noted by Apinis *et al.* [1] at their most thoroughly studied site (27 taxa). Although both study sites are geographically close (southern England and Dutch–Belgian coast), they had less than one third of the taxa in common and only two of the 10 common genera sporulating on *P. australis* leaf blades in the canopy at our study site (*Cladosporium* and *Phaeosphaeria*) were also recorded by Apinis *et al.* [1]. This low degree of similarity may to some extent reflect methodological differences. Part of the records by Apinis *et al.* [1] was obtained from leaf disks placed on agar plates, an indirect cultivation method favoring the development of Hyphomycetes that grow rapidly on laboratory media [17, 29]. In contrast, our direct observation of sporulation structures was likely to reveal a greater proportion of sexual stages of species, which often develop slowly but may have been more active in the field. Storage of our samples at 4°C for up 2 weeks did not bias our results, as suggested by the similar community structure noted on a collection of middle-height leaves screened in August 1999 both immediately after collection and again 2 weeks later: A total of 9 taxa, 8 each before and after incubation, occurred at similar frequencies (paired t test, $P = 0.40$, $N = 9$).

In addition to possible methodological differences, the dissimilarity in fungal community composition between studies is likely to be related to differences in environmental conditions or plant characteristics or both. For example, Van Ryckegem and Verbeken [69] found large differences in community composition among four sites along a salinity gradient: Only 13% of the sporulating taxa in the litter layer were shared among all sites with a Jaccard similarity index between 0% and 40% for any two sites (see Table 3 in Van Ryckegem and Verbeken [69]).

Vertical Variation. Results of the present study support the contention that middle-canopy leaves are a more benign habitat for fungi than the more exposed, tougher leaves in the upper shoot regions. As hypothesized, species diversity and especially the number of sporulation structures were higher at middle-canopy height (Fig. 1). The virtual restriction of sexual stages of Ascomycetes to the middle canopy and the dominance of Coelomycetes in the top canopy has also been found on leaf sheaths [70] and is consistent with the higher fungal biomass observed in lower regions of the canopy in other reed marshes [21].

Our records of sporulation structures only revealed relative abundances of sporulating taxa and disregard variation in terms of the number of sporulation structures, spore release, or biomass. Different taxa recorded as dominant may vary substantially in their activity and importance. For example, *Cladosporium* and *Septoriella* were both dominant with a comparable percentage of occurrence during the first 3 months in the middle canopy. However, *Cladosporium* spp. infect their host via stomata and, as seen on reed leaves in the present study, the initial patches they occupy are restricted to a single stoma. Patches colonized by *Septoriella*, in contrast,

appeared to be much larger and to contain multiple and larger fruitbodies, suggesting that both biomass and spore expulsion of *Septoriella* were much higher than for *Cladosporium*. Our data on fungal diversity in the plant canopy should therefore not be interpreted in terms of fungal biomass or activity. Consequently, the greater fungal biomass, activity, and ascospore expulsion of decaying upper leaves of standing-dead cordgrass shoots [47, 48] are not necessarily at variance with our observation of lower diversity in the top canopy. However, a notable difference between the sporulating fungal communities on *Spartina* and *Phragmites* is the low diversity and scarcity of asexual fungi on *Spartina*; one exception is *Stagonospora* spp., which are relatively common during early decay stages [26].

Most fungal sporulation appeared to be restricted to either the middle or top canopy. This tendency, which was also noted by Apinis *et al.* [1] and Van Ryckegem and Verbeken [69, 70], results in two distinct subcommunities of sporulating fungi within the plant canopy. Such distinct vertical distribution patterns may be explained by vertical differences in habitat conditions [56], because both leaf tissue quality and environmental conditions change from the shoot base to its tip (e.g., stomata density, cell wall thickness, temperature, and humidity regime; see Introduction). A time lag in leaf senescence of the upper shoot region [21], which was also noted in the present study, was apparently unimportant in structuring fungal communities, because only a few taxa (e.g., *Septoriella*) were found to first sporulate on leaves in the middle canopy and later in the top canopy as leaf senescence and death proceeded from the shoot base toward the tip.

Fungal Succession. Growth, senescence, and decomposition of leaves on *P. australis* shoots is a continuous process [27] that is accompanied by changes in the sporulation of fungal taxa (Fig. 4). In the present study, these temporal changes are reflected in both species diversity and relative frequency of occurrence of the sporulating taxa in leaves attached to shoots at all stages of growth, senescence, and decomposition (Fig. 4). However, the temporal sequence of sporulating fungi on attached leaf blades suggests gradual changes rather than replacement of the sporulating taxa (cf. [19, 25]), and the different successional phases are not well characterized on leaf blades. This is in contrast to the more clearly defined sporulation stages on leaf sheaths and stems [70, 71]. The latter could be attributable to the higher diversity of taxa of which several have a common sporulation period. Additionally, stems are broken down slowly and harbor more species that mature slowly and sporulate over longer periods, making it easier to map changes in sporulation over time.

When leaves fall on the sediment [Figs. 4 (black arrows) and 5C], successional events involving distinct shifts in sporulating species are initiated (Figs. 2 and 4), as has been noted by Apinis *et al.* [1]. This shift in species composition after leaves enter the litter layer was associated with a rapid disappearance of sporulation structures of most taxa previously present, even though a few continued to sporulate for a few months (Figs. 4 and 5C). Concomitantly, fungal biomass (ergosterol) rapidly decreased by as much as 65% (Fig. 5C). Comparable deleterious effects have been reported for fungi on *P. australis* leaf blades [65, 66] and other emergent macrophytes such as *Spartina alterniflora* [52] and *J. effusus* [38]. In a study with *P. australis* in a freshwater marsh, fungal biomass decreased only after an initial increase, although fungal growth rate did decline sharply immediately after submergence of leaves collected from decaying standing shoots [33]. Furthermore, under submerged conditions, bacterial productivity exceeded that of fungi [6]. Thus, it is clear that fungal communities can undergo drastic changes as leaves colonized during attachment to standing shoots move to a periodically or permanently aquatic environment on the sediment surface of marshes.

