Microbial Ecology

The Estimated Impact of Fungi on Nutrient Dynamics During Decomposition of *Phragmites australis* Leaf Sheaths and Stems

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Abstract

Decomposition of culms (sheaths and stems) of the emergent macrophyte *Phragmites australis* (common reed) was followed for 16 months in the litter layer of a brackish tidal marsh along the river Scheldt (the Netherlands). Stems and leaf sheaths were separately analyzed for mass loss, litter-associated fungal biomass (ergosterol), nutrient (N and P), and cell wall polymer concentrations (cellulose and lignin). The role of fungal biomass in litter nutrient dynamics was evaluated by estimating nutrient incorporation within the living fungal mass. After 1 year of standing stem decay, substantial fungal colonization was found. This corresponded to an overall fungal biomass of 49 ± 8.7 mg g^{-1} dry mass. A vertical pattern of fungal colonization on stems in the canopy is suggested. The litter bag experiment showed that mass loss of stems was negligible during the first 6 months, whereas leaf sheaths lost almost 50% of their initial mass during that time. Exponential breakdown rates were −0.0039 ± 0.0004 and −0.0026 ± 0.0003 day^{-1} for leaf sheaths and stems, respectively (excluding the initial lag period). In contrast to the stem tissue—which had no fungal colonization—leaf sheaths were heavily colonized by fungi (93 ± 10 mg fungal biomass g^{-1} dry mass) prior to placement in the litter layer. Once being on the sediment surface, 30% of leaf sheath's associated fungal biomass was lost, but ergosterol concentrations recovered the following months. In the stems, fungal biomass increased steadily after an initial lag period to reach a maximal biomass of about 120 mg fungal biomass g^{-1} dry mass for both plant parts at the end of the experiment. Fungal colonizers are considered to contain an important fraction of nutrients within the decaying plant matter. Fungal N incorporation was estimated to be 64 ± 13 and 102 ± 15% of total available N pool during decomposition for leaf sheaths and stems, respectively. Fungal P incorporation was estimated to be 37 ± 9 and 52 ± 15% of total available P during decomposition for leaf sheaths and stems, respectively. Furthermore, within the stem tissue, fungi are suggested to be active immobilizers of nutrients from the external environment because fungi were often estimated to contain more than 100% of the original nutrient stock.

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Introduction

Decomposition of plant litter is an important component of nutrient cycling in wetlands [32]. After an initial phase of releasing soluble nutrients by leaching, litter often immobilizes nutrients [e.g., 11, 22, 42, 43]. Nutrient immobilization refers to an increase in concentrations or even a net increase in nutrient amount compared to the original stock present at the onset of decomposition. The process of nutrient immobilization is realized by external nutrient incorporation and by the conservation of nutrients within the resource. These processes could be mediated by microbial decomposers or by adsorption [12, 60]. In a later phase of decomposition, mineralization predominates and nutrients are released in the environment. By immobilizing nutrients, litter may be a significant sink for nutrients and may increase the nutrient retention in wetlands [32, 60].

Of all wetlands, common reed [*Phragmites australis* (Cav.) Trin. ex Steud.] dominated wetlands are among

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the most geographically widespread and productive systems [32]. Most of this biomass enters the detrital pool, where it is transformed and mineralized by microbial assemblages and detritus-feeding organisms by means of two major pathways [11, 22, 43, 60]. Those pathways comprise a variable time of standing decay and eventually decay in the litter layer [11, 12, 26].

Apinis *et al.* [1, 2] were the first to describe the fungal species richness both in the reed canopy and in the litter layer. Since that time it has been shown that fungi constitute the major fraction of microbial mass and productivity in the standing dead shoots and in the litter layer, at least until reeds are fragmented or buried in the sediment [10, 29]. Those litter-associated fungi could contain considerable amounts of nutrients [e.g., 9] and they may be active in heavy metal retention [8]. The fungal species composition is influenced by environmental variables such as salinity and flooding (height and frequency) [54], and distinct communities are recognized on different plant parts (leaf blades, leaf sheaths, and stems) that change in time [1, 2, 55, 56]. Furthermore, within the canopy, fungal communities recognized on the plant parts differ along the vertical axes of shoots [2, 52, 55, 56].

However, considering this ample evidence of fungal importance, current knowledge on the involvement of fungi in carbon and nutrient cycles and their impact on the food chain and influence on the plant's life history is very limited and clearly a vacuum in microbial wetland research [e.g., 15, 17].

This study reports on the decomposition of *P. australis* leaf sheaths and stems in the litter layer of a brackish tidal marsh by determining mass loss, nitrogen, phosphorus, and plant polymer dynamics. We assess specifically the involvement of fungal decomposers by estimating fungal biomass and immobilization of N and P in fungal biomass.

Methods

Study Site. The study was conducted in a brackish tidal reed stand in the Scheldt estuary on the Dutch–Belgian border in the Netherlands at the edge of Saeftinghe Marsh (51°21′N, 4°14′E). Common reed is the dominant emergent macrophyte on the marsh, forming nearly monospecific stands. Reed biomass and carbon allocation in this reed bed has been modeled by Soetaert *et al.* [45], and selected site and reed characteristics are presented in Table 1. Meteorological data during the study period are presented in Fig. 1.

