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Autumnal Biomass and Potential Productivity of Salt Marsh Fungi from 29° to 43° North Latitude along the United States Atlantic Coast†

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It has been established that substantial amounts of fungal mass accumulate in standing decaying smooth cordgrass (*Spartina alterniflora*) marshes in the southeastern United States (e.g., in standing decaying leaf blades with a total fungal organic mass that accounts for about 20% of the decay system organic mass), but it has been hypothesized that in marshes farther north this is not true. We obtained samples of autumnal standing decaying smooth cordgrass from sites in Florida to Maine over a 3-year period. The variation in latitude could not explain any of the variation in the living fungal standing crop (as determined by ergosterol content) or in the instantaneous rates of fungal growth (as determined by acetate incorporation into ergosterol at a standard temperature, 20°C), which led to the conclusion that the potential levels of fungal production per unit of naturally decaying grass are not different in northern and southern marshes. Twenty-one percent of the variation in the size of the living fungal standing crop could be explained by variation in the C/N ratio (the higher the C/N ratio the smaller the fungal crop), but the C/P ratio was not related to the size of the fungal crop. Instantaneous rates of fungal growth were negatively related to the size of the living fungal crop ($r = -0.35$), but these rates were not correlated with C/nutrient ratios. The same two predominant species of ascomycetes (one *Phaeosphaeria* species and one *Mycosphaerella* species) were found ejecting ascospores from standing decaying smooth cordgrass blades at all of the sites examined from Florida to Maine.

Ascomycetous fungi are the principal microbial secondary producers in standing decaying salt marsh grasses (40). This conclusion has been reached by using transmission electron microscopy and direct epifluorescence microscopy and by the dynamics of a fungal index sterol, ergosterol (37, 44, 47). Most monitoring of the fungal mass dynamics in standing decaying smooth cordgrass (*Spartina alterniflora*), a major salt marsh grass of the western Atlantic Ocean (4, 10, 11, 29), has been performed in Georgia salt marshes (40), although the range of smooth cordgrass extends north into Canada and south into Florida (30, 31).

One examination of fungal mass dynamics in standing decaying smooth cordgrass in New Brunswick, Canada, led to the conclusion that at the northern end of the range, the fungal standing crops are smaller than the fungal standing crops in Georgia (51). Newell (33) and Samiaji and Bärlocher (51) hypothesized that smaller fungal standing crops occur in marshes that are farther north on the basis of visual estimates of the density of fungal sexual structures (ascomata). It has been reported that there are substantial differences in microbial grass decomposition activities over north-to-south gradients (e.g., threefold greater maximum microbial incorporation of ^{14}C-labeled wheat straw carbon at 43°N compared to incorporation at 61°N in Europe [6]) (1, 59). Here we describe our examination of the hypothesis that the level of fungal decomposition activity in smooth cordgrass is lower at higher latitudes. We conducted our examination by obtaining samples of living fungal standing crops and determining rates of fungal production in standing decaying smooth cordgrass in southern and northern temperate marshes in the autumn, when large standing crops of decaying shoots were present (45) before ice could shear away northern shoots (33). We obtained samples over a 3-year period because of the possibility that there could be interyear differences in marsh function (32, 51, 58), and as a subsidiary project, we obtained samples from standing decaying black needlerush (*Juncus roemerianus*) and black grass (*Juncus gerardi*). In addition, we examined variations in fungal standing crop size and activity in relation to nitrogen and phosphorus contents of decaying cordgrass in order to look for indications of potential control of fungi by these nutrients (20, 44).

MATERIALS AND METHODS

Sites. Samples were obtained from eight sites over a 3-year period (1996 to 1998) (Table 1). The sites from which samples were obtained are as follows: site ME in the Wells National Estuarine Research Reserve in Maine (western part of Drakes Island marshes) (3); site MA2 at the Plum Island Sound Long Term Ecological Research Site in Massachusetts (south side of Rowley River marshes) (14); site MA1 in the Waquoit Bay National Estuarine Research Reserve in Massachusetts (marshes on the eastern border of Sage Lot Pond) (3, 13, 49, 52); site VA1 at the Virginia Coast Reserve & Long Term Ecological Research Site in Virginia (upper Phillips Creek marshes) (7, 28); site VA2 near the Chesapeake Bay-Virginia National Estuarine Research Reserve in Virginia (York River marshes along Chesapeake Bay, about 5 km east-northeast of station LE4.3 [12]); site FL1 at the Florida State University Marine Laboratory in Florida (inlet marsh just to the east of the laboratory behind the beach dunes, on St. George Sound, Gulf of Mexico); site FL2 in the Anastasia State Recreation Area east of St. Augustine, Fla. (marsh at the south end of Salt Run inlet); and site FL3 at the Halifax River inlet at Ormond Beach in Florida (marsh strip on the west side of

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TABLE 1. Basic characteristics of sites from which samples were obtained in midautumn (near 15 November) in 1996 to 1998

Location[a]	Site	Year(s) samples were obtained	Latitude (°N)	Creek water temp (°C)	Creek water salinity (mg of salt liter^{-1})	Smooth cordgrass canopy height (cm)	Snail density (no./0.25 m^2)[b]
Wells Reserve	ME	1997, 1998	43.3	3–8	27–33	60–68	28–40
Plum Island	MA2	1998	42.7	8	27	50	1
Waquoit Bay	MA1	1996, 1997, 1998	41.5	7–10	17–24	75	0
Virginia coast	VA1	1996, 1997, 1998	37.5	9–15	26–30	130–180	0
Chesapeake Reserve	VA2	1996, 1997, 1998	37.2	9–14	17–22	80–110	7–13
Florida Gulf Coast	FL1	1996	29.9	19	28	60	3
Anastasia State Recreation Area	FL2	1997	29.9	13	21	127	0
Ormond Beach	FL3	1998	29.2	25	22	97	0

[a] For more information see the text.
[b] The snails at sites VA1, VA2, FL1, FL2, and FL3 were *Littoraria irrorata*, and the snails at sites ME, MA1, and MA2 were *Littorina littorea*.

the river at Riviera Park). Montague and Wiegert (31) have described the Florida marshes previously.

