THE ROLE OF SULFUR IN SALT MARSH METABOLISM

by

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THE ROLE OF SULFUR IN SALT MARSH METABOLISM

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ABSTRACT

The rate of sulfate reduction in stands of dwarf Spartina alterniflora in the Great Sippewissett Salt Marsh is approximately 75 moles $SO_4^=$ m^{-2} $year^{-1}$. This is the highest rate reported for any natural ecosystem. Sulfate reduction is the most important form of respiration in the marsh and results in the annual consumption of 1800 g C m^{-2}, approximately equivalent to net primary production. Sulfate reduction rates in the peat are high for at least three reasons: 1) the below-ground production of Spartina alterniflora provides a large, annual input of organic substrates over a depth of some 20 cm, 2) sulfate is rapidly resupplied to the peat in infiltrating tidal waters, so low sulfate concentrations never limit the rate of sulfate reduction, and 3) sulfide concentrations remain below toxic levels.

The stable mineral pyrite is a major end-product of sulfate reduction in salt marsh peat while iron mono-sulfides are not. This is unlike most anoxic marine sediments and apparently results because iron mono-sulfides are undersaturated. The iron mono-sulfides are undersaturated in part because of the relatively low concentration of total soluble sulfides and in part because of the fairly low pH of the peat. Both of these conditions probably result from the activity of the Spartina roots. If the incorporation of ^{35}S into pyrite were not measured, the $S^{35}O_4^=$ reduction measurements would greatly underestimate the true rate of sulfate reduction.

Pyrite acts largely as a temporary store of reduced sulfur. The pyrite concentration of the peat undergoes seasonal changes. On an annual basis, the reduced sulfur which results from sulfate reduction is either re-oxidized to sulfate within the peat or is exported, much of it as thiosulfate or a similar intermediately reduced compound.

Most of the energy which is originally in organic matters is stored in reduced sulfur compounds when the organic matter is respired by sulfate reducing bacteria. Consequently, the export of reduced sulfur compounds from the peat represents an energy export. The export of energy as reduced inorganic sulfur compounds is probably larger than the net above-ground production by Spartina. This is an important vector for moving some of the energy trapped by the below-ground production of Spartina to zones where it is available for coastal food webs.

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My research was part of the Great Sippewissett Salt Marsh project. I would like to thank all of the people who have been involved with that study, particularly Ivan Valiela, who with John Teal organized the project, and Brian Howes and Ann Giblin, with whom I have often worked. Dale Goehringer's cheerfulness and aid are appreciated. The late Arnold Gifford gave me access to his marshlands, where most of this work was performed. C. C. Woo has been of great help in my studies on pyrite and phosphides. I have benefited from discussions with my colleagues at the Woods Hole Oceanographic Institution, the Ecosystems Center, and elsewhere. I also thank Mike Connor, Charlie Hall, and others with whom I have worked on auxiliary projects. I thank Judi DeSanti and Betsy Davies for typing this thesis on rather short notice. And last but not least, I thank Cindy Lee whose support has been crucial to this work.

CHAPTER 1

INTRODUCTION AND OVERVIEW

Ecologists have long been interested in energy flows within salt marsh ecosystems and in the export of energy from marshes to adjacent estuaries. Previous studies (Teal, 1962; Day, et al., 1973) measured sediment oxygen uptake as an indication of total system activity. Reduced inorganic compounds such as hydrogen sulfide are abundant in the anoxic peat and muds of salt marshes, and so it was recognized that anaerobic respiratory processes such as sulfate reduction must be occurring (Teal and Kanwisher, 1961). Nonetheless, it was felt that most all of the reduced inorganic compounds formed by anaerobic respiration did not leave the marsh but rather were oxidized at the marsh surface, consuming oxygen. Consequently, workers assumed that oxygen uptake measurements gave a good estimate of total energy flow (Teal and Kanwisher, 1961).

This assumption is not entirely justified. When aerobic heterotrophs oxidize 1 mole C carbohydrate, 1 mole O_2 is consumed. When sulfate-reducing bacteria and associated microflora oxidize 1 mole C, 0.5 moles H_2S are produced. Were this H_2S to diffuse to an oxidized environment and be oxidized, 1 mole O_2 would be consumed. If the oxidation were purely chemical, the net effect is the oxidation of 1 mole C and the consumption of 1 mole O_2, just as for the aerobic heterotrophs. But if the oxidation were chemoautotrophic, as much as 0.18 moles organic C of new bacterial biomass could be produced for each mole of O_2 consumed. The net effect would be the consumption of 1 mole O_2 for the net oxidation of 0.82 moles C.

