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Multiyear patterns of fungal biomass dynamics and productivity within naturally decaying smooth cordgrass shoots

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Abstract

Ascomycetous fungi are predominant secondary microbial producers of the smooth cordgrass (*Spartina alterniflora*) shoot decay system. A 3-yr examination of concentrations of living fungal mass (as ergosterol content) in naturally decaying cordgrass, and of instantaneous rates of cordgrass–fungal production (as rates of incorporation of radiolabeled acetate into ergosterol at a standard temperature of 20°C), was conducted in three salt-marsh watersheds of Sapelo Island. Though the years of study were climatologically different (e.g., rainfall ranging over a factor ~2), ergosterol content of decaying leaves was not different from year to year, with a grand mean of 371 μg ergosterol g^{-1} organic mass of decaying system, and there was little difference among yearly average fungal productivities (range = 155–217 μg fungal organic mass g^{-1} system organic mass h^{-1} for autumn–spring data). Significant differences in ergosterol content of decaying leaves were found among marsh watersheds, probably due to differences in nitrogen (but not phosphorus) availability, but these were not large (maximum 1.3-fold for multiseasonal data), and differences were not found among marsh subsites (short to tall shoot) nor, for the most part, among types or parts of decaying leaves. Season of sampling, however, had a large effect upon fungal biomass and fungal productivity: average ergosterol contents were significantly higher in winter and spring (e.g., 416–554 μg g^{-1} organic mass of decaying blades) than in summer or autumn (<310 μg g^{-1}). Among the potential reasons for this unexpected pattern of seasonality might be (1) greater access of mycophagous invertebrates and/or bacterial competitors due to higher tides and lower elevation of leaves in late summer and fall, and (2) leaching of leaf digestate during periods of high rainfall and high spring tides. Significant correlations were found between ergosterol content of decaying leaves and mean tidal height for the 3 months before sampling ($r = -0.77$) and for fungal productivities and 3-month rainfall ($r = -0.62$). Grazing of decaying leaves by salt-marsh periwinkles over the range of snail densities of the study sites (0–85 m^{-2}) gave no clear evidence of depression of fungal biomass or repression of fungal activity, contrary to previous findings for higher snail densities. The calculated seasonal change in living fungal percentage of decaying leaves was from ~6 (summer–autumn) to 9% (winter–spring), and in 6-month fungal production, the change was from 101 to 434 g organic fungal mass m^{-2} marsh surface.

Gallagher and Pfeiffer (1977), with their publication of data for high rates of metabolism by the ''dead plant community'' on/in standing dead parts of shoots of smooth cordgrass (*Spartina alterniflora*), including greater retention of dissolved organic carbon when respiration rates were high, sparked interest in this formerly inert-seeming component of salt-marsh ecosystems of the southeastern U.S.A. The ''dead plant community'' consists primarily of a small group of species of ascomycetes that is invariably associated with the natural decay of leaves of smooth cordgrass, especially *Phaeosphaeria spartinicola, Phaeosphaeria halima,* an unnamed species of *Mycosphaerella,* and *Buergenerula spartinae* (Gessner 1977; Newell et al. 2000; Newell and Porter 2000; Newell 2001*a*). Lee et al. (1980) introduced measurement of fungal mass within decaying shoot material of smooth cordgrass using ergosterol as a biomass proxy. This was a major step forward, because the mass that fungi produce during marshgrass decay is located primarily within the cells and cell walls of the opaque substrate (Newell et al. 1996*b*). ''The polarized activities of wall synthesis and protein secretion, together with the enormous penetrating force that can be generated by turgor pressure[,]. . . make fungal hyphae great tunneling devices . . . ideally equipped for invading dead and living solid substrata . . .'' (Wessels 1999). Fungal mycelia and their hyphal sheaths become an integral and inseparable part of the grass decay system (Newell et al. 1996*b*), but the ergosterol of their membranes is readily extractable and measurable (Newell 2000, 2001*b*). An additional indispensable methodological advancement involved recognition that marshgrasses do not abscise their leaves or stems—the dead parts of shoots undergo substantial, primarily fungal, decay while standing in the canopy (Newell 1993*b*; Bärlocher 1997; Gessner et al. 1997; Kuehn et al. 2000; Newell and Porter 2000).

The ergosterol technique was subsequently expanded to enable recording of instantaneous rates of fungal growth (Newell 1993*a*, 2001*b*). This expansion made possible the measurement of fungal productivities in essentially the same way that radioisotopic-CO_2 and -DNA or -protein-precursor techniques had earlier permitted measurement of phytoplankton and bacterioplankton productivity (Newell and Fallon 1991; Gessner and Chauvet 1997; Suberkropp 1998; Kuehn et al. 2000). With application of the fungal-productivity technique, more accurate models can be constructed of the flow of carbon through detrital-based wetland systems

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Table 1. Basic environmental characteristics (ranges in averages for years and seasons) of the three stations.*

	Creek temp.	Creek sal.	Snails
Doboy (DS)			
Years	20–26	22–27	7–10
Seasons	17–30	14–29	8–9
Flume (FL)			
Years	22–27	15–26	9–12
Seasons	17–31	15–24	9–13
Odum/Teal (OT)			
Years	20–24	17–25	1–2
Seasons	17–29	15–25	1–2

* Temperature in °C; salinity in mg salt L^{-1}; snails = visible (not hidden in furled leaves) *L. irrorata* per 0.25 m^2; sampling once per season at mid-season at three stations for 3 yr.

(e.g., salt marshes: Montague and Wiegert 1990; reconfigured model in Newell and Porter 2000).

Ergosterol-based techniques were applied here to obtain multiannual values for biomass of living fungi and rates of fungal production within naturally decaying smooth cordgrass in south-temperate salt marshes. The goal of the research was to obtain seasonal and annual information for cordgrass–fungal mass and productivity per unit representative decaying shoot mass and per unit marsh area—not for individual shoot parts as they decomposed over time.

Methods

Sites—Sampling stations were established within three salt-marsh watersheds on Sapelo Island, Georgia, within the Sapelo Island National Estuarine Research Reserve (Chalmers 1997). The Doboy Sound station (DS; 31°23′54.7″N, 81°17′18.7″W) received Doboy Sound water with flooding tides and was adjacent to the former study site of Newell et al. (1989) and Newell and Wasowski (1995). DS was the nearest of the stations to the Atlantic Ocean (2.4 km from the mouth of Doboy Sound). The Odum/Teal station (OT; 31°23′42.2″N, 81°16′43.6″W) was alongside the Odum/Teal Boardwalk (Newell et al. 1996*a*), which is located alongside the banks of Southend Creek ~0.9 km upstream from the mouth of Southend Creek where it empties into Doboy Sound (~1.8 km from the Atlantic mouth of Doboy Sound). The Flume station (FL; 31°28′51.5″N, 81°16′3.1″W; also used by Dai and Wiegert 1996*a*) was in the upper reaches of the watershed of the Duplin River (a tidal channel, not a true river: Newell et al. 1988; FL was 10 km upstream from the mouth of the Duplin at Doboy Sound, which is ~4.8 km from the Atlantic mouth of Doboy Sound). Because of the differences among the stations in spatiotemporal distances from ocean-water mixing, the three stations represent a gradient of degree of marsh influence upon tidal-flooding water, increasing in the order DS < OT < FL (Newell et al. 1988).