Such large changes in fungal community structure, biomass, growth, and reproduction are likely to be related to the distinct environmental conditions in the litter layer compared to the plant canopy. The strong decline in ergosterol suggests that ergosterol was not particularly persistent in litter after fungal death. This is in contrast to the relatively high persistence observed in laboratory experiments [45]. Fungi in the litter layer of our study marsh experience periodic inundation by tidal water (Table 1), which causes sudden salinity shifts and regular smothering of leaf surfaces by a fine mud layer resulting from often high loads of suspended clay particles in the floodwater. Sediments are deposited at the margin of the marsh at an estimated rate of 34 mm $year^{-1}$ [68]. Shear stress by tidal floodwater aggravates these unfavorable habitat conditions for fungi in the litter layer, particularly for terrestrial Hyphomycetes with their fragile conidiophores. Tidal wetting yet appears to be too infrequent, or smothering by sediment deposits too important, for truly aquatic fungi to colonize the litter layer [69]. Thus, it is likely that the large changes we observed when leaf blades fell on the marsh surface were at least partly attributable to the harsh conditions to which leaf blades and their fungal colonizers became exposed in the litter layer.

Such reductions in biomass and sporulation observed after 1 month on the marsh surface are not at variance with a short-term stimulation of fungal activity when decaying leaves of marsh plants are periodically wetted [36, 39, 53, 49]. In contrast to short-term wetting events, the leaves incorporated in the litter layer become permanently exposed to very different environmental conditions compared to the conditions prevalent in the

canopy. That even less dramatic changes in environmental conditions can lead to both a significantly reduced fungal productivity and sporulation was shown by Newell *et al.* [50] via a prolonged misting experiment with standing-dead cordgrass shoots.

Temporal changes in fungal biomass (i.e., ergosterol concentration) and the number of sporulation structures were largely synchronized between October and May, although sporulation lagged behind fungal biomass during spring (Fig. 5C). Newell [47] also observed a significant positive relationship between rates of ascospore release from ascomata and fungal biomass within cordgrass leaves. This suggests that growth and reproduction of fungi can be tightly coupled on marsh plants both in leaves attached to standing shoots [47] and on the sediment surface (this study). In advanced stages of leaf decomposition, ergosterol concentrations of about 100–200 $\mu g\ g^{-1}$ AFDM suggested continued fungal presence throughout the present study, although no sporulation was observed on the rather fragile remaining leaf pieces (Fig. 5C). Therefore, it is possible that the fungi present at this stage were largely inactive, or that ergosterol persisted after fungal death [45], or that more typical soil fungi had become established but had not started to sporulate [19].

Litter Decomposition and Nutrient Dynamics. The decay rate of *P. australis* leaf blades observed in the present study is within the upper range of values reported from both brackish [42, 65] and freshwater marshes ([10, 33, 40, 41, 74]; [20] and references herein). A significant portion of litter mass loss could be accounted for by fungal activity: The net fungal biomass increase that occurred between December and February corresponds to a fungal dry mass production of at least 11.9 mg per g leaf dry mass present in litter bags in December. Leaf mass loss during that period was 9%, and if fungal growth efficiency was 35% [63], then 37% of this litter mass loss could be accounted for by fungal activity. Although the evidence presented above indicates that fungal importance in leaf litter decomposition may vary with decomposition stage, and possibly with season, the conservative estimate of 37% illustrates that fungi can contribute notably to leaf decomposition in the litter layer of marshes at least during some decomposition stages.

The poor correlation between fungal biomass and nitrogen concentration of leaves decomposing in the litter layer ($r = -0.45$) indicates that fungi immobilize little nutrients in the litter layer. Based on a nitrogen concentration of 6.5% in fungal biomass [18], living fungi could account for as much as 34% of the total N in leaf blades from the middle canopy just before shedding. This percentage was reduced to 12%, however, after 1 month in the litter layer and accounted on average for 10 ± 4% (mean ± SD) over the entire decay period in the litter layer. This suggests that (1) a significant fraction of N was bound to dead fungal mass (e.g., as glucosamine in the cell walls of dead hyphae) [52, 54], or that (2) a fraction was released from fungi but retained by other chemical or microbial mechanisms, or that (3) N losses were compensated by fresh inputs. One possible source of such fresh inputs was the fine mud transported with the tides and regularly deposited on leaves. As described by Newell *et al.* [52], part of the N associated with these fine particles may have infiltrated the increasingly porous leaf tissue as decomposition proceeded.

Conclusion

In conclusion, our results show that fungi are a rich component of the microbiota on leaves attached to *P. australis* in a temperate tidal marsh, with strikingly different communities sporulating in different vertical layers of the plant canopy. As dead brown leaves fall to the sediment surface, fungal communities experience dramatic declines in both fungal biomass and reproduction. Subsequently, after a lag phase, a characteristic species-poor community begins to sporulate in the litter layer. The litter fungi present at this stage, in particular, *P. berkeleyi* and *Phoma* sp. III, are likely to contribute significantly to litter decay on the tidal marsh surface. Additionally, fungi may be instrumental in nitrogen retention during leaf senescence and early aerial decomposition, although living fungi appear not to play a major role in litter N dynamics during decay in the litter layer of the marsh.

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