Further, the belief that almost none of the reduced inorganic compounds formed by anaerobic respiration leave the marsh is probably incorrect. This belief was based on a comparison of oxygen and carbon

dioxide exchanges across the surface of cores taken from marsh peat (Teal and Kanwisher, 1961). The cores consistently had respiratory quotients (the ratio of CO_2 released to O_2 consumed, or R.Q.) of approximately 1 or less than 1, suggesting to Teal and Kanwisher (1961) that organic carbon was not being degraded in excess of oxygen consumption. Infiltrating tidal waters which flow down through the peat and out into creeks carry out much more carbon dioxide than the amount of oxygen which they carry into the peat. These exchanges were not included in the core gas exchange measurements used to estimate the respiratory quotients. Further, carbon dioxide in the peat may be refixed by Spartina, producing organic carbon (Teal and Kanwisher, 1966). The R.Q.'s measured by Teal and Kanwisher (1961) probably reflect activities and reactions just near the very surface of the core and greatly underestimate the true R.Q.'s of the entire peat. To the extent that this is true, oxygen uptake measurements underestimate total system activity.

Even if oxygen uptake measurements were a good estimator of system activity, measurements of anaerobic activity would be desirable. Without an understanding of the specific processes whereby organic matter is produced and degraded and without an appreciation of where these processes occur, it would be impossible to understand the controls on marsh metabolism.

Teal (1962) used the difference between marsh primary production and the degradation of organic matter (as estimated from oxygen uptake data) to estimate the export of energy as organic carbon from a salt marsh. He estimated that 45% of the net primary production was exported. Teal (1962) argued that the exported organic matter was of major importance in supporting coastal food chains. This argument has been instru-

mental in efforts to preserve and protect salt marshes. Day (1973) using the same approach estimated that a little over 50% of the net primary production in another marsh was exported as organic carbon. Teal (1962) and Day, et al. (1973) used only the above-ground production in their calculations and ignored below-ground production. Their calculated export should have underestimated true export by an amount equal to the below-ground production. Actual energy export may be much larger than they estimated since below-ground production can be very high (Valiela, et al., 1976). Yet attempts to directly measure the export of organic carbon compounds from salt marshes have found rather small net exports or even net imports (Sottile, 1973; Woodwell, et al., in press; Mann, 1975; Heinle and Flemer, 1976; Valiela, et al., 1978). The discrepancy between the direct measurements of organic carbon export and the calculated energy exports suggests that most of the energy is not exported as organic carbon compounds but rather as reduced inorganic compounds, the end-products of anaerobic metabolism. The evidence presented in this thesis suggests that this is indeed the case. Most energy export in the Great Sippewissett Salt Marsh is as reduced inorganic sulfur compounds. Such an energy export can still be used to support coastal food chains. In fact, the energy of inorganic reduced compounds may be more readily used than is the energy of organic detritus, much of which is rather refractory.

The major forms of anoxic respiration are denitrification, sulfate reduction, and methanogenesis. Our preliminary data suggested that the export of methane from the Sippewissett marsh is quite small, and Kaplan, et al. (1978) have demonstrated that denitrification is unimportant in the energy budget of the marsh. This thesis is a collection of three papers which deal with sulfate reduction and the

fate of reduced inorganic compounds in the Great Sippewissett Salt Marsh.

Chapter 2 (Howarth, 1979) documents the rapid formation of pyrite (FeS_2) in salt marsh peat. In most anoxic marine sediments, pyrite forms very slowly over a period of months to decades as amorphous iron monosulfides (FeS) react with elemental sulfur. In salt marsh peat pyrite forms directly from the solution without iron monosulfides as intermediates. This apparently occurs because iron monosulfides are undersaturated in the peat. The incorporation of S^{35} into pyrite must be considered when measuring sulfate reduction rates with $S^{35}O_4^=$.

Chapter 3 (Howarth and Teal, manuscript-a) deals with the spatial and temporal patterns of sulfate reduction in marsh peat, shows the importance of sulfate reduction to organic carbon degradation, discusses the relationship between sulfate reduction and eutrophication, considers some of the differences between organic carbon degradation and energy flow, and examines the fate of the reduced sulfur compounds which results from sulfate reduction.