Collecting and handling. At each site, our goal was to collect standing dead shoots of smooth cordgrass (*S. alterniflora*) in a median-height, representative marsh plot in an area where tidal flood water should have a salinity in the range from 15 to 30 mg liter^{-1}. When samples were collected at a site in more than one year, the same plots were used each year. On a day between 15 November and 1 December, we selected four shoots that bore at least two attached, dead, intact leaf blades (completely brown, not shredded to the extent that they were split lengthwise more than once over more than 10 cm of the length, and not collapsed onto the sediment). Electrical cable ties were placed tightly around the shoots at the base (to retain the decayed sheaths), and the shoots were cut at the sediment level. The severed shoots were air dried in an air-conditioned room for 1 week. The shoots were then express shipped to Sapelo Island, Ga., in plastic bags containing freshly opened desiccant and processed at the University of Georgia Marine Institute.

Two leaf blades were cut at the ligule from each air-dried shoot. One blade (designated the upper blade) was obtained from the region that corresponded to two-thirds to three-quarters of the total height, and the other blade (designated the mid blade) was obtained from the region that corresponded to one-third to one-half of the total height. The height of the ligule of each blade was recorded. The lower 11.5-cm piece of each blade was cut free, and the tips were discarded. The 11.5-cm pieces were rinsed in running tap water to remove any clay present (39), and the widths 1 and 10 cm from the ligule were measured. The pieces were then air dried again at a relative humidity of approximately 50% and 23 to 25°C. Air drying to a water content of less than 15% of the water content of decaying leaves stops marsh grass fungal activity without reducing the fungal mass (35). We recognize that air drying could have an effect on fungal productivity (i.e., the rate of acetate incorporation into ergosterol [see below]) even though it does not reduce living fungal mass in decaying marsh grass blades and that this possibility should be studied empirically. However, since all of the samples used in the present study were treated in the same manner, including a 3-h seawater adaptation period before radiolabel was added to measure productivity (see below), this potential problem does not invalidate our multilatitudinal comparison of potential fungal productivities. Furthermore, intermittent wetting and thorough air drying take place naturally and routinely in the salt marsh grass ecosystem, and natural air drying does not alter microbial respiratory activity in subsequently wetted decaying marsh grass blades (33, 35, 37, 44, 45).

We also collected leaf blades or shoots of black needlerush (*J. roemerianus*) at the Virginia and Florida sites and of black grass (*J. gerardi*) at the Massachusetts and Maine sites from the upper portions of the marshes where smooth cordgrass was collected. For needlerush, four dead standing leaf blades that were approximately average height were cut at the sediment surface. The blades selected were brown from the base up to about 75 to 100% of total height, and 0 to 25% of the top portion was grey (more decayed). For black grass, four completely brown, dead, standing, flower-bearing shoots were used.

Fungal standing crop and productivity. The size of the standing crop of living fungi was measured by determining the ergosterol content of blades (15, 16, 19, 38). A high-pressure liquid chromatography assay method was used; this method included preincubation in a [^{14}C]acetate solution and measurement of the ^{14}C incorporated into ergosterol (34). The rates of acetate incorporation (fungal membrane synthesis) were used as an index of fungal productivity (16, 18, 19, 56). Pieces (1.5 cm) were cut from the nonligule end of each of the four air-dried blades obtained from a site for each height (upper or mid) on the shoots and were pooled to obtain one 6-cm sample, which was used for a fungal productivity assay, as described by Newell (36) and Newell et al. (44). Adaptation to seawater (salinity, 15 mg liter^{-1}; filtered with a 0.2-μm-pore-size filter) submergence was accomplished by incubation for 3 h with slow agitation at 20°C under 30 μmol of photosynthetically available radiation m^{-2} s^{-1} (36). The final specific activities of the 5 mM acetate solutions used for incubation were 2.5 to 4.4 dpm pmol of acetate^{-1} (1 Bq = 1 dps), and preparations containing radiolabeled acetate were incubated for 2 to 2.5 h under the conditions used to allow adaptation to submergence. Reactions were terminated by rinsing away the radioisotope and immediately submerging the preparations in ethanol and storing them at 4°C in the dark (36). The liquid chromatographic conditions used were the conditions described by Newell et al. (43); the ergosterol standards used were kept desiccated under nitrogen at 4°C in the dark (38). Ergosterol peaks were collected and scintillation counting was performed as described by Newell (34, 36).

Needlerush and black grass ergosterol analyses were performed by using four 1.5-cm pieces of the mid blade (*J. roemerianus*) or four 1.5-cm pieces of the leaf sheath and stem (*J. gerardi*; the leaf blades of black grass are very narrow and easily lost during handling).

It is possible to convert the fungal membrane synthesis rates reported here into total fungal production rates; however, a consistent functional peculiarity of the injection valve used in the work of Newell (36) resulted in 1.5-fold exaggeration of the conversion factors for marsh grass fungi. The corrected empirical factors are as follows: for smooth cordgrass, 12.6 μg of fungal organic matter per nmol of acetate incorporated into ergosterol; and for black needlerush, 17.8 μg of fungal organic matter per nmol of acetate incorporated (no empirical conversion factors are available for black grass). Ergosterol contents can be converted to fungal matter contents by using a broadly applicable empirical factor (200 U of organic matter per U of ergosterol) (16, 19, 38).