Field Procedures. Dead standing culm sections from that year's growing season were collected on 3 December 2001 1 m above the sediment. Culm sections comprised two nodes and one internode, with the leaf sheath still surrounding the stem. This approach allowed one to study the decay of stem and sheathing material in a state close to their natural association. It avoided quick penetration of water in the hollow internodium similar to natural conditions (cf. [11]). The samples were placed in a cool box, transported to the laboratory and used to fill 45 plastic litter bags (35 × 20 cm, 4-mm mesh) with 50.0 g fresh mass of cut culm sections. Thirty-five bags were placed in the tidal marsh reed stand the next morning. Litter bags were positioned flat on the sediment surface and anchored by hooked bars, and were left to be flooded regularly at high tides, about 15% of the river high tides. The remaining 10 litter bags were immediately dried at 40°C for 72 h to determine the initial dry mass of the litterbag contents. After drying, stems and sheaths were separated and processed separately. The initial dry mass (g) was 21.74 ± 0.85 and 3.10 ± 0.34 (mean ± SD) for stems and leaf sheaths, respectively. Initial ash was determined for five stems and sheaths. Two litter bags were retrieved at monthly intervals during the next 16 months.

Table 1. Selected characteristics of the investigated tidal marsh

Variable	*Mean ± SD*	*n*
Tidal marsh		
Flooding frequency (%)[a]	15	
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)	2455 ± 1510	19
NH_4^+-N (mg/L)	0.46 ± 0.35	21
NO_2^--N (mg/L)	0.06 ± 0.03	21
NO_3^--N (mg/L)	4.9 ± 0.65	21
Total P (mg/L)	0.73 ± 039	21
PO_4^{3-} (mg/L)	0.16 ± 0.05	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
Culm biomass (g m^{-2})[e]	570 ± 216	6
of which stems (g m^{-2})[f]	445 ± 16	5
of which leaf sheaths (g m^{-2})[f]	125 ± 16	5

[a]Percentage of flooding is the relative number of times the total high tides are higher than the marsh surface and are supposed to flood the marsh [30].
[b]From [30].
[c]Calculated as 157 × (flooding frequency) + 10, $r^2 = 0.52$, $P < 0.001$ [51].
[d]Annual average in 2002 (Flemish Environment Agency, Belgium, site code 154100, http://www2.vmm.be).
[e]Estimated at end of the growing season (10 September 1997) by harvesting all aboveground living reed matter in six 0.25-m^2 quadrats; biomass ≈ annual above net above-ground production. Note: after mowing, density of living shoots was found to increase the next year (~×2.5 in our study site) (Hoffmann, pers. comm.).
[f]Estimate based on average of 22% (19–26%) leaf sheath dry mass and 78% stem dry mass (five whole culms without inflorescence).

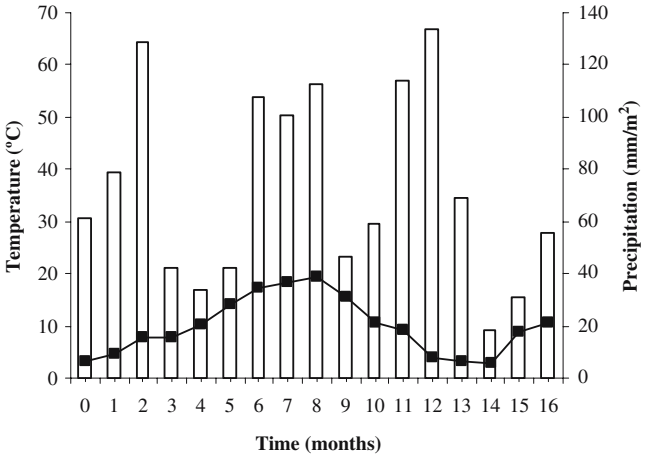

Figure 1. Average daily air temperature (*line*) and monthly precipitation (*bars*) during the study period. The first month (0) corresponds to December 2001. Both temperature and precipitation were measured in a nearby official weather station of the Royal Meteorological Institute of Belgium (Stabroek, Antwerp).

After collection, samples were immediately transported in a cool box to the laboratory and processed the same day. Litter samples were rinsed with distilled water as thoroughly as possible without fragmenting to remove adhering clay and macroinvertebrates. Sheath and stem material was separated, making it possible to follow the decomposition process of both plant parts [11]. Subsamples were taken for fungal biomass quantification and the remaining material was dried at 40°C for 72 h, ground to pass a 2-mm mesh, and used for analyses as detailed below.

Mass Loss, Nutrient Concentration, and Lignocellulose. The ash-free dry mass (AFDM) was determined following combustion (4 h at 550°C) of 250-mg subsamples and dry mass was corrected for the mass of subsamples removed for ergosterol analysis. Mass loss data were fitted to a simple exponential model, $m_t = m_o e^{-kt}$, where m_t is litter AFDM remaining after time t, m_o is the original AFDM, and k is the breakdown coefficient [6]. Data were not transformed and nonlinear regression analysis was used to estimate parameters using the default settings in SYSTAT, version 10.2.

Total N and P concentrations were measured using an automatic Skalar chain (segmented flow analyzer; SAN PLUS Analyzer, SKALAR) after digestion of approximately 100 mg plant material with sulfuric acid and potassium sulfate at 360°C. Initial C concentration and C concentration after 7 months (leaf sheaths) and 14 months of breakdown (stems) were determined with a Carlo-Erba elemental analyzer, type NA-1500.

Leaf sheaths and stems were analyzed for content of structural plant polymers (cellulose and lignin) according to the acid–detergent method [25, 57, 58]. This method adds an additional step, preceding the Klason method by pretreatment of samples with an acid detergent to remove protein, hemicellulose, and other components associated with lignocellulose, and is favored for plant quality characterization during decomposition [40] (but see [21, 24] for discussion on possible inaccuracies). All plant cell wall constituents were corrected for ash content.

A generalized linear model (GLM) procedure (SAS Statistical Package version 8.2, SAS 1999) was used to compare trends between plant parts for measured elements (N, P, cellulose, and lignin) with plant parts, time, and the two-way interaction of plant part and time of decomposition in the model. Significance of Plant Part × Time interaction was tested by F tests.