At each station, three subsites were selected: an area of short shoots (~0.5-m mature canopy height) of smooth cordgrass (*S. alterniflora*), an area of tall cordgrass shoots (~1.5-m canopy height) on a creekbank, and an area of intermediate-height cordgrass shoots (~1.0-m canopy height). Each subsite was marked, and all subsequent sampling was performed within a 5-m radius of the marker stake.

Collection of samples and environmental data—Each station and subsite were sampled at mid-season (15 Feb, May, Aug, and Nov) beginning in August 1996 and ending in May 1999. Visible salt-marsh periwinkles (*Littoraria irrorata*) were counted (because they are mycophagous leaf shredders: Graça et al. 2000) within three ¼-m^2 frames inserted nonselectively into the marshgrass swards (except during Feb samplings when periwinkles are dormant; Graça et al. 2000) (Tables 1, 2). Temperature and salinity of nearby creek water were measured (Table 1), and approximate height of the marshgrass canopy was recorded (Table 2).

Rainfall and tidal-height information was obtained because of the potential importance of these phenomena as wetting agents for fungal decomposers (Newell et al. 1989, 1996*b*, 1998; Newell and Porter 2000). Rainfall data were provided by the Sapelo Island National Estuarine Research

Table 2. Basic cordgrass shoot (*S. alterniflora*) characteristics ($\bar{x}$ ± SD, approximate height of canopy above sediment, height of ligules of sampled leaves, leaf blade width at 10 cm distal to the ligule, all in cm) and snail (*L. irrorata*) distributions ($\bar{x}$ ± SD, number per 0.25 m^2).*

Season	Sum	Aut	Win	Spr
Canopy	93 ± 35	116 ± 40	103 ± 39	99 ± 46
Ligule	18^{A} ± 11	27^{B} ± 18	40^{C} ± 23	46^{C} ± 26
Width	1.02^{AB} ± 0.28	1.15^{B} ± 0.31	1.05^{AB} ± 0.31	0.97^{A} ± 0.31
Snails	7.4 ± 9.4	7.2 ± 7.8	no data	6.6 ± 6.7
Station	DS	FL	OT	
Canopy	95^{A} ± 42	119^{B} ± 40	94^{A} ± 36	
Ligule	28^{A} ± 23	46^{B} ± 23	24^{A} ± 17	
Width	1.01^{A} ± 0.36	0.96^{A} ± 0.21	1.18^{B} ± 0.30	
Snails	8.4^{B} ± 7.8^{B}	11.5^{B} ± 8.4	1.3^{A} ± 2.8	
Subsite	Tall	Int	Shrt	
Canopy	145^{C} ± 25	101^{B} ± 26	63^{A} ± 19	
Ligule	51^{C} ± 26	27^{B} ± 18	20^{A} ± 11	
Width	1.35^{C} ± 0.25	1.04^{B} ± 0.15	0.76^{A} ± 0.17	
Snails	1.5^{A} ± 2.1	7.9^{B} ± 7.3	11.8^{C} ± 9.1	

* Within rows, mean values with different capital superscript letters are statistically significantly different (ANOVA + SNK, $P < 0.05$).

Reserve (SINERR) Monitoring Program. Average cumulative rainfall in the 3 months before sampling was highest before the 15 November sampling ($\bar{x} \pm$ SD = 41 ± 17 cm) and lowest before 15 May ($\bar{x}$ = 17 ± 11 cm), as expected (Chalmers 1997), but the differences among seasons were not significant (ANOVA, $P = 0.26$). Rainfall per year (summer–summer) ranged from 80 cm (1998–1999) to 147 cm (1997–1998). Tidal-height information was obtained from published tables for Savannah River Entrance (NOS 1996–1999). Average higher diurnal tidal heights (cm above mean lower low water) for the 3 months before sampling were significantly (ANOVA, $P < 0.0001$) different among seasons, being highest before the 15 November sampling ($\bar{x} \pm$ SD = 240 ± 1.5 cm), 9 cm lower for 15 August, and ~17 cm lower for the February and May samplings. Average tidal height per year-period was very similar from year to year (range = 229–230 cm). SINERR data showed subfreezing air temperatures in winter 1996–1997 and 1998–1999 (−2.0 to −4.0°C) but not in winter 1997–1998.

At each cordgrass subsite, three representative canopy-height shoots were severed at sediment level, after securing (if necessary) the decaying leaf sheaths near the shoot base. In mid-summer, the predominant type of canopy-height cordgrass shoot was young and green, with decaying blades at the base but few to no decaying leaf sheaths. At subsequent seasons, this prominent cohort of shoots was more and more decayed; it was entirely brown by the mid-spring sampling (Newell et al. 1998).

Sample preparation—Because leaf blades of smooth cordgrass decay before their attached leaf sheaths (unpubl. data), the samples taken from young shoots in mid-summer consisted only of leaf blades. At the other sampling times, two samples were taken from standing-decaying blades and one from the decaying sheath attached to the lower of the two blades sampled. The blades sampled were the uppermost fully brown blade and the next one or two blades below this one (blades die in succession from low to high on shoots: Newell et al. 1998). Blades were cut from sheaths at the ligule, and the 11.5-cm portion nearest the ligule was cut free from the tip. Tips were discarded, and the 11.5-cm portions were rinsed free of clay under running cold tap water. Rinsed blades were drained, and their widths were measured at 1 and 10 cm from the ligule end. Blade pieces were then allowed to air dry (~23°C and 50% RH; drying to less than −7.5 MPa water potential in <1 h: Newell et al. 1991) as a means of inactivating and preserving living fungal content (Newell et al. 2000). Sheaths were processed as above, except that they were first wetted to make them pliable, and then peeled gently from their positions tightly enwrapped around the inner sheaths and the true stem. Sheath widths were measured at 1, 3, and 6 cm from the ligule, and entire sheaths were air dried.

At winter and spring samplings, the uppermost dead cordgrass blade was often a diminutive one (too small for subsampling) just below the seedhead. Therefore, a representative upper blade was taken from the span two-thirds to three-fourths of the shoot height and a representative mid-height blade from the span one-third to one-half shoot height.

Carbon, nitrogen, and phosphorus analyses—Cordgrass leaf samples were dried and ground, and C, N, and P were measured by combustive autoanalyzer (C, N) or nutrient-solution autoanalyzer (P) after persulfate digestion as described by Newell et al. (2000).

Fungal biomass measurement—Living fungal mass was measured as ergosterol content of samples (Newell 1993*a*, 2000). A 2-cm piece was cut from the nonligule end of each of the three blades of each type from each subsite. This 6-cm total blade length was pooled in a 20-ml screw-cap vial, 5 ml reagent ethanol was added, and the vial was stored at 4°C in darkness. Sheath pieces were treated similarly, except that a 0.5-cm length including the ligule was cut away, and the next 1.0-cm piece was used for pooling to a total of 3-cm total length. Samples were subsequently reflux extracted in methanol, partitioned into pentane, and taken though high-performance liquid chromatography (HPLC) along with procedural standards of pure ergosterol as described by Newell (1993*a*, 2000).