Ascospores in cordgrass. The rate of expulsion of ascospores was measured (with mid blades; two replicates per site) as described by Newell and Wall (41) and Newell and Wasowski (42). To do this, ascospores were captured on a target coverslip placed below a wet blade sample and counted with a dissecting microscope (×100), and species identities were confirmed at a higher magnification (×400). Preparations were incubated for 72 h at 20°C under 30 μmol of photosynthetically available radiation m^{-2} s^{-1} (12 h of light and 12 h of darkness).

Organic matter content. Blade pieces were dried with a microwave oven, weighted, ignited (450°C, 4 h), and reweighed to determine the organic matter content (34).

Carbon, nitrogen, and phosphorus contents of cordgrass. C and N contents of blades were determined by using a combustive autoanalyzer (Perkin-Elmer model 2400 CHN analyzer) (44). The total phosphorus content of blades was determined by using an inorganic nutrient autoanalyzer (Braun-LuebbeAutoAnalyzerII) after persulfate-autoclaving digestion (2, 22). The efficiency of persulfate extraction was determined by using apple and citrus leaf standards obtained from the National Institute of Standards and Technology, U.S. Department of Commerce. C/N and C/P ratios were calculated as mass ratios.

Statistical procedures. An SPSS statistical software package (48) was used for statistical processing of data. Angular or logarithmic transformations were used with data that were in ratio form or that exhibited statistically significant heteroscedasticity (54). The word "significant" is used below to mean statistically significant (probability of type I error, <0.05 [54]). Least-significant-range testing was performed by using the Student-Newman-Keuls method (54). Means ± standard deviations are given below.

RESULTS

Ergosterol content of cordgrass. Analysis of variance (ANOVA) revealed that the ergosterol contents of upper and mid decaying smooth cordgrass blades were not significantly different ($\bar{x}$ for upper blades, 521 ± 251 μg g of organic matter^{-1}; $\bar{x}$ for mid blades, 622 ± 219 μg g^{-1}), so the data for the blade categories were pooled for further analysis. There was not a significant difference in the ergosterol contents of smooth cordgrass blades when sites were compared ($P = 0.88$) (Table 2). Overall, the mean content was 572 ± 237 μg g of organic matter^{-1}. The ergosterol contents of blades were not correlated with latitude ($r = 0.13$; $P = 0.50$) (Table 2).

TABLE 2. Ergosterol contents, acetate incorporation rates, C/nutrient ratios, and organic matter densities for standing decaying smooth cordgrass blades at sites from Maine to Florida

Site	Ergosterol content (μg g of organic matter^{-1})	Rate of acetate incorporation (pmol μg of ergosterol^{-1} h^{-1})	C/N ratio[a]	C/P ratio[a]	Leaf blade organic matter density (mg/cm^2 of leaf)
ME	597 ± 136[b]	43 ± 16	32 ± 3	311 ± 23	6.0 ± 0.8
MA2	714 ± 339	11 ± 3	34 ± 4	405 ± 18	6.5 ± 3.0
MA1	517 ± 332	23 ± 16	53 ± 10	599 ± 97	12.6 ± 1.9
VA1	573 ± 259	14 ± 9	74 ± 21	481 ± 25	11.7 ± 2.1
VA2	632 ± 270	21 ± 9	41 ± 10	378 ± 23	10.2 ± 1.6
FL1	607 ± 54	18 ± 11	56 ± 10	602 ± 75	14.2 ± 1.2
FL2	560 ± 63	22 ± 9	52 ± 18	324 ± 22	13.1 ± 2.7
FL3	337 ± 95	55 ± 20	68 ± 16	419 ± 69	13.4 ± 4.1
P, ANOVA[c]	0.876	0.005	0.002	0.000	0.000
r, with latitude[d]	0.128	−0.053	−0.363	0.009	−0.555
P, *r* with latitude	0.499	0.779	0.049	0.963	0.001

[a] Mass ratio.
[b] Mean ± standard deviation.
[c] *P*, among sites.
[d] Correlation with latitude ($n = 30$).

Blade ergosterol contents were significantly different in different years for the three sites (sites MA1, VA1, and VA2) from which samples were collected in all 3 years ($\bar{x}$ for 1996, 847 ± 151 μg g of organic matter^{-1}; $\bar{x}$ for 1997, 520 ± 268 μg g of organic matter^{-1}; $\bar{x}$ for 1998, 355 ± 110 μg g of organic matter^{-1}; the values for 1997 and 1998 were not significantly different as determined by the Student-Newman-Keuls method). There was not a significant interaction between year and site ($P = 0.24$).

The ergosterol content of blades was significantly negatively correlated with the C/N ratio ($r = -0.46$; $P = 0.01$) but not with the C/P ratio ($r = -0.09$; $P = 0.64$). The average C/N ratios for decaying smooth cordgrass blades at the sites ranged from 32 ± 3 at site ME to 74 ± 21 at site VA1; the C/N ratios were significantly different at different sites ($P < 0.01$), and the values were significantly negatively correlated with latitude ($r = -0.36$; $P < 0.05$) (Table 2). The average C/P ratios for cordgrass blades ranged from 311 ± 23 at site ME to 602 ± 75 at site FL1, and these ratios were also significantly different at different sites ($P < 0.001$), but the values were not correlated with latitude (Table 2). There were not significant differences in either C/N ratios or C/P ratios when values for different years were compared.