Chapter 4 (Howarth and Teal, manuscript-b) examines in more detail the differences between organic carbon mineralization and energy flow and constructs a preliminary model to illustrate the importance of reduced inorganic sulfur compounds in energy flow and export for a salt marsh ecosystem. In that chapter, the marsh ecosystem is defined just as the vegetated peat and does not include adjacent embayments and mudflats. Thus, "export" is used in the same manner as used by Teal (1962) and by Day, et al. (1973). Other authors (Woodwell, et al., in press; Valiela, et al., 1978) include mudflats and embayments when they refer to marsh ecosystems, so their use of the phrase "ecosystem export" is different. We would expect less export from the marsh-embayment

system than from the vegetated portion of the marsh alone. Chapter 4 concludes that perhaps 29% of the net primary production of the marsh is exported from the peat, most of it as reduced inorganic sulfur compounds.

Six appendices are included as part of this thesis. The first is a method for measuring sulfate in pore waters (Howarth, 1978). The second appendix discusses ways in which Spartina alterniflora maintains a relatively oxidized rhizosphere. Other appendices deal with the relationship between sulfate reduction and methanogenesis, the possibility that reduced phosphorus compounds such as iron phosphides may be biologically formed in anoxic sediments, the relationship between the organic content of marsh sediments and the rate of oxygen uptake, and the interference of reduced sulfur compounds with the measurement of oxygen by the Winkler technique.

This thesis deals only with the Great Sippewissett Salt Marsh. The results should be applicable to many other marshes but probably not all. The peat of the Sippewissett marsh has an organic content of approximately 50%, and most of the primary production is in the below-ground production of roots and rhizomes (Valiela, et al., 1976). Some other marshes have a much lower organic content and probably have a much higher percentage of their primary production in above-ground production. For example, the organic content of the peat at Sapelo Island, Georgia, is approximately 5% (Teal and Kanwisher, 1961). Since sulfate reduction rates seem to be tightly coupled to the below-ground production and seem to have little relation to the above-ground production, marshes with a lower below-ground production and a higher above-ground production probably have a lower rate of sulfate reduction.

The sulfate concentrations in the Sippewissett marsh are always sufficiently high that sulfate is not limiting to sulfate reduction. Sulfate is more likely to be limiting in a marsh which has a lower salinity or in a marsh which has a lower infiltration rate. We have some data which indicate an inverse relationship between the organic content of peat and the percolation rate of that peat. Consequently, the infiltration rate may be lower in a low-organic peat such as that at Sapelo Island than in a high-organic peat such as we find at Sippewissett. If so, low sulfate concentrations may limit the rate of sulfate reduction at Sapelo Island.

The pH of the Great Sippewissett Marsh is usually 5.0-6.5. This seems to be typical of other Cape Cod marshes, and similar pH's are found in New Jersey marshes (Meyerson and Luther, 1977; personal communication from G. W. Luther). However, salt marshes along the coast of Delaware often have much lower pH's (personal communication from T. Church), and the Sapelo Island marshes can have much higher pH's; 8.5-9.0, occasionally 10 (Pomeroy, 1959). A higher pH than that found at Sippewissett might tend to make methane export much more important, while a lower pH might make methane export even less important than at Sippewissett (see Appendix 3). A higher pH would also make pyrite formation less likely and the formation of iron monosulfides more likely (see Chapter 2).

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CHAPTER 2

PYRITE: ITS RAPID FORMATION IN A SALT MARSH AND ITS IMPORTANCE IN ECOSYSTEM METABOLISM

PYRITE: ITS RAPID FORMATION IN A SALT MARSH AND ITS IMPORTANCE IN ECOSYSTEM METABOLISM*

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ABSTRACT

Pyrite formation in salt-marsh peat occurs more rapidly than is generally thought for any natural system. Pyrite is the major end product of sulfate reduction, and sulfate reduction is the major form of respiration in the salt-marsh ecosystem. When the rapid formation of pyrite is ignored, the rates of sulfate reduction and ecosystem respiration may be grossly underestimated.