Fungal Standing Crop. Fungal biomass was estimated by measuring ergosterol concentration as adapted from the work of Gessner and Schmitt [14]. Freeze-dried stem and leaf sheath samples were kept in a 0.8% KOH MeOH solution for maximally 1 month prior to solid-phase extraction, followed by injection into a high-performance liquid chromatography (HPLC) system (Kratos Analytical Instruments Spectroflow 400): flow rate of 1.5 mL/min, constant column temperature of 30°C, and ergosterol eluted at ~ 8 min. Ergosterol concentration was estimated with a UV Spectroflow 757 detector at 282 nm. The software used to quantify peak area was Applied Biosystems Model 610A, a data analysis system for protein characterization, version 1.2.2. Conversion factors for calculating fungal biomass, % fungal C, % fungal N, and % fungal P were 5.8 mg ergosterol/g fungal dry mass, 431 mg C/g fungal biomass, 65 mg N/g fungal biomass [10], and 4 mg P/g fungal biomass [3], respectively.

Results

Decay and Nutrient Dynamics. Decomposition of *P. australis* leaf sheaths proceeded quickly after placement of litter bags on the sediment surface. Leaf sheaths lost 50% of their initial ash-free dry mass in 7 months (Fig. 2), corresponding to an exponential decay coefficient k of -0.0039 ± 0.0004 day^{-1} [mean ± asymptotic standard error (ASE); corrected $r^2 = 0.86$, $N = 23$; estimated $m_0 = 102.1 \pm 4\%$ (± ASE)]. In contrast to the surrounding leaf sheaths, the mass loss from stems was nondetectable during the first 6 months of the experiment (Fig. 2). Subsequently, breakdown of the stems followed a smooth exponential pattern with a loss of 50% ash-free dry mass after an exposure of about 15 months in the litter layer. When the initial 6-month lag period was eliminated, an exponential decay coefficient k of -0.0026 ± 0.0003 day^{-1} was found [mean ± ASE; $r^2 = 0.81$; $N = 22$; estimated $m_0 = 96 \pm 3.7\%$ (± ASE)].

During decay, ash content varied little: 13.5 ± 2.3 and 4.6 ± 1.2% for leaf sheaths and stems, respectively. Higher

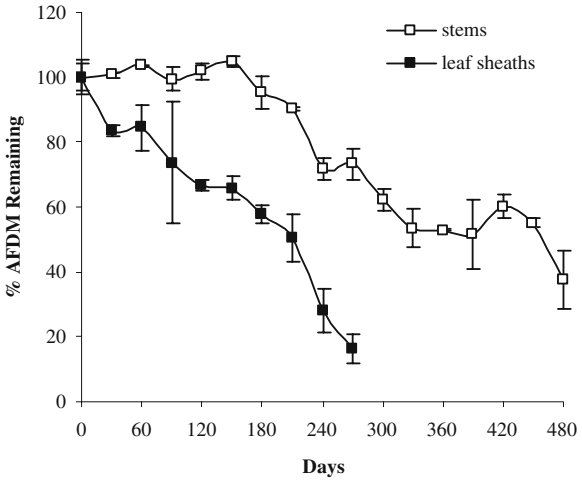

Figure 2. Ash-free dry mass remaining of leaf sheaths and stems of *P. australis* in the litter layer of a brackish tidal marsh during decomposition. Error bars indicate mean ± SD ($N = 2$, except for the start of the experiment, $N = 10$).

ash content was noticed during the last two experimental months, both for leaf sheaths (ash 16–18%) and stems (ash 6–9%).

Similar to the mass loss, nutrients in stems showed no changes in concentration during the first 6 months of the experiment. This lag period was followed by a sharp rise and then leveled off (Fig. 3B). Nitrogen and phosphorus concentrations (% of dry mass) increased over the entire study period for both leaf sheaths (Fig. 3A) and stems (Fig. 3B). Initial N and P concentrations in leaf sheaths exceeded those in stems by a factor >2. Initial differences in nutrient concentrations were roughly maintained among both litter types during decay. However, patterns were different for both plant parts, with a different increase rate, even when the initial lag period for stems was neglected (for N, $F_{1,32} = 7.42$, $P = 0.0104$; for P, $F_{1,32} = 17.82$, $P = 0.0002$, Plant Part × Time, GLM-ANCOVA) (Fig. 3A, B). Nutrient concentrations in leaf sheaths fitted best an exponential function for N (r^2 =