The ergosterol content of cordgrass blades was significantly negatively correlated with the organic matter density of blades (milligrams of total organic matter per square centimeter of abaxial leaf blade) ($r = -0.42$; $P = 0.02$). However, the organic matter density of blades was significantly correlated with the C/N ratio (i.e., denser blades had higher C/N ratios; $r = 0.48$; $P = 0.01$), and when both the C/N ratio and organic density were used as stepwise multiple-regression variables with ergosterol content as the dependent variable, only the C/N ratio was significantly related to ergosterol content ($P = 0.01$); the *P* value for organic matter density was reduced to 0.19. The organic matter density of decaying blades was significantly different at different sites, differing by a factor of about 2 from lowest to highest, and the major break occurred between the stations north and south of Cape Cod, Mass. (about 42°N) (Table 2).

Ergosterol synthesis in cordgrass. The rates of acetate incorporation into ergosterol per unit of ergosterol were not significantly different in different types of blades ($\bar{x}$ for upper blades, 28 ± 18 pmol μg of ergosterol^{-1} h^{-1}; $\bar{x}$ for mid blades, 21 ± 14 pmol μg^{-1} h^{-1}). The rates at different sites were highly significantly different, in contrast to ergosterol contents (Table 2), but like the ergosterol contents, the rates of ergosterol synthesis were not correlated with latitude. The mean rates of ergosterol synthesis were lowest at the site with the highest mean ergosterol content (site MA2) (Table 2) and highest at the site with the lowest mean ergosterol content (site FL3); there was a significant negative correlation between synthesis rate and ergosterol content ($r = -0.35$; $P = 0.03$).

The rates of ergosterol synthesis were different in different years for the three sites from which samples were obtained in all 3 years ($P = 0.001$), but the difference was opposite that found (see above) for ergosterol content ($\bar{x}$ for 1996 and 1997, 13 ± 5 pmol μg of ergosterol^{-1} h^{-1}; $\bar{x}$ for 1998, 32 ± 12 pmol μg^{-1} h^{-1}). There was not a significant interaction between year and site.

The synthesis rates were not correlated with C/N ratios ($P = 0.88$) and were more closely correlated with C/P ratios, although not significantly so ($r = -0.30$; $P = 0.11$). The synthesis rates were not correlated with the organic matter densities of decaying blades ($P = 0.79$).

Ascospores. Because ascospore expulsion values were highly variable for site and year, we pooled the data obtained for Florida and for the two sites north of Cape Cod. An ANOVA revealed that the values for individual or pooled sites were not significantly different ($P = 0.58$), and there was not a significant correlation between ascospore expulsion values and latitude ($r = -0.31$; $P = 0.25$). The coefficient of variation for overall mean ascospore expulsion values was more than 100% (overall $\bar{x}$, 60 ± 91 spores per mm^2 of abaxial blade surface per 72 h). The mean ascospore expulsion value was highest in 1996 (158 ± 134 spores mm^{-2} 72 h^{-1}) ($P = 0.08$, as determined by ANOVA). There was not an apparent shift in the species composition of the captured ascospores. The same two major ascomycetous blade decomposers and the same two regular but rarer ascomycetous blade decomposers that were previously found on Sapelo Island (40) were found at all of the sites. The major species were *Phaeosphaeria spartinicola* and *Mycosphaerella* species 2 of Kohlmeyer and Kohlmeyer (24), and the regular but rarer species were *Phaeosphaeria halima* and *Buergenerula spartinae*; however, no *P. halima* or *B. spartinae* was found at sites ME and MA2.

***Juncus* species.** Because two species were involved, *J. roemerianus* at sites VA1 and VA2 and sites south of these sites and *J. gerardi* at sites MA1, MA2, and ME, the data for sites were pooled before analysis as follows: data for sites MA1, MA2, and ME; data for sites VA1 and VA2; and data for sites FL1, FL2, and FL3. The ergosterol contents of *Juncus* samples obtained at different sites were not significantly different (overall $\bar{x}$, 267 ± 132 μg g of organic matter^{-1}; $P = 0.50$, as determined by ANOVA). The mean rates of acetate incorporation, however, were more than twofold higher for *J. gerardi* than for *J. roemerianus* (but the rates at the sites in Virginia and Florida for *J. roemerianus* were not significantly different) (*J. gerardi* rate, 81 ± 38 pmol μg of ergosterol^{-1} h^{-1}; *J. roemerianus* rate, 31 ± 20 pmol μg^{-1} h^{-1}). Although the leaf blades at sites FL1, FL2, and FL3 were larger (diameter, 3.4 ± 0.6 mm) than the leaf blades at sites VA1 and VA2 (diameter, 2.4 ± 0.2 mm), the organic matter density was lower ($\bar{x}$, 0.18 ± 0.03 mg mm^{-3}) for the *Juncus* blades at sites FL1, FL2, and FL3 than for the blades at sites VA1 and VA2 (0.30 ± 0.04 mg mm^{-3}) ($P < 0.001$). The organic matter density of *J. roemerianus* at sites VA1 and VA2 was equivalent to the organic matter density of *J. gerardi* sheaths and stems ($\bar{x}$ for *J. gerardi*, 0.31 ± 0.02 mg mm^{-3}; diameter, 1.1 ± 0.1 mm).

