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Nitrogen Fixation (Acetylene Reduction) in a Salt Marsh Amended with Sewage Sludge and Organic Carbon and Nitrogen Compounds[1]

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Seasonal distribution of nitrogen fixation by *Spartina alterniflora* epiphytes and in surface and soil samples was investigated in a Georgia salt marsh which was amended with sewage sludge or with glucose and/or ammonium nitrate. There was no significant difference between the rates of fixation in the unamended and sewage sludge plots. Additional perturbation experiments suggested that nitrogen addition indirectly stimulates nitrogen fixation by enhancing *Spartina* production and root exudation. Glucose additions, on the other hand, suppressed nitrogen fixation on a long-term basis. It is suggested that the microbial population in the soil out-competed the plants for the available nitrogen and in turn suppressed plant production and possibly root exudation. A comparison of nitrogen fixation in clipped and unclipped *Spartina* plots substantiated the suggestion that root exudation probably supports nitrogen fixation. Fixation in the clipped plots was significantly lower ($P < 0.05$) than the rates in the unclipped plots.

Marshlands on the east coast of the United States represent nearly 600 thousand hectares (22). They have many functions, one of which is providing a nursery and habitat for many migratory and indigenous fish populations. The economic importance of marshes is quite obvious. Therefore, future usage of these lands must not disrupt the function of the various trophic levels which exist in the ecosystem.

Gosselink et al. (7) proposed using the marshes as a potential natural tertiary treatment plant. However, studies of the effects of secondary sewage on marsh production and microbial ecosystem have only recently been initiated (see 3, 31, 32). The results from these studies indicate that the growth of *Spartina alterniflora* is stimulated. Other biological processes (e.g., nitrogen fixation and dentrification) are either stimulated or inhibited. To understand the possible consequence of sewage sludge disposal on the salt marsh metabolism, many intricate and connected biological and chemical processes require investigation. In this paper the results of a 12-month study on nitrogen fixation in a natural and sewage sludge-amended short *S. alterniflora* salt marsh are examined.

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MATERIALS AND METHODS

Sewage sludge sites. The study sites were in the Duplin River salt marsh contiguous to Sapelo Island, Ga. Four separate blocks (100-m^2 plots) were chosen in the short *S. alterniflora* marsh for this study (see 4 and 5 for a description of the plant zones around Sapelo Island). Two of the blocks received ground-up dried sewage sludge twice monthly at a rate of 200 g/m^2, equivalent to 4.0 g of N/m^2 (3). The sludge was applied by hand. The other two control blocks were separated from the sewage sludge plots by a road so as not to be influenced by the applied sewage sludge.

High-marsh nutrient amendments. Four replicate blocks in the short *Spartina* marsh were divided into ten 0.1-m^2 plots. The plots were randomized and treated in 10 different ways. *Spartina* in one-half of the plots in each replicate was clipped and lawn edgings were pushed down into the marsh to a depth of 15 cm; the other half remained unclipped with no lawn edging. The lawn edgings were used to prevent any lateral root movement into the experimental plots from adjacent zones. The clipped and unclipped plots were then treated with: (i) glucose (50 g/m^2 per month), (ii) ammonium nitrate (10 g/m^2 per month), (iii) glucose plus ammonium nitrate, (iv) rhodamine WD, and (v) no addition. Beginning in February, the nutrients were applied at a rate of 250 ml (50 g of glucose/liter, 11.4 g of ammonium nitrate/liter) per 0.1 m^2 once a month for 5 months. The nutrients were injected into the plots (0 to 15 cm) with a 50-ml syringe with a 1/16-inch (1.6 mm) gauge stainless-steel tube (20 cm long). As a control on nutrient injections, two plots (clipped and unclipped) were

injected with distilled water. There was no difference in salinities between the distilled water controls and the adjacent marsh at the end of the fifth month (Robert R. Christian, Drexel University, unpublished data). The blocks were sampled in July, and duplicate cores were taken from each plot except the rhodamine plots. The 0- to 5-cm and the 10- to 15-cm zones from each core were cut in half vertically and placed in bottles for acetylene reduction assay as described below. Cores from outside each replicate were also sampled and run simultaneously with the experimental cores on each sampling day.