For the purpose of maximizing comparability with other studies, a conversion factor of 200 units fungal organic mass per unit ergosterol was used to calculate living fungal mass (Newell 2000). This commonly used factor lies above the 95% confidence interval (154–172; $\bar{x}$ = 161) for five species of ascomycetes (four different genera in two different orders) of the smooth cordgrass and black needlerush (*Juncus roemerianus*) decomposition systems (Newell 1996). I present unconverted along with converted ergosterol concentrations and rates of acetate incorporation below, because I recognize that the conversion factor for ergosterol to living fungal mass, and the factor for conversion from rate of acetate incorporation to rate of fungal production (next paragraph), need further characterization, as discussed in Newell (2001*b*, and references therein).

Fungal productivity—Rates of fungal production at one standard temperature were determined based on measurement of rates of incorporation of [1-^{14}C]acetate into ergosterol (Newell 1993*a*, 2001*b*). The standard temperature chosen was 20°C, not far from the overall mean creek-water temperature measured at sampling times in the present work ($\bar{x} \pm$ SD = 23 ± 6°C; Table 1) and a temperature commonly reached during all seasons in Sapelo marshes (Chalmers 1997). At each sampling time, fungal productivity was determined for each type of leaf sample (upper, mid-, lowest, or sheath) for smooth cordgrass by pooling samples over stations and subsites. For each of two fungal-productivity replicates per smooth cordgrass leaf type, a 0.7-cm length was cut from the nonligule end of one blade piece (ligule end for sheaths) from each of the nine subsites, and the 6.3-cm total length was pooled in a 20-ml glass vial (vial cleaned and predried at 100°C, 5 h).

Fungal productivity was measured for the pooled samples as described by Newell (1993*a*, 2001*b*). The pooled pieces were submerged in 5 ml freshly prepared, 0.2-μm filtered Sapelo creek water (high slack tide, Southend Creek, settled for 24 h, diluted to 15 g L^{-1} salinity) and were incubated for 3 h at 20°C under fluorescent lighting (30 μE m^{-2} s^{-1} PAR) with slow agitation (60 reciprocations min^{-1} through

0.5 cm) to permit adaptation to submergence. Radiolabeled ([1-^{14}C]acetate, ICN) and nonlabeled acetate portions were then added to a final concentration of 5 mM (specific activity ~2.5 mCi mmol^{-1}) (1 mCi = 37 MBq), and incubations were continued for 2 h. Incubations with radioisotope were terminated by rinsing (×2, 20 ml of the filtered creek water) and immediate submergence in 5 ml reagent ethanol. Ergosterol was extracted and taken through HPLC as above, except that the ergosterol peaks were captured in scintillation vials and mixed with 10 ml scintillation fluor (Ecolume, ICN) for determination of radioacetate incorporation into ergosterol via scintillation counting (Beckman LS7500).

Newell and Fallon (1991) discovered that killed controls for neither mycelium of smooth cordgrass ascomycetes nor for decaying smooth cordgrass (brought to 2% formaldehyde before addition of radioacetate) exhibited detectable [^{14}C]acetate in the ergosterol fraction of HPLC collected as above. During the August 1996 fungal-productivity measurements, a set of formaldehyde-killed controls was processed alongside the corresponding living samples, and the result was that killed-control replicates were equivalent to background (average = 1.8 CPM over background vs. 10^2–10^3 CPM over background for live samples).

Fungal productivities for smooth cordgrass samples were calculated from rates of acetate incorporation into ergosterol using the factor 12.6 μg fungal organic mass per nmol acetate incorporated, measured in experiments conducted at 20°C (Newell 2000, 2001*b*). This is a corrected figure from the value given in Newell (1996), which was high due to a functional peculiarity of the HPLC injection valve (Newell 2000, 2001*b*; Newell and Porter 2000).

Density of organic matter in leaves—One-centimeter lengths were cut from the nonligule end of blades (or ligule end of sheaths) from each of two leaf pieces per station and subsite, and the 2-cm total length was placed in a glass dish. The leaf samples were microwave dried in a 1,000-W microwave oven for 6 min at the high (''cook'') setting (Newell et al. 1991) and immediately weighed. Dried samples were muffled (450°C, 4 h) and reweighed to find loss on ignition (=organic content).

Statistical analysis—SPSS/PC+ Version 5.0 (Norušis 1992) was used to process all data. Angular or logarithmic transformations were used with data that were in ratio form or that exhibited statistically significant heteroscedasticity (Sokal and Rohlf 1995). The word ''significant'' is used below exclusively to mean ''statistically significant'' (probability of type I error <0.05; Sokal and Rohlf 1995). Least-significant range testing was performed using the Student–Newman–Keuls method (SNK; Sokal and Rohlf 1995). Means are shown below ±1 standard deviation (SD).

It was anticipated that many readers would want multiyear and/or multisite average values for fungal crop and productivity for use in modeling of fluxes of marsh organic matter. Therefore, for the most part, in the tables that follow, means are given for main effects, and interaction effects are described in the text.

Table 3. Carbon-to-nitrogen (C : N) and carbon-to-phosphorus (C : P) mass ratios ($\bar{x}$ ± SD) of leaf samples of smooth cordgrass (*S. alterniflora*) from 1998–1999 period.*

	Summer	Autumn	Winter	Spring
C : N	$78^{B} \pm 31$	$50^{A} \pm 16$	$45^{A} \pm 16$	$40^{A} \pm 11$
C : P	$597^{AB} \pm 111$	$558^{A} \pm 129$	$620^{AB} \pm 145$	$723^{B} \pm 251$

* Data for upper plus mid-leaf blades only (no sheath data included) (n = 18 per $\bar{x}$); mean values within rows with different capital superscript letters significantly different (ANOVA + SNK, $P \leq 0.05$).

Results

Shoot characteristics and shredder-snail distribution—Cordgrass shoots were significantly taller at the FL station than at the DS or OT station, which resulted in the sampling heights being more elevated at FL (Table 2). The shoots at FL were also significantly smaller in diameter than at the other two stations, as shown by the average widths of leaf sheaths (which wrap around the stem) (FL, 1.44 cm; DS, 1.64 cm; and OT, 1.86 cm). The thick shoots of the OT station also had the widest leaf blades. Shoot morphologies at the three subsites (tall, intermediate-height, and short shoots) differed significantly in the expected directions ($\bar{x}$, sheath widths: tall, 2.10 cm; int., 1.67 cm; and short, 1.17 cm).

Shoot characteristics were equivalent from year to year (e.g., range of only 1.02–1.09 cm in annual mean blade width among years). Because shoots had attained nearly full mature height by mid-August, there was also no significant difference among seasons in shoot height, although the decline in averages after autumn was possibly real and caused by breaking off of tops of wholly dead shoots (unpubl. data) (Table 2). Ligule height of sampled leaves rose with season, as expected, because leaves die in sequence from low to high on shoots (Newell et al. 1998). Correspondingly, widths of sampled blades fell from autumn to spring, because leaves higher on shoots are smaller.