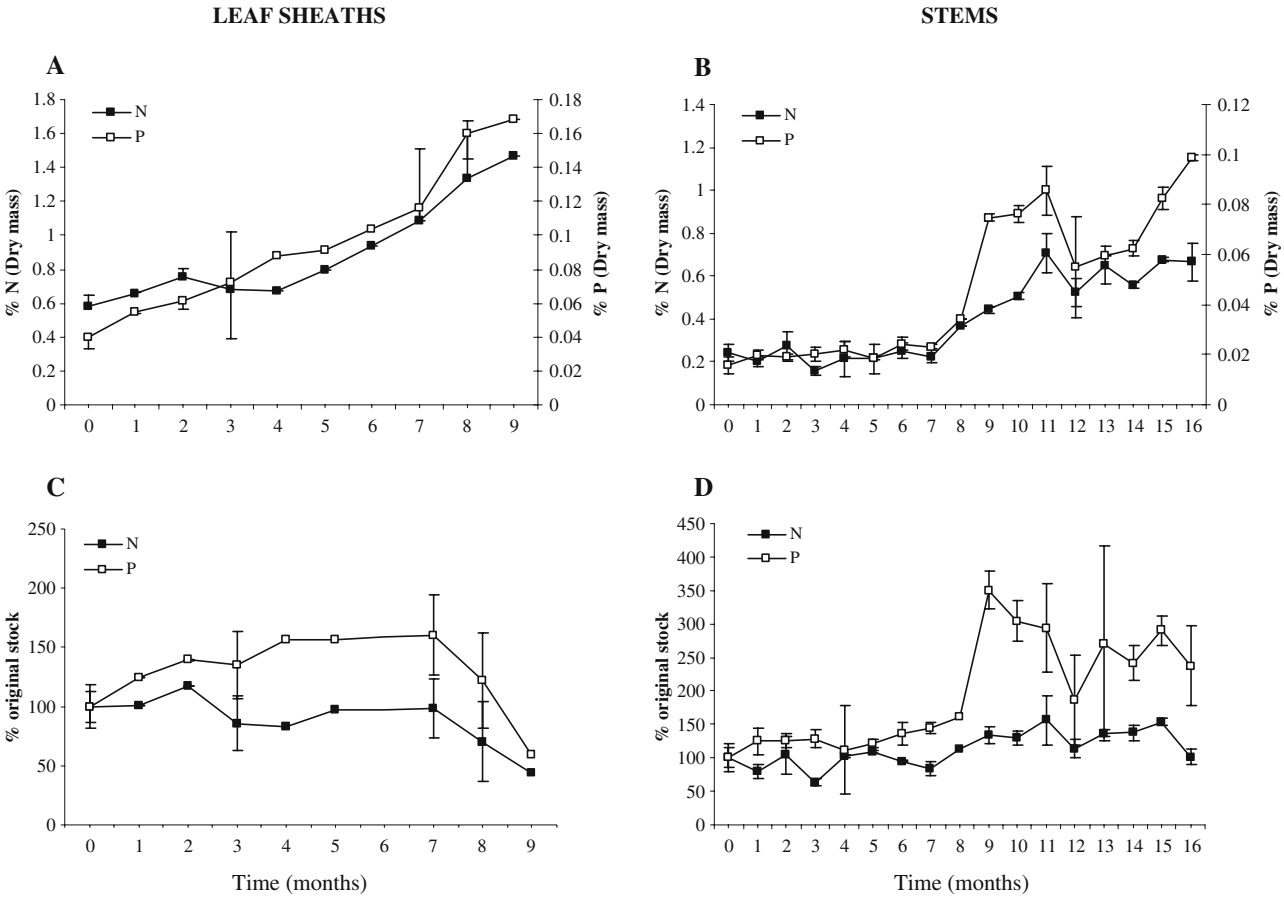

Figure 3. Dynamics of nitrogen and phosphorus concentrations of leaf sheaths (A) and stems (B) and changes in amounts of nitrogen and phosphorus per litter bag of leaf sheaths (C) and stems (D) of *P. australis* litter enclosed in litter bags on the sediment surface of a tidal brackish marsh. Error bars indicate mean ± SD ($N = 1$–2 for leaf sheath data, $N = 2$ for stem data, except for initial measurements, $N = 3$).