Nitrogen fixation: marsh surface. Rates of fixation were estimated in the short *Spartina* marsh by the acetylene reduction method (13).

For in situ measurement of surface nitrogen fixation, a relatively low-volume (1,100 cc), large-surface-area (0.043 m^2) dome incubator was used. After clipping and removing *Spartina* plants from a 0.05-m^2 area of the marsh, the incubator was pushed down into the soil. During the summer, two layers of 1-mm mesh screen were placed over the incubator to prevent a temperature increase inside the incubator. Temperatures never increased by more than 5°C. Acetylene and ethane (used as an internal standard) were added, yielding an atmosphere of 15% acetylene and 0.1% ethane. After an equilibrium period of 45 to 60 min, three gas samples were removed for analysis every hour for 4 h. Concentrations of ethylene and ethane were analyzed by use of a flame ionization detector gas chromatograph with a Porpak N column. The ethylene concentration was normalized to the ethane in the incubator. In control experiments, in situ ethylene and ethane production were not found in the absence of added acetylene.

After each run, surface sediment (0 to 1 cm) was removed from beneath the dome incubators and examined for total carbon and nitrogen. Sediment samples were dried at 65°C, ground in a mortar and pestle, sieved through a 0.5-mm mesh screen, and dried to constant weight. Soil carbon content was analyzed with a Coleman model 29 nitrogen analyzer. The percent carbon and nitrogen values were used to calculate the C-N ratio (wt/wt).

Soil depth profile. Three intact cores 2.6 cm in diameter were taken to 25 cm. Slices 2 cm thick from selected depths were placed in small incubation chambers with an internal gas space of 50 cc. No disturbance of core geometry was caused by this procedure. Acetylene and ethane were added to yield the concentrations described above, and the chambers were incubated in the dark at in situ temperatures. Gas samples were analyzed for ethylene and ethane at 4- to 8-h intervals for 30 h. Ethylene production generally became linear after an initial period of 10 h and remained linear up to 36 h. Control jars (sediment plus ethane) had only traces of ethylene. Sediment cores were analyzed for nitrogen as described above.

Spartina plants. Nitrogen fixation by epiphytes on aerial *Spartina* (leaves and stems) was determined on the culms clipped from the 0.05-m^2 plot used for the surface incubator. The plant material, both live and dead, was placed in wide-mouth jars. The same gas concentrations (percentage of internal gas volume) were used as for the soil samples. The jars were incubated under full sunlight in a tube of water (25 to 30°C). Gas samples were analyzed every hour for 4 h, and ethylene production was calculated from the linear portion of the curve. In the control jars without added acetylene, plants produced no ethylene over the incubation period.

RESULTS

Marsh surface fixation. Monthly rates of nitrogen fixation were measured for the salt marsh soil surface (Table 1). The results indicate temporal variations, with low in situ rates (1 to 10 μmol of C_2H_4/m^2 per h) during the winter and high rates (10 to 100 μmol/m^2 per h) during the summer. There was no significant

TABLE 1. *In situ nitrogen fixation rates ($\bar{X}$ ± standard error) in control and sewage sludge plots in the high marsh, Sapelo Island, Ga.*[a]