Averaged distributions of salt-marsh periwinkles (*L. irrorata*) also did not vary with year or season (Table 2), but snail concentrations were sharply different among stations and subsites (Tables 1, 2). The lowest concentration was at the OT station, and the concentration increased from tall toward short subsites, as expected (Newell 1993*b*).

Carbon : nutrient mass ratios—The C : N ratio of standing-decaying cordgrass leaves changed significantly with season, e.g., for upper and mid-shoot blades, falling from a summer level of 78 to a spring level of 40 (Table 3). The C : P ratio exhibited an opposite significant seasonal pattern, rising from summer–autumn to spring (Table 3), resulting in a rising N : P (mass ratio) from 8 in summer to 18 in spring.

The three stations were not significantly different for the overall data set with respect to C : N of decaying leaves. There was significant interaction between station and season, as a result of exceptionally low mean C : N (36) for the OT station for winter and spring. For the autumn–spring data, C : P was significantly lower at DS than at the other two stations, resulting in low N : P (12) relative to the other two

Table 4. Ergosterol content ($\bar{x}$ ± SD, μg g^{-1} organic mass) of leaf samples of smooth cordgrass (*S. alterniflora*), shown separately for autumn–spring and for summer, because of the difference in types of samples collected in these seasonal sets.*

	Autumn–Spring			Summer only		
Year	96/97	97/98	98/99	96/97	97/98	98/99
	397 ± 171	384 ± 132	401 ± 176	364 ± 301	279 ± 98	262 ± 97
Season	Aut	Win	Spr	N/A	N/A	N/A
	279^{A} ± 119	390^{B} ± 120	514^{C} ± 146			
Station	DS	FL	OT	DS	FL	OT
	390^{A} ± 137	351^{A} ± 123	442^{B} ± 200	361^{B} ± 301	205^{A} ± 48	333^{B} ± 70
Subsite†	Tall	Intm	Short	Tall	Int	Short
	420 ± 153	365 ± 127	398 ± 193	312 ± 81	311 ± 157	279 ± 289
Sample*	Upper	Mid	Sheath	Upper	Mid	Lower
	411^{B} ± 171	442^{B} ± 136	330^{A} ± 153	290 ± 279	294 ± 108	318 ± 158

* Within rows for autumn–winter (n = 81 per $\bar{x}$) or for summer (n = 27 per $\bar{x}$) sets, mean values with different capital superscript letters are statistically significantly different (ANOVA + SNK, $P < 0.05$). *See* text for descriptions of significant interactions (subsite × sample type [both seasonal sets], station × subsite [autumn-spring], year × station [summer]). For autumn-spring, ''upper'' and ''mid'' are leaf blades at different heights on shoots, and ''sheath'' is the leaf sheath attached to the mid-blade; for summer, all samples are leaf blades (decaying sheaths become prominent components of the standing-dead material after summer: Newell et al. 1998).

† Subsites with differing canopy heights (tall, ~1.5 m; intermediate, 1.0; short, 0.5).

stations (17–18). Among subsites, C : nutrient ratios varied little for the overall data set (e.g., tall–short for autumn–spring = 42–46 for C : N). Leaf sheaths were equivalent in C : N to leaf blades for the autumn–spring data, but in summer, lowermost blades were significantly lower in mean C : N (47) than blades decaying highest on shoots (81). For the autumn–spring data, mean C : P increased significantly in the order upper blades (580), mid-blades (687), and sheaths (787), but in summer, all blades had equivalent average C : P ratios ($\bar{x}$ = 580).

Ergosterol content—Living fungal mass (as ergosterol) in naturally decaying leaves of smooth cordgrass was stable from year to year (Table 4), with an overall mean of 371 ± 173 μg ergosterol g^{-1} organic decay system mass (seasonal maximal individual values ranged from 752 to 1,626 μg g^{-1}, and minimal values ranged from 61 to 259 μg g^{-1}). However, ergosterol content rose ~1.5-fold from summer–autumn through winter–spring to a mean for leaf blades of 554 ± 144 μg g^{-1} (Tables 4, 5).

Ergosterol content at the OT station was consistently higher than that at the FL site (Table 4). The highest mean value for leaf blade ergosterol content over all 3 yr (871 μg g^{-1} organic mass of decay system) was found for short shoots at the OT station in spring 1998–1999, and the corresponding lowest value (148 μg g^{-1}) was for FL, short, summer 1998–1999. An exceptionally high ergosterol content for DS in 1996–1997 (549 μg g^{-1}) and an exceptionally low content for DS in 1998–1999 (221 μg g^{-1}) led to the significant interaction between year and site (summer only; Table 4).

For the overall sampling set, ergosterol content was equivalent among subsites (tall–short shoots) (Table 4). A significant interaction effect between station and subsite (autumn–spring data) was the result of (1) the highest mean ergosterol contents (408–505 μg g^{-1}) being at the tall subsite at the DS and OT stations but not at FL (346 μg g^{-1}); and (2) an exceptionally high mean ergosterol content (469 μg g^{-1}) for OT short.

Standing-decaying leaf sheaths (which were not sampled in summer because of their scarcity) exhibited lower (×0.8) mean ergosterol contents than leaf blades (Table 4). Height of blade on shoots (upper, mid-, and lower) did not significantly affect ergosterol content. Significant interaction between subsite and type of sample (autumn–spring data) was due to exceptionally high ergosterol content for sheaths at tall-shoot subsites (418 μg g^{-1}). The same interaction for summer data was related to a reversal at the short-shoot subsites in direction of change in ergosterol contents: at tall and

Table 5. Ergosterol content ($\bar{x}$ ± SD, μg g^{-1} organic mass), rate of incorporation of acetate into ergosterol per unit ergosterol ($\bar{x}$ ± SD, pmol μg^{-1} ergosterol h^{-1}), and fungal productivity (''Fungprod''; $\bar{x}$ ± SD, μg fungal organic mass g^{-1} system organic mass h^{-1}) for standing-decaying leaf blades of smooth cordgrass (*S. alterniflora*) in all four seasons.*

	Summer	Autumn	Winter	Spring
Ergosterol, all yr	292^{A} ± 208	310^{A} ± 98	416^{B} ± 110	554^{C} ± 144
Acetate, 96/97	15.4^{A} ± 5.4	19.7^{A} ± 4.5	40.3^{C} ± 20.9	18.8^{A} ± 4.0
Acetate, 97/98	24.5^{ABC} ± 7.1	25.1^{ABC} ± 5.6	19.8^{A} ± 9.9	22.2^{AB} ± 3.9
Acetate, 98/99	19.2^{A} ± 3.5	38.0^{BC} ± 1.2	21.1^{AB} ± 6.6	30.9^{ABC} ± 2.7
Fungprod, 96/97	70^{A} ± 22	113^{AB} ± 10	288^{CD} ± 135	176^{ABC} ± 38
Fungprod, 97/98	125^{AB} ± 29	159^{AB} ± 38	147^{AB} ± 76	184^{ABC} ± 31
Fungprod, 98/99	109^{AB} ± 20	216^{BC} ± 41	171^{ABC} ± 69	329^{D} ± 29

* No significant interaction between year × season for ergosterol content; significant interaction (ANOVA, $P < 0.001$) between year × season for acetate incorporation and for fungal productivity. Mean values with different capital superscript letters within each of the three variables indicate means that are significantly different (SNK, $P < 0.05$).