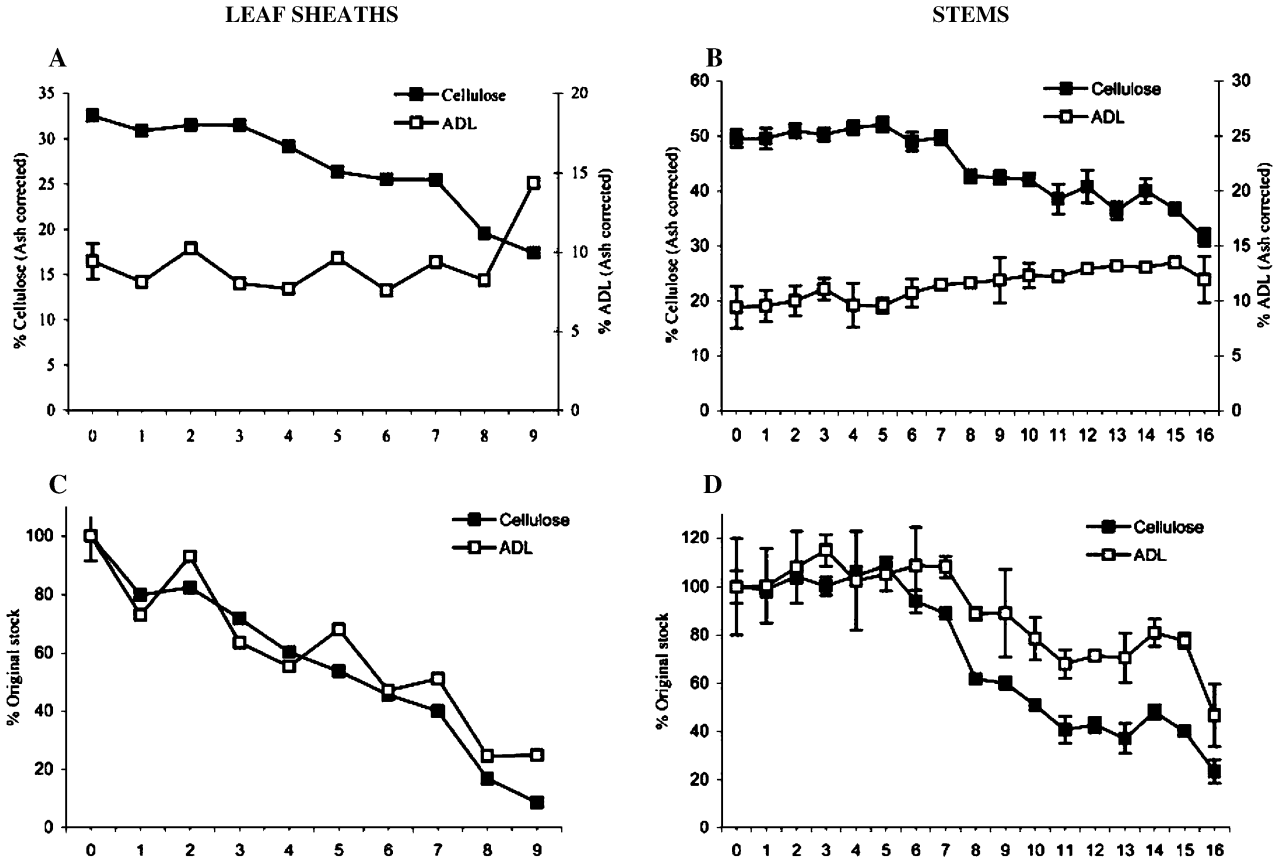

Figure 4. Dynamics in cellulose and acid detergent lignin concentrations of leaf sheaths (A) and stems (B) and changes in amounts of cellulose and acid detergent lignin per litter bag of leaf sheaths (C) and stems (D) of *P. australis* litter enclosed in litter bags on the sediment surface of a tidal brackish marsh. Error bars indicate mean ± SD (N = 1–2 for leaf sheath data, N = 4 for stem data except for initial measurements, N = 5).

0.89) and a linear for P (r^2 = 0.94) up to the end of the study.

The N stock increases in the litter bags during the study (Fig. 3C, D). Stems showed a net immobilization during decay, with a significant rise in N stock if we compare the period with mass loss with the initial lag period ($P < 0.01$, ANOVA) (Fig. 3D). This resulted in an increased N stock of about 155% of the original stock (Fig. 3D). The C/N ratio of leaf sheaths decreased from 72 ± 7 to 43 ± 17 in July after 7 months of decay, and from 176 ± 24 to 86 ± 3 for stems after 14 months of decay (mean ± SD, N = 4). However, at the end of the 9-month study the leaf sheaths showed a tendency of net N mineralization (Fig. 3C).

Within stems, the P stock increased significantly after the initial lag period ($P < 0.01$, ANOVA) (Fig. 3D). Net immobilization of P was higher for stems than for leaf sheaths, resulting in a reduced difference in C/P proportion between litter types. The C/P ratio of leaf sheaths decreased from 1063 ± 193 to 371 ± 25 after 7 months of decay and for stems from 2676 ± 429 to 763 ± 42 after 14 months of decay (mean ± SD, N = 4). During the entire study, a net P mineralization was noticed only for leaf sheaths during the last month of the study period (Fig. 3C).

Cellulose concentrations in both leaf sheaths and stems (once decay started) (Fig. 4A, B) tended to show a gradual and comparable decrease pattern ($F_{1,60}$ = 0.06, P = 0.81, Plant Part × Time interaction, GLM-ANCOVA). Lignin concentrations tended to increase in stems (Fig. 4B), whereas this was not observed for leaf sheaths (except for a sharp rise in the last month, probably due to sediment infiltration in the soft leaf tissue). However, lignin concentration patterns were not significantly different between both plant parts during decomposition ($F_{1,60}$ = 0.61, P = 0.44, Plant Part × Time interaction, GLM-ANCOVA). Parallel to the pattern of mass loss, standing stocks of both cellulose and lignin decreased, with a higher loss of the original stock of cellulose compared to lignin for both plant parts (Fig. 4C, D).

Fungal Biomass and Nutrient Immobilization. In contrast to the leaf sheaths, which showed high fungal biomass present (93 ± 10 mg fungal biomass g^{-1} dry mass), middle height stem sections showed little de-

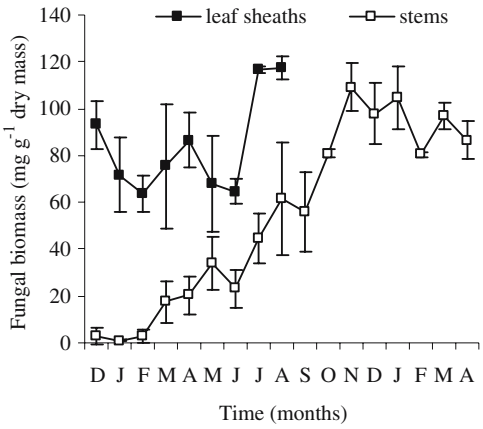

Figure 5. Dynamics of fungal biomass (ergosterol) associated with leaf sheaths and stems of *P. australis* placed on the sediment surface in December 2001 in a brackish tidal marsh. Error bars indicate mean ± SD (N = 2 to 4 except for initial values, N = 5).

tectable ergosterol at the start of the experiment (Fig. 5). After placement of the litter bags on the sediment, ergosterol concentrations in the leaf sheaths decreased by about 30%, but this difference was not significant ($P > 0.05$, $t = 1.42$, $N = 3$, paired t test) (Fig. 5) and subsequently increased again to initial values. Maximal ergosterol values were obtained for leaf sheaths largely decomposed (117 ± 5 mg fungal biomass g^{-1} dry mass). Ergosterol concentrations in stems gradually increased over time to reach a maximum (118 ± 10 mg fungal biomass g^{-1} dry mass) and then leveled off after about 11 months of decay.

To check whether fungi are also important in the standing decay phase of stems, fungal biomass was measured for two comparable standing dead stems still carrying their panicle in November 2002, after 1 year standing decay. Stem tissue showed appreciable fungal colonization with an overall mass recovery of 49 ± 8.7 mg fungal biomass g dry mass, corresponding to 20 ± 8 mg fungal C g^{-1} dry mass. A vertical pattern in ergosterol concentration was consistent between both selected stems, using the paired t test ($P = 0.90$, $t = -0.13$, $N = 32$) (Fig. 6). Fungal colonization was minimal for the lowest internode but increased steeply a few centimeters higher up the stem. Fungal biomass decreased again toward the middle height of the standing stems, and subsequently increased to reach maximal fungal biomass near the apex.