Month in 1975	Surface nitrogen fixation (μmol of C_2H_4/m^2 per h)		Carbon and nitrogen (mean % dry wt)					
			Control			Sewage sludge		
	Control	Sewage sludge	C	N	C-N	C	N	C-N
January	1.33 ± 0.56 (3)	3.51 (2)	ND[b]	ND				
February	6.46 (2)	7.37 (2)	4.38	.32	13.7:1	6.93	1.04	6.66:1
March	9.80 ± 3.25 (4)	7.22 ± 1.17 (4)	3.74	.30	12.5:1	7.15	0.63	11.4:1
April	9.95 (2)	12.7 (2)	3.99	.40	10.0:1	9.9	1.21	8.18:1
May	10.1 (2)	98.2 (2)	4.09	.37	11.1:1	8.59	0.85	10.1:1
June	46.2 (2)	15.4 (2)	4.23	.39	10.9:1	10.1	1.23	8.21:1
July	ND	ND	ND	ND		ND	ND	
August	5.24 ± .82 (4)	12.2 (2)	3.81	.28	13.1:1	11.5	1.20	9.58:1
September ...	14.7 ± 8.08 (4)	11.8 ± 1.61 (4)	5.41	.51	10.6:1	10.0	1.05	9.52:1
October	0.610 ± 0.350 (4)	4.26 ± 1.45 (4)	ND	ND		ND	ND	
November	1.82 ± 0.82 (4)	0.670 ± 0.287 (4)	4.82	.73	6.60:1	10.1	1.16	8.71:1
December	0 ± 0 (4)	2.25 ± 0.78 (4)	3.92	.48	8.16:1	9.84	0.97	10.1:1

[a] Carbon and nitrogen values are means of duplicates, and the C-N ratios are based on dry weights. Values in parentheses are replicate numbers.

[b] Not determined.

difference ($P > 0.05$) between the rates measured in the sewage sludge plots and the control plots. The integrated annual rate for the sewage sludge plots was slightly higher (5.64 mmol/m² per h), but not significantly higher ($P > 0.05$) than the control plots (4.09 mmol/m² per h).

The carbon and nitrogen content for the surface soil in the control plots ranged from 3.74 to 5.41% and 0.28 to 0.73%, respectively (Table 1), with a mean C-N (wt/wt) ratio of 10.7:1 (range, 6.6:1 to 13.7:1). On the other hand, carbon and nitrogen content for the surface soil in the sewage sludge plots ranged from 6.93 to 11.5% and 0.63 to 1.23%, respectively, with a mean C-N ratio of 9.2:1 (range, 6.6:1 to 11.4:1).

***Spartina* epiphytes.** Epiphytic nitrogen fixation in the sewage sludge plots and the control plots was measured from March to December 1975 (Table 2). The results indicate that high fixation rates occurred during the later part of the growing season (August and September) in both areas. As was found for the surface fixation, there was no significant ($P > 0.05$) difference between the rates measured in the control and sewage sludge plots. The integrated annual rates for the control and sewage sludge plots were 2.13 and 2.32 mmol of C_2H_4/m² per h, respectively, about one-half of the surface values.

TABLE 2. *Nitrogen fixation associated with high-marsh Spartina alterniflora*[a]

Month in 1975	Epiphytic nitrogen fixation (μmol of C_2H_4/m² per h)	
	Control plots	Sewage sludge plots
January	ND[b]	ND
February ...	ND	ND
March	8.13 ± 3.00 (4)	7.23 ± 1.54 (4)
April	5.57 (2)	7.93 ± 2.44 (4)
May	5.53 (2)	ND
June	5.24 (2)	1.83 (2)
July	ND	ND
August	5.58 (2)	20.5 (2)
September ..	25.8 (2)	16.1 (2)
October	4.53 ± 2.81 (4)	0.661 ± 0.298 (3)
November ..	2.10 ± 0.81 (4)	0.931 ± 0.525 (4)
December ..	4.27 ± 2.06 (4)	4.99 ± 1.25 (4)

[a] The mean rate ± the standard error is given, with the number of replicates in parentheses.
[b] Not determined.

Soil depth profile. Subsurface nitrogen fixation (0 to 21 cm) was measured over a 7-month period (Table 3). The rates were integrated over depth, and the estimated monthly rates were used to calculate the annual rates. Values for the control and sewage sludge plots were 228 and 252 mmol of C_2H_4/m² per h, respectively. Again, there was no significant difference between treatments.