INTRODUCTION

The formation of pyrite (FeS_2) in nature is generally thought to be a very slow process, taking months, years, or decades as amorphous iron monosulfides (FeS) react with elemental sulfur (S^0)(1). In salt-marsh peat, pyrite can form in a day or less without iron monosulfides as intermediates. Measurements of sulfate reduction determined from the turnover of tracer amounts of $^{35}SO_4^{2-}$ (2) in the surface peat of a Cape Cod salt marsh show that pyrite is a major end product. Very little of the resulting ^{35}S label, at most 30 percent, ends up in soluble (H_2S, HS^-) or acid-volatile (FeS) pools (3, 4). If the ^{35}S in the pyrite fraction were not measured, the rate of sulfate reduction would be grossly underestimated. My measurements indicate that the rates of sulfate reduction are very high in the salt-marsh peat throughout much of the year and that the sulfate-reducing bacteria annually respire approximately 1800 g of carbon m^{-2}, an amount of organic carbon equivalent to the major fraction of net primary production in the marsh (3). Other terminal electron acceptors such as oxygen and nitrate are much less important in the total respiration of the salt-marsh ecosystem (3).

Pyrite is normally detected by x-ray diffraction and is quantified on the basis of the amount of sulfur released by digestion with aqua regia (1, 5, 6). Neither approach is sufficient to prove that the ^{35}S is being reduced and incorporated into pyrite in marsh peat. X-ray diffraction analysis of marsh peat has repeatedly demonstrated pyrite as a major mineral phase, but x-ray diffraction cannot show that the ^{35}S is associated with pyrite. The ^{35}S that remains in the sediment after acid treatment to free acid-volatile sulfides is not

extracted by refluxing with 6N HCl but is extracted by aqua regia (boiling 1:1 HCl-HNO_3). That aqua regia but not refluxing HCL frees ^{35}S strongly suggests that it is in pyrite and proves that the ^{35}S is not in sulfate esters, amino acids, or proteins (7). However, refluxing with HCl may not extract ^{35}S from elemental sulfur or from humic or fulvic acids, and these possible sources must be examined by other approaches. Organic solvents such as CS_2 extract little or no ^{35}S, and thus no ^{35}S is in elemental sulfur. But some ^{35}S may be in fulvic acids (8): successive extractions with 0.1N NaOH release small but significant quantities of ^{35}S, approximately 5 percent of that extracted by aqua regia. Yet none of the ^{35}S is in humic acids (8), for when the alkaline extracts are acidified and centrifuged, all of the ^{35}S remains in solution (8). Since it seems unlikely that sulfur would be rapidly incorporated into fulvic acids but not humic acids, the labeled sulfur is probably incorporated into pyrite, which is then partially oxidized by the NaOH extraction procedure. Investigations with pyrite standards have confirmed that pyrite can be oxidized, although not quantitatively, by the extraction procedure (9).

Pyrite (specific gravity, 5.0) is considerably denser than most sediment materials. Separation of radiolabeled sediments by density in tetrabromomethane (specific gravity, 2.96) confirms that some pyrite is being formed rapidly in the marsh sediments. The denser pyrite-containing fraction (confirmed by x-ray diffraction) is virtually free of organic matter as shown by carbon-hydrogen-nitrogen analysis and has 10 percent of the ^{35}S. The lighter fraction, having 90 percent of the ^{35}S is largely organic matter which probably has trapped some very fine-grained pyrite. The ^{35}S in this fraction is probably associated with

such fine-grained pyrite, although a small percentage of it may be in fulvic acids (10).

To obtain additional evidence that pyrite can form rapidly under conditions such as those found in the marsh, I buried Teflon bags containing approximately 200 ml of 1mM $FeSO_4$ (the pH was adjusted to 5.0 with citrate buffers) in the marsh sediments. The Teflon bags are permeable to gases but not to ions, and so the total sulfide concentrations and activities of S^{2-} were controlled by the external partial pressure of H_2S. No attempt was made to intially exclude air from the bags, and some ferrous iron was undoubtedly oxidized. Within 48 hours, pyrite, confirmed by x-ray diffraction, had formed in these bags. This pyrite was insoluble and stable in refluxing 6N HCl but was significantly oxidized in 0.1N NaOH.