We estimated that nearly all plant nitrogen was present as fungal biomass in leaf sheaths from the canopy at the start of the experiment. Once in the litter layer, amounts of fungal N leveled off to an average proportion of 64 ± 13% (mean ± SD, N = 33) (Fig. 7A). Within stems the contribution of fungal N to the total N pool in litter gradually increases and is estimated to retain most of the available nitrogen (102 ± 15%; mean fungal N for month 6–16, ± SD, N = 37) in the decomposing stem litter (Fig. 7B).

Similar to nitrogen, total litter phosphorus was also estimated for fungal P amount, and it was estimated that nearly all of the litter P was incorporated in fungal tissue in leaf sheaths from the canopy. After placement in the litter layer, the average proportion of fungal P was reduced and estimated to be, on average, 37 ± 9% (mean ± SD, N = 33) (Fig. 7C). In stem tissue, the proportion of P incorporated in fungal biomass reflects the increasing fungal biomass (Fig. 5). This resulted in a maximal P capture of 75 ± 25% in May by the fungal hyphae in the litter (mean ± SD, N = 4) (Fig. 7D). As total P concentrations gradually increased, the proportion of P in fungal living mass during breakdown was slightly reduced and accounted for 52 ± 15% (mean ± SD, N = 37) of total P during decay.

Discussion

Decomposition Process. Observed breakdown rates for both leaf sheaths and stems are close to the rates of those mentioned by Gessner [11] in a eutrophic hard-water lake. As reported by Gessner [11], stems resisted detectable breakdown for up to 6 months after placement in the litter layer. Because of the increasing fungal biomass during the decay, the monthly litter mass loss was underestimated by 8 ± 3 and 8 ± 2% for stems (excluding lag period) and leaf sheaths, respectively (e.g., see also [13], their Fig. 2). These underestimates are significant ($P < 0.01$, $t = 16.27$ and 7.03 for stems and leaf sheaths, respectively; paired t test), and correction for microbial biomass resulted in a 3.5 and 1.7% higher breakdown coefficient for stems and leaf sheaths, respectively. The effective breakdown coefficient could be even higher, as

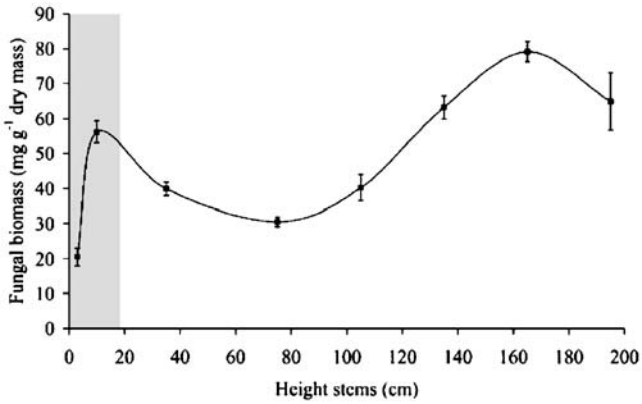

Figure 6. Vertical pattern in fungal biomass (ergosterol) after 1 year standing dead decay along the vertical axis of stems of *P. australis* in a brackish tidal marsh. Error bars indicate mean ± SD (N = 2). The grey section indicates the part of the stems regularly inundated by tidal flooding water.