DISCUSSION

The hypothesis of Newell (33) and Samiaji and Bärlocher (51) that there should be a south-to-north decrease in the living fungal standing crop and, by extension, a decrease in the potential fungal productivity in standing decaying smooth-cordgrass (*S. alterniflora*) was shown to be false by the findings described here. The hypothesis was based on visually estimated densities of ascomata (fungal sexual structures) in decaying blades from latitudes north of 44°N. We observed no statistically significant decreases in ascospore expulsion rates (which presumably reflected densities of mature ascomata), fungal standing crop sizes, or rates of ergosterol synthesis at 20°C moving north at sites located from 29 to 43°N. Samiaji and Bärlocher (51) observed small fungal standing crops (maximum mean, 125 μg per g of organic matter consisting of smooth cordgrass leaf blades) (cf. Table 2) for Bay of Fundy marshes (45°N), so it is still possible that a sharp decrease in fungal mass accumulation occurs in standing decaying smooth cordgrass blades at the northern end of the range of this marsh grass. A point at which there could have been a change in fungal activity, based on the known marked shifts in biovariables that occur there, was the boundary between the Acadian and Virginian Provinces (Cape Cod, Mass.; 42°N) (3). However, we obtained mean values for ergosterol content and the ergosterol synthesis rate at 20°C in the Acadian Province that were as high as or higher than some of the mean values obtained for Virginia marshes and marshes that are farther south (Table 2), including the area of maximum development of smooth cordgrass salt marshes in Georgia (40). It appears that substantial potential ascomycetous secondary production based on decay of shoots is the rule wherever smooth cordgrass marshes occur. High rates of autumnal fungal production in northern marshes may be the result of adaptation to short periods when the temperatures permit fungal activity (59). We need to determine levels of fungal productivity at field temperatures at a range of latitudes and in different seasons (instead of our standard 20°C temperature with autumn blades) before we will have a clear picture of the latitudinal range of realized levels of fungal productivity.

Primary productivity of smooth cordgrass is generally limited by nitrogen availability interacting with sediment redox stress (10, 29). There is evidence that nitrogen can limit the efficiency of heterotrophic microbial yields (5) and can limit accumulation of living matter by cordgrass fungi (44). Consistent with these findings was the explanation for part (21%) of the variation in our multilatitudinal data set for the ergosterol content of standing decaying cordgrass. The higher the level of available nitrogen, the more fungal growth and the greater the immobilization of nitrogen, which results in a lower C/N ratio for decaying blades. Higher nitrogen contents of decaying blades were not related to higher instantaneous rates of fungal growth (as determined by acetate incorporation), probably because a higher N content was largely a result of previous fungal immobilization. It has been found that adding nitrogen leads to higher levels of cordgrass fungal productivity, but only at a relatively early point in the decay process (44). There was a hint ($P = 0.11$) in our data that a low C/P ratio rather than a low C/N ratio was weakly ($r^2 = 0.09$) related to higher fungal growth rates; we speculate that this may be a sign that phosphorus can occasionally limit the growth of cordgrass ascomycetes when sufficient nitrogen is present later in the standing decay process (20, 57).

Eighteen percent of the variation ($r^2 = 0.18$) in the ergosterol content of our cordgrass blade samples was attributable to variation in the organic matter density of the blades, but this relationship disappeared when the variation attributable to differences in the C/N ratio was factored out. Thus, the relationship between high ergosterol content and low organic matter density was partly due to the tendency of leaves with low organic matter densities to have high nitrogen contents. The relationship between high ergosterol content and low organic matter density could be (i) the result of blades that are more decayed (and thus have lower organic matter densities) having accumulated more fungal matter per unit of remaining decaying system matter (but we did not find a significant difference in ergosterol content between our less-decayed upper blades and more decayed mid blades), and/or (ii) the result of the capacity of leaves with lower lignocellulose contents to permit accumulation of higher living fungal contents. The much lower organic matter densities of the decaying Acadian leaves (sites ME and MA2) (Table 2) probably could be attributed to the trend toward the lower structural carbohydrate contents in living material in more northern cordgrass stands (however, the evidence that this occurs is based on belowground structures [17]). The relative flimsiness of the Acadian cordgrass blades was obvious even to the naked eye and was reflected in the greater flexibility observed during handling of the blades, which implied that the lignin content was low. If the ascomycetes had had less need to lyse very complex lignocellulose polymers in the Acadian blades, they might have had more energy to spend in accumulating mass (per unit of leaf mass) and preparing to expel ascospores. This implies that the ascomycetes in the Acadian blades could have immobilized more nitrogen per unit of decaying leaf matter (40) and so could be consistent with the finding that the ergosterol content was related to the C/N ratio of decaying blades. The linkage between low decaying blade C/N ratios and high latitudes may also be related to the greater (about twofold) output of N into the coastal Atlantic Ocean in the northeastern United States than in the southeastern United States (23).

Since neither latitude nor phosphorus content was associated with differences in ergosterol content, much of the variation in ergosterol content remains unexplained. Part of the variation was due to year-to-year variation (the ergosterol content in 1996 was twofold higher than the ergosterol content in the following 2 years at the three sites sampled in all 3 years). The interannual variation in secondary microbial productivity

may have some of the same causes as the interannual variation in the productivity of smooth cordgrass that has been observed (32, 51, 58). Additional potential sources of variation include (i) differences in the duration of decay for the leaves used (i.e., leaf blades may senesce, die, decay, and be lost by breakage at different rates at different sites, so that all of the blades obtained at different sites were not at approximately the same point in the standing decay process, as we had intended); (ii) differences in time spans required for different strains of cordgrass ascomyetes to complete digestion of the blades and/or production of ascomata and/or expulsion of ascospores; and (iii) differences in the impacts of invertebrate leaf grazers on cordgrass ascomycetes. Regarding the third possibility, Graça et al. (21) found that leaf-grazing cordgrass marsh invertebrates have distinctly different effects on cordgrass ascomycetes, ranging from no growth enhancement (periwinkles [*Littoraria irrorata*]) to twofold enhancement (salt marsh coffeebean snails [*Melampus bidentatus*]), so different shredder invertebrate compositions (perhaps including species not yet recognized as shredders [40]) could clearly result in variations in the fungal contents of decaying leaves.