A number of laboratory studies have demonstrated that pyrite can be synthesized rapidly, in 1 day to a few days, from inorganic solution under suitable conditions [see Table 1 and (11, 12). It is tempting to conclude from these studies that pyrite will form rapidly under suitable acidic conditions whereas iron monosulfides such as mackinawite or greigite will form under more alkaline conditions. This would explain the rapid formation of pyrite in the salt marsh where the pH is usually between 5.0 and 6.5. However, it can be easily demonstrated that pH itself is not the key variable. Roberts et al. (12) mixed FeO · OH and H_2S at pH 7 while vigorously excluding air and produced iron monosulfides but no pyrite. I repeated their experiment at pH 7.5 while maintaining the partial pressure of H_2S at 1 atm and achieved similar results. But, when the partial pressure of H_2S was maintained at 10^{-4} atm in another experiment, pyrite and not the iron monosulf e was the product (Table 1). The formation of mackinawite

(and other iron monosulfides) is kinetically favored over the formation of the thermodynamically more stable pyrite, and, once iron monosulfides form, they are only gradually converted to pyrite. My results support the hypothesis that, if the iron monosulfides are undersaturated, pyrite (which is still likely to be supersaturated because of its much lower solubility product) can precipitate rapidly, without competition from iron monosulfides (5). If this hypothesis is true, then the effect of changing the partial pressure of H_2S from 1 to 10^{-4} atm was to change the iron monosulfides from supersaturated to undersaturated.

The observed trend for pyrite to form at lower pH and iron monosulfides at higher pH may just reflect the effect of pH on S^{2-} activity. For a constant concentration of total soluble sulfides (H_2S, HS^-, S^{2-},) decreasing the pH will decrease the concentration and activity of S^{2-} and thus iron monosulfides are more likely to be undersaturated at lower pH.

Measurements of Fe^{2+} and of the S^{2-} activity in the pore waters of marsh peat indicate that the ion product $[Fe^{2+}][S^{2-}]$ is almost always less than the solubility product of mackinawite, 2.75×10^{-18} (13) (Table 2); that is, mackinawite is undersaturated. But the solubility product of pyrite is approximately 2.4×10^{-28} (5, 14), and so it can be assumed that pyrite is supersaturated. This finding is consistent with the hypothesis that pyrite forms rapidly at low temperatures only when iron monosulfides are undersaturated. Since mackinawite cannot form under the undersaturated conditions found in the marsh, pyrite forms quite rapidly, although not as rapidly as mackinawite or other iron monosulfides would form were the conditions suitable. Iron

monosulfides were also undersaturated and pyrite supersaturated in the Teflon bag experiment (Table 2).

If pyrite can form rapidly whenever soluble sulfides are present but iron monosulfides are undersaturated, then it may be forming more rapidly than has been thought in some marine sediments other than salt marshes. Observations made in the Santa Catalina Basin and some other locations tend to support such a hypothesis. Sediments from these locations have significant concentrations of pyrite but not of iron monosulfides occurring in the surface sediments (6). This pattern is also found in marsh sediments, and, like the salt-marsh peat, these sediments have no major increase in pyrite concentration with depth. Such observations contrast with those made in sediments where pyrite forms slowly by conversion of iron monosulfides. There, the pyrite content increases with depth and the content of iron monosulfides decreases with depth, as in most anoxic marine sediments (5). The sediments from the Santa Catalina Basin are fairly oxidized, and sulfide concentrations are usually undetectably low (6). If sulfides were present only in very low concentrations, the iron monosulfides could be undersaturated and pyrite supersaturated even at the high pH found in these sediments. In light of the rapid formation of pyrite in salt marshes and its importance to ecosystem respiration there, the process should be more closely investigated in other likely systems.

Table 1. Some attempted low-temperature syntheses of pyrite

Reaction (partial) pressure of H_2S)	pH	Time (days)	Temperature (°C)	Products	References
$FeO \cdot OH + H_2S$ (1 atm)	4	0.6	20 to 25	FeS_2, FeS	(11)
$FeO \cdot OH + H_2S$*	3.8 to 6.5	1	25	FeS_2	(12)
$FeO \cdot OH + H_2S$*	7	2.5	25	FeS (no FeS_2)	(12)
$FeO \cdot OH + H_2S$ (1 atm)	7.5	3	Room	FeS	This study
$FeO \cdot OH$ H_2S (10^{-4} atm)	7.5	3	Room	FeS_2 (small yield)	This study
$FeS + S^0$	4.5	7	Room	No FeS_2	(12)
$FeSO_4(NH_4)_2SO_4 + H_2S$ (1 atm)	3	6	20 to 25	FeS	(11)

*The partial pressure of H_2S was not reported.