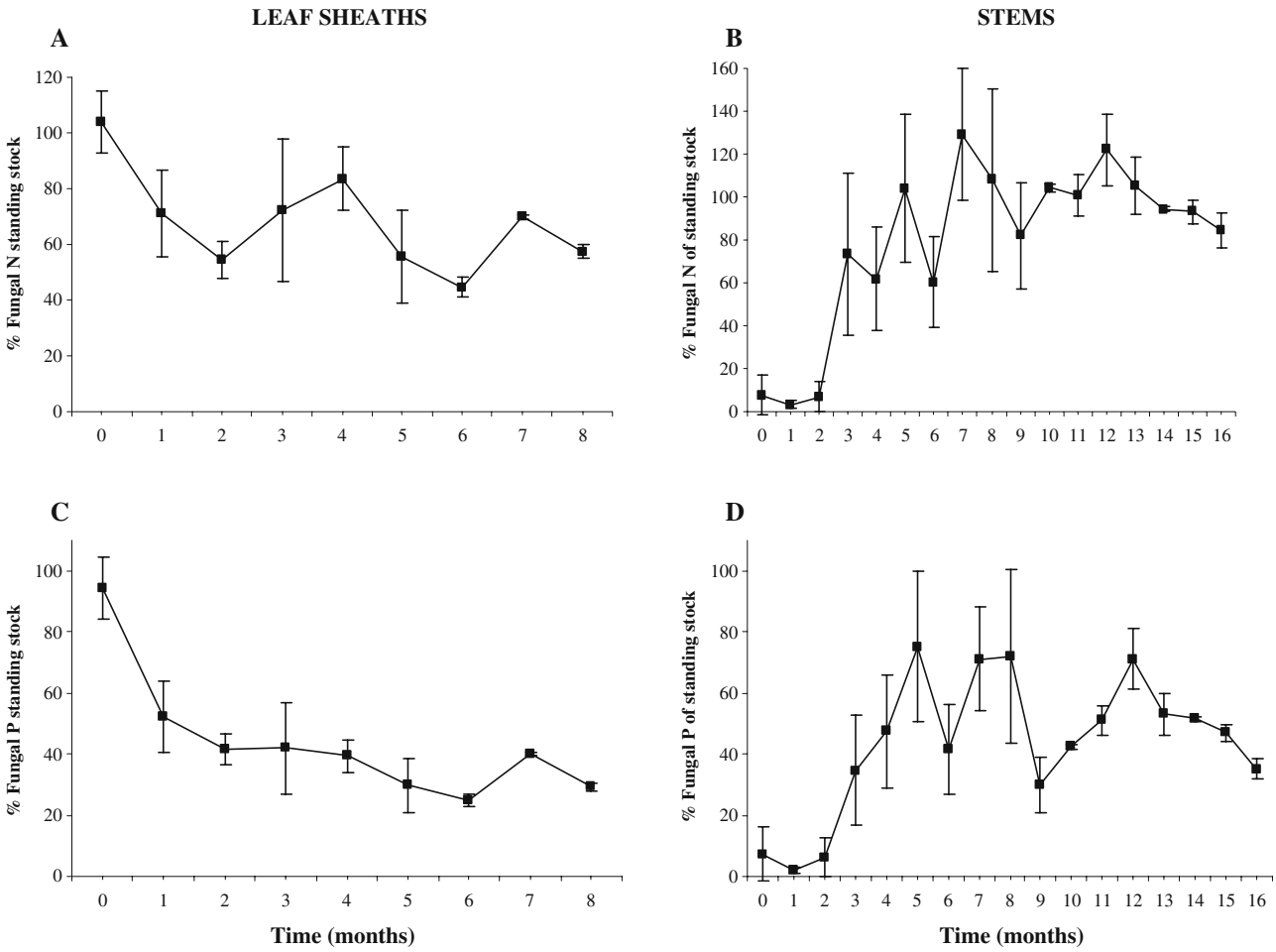

Figure 7. Estimated amount of the standing stock of total detrital nitrogen incorporated in fungal biomass in leaf sheaths (A) and stems (B) and estimated amount of the standing stock of total detrital phosphorus incorporated in fungal biomass in leaf sheaths (C) and stems (D) of *P. australis* incubated on the sediment surface of a tidal brackish marsh. Error bars indicate mean ± SD [N = 1 (no error bar) to 4].

the biomass is only a fraction of the accumulating fungal carbon present [23, 24, 38].

During decay, cellulose concentrations decreased, whereas lignin concentrations tended to increase or remained stable (sheaths) (Fig. 4A, B). Therefore, cellulose seems better available to the active decomposers compared to the more recalcitrant lignin. However, both plant polymers were decomposed (Fig. 4C, D) and might be well available for fungal colonizers [4]. Some genera (*Phaeosphaeria*, *Mycosphaerella*, *Buergenerula*, *Passeriniella*, *Stagonospora*, and *Helicomyces*) were shown to degrade lignocellulose, and the lignin components on *Carex* and *Spartina* resources [4, 5] were observed on *P. australis* during this study (see http://intramar.ugent.be/nemys/fungi/web/Phragmiticolous%20fungi.asp) [52].

Because of the variable time of aerial breakdown, the plant parts that continuously enter the litter layer have a different chemical composition, differ in microbial colonization [55, 56], and are confronted with different environmental (seasonal) conditions. Hence, any litter bag experiment following litter layer decay describes only one of the multiple patterns present in a reed stand. Within the studied site, the estimated proportion of stems following the studied decay pattern in the litter layer is about 25%, whereas another 35–40% of the culms fall gradually on the sediment surface during the following year [45, see their Fig. 4]. Hence, described patterns are representative for a considerable fraction of the decaying plant matter.

Fungal Dynamics During Natural Decay. Analysis of the standing dead reed culms prior to placing the litter bags on the marsh surface indicates appreciable fungal colonization of the leaf sheaths (40 ± 4 mg C g^{-1} dry mass), whereas fungal presence was nearly undetectable in the stem tissue. This high fungal biomass and the observed high species richness on standing dead leaf sheaths [55] suggest that fungi are an important metabolic

component within standing reed litter, as was recently reported by Kuehn *et al.* [29]. Because a large proportion of culms stays in an upright position for at least several months [18, 20] and leaf sheaths have a relatively fast decay compared to stems (Fig. 2; [11]), leaf sheaths are among plant parts that experience highest mass loss within the canopy. Moreover, a complete breakdown of leaf sheaths was noticed in the standing position [55]. Consequently, if standing decay is incorporated in carbon and nutrient budgets [12, 16, 41], leaf sheaths, accounting for 14% of total above ground biomass (Table 1), have an appreciable impact on the organic matter dynamics within *P. australis* stands in a relatively short term.

The high fungal biomass measured in sheaths [29; Fig. 5] and the proportionally larger sheath mass higher in the canopy might substantially impact fungal colonization patterns described for emergent macrophyte shoots. For example, the vertical ergosterol patterns described during the first 6 months of standing dead decay of both *P. australis* [10] and *Erianthus giganteus* culms [27] are possibly due to high fungal biomass in leaf sheaths and nearly fungal absence within the stem tissue. This demonstrates the argued need to separate plant parts in order to understand observed decay patterns in aquatic macrophytes [e.g., 11].

There is an appreciable fungal biomass measured after 1 year in a standing dead position (Fig. 6). This indicates microbial mineralization for those plant parts in an upright position [see also 26, 29]. The observed fungal biomass pattern along the axis of standing stems (Fig. 6) could be caused by a combination of external conditions and both physical and chemical characteristics of the resource. The lowest internode stays viable for several more months (as being greenish in color) and showed significant lower ergosterol concentration compared to the second internode (about 10 cm above sediment surface) (t test, $P < 0.05$, $N = 7$). This delayed senescence probably detains fungal colonization. The zone above the first internode shows higher fungal biomass compared to the region above. It appears that fungal growth is promoted through the periodical wetting of the basal stem parts (except for the lowest part). Higher up the stems, ergosterol concentrations gradually increased as stems become thinner and less refractory. This may be related to the absence of or thin sclerenchymatous layers and thinner cuticle under the leaf sheaths [44, 59], resulting in a less restrained growth and/or a higher level of dew set at the top of the shoot canopy [36]. This dew set could be well incorporated in the thicker layer of sheaths present in the upper canopy and create more benign conditions for fungal stem colonization.