The distribution of nitrogen fixation over depth was investigated. The monthly rates were normalized on a gram (dry weight) per hour and milligram of N per hour basis, and the mean monthly rates were plotted along with the mean monthly percent N in the soil profile (Fig. 1a and b). The results clearly indicate a definite zone of activity at 5 to 7 cm. Below 5 to 7 cm the activity decreased rapidly. The distribution of activity approximates the root density distribution in the short *Spartina* marsh (8). The average coefficient of variation for the normalized data (grams, dry weight) was 92 ± 20% (standard deviation), which reflects both seasonal variation and heterogeneity in the system.

Two different activity patterns were found for the control (Fig. 1a) and sewage sludge (Fig. 1b) plots when the rates were normalized on a milligram of N basis. Significantly more ($P < 0.05$) nitrogen was fixed at 5 to 7 cm in the sewage sludge plots than in the control plots.

TABLE 3. *Nitrogen fixation soil depth profile in the high marsh*[a]

Treatment	Depth (cm)	Nitrogen fixation (μmol of C_2H_4/m² per h)					
		September	October	November	December	January	March
Control plots	0–2	29.6 ± 1.46	24.9 ± 10.1	35.9 ± 7.55	99.9 ± 65.4	22.3 ± 12.1	1.65 ± 0.71
	5–7	116.7 ± 65.7	74.9 ± 24.4	30.4 ± 6.74	46.9 ± 1.50	27.8 ± 18.4	1.94 ± 0.37
	10–22	62.8 ± 53.6	44.3 ± 18.4	25.8 ± 4.28	41.4 ± 11.0	39.4 ± 25.5	16.3 ± 3.11
	20–22	17.8 ± 0.63	28.6 ± 15.7	10.6 ± 6.85	57.9 ± 40.9	51.3 ± 26.0	11.5 ± 2.11
Integrated	0–22	1,208	912	488	1,084	744	194
Sewage sludge plots	0–2	50.3 ± 10.7	15.3 ± 4.60	89.9 ± 84.5	32.6 ± 11.3	10.4 ± 8.96	0.79 ± 0.34
	5–7	50.3 ± 37.2	18.8 ± 0.43	291.1 ± 144.3	39.6 ± 14.7	5.01 ± 1.84	3.79 ± 1.18
	10–12	68.3 ± 28.3	10.8 ± 8.45	100.1 ± 60.9	53.2 ± 13.8	14.9 ± 7.3	4.05 ± 1.28
	20–22	91.8 ± 62.2	38.9 ± 26.6	34.2 ± 18.1	19.6 ± 5.56	10.4 ± 4.67	5.07 ± 1.58
Integrated	0–22	1,349	408	2,529	776	214	77

[a] The values are $\bar{X}$ ± standard error.

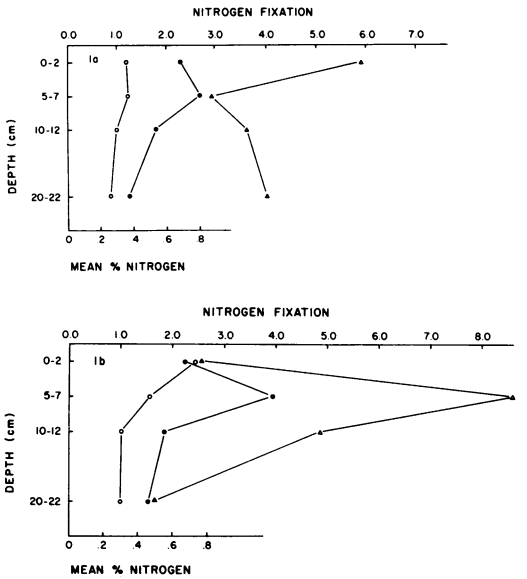

FIG. 1. *Mean monthly nitrogen fixation rate versus depth in the control (a) and in the sewage sludge plots (b). The mean rates were normalized to nanomoles of C_2H_4 per gram per hour (●) and nanomoles of C_2H_4 per milligram of N per hour (△) and plotted with the mean monthly percent N (○).*

The results suggest a stimulation of nitrogen fixation by root exudation (see Discussion).

