Effect of the *Spartina alterniflora* Root-Rhizome System on Salt Marsh Soil Denitrifying Bacteria†

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Nitrous oxide (N_2O) reductase activity was used as an index of the denitrification potential in salt marsh soils. In a short *Spartina alterniflora* marsh, the seasonal distribution of N_2O reductase activity indicated a causal relationship between *S. alterniflora* root-rhizome production and the denitrification potential of the soil system. The relationship was not discerned in samples from a tall *S. alterniflora* marsh. To further examine the in situ plant-denitrifier interaction in the short *S. alterniflora* marsh, plots with and without living *S. alterniflora* were established and analyzed for N_2O reductase activity 5 and 18 months later. In the plots without living *Spartina* there was a significant reduction in the soil denitrification potential after 18 months, indicating that in the SS marsh the denitrifiers are tightly coupled to the seasonal production of below-ground *Spartina* macroorganic matter. In plots with intact *Spartina*, the soil denitrification potential was not altered by NH_4NO_3 or glucose enrichment. However, in plots without living *Spartina*, there were significant changes in soil N_2O reductase activity, thus indicating that the plants can serve as a "buffer" against this form of pulse perturbation.

The interactions between the root systems of higher plants and soil microorganisms involved in carbon, nitrogen, and sulfur cycling are currently receiving considerable attention. Among the soil bacterial populations involved in the nitrogen cycle, nitrogen fixers have been studied most extensively—the denitrifiers least. In this report we present preliminary evidence for a causal relationship between the seasonal below-ground growth pattern of the salt marsh grass *Spartina alterniflora* and the soil-denitrifying bacterial population.

To assess the denitrification "potential" of the experimental soil systems, we used a modification of a technique for quantitative measurement of the rate of nitrous oxide (N_2O) reduction in soil slurries (8). With the proper controls, N_2O reductase activity indicates the relative concentrations of the enzyme complex and, by implication, the existence of the other denitrifier reductase systems as well (i.e., those for nitrate, nitrite, and nitric oxide reduction). Reliance on N_2O reductase activity as an indicator reaction is based on the assumption that, in nature, environmental factors that initiate the synthesis of N_2O reductase are not likely to stimulate synthesis of just one denitrifying enzyme and not the others. In addition, laboratory studies with a marine bacterium indicated that, when aero-

bically grown denitrifying bacteria were subjected to anaerobic conditions, synthesis of all the denitrifying enzymes began simultaneously within 40 min (12). Consequently N_2O reductase activity is taken to be a dependable indicator of a complete and functional denitrification system.

MATERIALS AND METHODS

Our study was conducted in the salt marsh at Sapelo Island, Ga., in areas covered with tall (TS) and short (SS) *S. alterniflora*. The TS site, located next to the University of Georgia Marine Laboratory on the levee of a tertiary creek, is typical of several TS marshes in the vicinity of Sapelo Island described in detail by other investigators (e.g., 6, 10, 13, 14, 16). The SS marsh site is located about 300 m inland from the Duplin River and has also been extensively described (3-5).

Seasonal distribution of denitrifying bacteria. Periodically, over the interval from July 1975 to September 1976, the vertical distribution of N_2O reductase activity of the bacteria in the soil column in the TS and SS marshes was assessed. Four cores (each 7.5 by 40 cm) were collected from each area per sampling period and subsampled at six depths from 0 to 31 cm. Slurries were prepared from the subsamples and assayed for their ability to reduce N_2O (see below). The slurries were incubated at temperatures approximating those in the field at the time of collection. The salinity of the slurries also represented the average salinity of the in situ interstitial water at the time of collection.

In situ perturbation experiments. Field experi-

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ments were designed to test the hypothesis that living SS regulates the denitrifier portion of the soil microbial community through the influence of both root and rhizome exudation and utilizable underground plant production. The experimental plots consisted of: (i) control plots (C), which represented "normal" SS marsh; (ii) *S. alterniflora* shoot-clipped and root-pruned plots (C+P), representing a situation where there was no carbon input to the soil system by living plants on the plots (however, there could be lateral diffusion of carbon compounds to and from the surrounding soil as well as the movement of living roots and rhizomes into the plots [only experiment 1 included this group]); and (iii) clipped, pruned, and enclosed plots (C+P+E), which represented the same perturbation as (ii) except that lateral movement of carbon was inhibited.