Table 2. Solubility data for the salt-marsh pore waters and for the Teflon bag experiment.

pH	$[S^{2-}]$	$[Fe^{2+}]$	$[Fe^{2+}][S^{2-}]$	Interpretation
		Salt-marsh pore waters		
5.0 to 7.8 (usually 5 to 6.5)	Less than 10^{-14}*	2×10^{-4} to 2×10^{-5}	Usually much less than 2×10^{-18}	FeS undersaturated, FeS_2 supersaturated
		Teflon bag		
5.0	Less than 2×10^{-15}†	Less than 10^{-3}‡	Less than 2×10^{-18}	FeS undersaturated, FeS_2 supersaturated

*The S^{2-} activity varies greatly over time and space; 10^{-14} is a fairly high value. The activity is low even though the concentration of total soluble sulfides can be high because the pH is low (3). †The S^{2-} activity in the bag varies as the external partial pressure of H_2S varies; 2×10^{-15} is the highest value. ‡This was the starting concentration. Some Fe^{2+} was undoubtedly oxidized.

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2. The method is that of B. B. Jorgensen and T. Fenchel (Mar. Biol. 24, 189 (1974), modified as described in (3).

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4. In contrast to these marsh results, my measurements in anoxic subtidal marine sediments result in the recovery of roughly 80 to 100 percent of the reduced ^{35}S as water-soluble sulfides (H_2S, HS^-) and acid-soluble sulfides (presumed to be FeS (1, 5). The rest is elemental $^{35}S^0$, recoverable by CS_2 extraction. The $^{35}S^0$ forms from $Fe^{35}S$ during acidification and is an artifact of the method (3); R. A. Berner, Mem. Am. Assoc. Pet. Geol. 20 (1974).

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7. Proteins and sulfate esters are readily acid-hydrolyzed into water-soluble components (A. B. Roy and P. A. Trudinger, The Biology of Inorganic Compounds of Sulfur, (Cambridge Univ. Press, Cambridge, England, 1970)).

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9. The oxidation of both sulfides (M. Avrahami and R. M. Golding, J. Chem. Soc. 1968, 647 (1968))and ferrous ions (W. Stumm and J. H. Morgan, Aquatic Chemistry (Wiley-Interscience, New York, 1970))is greatly increased with increasing pH. Since the oxidation of pyrite is rate-limited by the oxidation of

ferrous ions and, perhaps under the conditions tested here, by the oxidation of sulfides, increasing the pH should increase the oxidation rate of pyrite.

10. Any ^{35}S incorporated into fulvic acids is almost certainly reduced from $^{35}SO_4^{2-}$ to $^{35}S^{2-}$ first and then incorporated as the sulfide, as Nissenbaum and Kaplan (8) have demonstrated for the introduction of sulfur into humic acids. Thus, even if some of the ^{35}S label in the "pyrite fraction" is not pyrite, it is reduced sulfur and must be considered when measuring sulfate reduction. For routine sulfate reduction measurements, there is no need to distinguish between pyrite sulfur and fulvic acid sulfur.

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12. W. M. B. Roberts, A. L. Walker, A. S. Buchanan, Miner. Deposita 4, 18 (1969).

13. R. A. Berner, Am. J. Sci. 265, 773 (1967).

14. D. T. Rickard's finding (ibid. 275, 636 (1975)) that pyrite can be formed rapidly through the direction reaction of aqueous ferrous ions and polysulfide ions strengthens my use of this theoretically calculated solubility product for pyrite if saturation with solid orthorhombic sulfur is assumed; elemental sulfur is abundant in marsh peat, and so saturation is likely.