It is not likely that differences in the ergosterol contents of ascomycete mycelia were major causes of variation in our ergosterol content data, since species shifting was not observed at different sites (see above) and the ergosterol contents of marsh grass ascomycete species (with no history of maintenance on artificial media) have been found to be homogeneous (the average coefficient of variation was 8% when five species belonging to two large ascomycetous taxa and two marsh grass species were used) (36). However, although temperature probably does not substantially influence the mycelial ergosterol content (19), we cannot rule out the possibility that growth temperature could have an effect on the ergosterol content of marsh grass ascomycetes.

Relatively high ergosterol contents were associated with relatively low rates of fungal production. This relationship has been observed previously with smooth cordgrass (44) and with riparian leaves decaying in streams (18, 55). This relationship probably occurs because the amount of living fungal matter increases until any additional increase in the living fungal matter content of decaying leaves is hindered by limited substrate availability or limited access to one or more nutrients, at which point the growth rate decreases and translocation of fungal organic compounds to points where spores are produced becomes the dominant fungal activity (8). This hypothesis is supported by the finding that as the fungal contents of smooth cordgrass plateaued, the amount of CO_2 released per unit of fungal mass decreased (44).

The high level of noise in our data for rates of ascospore expulsion per square centimeter of blade abaxial surface is curious, yet such noise has appeared (coefficients of variation, >100%) each time that these rates have been measured for standing decaying smooth cordgrass (41, 42). The variation reported for individual sites is variation from blade to blade, but patchiness on 25-mm^2 spore capture targets for individual blades has also been observed (unpublished data). Some variation in the rate of ascospore expulsion would be expected due to differences in (i) the time elapsed since the onset of fungal pervasion of blades, (ii) the time required to produce mature ascomata, and (iii) the degree of grazing by shredder invertebrates (21). Evidence that these explanations are valid was described by Newell and Wasowski (42). However, high coefficients of variation were obtained even for blades that presumably were essentially identical with respect to the potential three causal factors described above (42). Why should the rates be so patchy? Might it be that a high degree of spatiotemporal patchiness in the rates is advantageous because it helps ensure that a steady stream of new ascospores is released for colonization of new substrates (mature leaf blades)?

Recently, researchers have shown that it is likely that in general fungi dominate secondary microbial production in marine and freshwater standing decaying grass shoots (26, 27, 37, 46, 50, 53). Kohlmeyer et al. (25) found a diverse community of ascomycetous decomposers that are especially adapted to standing decaying blades of black needlerush (*J. roemerianus*). Therefore, it is not surprising that we found that *Juncus* species had ergosterol contents of hundreds of micrograms per gram of organic decaying material. Despite the fact that two species were involved (black needlerush and black grass [*J. gerardi*]), there were not significant differences among the means obtained for *Juncus* sites from 29°N to 43°N. However, the mean ergosterol content of the *Juncus* species (267 μg g of organic $matter^{-1}$) was only about one-half the mean ergosterol content of smooth cordgrass (572 $\mu g\ g^{-1}$), implying that the limits for accumulation of living fungal mass are lower in *Juncus* species. *J. roemerianus* decays slowly naturally (9); resistance to build-up of fungal mass may be the principal cause of the slow rate of decomposition. The mean rate of acetate incorporation into ergosterol for black needlerush (30 pmol μg of $ergosterol^{-1}\ h^{-1}$) was in the range of the values obtained for smooth cordgrass (Table 2), but the mean rate for black grass was more than twice as high. One potential explanation for this is that the conversion factor (from acetate incorporation to fungal mass production) for the principal species of fungi that decompose black grass is lower than the conversion factors for the fungi that decompose black needlerush and smooth cordgrass (19, 40).

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REFERENCES

1. **Aerts, R.** 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos **79:**439–449.
2. **Allen, S. E. (ed.).** 1989. Chemical analysis of ecological materials. Blackwell Scientific, Oxford, United Kingdom.
3. **Ayvazian, S. G., L. A. Deegan, and J. T. Finn.** 1992. Comparison of habitat use by estuarine fish assemblages in the Acadian and Virginian zoogeographic provinces. Estuaries **15:**368–383.
4. **Bertness, M. D., and S. C. Pennings.** Spatial variation in process and pattern in marsh plant communities. *In* M. P. Weinstein and D. A. Kreeger (ed.), Concepts and controversies in tidal marsh ecology, in press. Kluwer, Amsterdam, The Netherlands.
5. **Blagodatsky, S. A., I. V. Yevdokimov, A. A. Larionova, and J. Richter.** 1998. Microbial growth in soil and nitrogen turnover: model calibration with laboratory data. Soil Biol. Biochem. **30:**1757–1764.
6. **Bottner, P., F. Austrui, J. Cortez, G. Billès, and M. M. Coûteaux.** 1998. Decomposition of ^{14}C- and ^{15}N-labeled plant material, under controlled conditions, in coniferous forest soils from a north-south climatic sequence in western Europe. Soil Biol. Biochem. **30:**597–610.
7. **Brinson, M. M., R. R. Christian, and L. K. Blum.** 1995. Multiple states in the sea-level induced transition from terrestrial forest to estuary. Estuaries **18:** 648–659.
8. **Carlile, M. J., and S. C. Watkinson.** 1994. The fungi. Academic Press, New York, N.Y.
9. **Christian, R. R., W. L. Bryant, and M. M. Brinson.** 1990. *Juncus roemerianus* production and decomposition along gradients of salinity and hydroperiod. Mar. Ecol. Prog. Ser. **68:**137–145.
10. **Dai, T., and R. G. Wiegert.** 1996. Ramet population dynamics and net aerial primary productivity of *Spartina alterniflora*. Ecology **77:**276–288.
11. **Dardeau, M. R., R. F. Modlin, W. M. Schroeder, and J. P. Stout.** 1992.