Experiment 1. The experiment, making use of 12 plots (each 0.15 m²), was begun in February 1975, and terminated in August 1975. In four plots, the above-ground portions of *S. alterniflora* were clipped and the roots and rhizomes entering and leaving the plots were pruned to a depth of 25 cm (C+P plots). Four other plots were manipulated as above but were also enclosed to a depth of 15 cm with plastic garden edging. The top of the edging was inserted flush to the marsh surface (C+P+E plots). Four undisturbed plots served as C. N₂O reduction activity of bacteria in cores obtained from the plots was assayed (one core per plot, three depths per core between 0 and 10 cm).

Experiment 2. Eight plots (four C and four C+P+E) were established in February 1975. N₂O reduction activity of bacteria in cores obtained from the plots in August 1976, was assayed.

Experiment 3. In February 1976, a combination *S. alterniflora* root-pruning:nutrient-addition experiment was initiated. The experiment was designed as a randomized complete block with four blocks and eight treatment groups. The treatments were (i) C; (ii) C + NH₄NO₃; (iii) C + glucose; (iv) C + NH₄NO₃ + glucose; (v) C+P+E; (vi) C+P+E + NH₄NO₃; (vii) C+P+E + glucose; and (viii) C+P+E + NH₄NO₃ + glucose. The C were intact 0.1-m² plots (one per block). Beginning in February 1976, NH₄NO₃ and/or glucose solutions (a total of 250 ml per plot) were injected into the plots every month to a depth of 15 cm (see reference 6 for detailed description of the procedure). Injections were continued through June 1976. The solutions contained 11.4 g of NH₄NO₃ and 50 g of glucose per liter. In a combined solution, the compounds provided a carbon/nitrogen ratio of 5. Application was equivalent to supplementation with 50 g of carbon and 10 g of nitrogen/m² per month (or 0.08 mg of glucose and 0.07 mg of NH₄NO₃ per cm³ of soil per month). In July 1976, one core from each plot was assayed for the N₂O-reducing capacity of bacterial populations residing at 0-, 5-, and 10-cm depths.

Slurry preparation and N₂O reduction assay. From each core, 40-cm³ soil samples were obtained. Each of the samples was placed in a glass bottle (120-ml capacity) and diluted with 40 ml of artificial seawater (the salinity was adjusted to approximate that of the interstitial water). The bottles were stoppered, sealed with high-vacuum stopcock grease, and sparged with helium at 80 ml/min for 6 min. Each sample was

then violently agitated for 2 min to homogenize the slurry. The contents of the bottles were equilibrated for 1 h to a temperature approximating the in situ soil temperature. One milliliter of N₂O was then added to each sample, and the contents were agitated for 30 s for equilibrium partitioning of N₂O between the gas and liquid phases. Gas samples (0.3 ml) were withdrawn and analyzed at time zero and after 3 h with a Carle AGC 111 Analytical Gas Chromatograph fitted with a Porapak T column (10 ft by 1/8 inch; ca. 304.8 by 3.2 cm) and operated at 50°C. The chromatograph was equipped with a thermal conductivity microdetector. The carrier gas (helium) flow rate was 35 ml/min. A standard response curve was constructed by relating recorder peak heights to various quantities of N₂O, and the quantity of N₂O reduced was calculated by comparison of experimental values with a standard.

RESULTS

Seasonal distribution of N₂O reductase activity. N₂O reduction rates changed seasonally within the SS region. The rates were highest during spring, summer, and fall and lowest during winter months (Fig. 1). The potential activity of N₂O reductase in the TS soil profile, on the other hand, was uniform throughout the year and comparable to the winter rates in the SS site (Fig. 2). The seasonal depth distribution pattern also differed between the two marsh regions. In the SS marsh, there was a peak in N₂O reduction rates between 0 to 10 cm during the summer and fall (Fig. 1). The peak disappeared during the winter along with the decrease in overall activity throughout the soil column. The TS region did not exhibit a corresponding peak of activity at any depth during any season (Fig. 2).