In the surface layer the total nitrogen content was two to three times higher in the sewage sludge plots than in the control plots (Fig. 1a and b, respectively). The nitrogen content below 5 cm was similar in both areas.

High-marsh nutrient amendment. In addition to the high-marsh sewage sludge perturbation experiments, several *Spartina* plots were either clipped or left unclipped and the soil was enriched with glucose and/or ammonium nitrate. As shown in Fig. 2, nitrogen fixation was significantly ($P < 0.05$) influenced in the present *Spartina* culms. In soil plots which were clipped (Fig. 2a and 2b), fixation was significantly ($P < 0.05$) lower than in plots which were not clipped (Fig. 2c and 2d).

There was no significant ($P > 0.05$) difference between treatments within either the clipped or the unclipped plots (Fig. 2). However, in the unclipped 0- to 5-cm zone (Fig. 2a), ammonium nitrate appeared to stimulate nitrogen fixation, whereas fixation was low in the plots enriched with glucose and glucose plus ammonium nitrate (Fig. 2a). On the other hand, nitrogen fixation within the 10- to 15-cm zone was significantly greater ($P < 0.05$) in the ammonium nitrate-treated plot than in the other plots (Fig. 2b).

Nitrogen fixation in the outside control was lower only in the 0- to 5-cm zone (Fig. 2a). This may have been due to the injection holes which were produced when the various nutrient solutions were added to the soil. In any case, the clipped and unclipped nutrient treatments are directly comparable. The mean coefficient of variation for the entire data set was 43 ± 17% (standard deviation), again indicating heterogeneity.

Light and dark nitrogen fixation. Diel surface nitrogen fixation proceeded at a relatively constant rate except in areas where heterocystic nitrogen-fixing blue-green algae were present (Table 4). In those areas, dark fixation was less than 5% of the light fixation. However, dark fixation was 70 to 100% of the light fixation in the absence of heterocystic algae. In February, *Anabaena* patches covered extensive areas in one marsh, and in March a *Calothrix* crust layered a massive area in a sandy marsh. However, the relatively high fixation generally found was not always due to heterocystic algae, as was the case in July. The dominant alga was an *Oscillatoria*. It was not possible to determine whether it was fixing nitrogen or was an associated bacterial population.

The carbon and nitrogen content of the upper 0 to 5 mm was noted to be high when heterocystic nitrogen-fixing blue-green algae were present (Table 4).

DISCUSSION

S. alterniflora has been found to be nitrogen limited (2, 6, 19, 29, 30, 32). On the other hand,

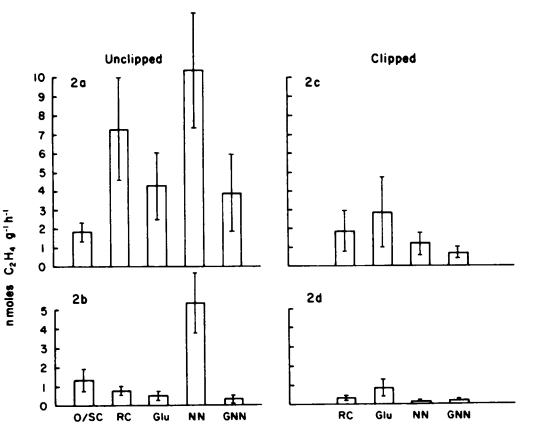

FIG. 2. *Nitrogen fixation rates in unclipped (2a and 2b) and clipped (2c and 2d) Spartina alterniflora plots in two depth zones: 0 to 5 cm (2a and 2c) and 10 to 15 cm (2b and 2d). Histogram bars represent the mean rates of eight cores from four replicated plots. Vertical bars are ± 1 standard error of the mean. O/SC = Outside control; RC = replicate control; Glu = glucose; NN = ammonium nitrate; and GNN = glucose and ammonium nitrate.*

TABLE 4. *Light and dark nitrogen fixation in various marshes contiguous to Sapelo Island, Ga.*