Estuaries, p. 615–744. *In* C. T. Hackney, S. M. Adams, and W. A. Martin (ed.), Biodiversity of southeastern United States/aquatic communities. Wiley, New York, N.Y.
12. **Dauer, D. M., A. J. Rodi, and J. A. Ranasinghe.** 1992. Effects of low dissolved oxygen events on the macrobenthos of the lower Chesapeake Bay. Estuaries **15:**384–391.
13. **D'Avanzo, C., J. N. Kremer, and S. C. Wainwright.** 1996. Ecosystem production and respiration in response to eutrophication in shallow temperate estuaries. Mar. Ecol. Prog. Ser. **141:**263–274.
14. **Deegan, L. A., and R. H. Garritt.** 1997. Evidence for spatial variability in estuarine food webs. Mar. Ecol. Prog. Ser. **147:**31–47.
15. **Ekblad, A., H. Wallander, and T. Näsholm.** 1998. Chitin and ergosterol combined to measure total and living fungal biomass in ectomycorrhizas. New Phytol. **138:**143–149.
16. **Fell, J. W., and S. Y. Newell.** 1998. Biochemical and molecular methods for the study of marine fungi, p. 259–283. *In* K. E. Cooksey (ed.), Molecular approaches to the study of the oceans. Chapman & Hall, London, United Kingdom.
17. **Gallagher, J. L., and D. M. Seliskar.** Functions of restored marshes: the role of genetic variation in plant morphology and physiology. *In* M. P. Weinstein and D. A. Kreeger (ed.), Concepts and controversies in tidal marsh ecology, in press. Kluwer, Amsterdam, The Netherlands.
18. **Gessner, M. O., and E. Chauvet.** 1997. Growth and production of aquatic hyphomycetes in decomposing leaf litter. Limnol. Oceanogr. **42:**496–505.
19. **Gessner, M. O., and S. Y. Newell.** 1997. Bulk quantitative methods for the examination of eukaryotic organoosmotrophs in plant litter, p. 295–308. *In* M. McInerney, L. Stetzenbach, C. J. Hurst, G. Knudsen, and M. Walter (ed.), Manual of environmental microbiology. ASM Press, Washington, D.C.
20. **Gessner, M. O., K. Suberkropp, and E. Chauvet.** 1997. Decomposition of plant litter by fungi in marine and freshwater ecosystems, p. 303–322. *In* D. T. Wicklow and B. Söderström (ed.), The mycota. IV. Environmental and microbial relationships. Springer-Verlag, Berlin, Germany.
21. **Graça, M. A. S., S. Y. Newell, and R. T. Kneib.** Consumption rates of organic matter and fungal mass of the *Spartina alterniflora* decay system by three species of saltmarsh invertebrates. Mar Biol., in press.
22. **Hansen, H. P., K. Grasshoff, P. J. Statham, and P. J. L. Williams.** 1983. Automated chemical analysis, p. 347–395. *In* K. Grasshoff, M. Ehrhardt, and K. Kremling (ed.), Methods of seawater analysis. Verlag Chemie, Deerfield Beach, Fla.
23. **Howarth, R. W., G. Billen, D. Swaney, A. Townsend, N. Jaworski, K. Lajtha, J. A. Downing, R. Elmgren, N. Caraco, T. Jordan, F. Berendse, J. Freney, V. Kudeyarov, P. Murdoch, and Z. Zhao-Liang.** 1996. Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: natural and human influences. Biogeochemistry **35:**75–139.
24. **Kohlmeyer, J., and E. Kohlmeyer.** 1979. Marine mycology. The higher fungi. Academic Press, New York, N.Y.
25. **Kohlmeyer, J., H.-O. Baral, and B. Volkmann-Kohlmeyer.** 1998. Fungi on *Juncus roemerianus*. 10. A new *Orbilia* with ingoldian anamorph. Mycologia **90:**303–309.
26. **Kuehn, K. A., and K. Suberkropp.** 1998. Diel fluctuations in rates of CO_2 evolution from standing dead leaf litter of the emergent macrophyte *Juncus effusus* L. Aquat. Microb. Ecol. **14:**171–182.
27. **Kuehn, K. A., P. F. Churchill, and K. Suberkropp.** 1998. Osmoregulatory responses of fungi inhabiting standing litter of the freshwater emergent macrophyte *Juncus effusus*. Appl. Environ. Microbiol. **64:**607–612.
28. **MacMillin, K., L. K. Blum, and A. L. Mills.** 1992. Comparison of bacterial dynamics in tidal creeks of the lower Delmarva Peninsula, Virginia, USA. Mar. Ecol. Prog. Ser. **86:**111–121.
29. **Mendelssohn, I. A., and J. T. Morris.** Eco-physiological controls on the primary productivity of *Spartina alterniflora. In* M. P. Weinstein and D. A. Kreeger (ed.), Concepts and controversies in tidal marsh ecology, in press. Kluwer, Amsterdam, The Netherlands.
30. **Mobberley, D. G.** 1956. Taxonomy and distribution of the genus *Spartina*. Iowa State College J. Sci. **30:**471–574.
31. **Montague, C. L., and R. G. Wiegert.** 1990. Salt marshes, p. 481–516. *In* R. L. Myers and J. J. Ewel (ed.), Ecosystems of Florida. University of Central Florida Press, Orlando.
32. **Morris, J. T., and B. Haskin.** 1990. A 5-yr record of aerial primary production and stand characteristics of *Spartina alterniflora*. Ecology **71:**2209–2217.
33. **Newell, S. Y.** 1993. Decomposition of shoots of a saltmarsh grass; methodology and dynamics of microbial assemblages. Adv. Microb. Ecol. **13:**301–326.