The data used to construct Fig. 1 and 2 were analyzed by analysis of variance (ANOVA). The

FIG. 1. Seasonal depth distribution of N₂O reductase activity in SS soils. Values in the columns are mean micrograms of N₂O reduced per cubic centimeter of soil per hour. Solid lines (isolines) indicate areas of figure with equal N₂O reduction rates; they provide a contour picture of regions of varying activity.

SS soils contained populations with significantly greater activity than those in the TS soils during the summer and fall months ($P < 0.001$). In December 1975, January 1976, and March 1976, however, the bacteria in the two regions did not differ significantly in their N_2O reductase activity ($P < 0.25$).

Statistical comparisons of N_2O reduction rates between months, within each depth interval in SS soils, showed that, with the exception of the 10- to 11-cm depth, there were highly significant seasonal differences in activity throughout the entire soil column. Similar analyses for the TS region showed that, with the exception of the 10- to 11-cm depth, there were no strong differences in activity between the various months anywhere in the soil column.

In situ experiment 1. After 5 months, there was no overall effect of treatments on N_2O reduction rates within the 10-cm profile as a whole

FIG. 2. Seasonal depth distribution of N_2O reductase activity in TS soils. Values in the columns are mean micrograms of N_2O reduced per cubic centimeter of soil per hour.

(Table 1). Separate analyses of the data within each depth interval revealed a significant effect in the surface centimeter at an α level of 0.1. However, the level of significance was not great enough for the relatively conservative Student-Newman-Keuls multiple-range test to distinguish differences between the means of the three treatments. Therefore, one must conclude that there is no strong evidence for perturbation of the denitrifier portion of the microbial community 5 months after the soil system was removed from the influence of *S. alterniflora*.

In situ experiment 2. A second 5-month root-pruning experiment was begun in February 1976 (Table 1). ANOVA showed that there was a slight overall effect of the perturbation on the N_2O reduction capacity of the soils ($P < 0.1$); i.e., the populations in C+P+E plots had slightly decreased capacity over the C plots. However, the effect was not evident when the activities at individual depths were analyzed separately. The response was not large enough to overcome the inherent variability between samples.

In situ experiment 3. (A) C+P+E versus C plots. Analysis showed (Table 1) that after 18 months, in the absence of *S. alterniflora*, the N_2O -reducing capacity of bacteria in the soils had significantly decreased ($P < 0.025$) relative to the C soils. Since there was no depth effect, the data were pooled across depths and reanalyzed with a single classification ANOVA to increase the test's power. The bacteria in soils in the C+P+E plots showed a marked decrease in denitrification potential as compared with those in the C soils ($P < 0.005$).

(B) Nutrient enrichment. For purposes of statistical analysis, the groups were lumped into two major categories (Table 2): group 1 consisted

TABLE 1. Effect of in situ clipping and pruning SS shoots and roots on N_2O reductase activity of bacteria in the soil^a

Expt no.	Treatments	Depths (cm)		
		0-1	5-6	10-11
1 ^b	C plots	1.00 (0.24)	1.57 (0.45)	1.93 (0.40)
	C+P plots	0.95 (0.18)	1.55 (0.22)	1.90 (0.57)
	C+P+E plots	2.02 (0.37)	2.10 (0.44)	1.90 (0.74)
2 ^c	C plots	1.88 (0.38)	3.48 (0.63)	2.55 (0.50)
	C+P+E plots	1.13 (0.12)	2.34 (0.81)	2.01 (0.46)
3 ^d	C plots	2.20 (0.21)	1.41 (0.32)	1.95 (0.27)
	C+P+E plots	0.99 (0.13)	1.35 (0.22)	1.18 (0.11)

^a Values in the columns are mean micrograms of N_2O reduced per cubic centimeter of soil per hour (1 standard error of the mean).