Table 6. Rate of incorporation of acetate into ergosterol per unit ergosterol ($\bar{x} \pm$ SD, pmol μg^{-1} ergosterol h^{-1}), and fungal productivity (''Fungprod''; $\bar{x} \pm$ SD, μg fungal organic mass g^{-1} system organic mass h^{-1}) for leaf samples of smooth cordgrass (*S. alterniflora*), shown separately for autumn–spring and for summer, because of the difference in types of samples collected in these seasonal sets.*

	Autumn–Spring			Summer only		
Year	96/97	97/98	98/99	96/97	97/98	98/99
Acetate	30.7 ± 17.0	25.6 ± 8.6	34.1 ± 14.3	12.8^{A} ± 5.8	22.3^{B} ± 6.6	19.0^{AB} ± 4.3
Fungprod	194 ± 102	155 ± 43	217 ± 83	59^{A} ± 24	120^{B} ± 30	115^{B} ± 35
Season	Aut	Win	Spr	N/A	N/A	N/A
Acetate	33.4 ± 15.8	31.0 ± 17.0	25.9 ± 5.9			
Fungprod	152 ± 56	197 ± 103	215 ± 72			
Sample*	Upper	Mid	Sheath	Upper	Mid	Lower
Acetate	30.3^{AB} ± 12.2	22.2^{A} ± 7.9	37.9^{B} ± 16.2	23.3^{B} ± 4.6	16.0^{AB} ± 5.9	14.8^{A} ± 6.7
Fungprod	221 ± 99	175 ± 65	169 ± 73	108 ± 24	94 ± 40	92 ± 56

* Within rows for autumn–spring ($n = 18$ per $\bar{x}$) or for summer ($n = 6$ per $\bar{x}$) sets, mean values with different capital superscript letters are statistically significantly different (ANOVA + SNK, $P < 0.05$). *See* text and Table 5 for descriptions of significant interactions (year × season [autumn-spring], season × sample type [autumn-spring]). *See* also footnotes for Table 4.

intermediate-height subsites, ergosterol content increased from high to low on shoots (253–364 $\mu g\ g^{-1}$), but at short-shoot subsites, ergosterol content increased from low to high on shoots (226–386 $\mu g\ g^{-1}$).

Ergosterol synthesis—For the autumn–spring data set (including leaf sheaths), there was no significant difference among years in the rate of incorporation of acetate into ergosterol per unit ergosterol (Table 6). In summer, the rate was nearly twice as great in 1997–1998 as in 1996–1997, and (for leaf blades only) for all seasons, there were significant interactions between year and season. This interaction was a consequence of different patterns for acetate incorporation (highest in winter in 1996–1997; no significant difference in 1997–1998; and lowest in summer in 1998–1999)

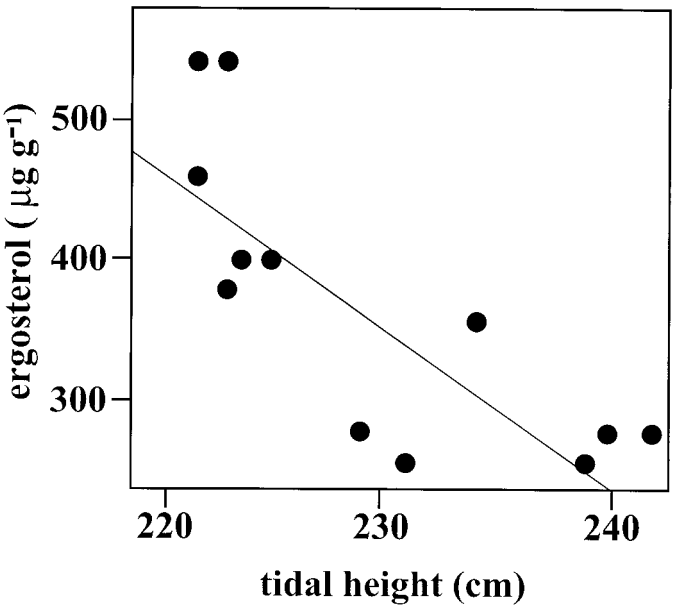

Fig. 1. Ergosterol content of standing-decaying leaves of smooth cordgrass (*S. alterniflora*), means for each seasonal sampling, versus the mean higher diurnal tidal height (cm above mean lower low water) over the 3 months before sampling ($r = -0.77$, $P = 0.004$; line = linear least-squares fit).

(Table 5). For the autumn–spring data set, there was no seasonal effect detected (Table 6).

Decaying leaf sheaths exhibited significantly higher (×1.7) rates of acetate incorporation than the mid-blades to which they were attached (Table 6). In summer, upper blades supported higher (×1.6) rates of acetate incorporation than lowermost blades. Significant interaction between season and sample type was the result of a distinct decline (by ×1.6) in rates from autumn to spring that was found for leaf sheaths only.

Fungal productivity—Rates of fungal organic production per unit organic mass of the decay system (converted from rates of acetate incorporation using an empirical conversion factor; *see* Methods) were equivalent among years, except for a low mean value for summer 1996–1997, just as for rates of acetate incorporation per unit ergosterol (Table 6). Season–year interactions were also found for fungal productivity, as they had been for acetate incorporation per unit ergosterol, but fungal-productivity patterns showed peaks in winter (1996–1997) and spring (1998–1999) and consistently low values in summer (Table 5).

For the overall autumn–spring or summer data sets, fungal productivities did not differ significantly among sample types (Table 6). Significant interaction between season and type of sample (autumn–spring) was the result of an exceptionally low mean value for winter mid-blades (142 $\mu g\ g^{-1}$ organic mass of decay system h^{-1} vs. 188–262 $\mu g\ g^{-1}$ for sheaths and upper blades in winter).