Month	Location	Blue-green algae present	Condition	Surface nitrogen fixation[a]	Carbon and nitrogen (mean % dry wt)		
					C	N	C–N
January	Airport Marsh	None	Daylight	1.62	ND[b]	ND	
			Night	1.64 (100)			
February	Laughing Gull Marsh	*Anabaena*	Daylight	818	5.04	.69	7.4:1
			Dark	48.2 (5)			
March	Cabretta Marsh	*Calothrix*	Daylight	72.8	1.00	.15	6.7:1
			Dark	12.7 (2)			
			Subsurface	2.89	0.22	.034	6.9:1
July	Cabretta Marsh	*Oscillatoria*	Daylight	79.9	0.58	.945	10.3:1
			Dark	57.4 (72)			

[a] Expressed as micromoles of C_2H_4 per square meter per hour. Values in parentheses are the percentage of daylight fixation rates.

phosphorus has been shown not to be limiting production as a consequence of the phosphorus reserves in marsh soils (21). Although *Spartina* and benthic algae support the detrital food chain, the large microbial community has been shown to be operating below its metabolic potential (i.e., carbon limited) (9, 10, 35; R. R. Christian, Ph.D. thesis, University of Georgia, Athens).

Valiela and Teal (30), Valiela et al. (32), and Chalmers et al. (3) found that sewage sludge increases *Spartina* and algal production as a result of the relatively high nitrogen content of sewage (2 to 3%). On the other hand, Van Raalte et al. (33) reported that nitrogen fixation by blue-green algae in Massachusetts was inhibited by high dosages (25.2 g/m^2 per week) but not low dosages (8.4 g/m^2 per week) of fertilizer (10% N). Although they did not investigate rhizosphere nitrogen fixation, the surface fixation data from the low-dose plots agree with the nitrogen fixation data reported here.

To investigate the enhancement of nitrogen fixation by sewage sludge in the Georgia marsh, a relatively simple field experiment was designed (see Fig. 2). In general, the results indicate that the energy source for nitrogen fixation in the soil was most likely *Spartina* carbon:

1. Nitrogen fixation was significantly higher in the unclipped than in the clipped soil plots. Thus, the removal of *Spartina* shoots prevented any carbon input via the roots, other than possibly the diffusion of surface algal carbon into the rhizosphere. In a system similar to salt marshes, it has been determined that nonsymbiotic nitrogen fixation was stimulated in the rhizosphere of rice plants (17, 24, 36).

2. It was found that the monthly nitrogen additions significantly increased plant production over the nonamended plots (Christian, unpublished data), substantiating other investigations (2, 6, 19, 29, 30, 32). The increased nitrogen fixation activity in the nitrogen-amended *Spartina* plots was apparently related to increased *Spartina* productivity and root exudation.

3. Nitrogen fixation was enhanced in the unclipped ammonium nitrate-enriched plots. It is well known that inorganic nitrogen (> 25 mg/liter has an immediate inhibitory effect on bacterial nitrogen fixation (10, 16, 18, 20). The immediate inhibitory effect of combined nitrogen on nitrogen fixation has no doubt decreased, via chemical or biological transformations, between nitrogen amendments and sampling of the marsh for nitrogen fixation activity. Therefore, the increase in *Spartina* productivity from the nitrogen amendment outweighed the inhibitory effect of inorganic nitrogen on nitrogen fixation in the field.

4. It was found that addition of glucose to the plots suppressed nitrogen fixation in the soil. However, nitrogen fixation in soil slurries was immediately stimulated by a number of different carbohydrates (10). This can be explained if the glucose was consumed along with the available soil nitrogen by the microbial community, thereby reducing the utilizable *Spartina* nitrogen pools in the soil. As a result, the plants were further nitrogen stressed. Plants in the glucose-amended plots appeared yellow, whereas plants in the nitrogen-amended plots were green. Therefore, *Spartina* production supported the energy requirements of heterotrophic nitrogen fixation in Georgia salt marsh soils.