^b Five-month experiment. No significant differences between treatments or depths ($P > 0.1$).

^c Five-month experiment. No significant differences between treatments or depths ($P > 0.1$).

^d Eighteen-month experiment. Overall significant difference between treatments ($P < 0.005$); no significant difference between depths ($P > 0.1$).

TABLE 2. *Effect of in situ NH₄NO₃ and glucose treatments on the N₂O reduction potential of bacteria in SS marsh soils*

Treatment	Depth-integrated N ₂ O reduction rates (0–10 cm) (μg of N ₂ O reduced/h)				Data averaged across blocks (1 SEM) ^a
Group 1 ^b					
C	43.5	32.0	18.2	32.3	31.7 (5.0)
+ NH ₄ NO ₃	16.5	30.3	37.8	26.9	27.9 (4.4)
+ Glucose	31.7	15.2	29.8	19.8	24.1 (3.9)
+ NH ₄ NO ₃ + glucose	38.2	24.5	23.9	45.3	35.5 (4.5)
Group 2 ^c					
C+P+E	38.2	15.2	13.9	19.6	21.8 (3.6)
+ NH ₄ NO ₃	41.0	38.4	29.5	29.8	34.7 (5.9)
+ Glucose	13.4	12.9	16.9	25.6	17.2 (2.9)
+ Glucose + NH ₄ NO ₃	57.6	45.2	34.8	47.0	46.2 (4.7)

^a SEM, Standard error of the mean.^b Group 1 is soil system with living SS. No significant differences between treatments ($P < 0.50$).^c Group 2 is soil system without living SS. Significant differences between treatments ($P < 0.005$) in the following order: C+P+E + glucose + NH₄NO₃ = C+P+E + NH₄NO₃ > C+P+E > C+P+E + glucose.

of plots containing living *S. alterniflora*; group 2 contained enclosed plots in which *S. alterniflora* shoots were clipped and the roots pruned.

Group 1. ANOVA (Table 2) showed that there was no overall treatment effect ($P < 0.50$) or block effect ($P < 0.75$). To increase the degrees of freedom and, hence, the discriminatory ability of the ANOVA, the blocks were pooled and analyzed by a single-classification ANOVA. No treatment effect was discernable ($P < 0.50$). Neither NH₄NO₃, glucose, or NH₄NO₃ + glucose amendments had any differential effect on the soil denitrification potential in plots containing intact living *S. alterniflora*.

Group 2. The treatment effects were highly significant ($P < 0.005$) (Table 2). Pooling the blocks did not increase the significance. Partitioning the sums of squares showed that the plots amended with NH₄NO₃ (with or without glucose) contained soils with greater N₂O reductase activity than those in C or glucose-amended plots. The bacteria in glucose-treated plots had significantly lower N₂O reductase activity than in C plots.

DISCUSSION

Distinct differences were found between TS and SS marshes with respect to the seasonal distribution of potential denitrifier activity. Whereas the N₂O reductase activity in the SS marsh peaked in the warmer seasons, there was no corresponding alteration of activity in the TS region. Christian et al. (3) and Christian (R. R. Christian, Ph.D. thesis, University of Georgia, Athens, 1976) found a similar seasonal pattern for adenosine 5'-triphosphate (ATP) concentration (indicative of microbial biomass), although there were observable increases in ATP during

the summer in the TS region. In general, ATP concentrations differed by a factor of 2.5 to 4.0 between the winter and summer. In the SS zone, the difference between the two seasons was more pronounced, being on the order of five to nine times greater in the summer. The pattern of release of methane from the soil surface also demonstrates the distinction between the two marsh types. Seasonal variability and summer peaking were seen in the SS but not the TS marsh (G. King, University of Georgia Marine Institute, Sapelo Island, personal communication).