Correlation analysis: Rain and tides—Ergosterol content of standing-decaying smooth cordgrass leaves was negatively correlated to cumulative rainfall amount in the 3 months before sampling ($r = -0.63$, $P = 0.03$), but when stepwise multiple regression was performed, with cumulative rainfall and mean higher diurnal high tide for the 3 months before sampling as independent variables, only tidal height showed a significant negative relationship to ergosterol content ($r = -0.77$, $P = 0.004$) (Fig. 1). There was no significant relationship between ergosterol-synthesis rate per unit ergosterol and cumulative rain or tidal height, but for fungal productivity (rate of organic fungal production per unit decay sys-

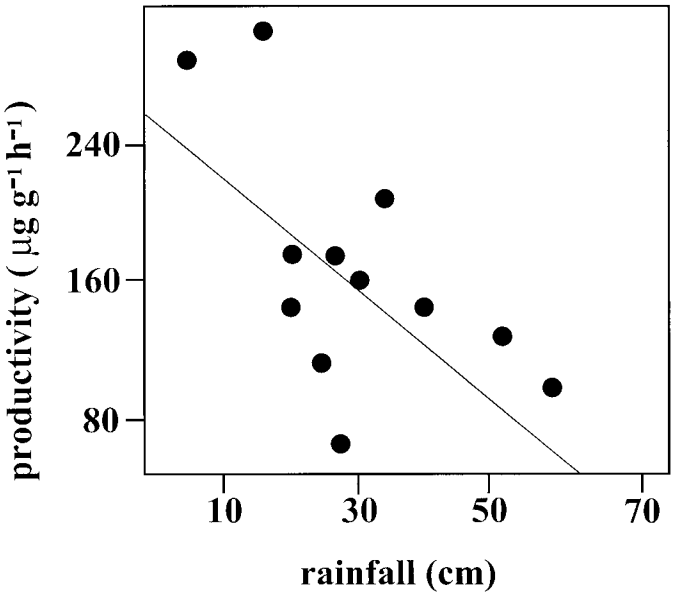

Fig. 2. Fungal productivity, organic fungal mass per unit organic decaying cordgrass (*S. alterniflora*) mass, means for each seasonal sampling, versus cumulative rainfall for the 3 months before sampling ($r = -0.62$, $P = 0.03$; line = linear least-squares fit).

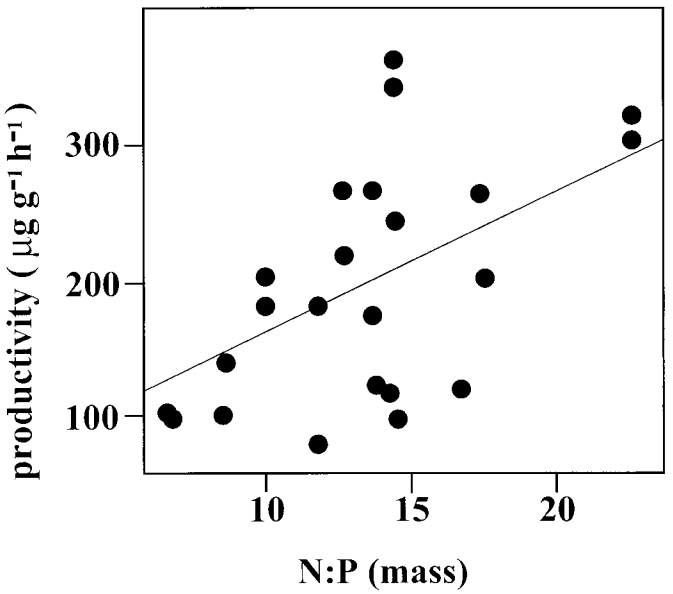

Fig. 3. Fungal productivity, organic fungal mass per unit organic decaying cordgrass (*S. alterniflora*) mass, versus the nitrogen-to-phosphorus mass ratio of the decaying leaf system ($r = 0.52$, $P = 0.009$; line = linear least-squares fit).

tem mass), cumulative rainfall was significantly correlated ($r = -0.62$, $P = 0.03$) (Fig. 2), and tidal height was not ($r = -0.38$, $P = 0.22$).

Correlation analysis: Snails, elevation, salinity, and temperature—No significant relationships were found for ergosterol content of cordgrass blades with snail (*L. irrorata*) densities, not even for the more snail-accessible (lower) blade sampled at short subsites where periwinkle numbers (Table 2) were highest ($r = 0.09$, $P = 0.66$). Height above sediment was not significantly correlated to ergosterol content of blades, not even over the whole range of heights of upper blades, from <10 cm to >100 cm ($r = 0.11$, $P = 0.26$). There were no significant relationships for ergosterol content, rate of ergosterol synthesis, or fungal productivity with salinity or temperature of creek water at the time of sampling. Salinity and 3-month rainfall before sampling were not correlated ($r = -0.08$, $P = 0.80$), presumably because salinity reflects only the most recent rainfall events.

Correlation analysis: Rates with biomass—Rate of ergosterol synthesis per unit ergosterol was significantly negatively related to ergosterol content of decaying leaves ($r = -0.24$, $P = 0.04$). Even so, fungal productivity per unit mass of decay system was significantly positively related to ergosterol content of decaying blades ($r = 0.50$, $P < 0.0001$).

Correlation analysis: C : nutrient ratios—C : N mass ratio was significantly negatively correlated to ergosterol content of decaying cordgrass leaves ($r = -0.37$, $P = 0.0001$), but C : P was not ($r = 0.01$). Rate of ergosterol synthesis per unit ergosterol was not significantly correlated to C : nutrient ratios. Rate of fungal production per unit decay system mass was significantly correlated to C : N ($r = -0.57$, $P = 0.004$) but not to C : P ($r = 0.19$, $P = 0.37$). There was no clear plateauing of rate of fungal production at the high end of the N : P range (Fig. 3).

Discussion

Annual and seasonal variability—Morris and Haskin (1990) and Morris (2000) found that primary productivity of smooth cordgrass in high marshes of South Carolina (33°N) is positively affected by year-to-year higher sea levels (sea-level anomalies) and cumulative rainfall, resulting in as much as 2.6-fold differences in annual production. In contrast, Teal and Howes (1996) found no statistically significant correlation, over a 22-yr period, between sea-level anomalies or rainfall and high-marsh smooth cordgrass annual production in a Massachusetts salt marsh (41°N), and a maximum difference among yearly production values of 1.5-fold. The summer-to-summer year-periods of the present work were climatologically distinct (e.g., rainfall ranging over a factor ~2, and absence of subzero air temperatures in only one winter; *see* Methods), but living ascomycete mass and ascomycete productivities per unit decaying cordgrass leaf system in Georgia salt marshes exhibited year-to-year evenness comparable to that found for cordgrass photosynthetic production by Teal and Howes (1996) (e.g., 4% difference between high and low yearly averages for autumn–spring data for ergosterol content; Tables 4–6). However, fungal biomasses and productivities per unit marsh area, because they are annually uniform per unit cordgrass substrate, would be expected to reflect the year-to-year variation found by Morris (2000) for primary productivity of salt marshes of the Georgia Bight.

Although living fungal biomass (ergosterol content) within standing-decaying smooth cordgrass leaves was signifi-

cantly different among the marsh watersheds sampled (low at FL, the upper Duplin station; Table 4), the difference from high to low was only 1.3-fold (autumn–spring data). Fungal mass content was stable across marsh subsites (tall- to short-shoot areas) and was low by 1.3-fold only for leaf sheaths versus the types of blades sampled. Fungal-specific activity (acetate incorporation per unit ergosterol) showed a weak tendency to be low among sample types with high ergosterol contents; nevertheless, mean fungal productivity per unit decay system mass was level across types of leaves or leaf parts. Thus, fungal crop and productivity per unit leaf substrate are stable not only among years but also across marsh subsystems, in spite of the marked differences in shoot characteristics of smooth cordgrass itself from short- to tall-form subsites (Dai and Wiegert 1996*a*; Table 2).