Movement of the *P. australis* leaf sheath litter from a standing dead position to the sediment surface results in a decrease in fungal biomass (Fig. 5). This decrease in living fungal mass was previously observed for *P. australis* leaf blades [48, 49, 53]. Those distinct changes in biomass are generally accompanied by a lower productivity rate [26, 28, 37] and a shift in dominant fungal taxa [55, 56]. However, following a decrease in fungal biomass after placement of the leaf sheaths on the sediment surface, fungal mass recovered and even surpassed initial standing dead amounts. Together with the steady increase in fungal biomass in stems, this suggests an ongoing dominance of fungal decomposers in the litter layer. Although not measured during the present study, bacterial biomass and productivity during initial submergence or decay in the litter layer is generally estimated to be less than 10% of fungal biomass and productivity for *P. australis* litter in freshwater tidal and submerged conditions [10, 26]. Furthermore, similar findings are reported for other emergent macrophytes in both freshwater and marine habitats [9, 28, 37, 38].

Nutrient Dynamics: Fungal Involvement. Increased total nutrient stocks in the litter bags and the absence of any substantial nutrient loss (by leaching and/or mineralization) (Fig. 3) suggest that nutrients were either immobilized into microbial biomass, bound to the remaining plant matter, or that losses were (over) compensated for by fresh inputs.

The large nitrogen sequestration by both plant parts may be largely accounted for by immobilization in fungal biomass (Fig. 7). Furthermore, litter N associated with fungal mycelia is probably higher because our reported fungal mass is measured as living biomass. A substantial portion of detrital N could be associated with residual dead fungal mycelium (i.e., *N*-acetyl-glucosamine). Nonliving fungal mass within decaying litter may be twice that of living fungal biomass [33, 34, 38]. The steady increase in the proportion of fungal N while total nitrogen concentrations remained stable in the stem tissue indicates a gradual translocation of plant litter nitrogen into fungal mycelia. Some of the high estimates for fungal N in stem litter could be due to conversion factors being slightly too high for our fungal community. Otherwise, estimates of living fungal biomass could be slightly too high because ergosterol has been found to be recalcitrant after death of fungi [e.g., 31].

Fungi can immobilize nutrients from the external environment [46, 47] or they manage their growth by translocation of internal nutrient sources. Our study shows no indications for nutrient immobilization from the environment within the leaf sheaths by fungi. Sheaths are nutrient rich compared to stems. Within this nutrient-rich tissue it appears that the amount of original nutrients stocks are always higher than the amounts of nutrients incorporated within fungal living mass during decay (Fig. 7A, C). Within leaf sheaths, fungal biomass

was estimated to contain, on average, 57 ± 11 and 51 ± 10% of the original stock of N and P, respectively. This suggests that leaf sheath mycota are not limited by nitrogen or phosphorus, and, possibly, other factors (such as C availability) limit the growth of fungi.

Contrary to leaf sheaths, stems with lower initial N and P concentrations seem to show a net immobilization from the external environment of both N and P. This could be due to fungal colonization. The latter is suggested by the higher nutrient concentrations in fungal biomass during decay compared to the initial nutrient pool available in reed stems. Fungal biomass was estimated to contain, on average, 129 ± 21 and 123 ± 20%, respectively, of the original stock of N and P. However, within the stems N seems to be the limiting factor for fungal growth, as all available N seems to be incorporated in the fungal tissue (see above). In contrast to N, the proportion of the standing stock of phosphorus incorporated in fungal biomass was determined to be far lower than the amounts present in the detrital mass (Fig. 7D). This suggests that other mechanisms than fungi are responsible for increased P concentrations in stems. Yet, the conversion factor used could have been too low, or the P demand and incorporation in fungal tissue might be variable during their life cycle. We used a conversion factor at the lower end of the values mentioned by Beever and Burns [3] for eumycotic mycelial growth in nutrient-poor conditions. However, during periods of abundant sporulation (during the summer in our site [56]) P demand could be higher, as ascocarps and spores have generally a higher phosphorus concentration compared to mycelium [3]. Furthermore, fungal tissues might incorporate more phosphorus with increased P availability in the surroundings [19]. In our study site, P availability from the external stem tissue was higher during summer because of an increased P load in the tidal exchange water [50]. Furthermore, the increase in P concentrations in the stems coincides with mineralization of leaf sheath phosphorus (Fig. 3C). However, other factors such as complexation and adsorption of P to the detrital mass may explain the summer rise in P concentrations in stems (Fig. 3A). Whichever factor or factors are responsible for the observed patterns, the above discussion illustrates the need to obtain, and test, available conversion factors during decomposition in relation to changing environmental circumstances.

Furthermore, fungi may impact carbon and nutrient dynamics within the studied site by their luxurious sporulation [55, 56]; hence a substantial part of the assimilated carbon and nutrients may be expulsed in the form of spores or conidia within the environment [7, 35, 39]. Newell and Wasowski [39] estimated the fraction of total fungal production allocated to ascomata at 9%, and Newell [35] estimated a minimal allocation of 4.5% fungal year production toward spores (7.5 g spore mass m^{-2} $year^{-1}$). Although the influence of carbon loss from an ecosystem by means of spore expulsion can thus be substantial, the impact on nutrient budgets is proportionally higher because N and P are incorporated in high concentrations within the conidia and spores [e.g., 3, 7].

In conclusion, this article demonstrates the important role of fungi during decay of aboveground plant parts of common reed. This is illustrated by a high fungal biomass present, which was estimated to contain a considerable proportion of the nutrients during the decay process, both in the canopy and in the litter layer. Moreover, litter-associated fungi may immobilize nutrients from the external environment. Hence, fungi have a considerable impact on the organic matter dynamics and should be incorporated in any study trying to understand nutrient cycling in wetland ecosystems.

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