The seasonal differences in the above microbial parameters between the two marsh types could not readily be accounted for on the basis of a direct temperature effect on bacterial metabolism. Rather, available evidence suggests that these seasonal disparities are related to the differential and seasonal growth patterns of *S. alterniflora* between SS and TS marshes. In the first 25 cm of the soil profile, the total amount of below-ground macroorganic matter (MOM—composed principally of living and dead *S. alterniflora* crowns, rhizomes, and roots) per unit volume of soil is lower in the TS than in the SS region (4). The difference results from the roots and rhizomes of living TS migrating down to a depth of 1 m with very little horizontal growth, whereas the horizontal growth pattern of the roots and rhizomes of the short form of the plant results in a dense mat of material within a depth range of 10 to 20 cm. The microbial community within this section of the SS soil profile appears to be more tightly coupled to the seasonal production of MOM than is the microbial community in the TS marsh and, hence, tends to track the seasonal availability of the MOM. In addition, ATP concentrations (3) as

well as N_2O reductase activity (Fig. 1) were maximized within the top 10 cm of SS soil, especially during the *S. alterniflora* growing season.

To further examine the plant-denitrifier interaction, field perturbation experiments were performed with the objective of manipulating the living *S. alterniflora* component of the soil system in order to remove the microbial community from its influence and then to examine the response of the denitrifiers to this perturbation. The potential N_2O reductase activity was measured 5 and 18 months after the initial clipping and pruning of the grass. There was no consistent trend after 5 months between C and perturbed (C+P and C+P+E) plots. However, at the end of 18 months there was a significant decrease in N_2O reductase activity in perturbed plots (C+P and C+P+E) as compared with C plots.

The soil microbial carbon sources are initially supplied by the plant and are necessary for the maintenance of the denitrifier population. On the basis of the relationship between ATP distribution and the root mat in the SS region, Christian et al. (3) suggested that one linkage between the plants and microbes is root exudation. Following in situ perturbation experiments, Christian (Ph.D. thesis, University of Georgia, Athens, 1976) concluded that two other important pools of organic carbon are the seasonal production of below-ground MOM and the large standing stock of soil organic matter. The soil organic matter is relatively refractory to microbial degradation and has a longer turnover time than MOM.

In light of Christian's work, the results of the present study may be similarly interpreted. If the maintenance of the denitrifier population depended primarily on root exudation, the N_2O reduction rate should have been affected the first 5 months after living plants were removed from the system. This could not necessarily be true if the population had initially relied on exudation but later switched to the decomposition products of the MOM. However, by sludge addition and transferred-sediment experiments, Christian (Ph.D. thesis, University of Georgia, Athens, 1976) showed that not to be the case for at least the total microbial community.

Gallagher (personal communication) demonstrated seasonal production and utilization of the MOM standing stock in the SS region. Thus, it is evident that the microbial community can utilize this pool of organic carbon on a seasonal basis. If MOM were the major source of carbon and energy for the denitrifier population, then inhibiting further yearly production by clipping

and pruning the plants should reduce the denitrification potential on a time scale of 1 year or more. The results of the 18-month perturbation experiment are consistent with that hypothesis. In addition to the significantly decreased N_2O reductase activity the C+P+E plots contained about 20 g (dry weight) of MOM per dm^2 (integrated to a depth of 35 cm) less than the controls.

Living *Spartina* appears to provide a "buffer" for the soil microbial community against one form of perturbation, which should tend to drive it away from steady-state conditions. In the in situ nutrient enrichment experiment, the plots with intact plants did not show any significant differential response to NH_4NO_3 and/or glucose enrichment, but did have a significantly altered denitrification potential in the absence of living *S. alterniflora* (Table 2). A similar trend was observed with indexes of total microbial biomass and growth state, i.e., total adenylates and energy charge, respectively (K. Bancroft, Ph.D. thesis, University of Georgia, Athens, 1977). In the intact plots, there was no difference in either parameter between the C and perturbed (C+P and C+P+E) soil systems. However, in the clipped plots both energy charge and total adenylates were significantly altered in response to chemical amendments and mechanical disruption of the plant component of the system. The mechanism by which *Spartina* minimizes the microbial community's response to the above chemical perturbations is unknown and requires further investigation.

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