Stability was found for fungal biomass and productivity on a multiannual basis, but there was considerable change in these variables among seasons. Contrary to what would be expected for marshgrass and microbial crops and photosynthetic or respiratory (carbon or sulfur based) rates (e.g., Morris and Haskin 1990; Dai and Wiegert 1996*a*; Teal and Howes 1996), shoot decay fungi did not show higher mass content or rates in the warmer months. Fungal biomass and 20°C productivities (per unit decay system mass) were generally high in winter and spring and low in summer and autumn (e.g., 1.6-fold higher living fungal mass in winter–spring blades than in summer–autumn blades and three- to fourfold higher fungal productivity in winter or spring than in the previous summer; Tables 4–6). Interestingly, high values for living fungal content were also found for salt-marsh grass (*Spartina maritima*) in Portugal in winter (but winter months were the rainiest) (Castro and Freitas 2000).

Gallagher and Pfeiffer (1977) found a summer depression (by twofold in the high-marsh subsite used) in respiration rates of standing-decaying parts of smooth cordgrass shoots that they submerged in laboratory microcosms. There was no obvious cause of this change from the May to July sampling, but the present data for fungal mass content and productivity offer a possible explanation. Because Gallagher and Pfeiffer (1977) sampled in the same manner as in the present work (sampling the standing-decaying shoots that were representative of the marsh at sampling times, rather than following temporal change in tagged shoots; *see* Newell et al. 1998), it seems likely that Gallagher and Pfeiffer were seeing the same shift (with similar magnitude) that appears in the present data, from high fungal mass in spring, high on wholly dead shoots, to lower masses on dead blades on partially living shoots in summer (Tables 4–6). There are several potential explanations of the unusual seasonality for Georgia salt-marsh ascomycetes, including (1) more frequent seawater submergence and higher rainfall in summer and fall; (2) more exposure to invertebrate shredding of the leaves in summer–autumn, in part because the standing-decaying leaves are higher on shoots in winter and spring (Table 2); and (3) fungal growth optimization at cool temperatures as part of an adaptation to use of dew as a major wetting phenomenon.

Submergence, invertebrates, and rainfall as factors—More frequent seawater submergence could be a factor producing summer–autumn minima in fungal variables, because decaying leaves are lower on shoots in summer–autumn than in winter–spring (Table 2), and tides are higher on average by 9–17 cm in summer–autumn. Living fungal biomass was negatively correlated to mean tidal heights in the 3-month interval before sampling (Fig. 1). Leaf blades low on short shoots in summer would be the ones receiving the most regular and persistent seawater influence (Newell et al. 1998), and it was these blades that exhibited a reversal of the trend toward higher ergosterol content lower on shoots. It may be that the benefits provided by seawater wetting are more than offset by the provision of easier access to decaying leaves by mycophagous invertebrates (gastropods, amphipods, crabs, and protozoa [large ciliates containing ingested ascospores of *P. spartinicola* have been seen on wet blades of smooth cordgrass: pers. comm.]; Newell and Porter 2000). Salt-marsh periwinkles (*L. irrorata*) are capable of reducing cordgrass–fungal biomass without a stimulation of fungal productivity (Graça et al. 2000), but two other shredding invertebrates (a gastropod and an amphipod) reduced living fungal mass and appeared to stimulate fungal productivity, so whether higher access of mycophagous invertebrates would be negative or positive for fungal productivity appears to depend on the invertebrates involved (and on their densities—*see* below). Because periwinkles become dormant on the marsh surface at shoot bases when temperatures fall below 15°C (Newell 1993*b*), there would be no periwinkle grazing during a portion of the winter and spring periods, perhaps resulting in a release of fungi from snail-grazing repression in the marsh subsites with the highest snail concentrations (Table 2) (but no evidence for this repression was found in this study—*see* below). Because tidal height varied strongly with season (*see* Collection of samples above; Kneib 1997), it is possible that some seasonally changing variable other than tides and grazers was the principal influence upon fungal mass.

Fungi digest hyphal tunnels into solid lignocellulosic substrates and nutritionally utilize the digestate, which they may complex in their hyphal sheaths (Newell et al. 1996*b*; Wessels 1999). In addition to prolonged shredder access, another potentially myco-negative effect of longer periods of seawater submergence might be increased leaching of fungal digestates of leaf substrate away from the fungal decay system. High rainfall rates could have this same negative effect; indeed, when standing-decaying smooth cordgrass leaves were experimentally maintained at water saturation by misting in the field, fungal mass contents and productivities were low, loss of organics was high (*see* also Halupa and Howes 1995), and there was increased presence of potential bacterial competitors relative to decaying leaves under a normal water-availability regime (Newell et al. 1996*a*; Newell and Palm 1998). Fungal productivity per unit decay system mass in the present work was lower when rainfall was high (Fig. 2), consistent with the 1996 finding. That high rainfall rates would be negative for shoot decay ascomycetes might be considered counterintuitive, because a very important nitrogen source in grasslands is precipitation (Blair et al. 1998; *see* Paerl et al. 1999; Seitzinger and Sanders 1999).

Newell and Bärlocher (1993) and Graça et al. (2000) found that shredding by salt-marsh periwinkles (*L. irrorata*)

was severe and fungal-selective enough to reduce ergosterol content of naturally decayed cordgrass blades by >50% over a 48-h period of constant water saturation of blades. However, this shredding was for periwinkle : blade ratios that were quite high (comparable to snail densities of >400 m^{-2}) (Newell and Bärlocher 1993). In the present work, no significant relationship was found between periwinkle densities and living fungal mass or fungal productivity (e.g., for ergosterol content, short subsites, and lower blades, $r = -0.008$; $P = 0.66$). Highest recorded periwinkle density in the present work was 85 m^{-2} (the FL station, short shoots), and mean periwinkle density overall was 28 m^{-2}. The fungal-repressive nature of periwinkle grazing found by Graça et al. (2000) may be characteristic of very high snail densities only; at average pan-marsh densities (~25 m^{-2}), periwinkle grazing could be fungal-stimulative, as was grazing by salt-marsh coffeebean snails (*Melampus bidentatus;* Graça et al. 2000). One caveat here is that sample selection may have tended to minimize the effect of periwinkles, in that shoots chosen for sampling were those that bore blades intact enough to permit sample processing; although snail-grazed blades were not excluded, there may have been selection against the most extensively periwinkle-grazed blades (reduced to loose tangled strings: *see* fig. 2 in Newell et al. 1989).

It has been previously concluded that dewset is the predominant wetting event for the smooth cordgrass shoot decay system (Newell et al. 1996*a*, 1998). Dew, which sets on the marsh canopy on most nights (Newell et al. 1989), would not be expected to result in high leaching rates from the decaying leaves, though it can permit periwinkle climbing and grazing (pers. comm.). The regularity of dewset, and fungal adaptation to dew-wetting and accompanying cool temperatures, may be part of the explanation for the stability of fungal crop among years, marsh types, and elevation above the sediment, for the high winter–spring fungal production, and for the negative reaction of fungi to high rainfall rates and persistent tidal submergence. A better definition of the relationship between marshgrass ascomycetes and dewset is needed (Newell and Porter 2000).