34. **Newell, S. Y.** 1993. Membrane-containing fungal mass and fungal specific growth rate in natural samples, p. 579–586. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole (ed.), Handbook of methods in aquatic microbial ecology. Lewis, Boca Raton, Fla.
35. **Newell, S. Y.** 1995. Minimizing ergosterol loss during preanalytical handling and shipping of samples of plant litter. Appl. Environ. Microbiol. **61:**2794–2797.
36. **Newell, S. Y.** 1996. The [^{14}C]acetate-to-ergosterol method: factors for conversion from acetate incorporated to organic fungal mass synthesized. Soil Biol. Biochem. **28:**681–683.
37. **Newell, S. Y.** 1996. Established and potential impacts of eukaryotic mycelial decomposers in marine/terrestrial ecotones. J. Exp. Mar. Biol. Ecol. **200:**187–206.
38. **Newell, S. Y.** Methods for determining biomass and productivity of mycelial marine fungi. *In* K. D. Hyde and S. B. Pointing (ed.), Techniques for the study of marine fungi, in press. Fungal Biodiversity Press, Hong Kong.
39. **Newell, S. Y., and L. A. Palm.** 1998. Responses of bacterial assemblages on standing decaying blades of smooth cordgrass to additions of water and nitrogen. Int. Rev. Hydrobiol. **83:**115–122.
40. **Newell, S. Y., and D. Porter.** Microbial secondary production from saltmarsh-grass shoots, and its known and potential fates. *In* M. P. Weinstein and D. A. Kreeger (ed.), Concepts and controversies in tidal marsh ecology, in press. Kluwer, Amsterdam, The Netherlands.
41. **Newell, S. Y., and V. D. Wall.** 1998. Response of saltmarsh fungi to presence of mercury and polychlorinated biphenyls at a Superfund site. Mycologia **90:**777–784.
42. **Newell, S. Y., and J. Wasowski.** 1995. Sexual productivity and spring intramarsh distribution of a key saltmarsh microbial secondary producer. Estuaries **18:**241–249.
43. **Newell, S. Y., T. L. Arsuffi, and R. D. Fallon.** 1988. Fundamental procedures for determining ergosterol content of decaying plant material by liquid chromatography. Appl. Environ. Microbiol. **54:**1876–1879.
44. **Newell, S. Y., T. L. Arsuffi, and L. A. Palm.** 1996. Misting and nitrogen fertilization of shoots of a saltmarsh grass: effects upon fungal decay of leaf blades. Oecologia (Berlin) **108:**495–502.
45. **Newell, S. Y., T. L. Arsuffi, and L. A. Palm.** 1998. Seasonal and vertical demography of dead portions of shoots of smooth cordgrass in a south-temperate saltmarsh. Aquat. Bot. **60:**325–335.
46. **Newell, S. Y., M. A. Moran, R. Wicks, and R. E. Hodson.** 1995. Productivities of microbial decomposers during early stages of decomposition of leaves of a freshwater sedge. Freshwater Biol. **34:**135–148.
47. **Newell, S. Y., D. Porter, and W. L. Lingle.** 1996. Lignocellulolysis by ascomycetes (Fungi) of a saltmarsh grass (smooth cordgrass). Microsc. Res. Technol. **33:**32–46.
48. **Norušis, M. J.** 1992. SPSS/PC+. Base system user's guide, version 5.0. SPSS Inc., Chicago, Ill.
49. **Orson, R. A., and B. L. Howes.** 1992. Salt marsh development studies at Waquoit Bay, Massachusetts: influence of geomorphology on long-term plant community structure. Estuarine Coastal Shelf Sci. **35:**453–471.
50. **Poon, M. O. K., and K. D. Hyde.** 1998. Biodiversity of intertidal estuarine fungi on *Phragmites* at Mai Po marshes, Hong Kong. Bot. Mar. **41:**141–155.
51. **Samiaji, J., and F. Bärlocher.** 1996. Geratology and decomposition of *Spartina alterniflora* Loisel in a New Brunswick saltmarsh. J. Exp. Mar. Biol. Ecol. **201:**233–252.
52. **Short, F. T., and D. M. Burdick.** 1996. Quantifying eelgrass habitat loss in relation to housing development and nitrogen loading in Waquoit Bay, Massachusetts. Estuaries **19:**730–739.
53. **Sinsabaugh, R. L., and S. Findlay.** 1995. Microbial production, enzyme activity, and carbon turnover in surface sediments of the Hudson River estuary. Microb. Ecol. **30:**127–141.
54. **Sokal, R. R., and F. J. Rohlf.** 1995. Biometry. Freeman, New York, N.Y.
55. **Suberkropp, K.** 1997. Annual production of leaf-decaying fungi in a woodland stream. Freshwater Biol. **38:**169–178.
56. **Suberkropp, K., and H. Weyers.** 1996. Application of fungal and bacterial production methodologies to decomposing leaves in streams. Appl. Environ. Microbiol. **62:**1610–1615.
57. **Tank, J. L., and J. R. Webster.** 1998. Interaction of substrate and nutrient availability on wood biofilm processes in streams. Ecology **79:**2168–2179.
58. **Teal, J. M., and B. L. Howes.** 1996. Interannual variability of a salt-marsh ecosystem. Limnol. Oceanogr. **41:**802–809.
59. **Wardle, D. A.** 1998. Controls of temporal variability of the soil microbial biomass: a global-scale synthesis. Soil Biol. Biochem. **13:**1627–1637.