Bacterial and blue-green algal nitrogen fixa-

tion has been reported in numerous other salt marshes and estuaries (1, 8, 11, 12, 14, 15, 18, 26, 28, 33, 34). As was reported here, most of these studies have shown seasonal trends: highest rates in late spring to early fall and lowest rates during the winter months, with the exception of the Rhodes River estuary where fixation was out of phase with the period of rapid plant growth (18). In addition to temporal variations, spatial variation of nitrogen fixation in soils and sediments has suggested that most of the energy and carbon compounds enter the system from the surface; that is, nitrogen fixation activity decreases with depth (1, 14, 34). But, in the short *Spartina* marshes contiguous to Sapelo Island, the highest activity has been found primarily in the 5- to 7-cm depth zone. Maximal short *Spartina* root density has also been reported between 0 and 10 cm (5). These results suggest rhizosphere interactions. In addition to the nitrogenase activity found on and in the marsh soils, it has also been reported on marsh plant leaves and stems (8), as has been found in this study. Plant exudates, which are collected between the *Spartina* leaf sheaths and stems, contain organic-rich metabolites to maintain heterotrophic nitrogen fixation (25).

Many nonsymbiotic nitrogen fixers have been reported, and they exist nearly everywhere (27). Between *Spartina* leaf and stem interfaces in Georgia, photosynthetic bacteria have been seen microscopically during the latter part of the growing season (September and October). Those that grew in nitrogen-free enrichment media resembled *Rhodospeudomonas* and *Chloropseudomonas* species. These photosynthetic bacteria were also present on the soil surface along with nitrogen-fixing *Calothrix* and *Anabaena* species. *Calothrix* and *Anabaena* were also present on the standing dead *Spartina* stems and leaves. Below the surface, acetylene-reducing *Desulfovibrio* and *Clostridium* species were enriched from soil samples in the high marsh.

Considerable effort has been made to estimate the relative importance of various nitrogen fluxes and nitrogen standing stocks in the marshes of Sapelo Island (9). As yet there are many fluxes still unaccountable (e.g., tidal nitrogen flux, nitrification, ammonification, ammonia volatilization, etc.). Many of those fluxes and standing stocks measured are only estimates, but they are the best estimates available. For example, the acetylene reduction method is the most sensitive and inexpensive technique available for measuring nitrogen fixation potential in various diverse ecosystems. However, $^{15}N_2$ tracer experiments are required for accurate conversion of ethylene units to nitrogen units, since conversion factors have been shown to range from the theoretical ratio of 3:1 to 18:1 (23). Nevertheless, extremely good estimates can be derived from the existing ethylene production data (Eldor A. Paul, University of Saskatchewan, personal communication). Therefore, rates of ethylene production were converted to annual potential nitrogen fixation rates based on a 3:1 conversion factor (13) (Table 5). The yearly nitrogen fixation rate of 20 to 50 g of N_2/m^2 could significantly support autotrophic production (plant and algae) in the marsh. Haines et al. (9) reported that the nitrogen flux through marsh autotrophs may be as high as 30 to 40 g of N/m^2 per year.

TABLE 5. *Estimated annual nitrogen fixation in control and sewage sludge-amended plots (experimental), Sapelo Island, Ga., 1975*

Sample	Annual N_2 fixation (g of N_2/m^2)[a]			
	Control plot		Experimental plot	
	10 h[b]	24 h	10 h	24 h
Soil surface	0.381	0.915	0.526	1.26
Spartina epiphytes	0.199	0.477	0.216	0.520
Soil profile (0–22 cm)	21.6	51.4	23.6	56.2
Total	22.2	52.4	24.3	58.0

[a] Based on a 3:1 (C_2H_4-N_2) conversion factor.
[b] Fixation period.

In conclusion, sewage sludge apparently enhances rhizosphere nitrogen fixation in the soil by stimulating *Spartina* production in the salt marshes of Georgia. However, its effect on other processes may be inhibitory. For example, dentrification in Georgia marshes was inhibited by sewage sludge (3).

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