Nitrogen, phosphorus, and fungi—Nitrogen is a principal growth-controlling nutrient for smooth cordgrass in salt marshes (e.g., Osgood and Zieman 1993; Dai and Wiegert 1996*a*; Mendelssohn and Morris 2000). Field-experimental manipulations have revealed that nitrogen availability also controls cordgrass–fungal production (asexual and sexual; Newell et al. 1996*a*). Nitrogen content of standing-decaying smooth cordgrass rose the most (resulting in C : N = 36, ~25% lower than the other stations) with season at the OT station (which is ~200 m from the mouth of a surface-drainage ditch that flows from alongside a septic field), and OT exhibited high ergosterol contents of decaying cordgrass (Table 4). The FL station, with the greatest potential marsh influence upon flooding-tidal nitrogen concentrations (Whitney et al. 1981; Newell et al. 1988; Childers 1994; Vörösmarty and Loder 1994; Cai et al. 2000) and potential signs of relatively low nitrogen availability (narrow stems and leaves, Table 2; Dai and Wiegert 1996*b*), had low leaf ergosterol contents (Table 4). Nitrogen content of decaying leaves (as C : N) explained 14% of the variation in ergosterol content and 32% of the variation in fungal productivity of the leaves ($P = 0.0001$–0.004), not very different from the 21% coefficient of determination found for ergosterol content versus C : N of standing-decaying smooth cordgrass leaves in marshes from Maine to Florida (Newell et al. 2000). Interestingly, dissolved N and P concentrations in tidal creeks of South Carolina marshes peak in summer and bottom out in winter, the opposite of the trends for cordgrass–fungal mass and productivity (Morris 2000). Because fungi within decaying cordgrass are likely to immobilize nitrogen to an extent dependent on its external and internal availability (Newell 1993*b*), the C : N of decaying leaves probably reflects the degree of previous availability of nitrogen, and the significant relationship between C : N and fungal variables is probably further evidence of participation of nitrogen in control of fungal production.

Phosphorus is unlikely to be a growth-controlling nutrient for smooth cordgrass, at least in part because of its low sorption in salt-marsh sediments (Whitney et al. 1981; Howarth 1988; Paludan and Morris 1999; Sundareshwar and Morris 1999; Mendelssohn and Morris 2000). Osgood and Zieman (1993) found that smooth cordgrass tissue phosphorus was constant across a range of high- and low-elevation marsh sites, and the marshgrass did not respond to nitrogen + phosphorus fertilization by increasing tissue-P concentrations (whereas nitrogen concentrations were sharply increased), suggesting that phosphorus was highly available relative to nitrogen. For the fungal decomposers of smooth cordgrass, the situation appears to be similar: there was no significant relationship between C : P of decaying cordgrass leaves and either living fungal content, specific rate of ergosterol synthesis, or fungal productivity per unit decay system mass. In spite of the relatively high N : P ratios of the decaying leaves (mean N : P mass ratio, autumn–spring data = 16; compare ~11 for living leaves of smooth cordgrass: Osgood and Zieman 1993), not even at the high end of the N : P range did any clear evidence appear for phosphorus limitation of fungal productivity (Fig. 3).

Calculations, unit-marsh basis—The 3-yr mean ergosterol contents and fungal productivities for summer–autumn and winter–spring can be used to roughly calculate average, cross-marsh fungal production per square meter Sapelo smooth cordgrass salt marsh (Table 7). Relatively small differences among sample types were ignored (e.g., 40% lower ergosterol content of leaf sheaths than blades [Table 4]; 58% higher fungal productivity for high blades and sheaths than lower blades in winter [*see* Results]), and it was assumed that fungal variables for stems would equal those for leaves (this is a very tenuous assumption that is in need of a challenge). Data for mass of standing-decaying smooth cordgrass are from Newell et al. (1998). Values for fungal productivity were obtained at 20°C, and these were not altered to match mean temperatures for the two half-years (Table 7), in part because it is not yet known when cordgrass ascomycetes do most of their growing—it may be at night, at cooler temperature, or during dewset (Newell and Porter 2000; *see* above). Fungi were assumed to be fully active (released from water-stress dormancy by dew, rain, and tides) for 50% of

Table 7. Average values for summer + autumn and winter + spring for variables used in rough calculation of g organic living fungal mass m^{-2} marsh and fungal productivity (g organic fungal mass per 6 month) m^{-2} marsh.*

	Summer–Autumn	Winter–Spring
Decaying shoots†	367 g m^{-2}	960 g m^{-2}
Percentage living fungal	5.80%	9.04%
Fungal mass	21 g m^{-2}	87 g m^{-2}
Percentage fungal N‡	33%	39%
Percentage fungal P‡	44%	88%
Fungal productivity§	125.3 μg g^{-1}	206.3 μg g^{-1}
Fungal productivity‖	101 g m^{-2}	434 g m^{-2}

* Averaging over tall- to short-form subsites and assuming equality among leaf blades, leaf sheaths, and stems.

† From Newell et al. (1998) for standing, attached material only; excludes upright-detached and detached-fallen material.

‡ The percentage of total N and P of the decay system estimated to be present in living fungal mass, using 4% N of fungal mass (Newell 1993*a*) and 0.05% P of living fungal mass (Lilly and Henson 1985, for low P conditions). Note that total fungal mass (living plus empty hyphal) may be twofold greater than living fungal mass (Newell and Porter 2000).

§ Micrograms of organic fungal mass g^{-1} organic system mass h^{-1} at 20°C.

‖ Per 6-month period, assuming average production rates in effect 50% of time, without correction for temperature difference from the 20°C used in productivity incubations (Newell et al. 1989; Newell and Porter 2000). Average creek-water temperature at sampling times, summer + autumn, 24.7°C; winter + spring, 21.2°C.

diel periods (another tenuous assumption needing thorough investigation) (Newell et al. 1989; Newell and Porter 2000). Resultant per-area values for fungal biomass range from 21 to 87 g m^{-2}, and 6-month fungal production ranges from 101 to 434 g m^{-2} (Table 7). The annual fungal production would then be 535 g m^{-2} (ratio of production to living fungal biomass, ~10 : 1), lower than the preliminary value (734 g m^{-2}) given by Newell and Porter (2000), which was calculated using less than one-third of the 3-yr data. The 535-g value lowers calculated yield to 41% of total potentially available cordgrass-shoot substrate (cross-marsh average annual production of smooth cordgrass shoots, 1,313 g m^{-2}: Newell and Porter 2000, from Dai and Wiegert 1996*a*). This yield (amounting to 6×10^3 metric tons in the 11-km^2 Duplin River marsh watershed) might still be considered a meaningfully high value with respect to potential output of secondary animal production (''trophic relay'': Kneib 1997) in a highly photosynthetically productive, detritus-based ecosystem such as the salt marshes of Georgia, especially when the annual production of bacteria is added to it (one rough calculation = 193 g m^{-2}, 96% in the form of sediment bacteria: Newell and Porter 2000; *see* Boschker et al. 1999; this estimated annual bacterial production does not include creek-water bacterioplanktonic productivity based on smooth cordgrass organics: Newell and Krambeck 1995; Shiah and Ducklow 1995; del Giorgio and Cole 1998).

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