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CHAPTER 3

SULFATE REDUCTION IN A NEW ENGLAND SALT MARSH

SULFATE REDUCTION IN A NEW ENGLAND SALT MARSH*

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ABSTRACT

Sulfate reduction rates were measured for 2 years in the peat of a salt marsh using a radio-tracer technique. Rates are high throughout the peat from the surface to more than 20 cm deep. The integrated annual rate is approximately 75 moles $SO_4^=$ m^{-2} yr^{-1}, the highest rate yet reported for any natural ecosystem. Sulfate reduction accounts for the consumption of 1800 g organic carbon m^{-2} yr^{-1}, which is approximately equal to net primary production in the marsh. Respiration using other electron acceptors such as oxygen or nitrate is much less important in the marsh. It is hypothesized that sulfate reduction rates in the peat of the salt marsh are high for at least three reasons: 1) the below-ground production of Spartina alterniflora provides a large, annual input of organic substrates over a depth of some 20 cm, 2) sulfate is rapidly resupplied to the peat in infiltrating tidal waters, so sulfate depletion never limits the rate of sulfate reduction and 3) sulfide concentrations remain below toxic levels. The stable mineral pyrite (FeS_2) is a major end-product of sulfate reduction in the marsh peat while iron monosulfide (FeS) is not. If the incorporation of ^{35}S into pyrite were not measured, our $S^{35}O_4^=$ reduction measurements would greatly underestimate the true rate of sulfate reduction. Pyrite acts largely as a temporary store of reduced sulfur; seasonal changes occur in the pyrite concentration of the peat.

INTRODUCTION

Using energy flow to analyze salt marsh ecosystems, Teal (1962) and later workers (i.e. Day et al. 1973) measured sediment oxygen uptake as an indication of total system activity. The possible importance of anaerobic respiratory processes such as denitrification and sulfate reduction was recognized, but it was felt that oxygen measurements gave a good measure of total energy flow (Teal and Kanwisher 1961). This assumption was based largely on the finding of "respiratory quotients" (ratio of CO_2 released to O_2 consumed or R.Q.) of approximately one or less from measurements of gas exchange on cores, suggesting a balance between reduction at depth and oxidation near the surface (Teal and Kanwisher 1961). However, there are reasons to suspect that the R.Q. measured on cores is not representative of the actual R.Q. in nature. Carbon dioxide produced in the peat may not leave the peat solely by diffusion across the surface. It may also be exported in pore waters draining from the peat at low tide. We have preliminary evidence that the molar amount of CO_2 exported from the peat in this manner is much larger than the amount of O_2 delivered in infiltrating water. Also, respiratory CO_2 in the peat may be refixed by Spartina (Teal and Kanwisher 1966) or by other organisms, producing organic carbon. To the extent that such processes are occurring, the R.Q. is underestimated, and measurement of oxygen uptake underestimates total respiration.

Comparison of net primary production with oxygen uptake measurements have been used to estimate the export of energy or organic carbon from salt marshes (Teal 1962; Day et al. 1973). Although this approach gives an indication of energy export, the energy is not

necessarily exported in the form of organic carbon. Rather, some of the energy could be exported as the inorganic reduced end-products of anaerobic respiration (H_2S, CH_4, etc.). The estimates of energy export made by the difference between net above-ground primary production and total-community oxygen uptake ignored the below-ground production of Spartina alterniflora and other marsh grasses. So the recent finding that the below-ground production can be significantly greater than the above-ground production suggests that energy export may be much greater than the 15.4 MJ (3671 Kcal) m^{-2} yr^{-1} estimated for Georgia marshes (Teal 1962) or the 15.3 MJ (3667 Kcal) m^{-2} yr^{-1} estimated for Louisiana marshes (Day et al. 1973, recalculated assuming 20.1 KJ per gram organic matter). Yet most attempts to directly measure the export of energy in the form of organic carbon have found much smaller net exports or even net imports (Sottile 1973; Woodwell et al. in press; Mann 1975; Heinle and Flemer 1976). Valiela et al. (1978) found a net export of particulate organic carbon from the Great Sippewissett Salt Marsh which was 35% of above-ground production but only 3 to 4 % of total net primary production of S. alterniflora (calculated from data in Valiela et al. 1976). This discrepancy between 1) estimates of energy export based on differences between oxygen uptake and primary production, and 2) direct measures of organic carbon export, suggests that much energy is exported as reduced inorganic compounds. A study of anoxic respiration is a first step in analyzing this possibility. Also, studies of sulfate reduction and other forms of anaerobic respiration lead to a better understanding of controls on marsh metabolism than studies of oxygen uptake alone.

The major forms of anoxic respiration are denitrification, sulfate reduction, and methanogenesis. Denitrification in the Great

Sippewissett Marsh oxidizes only 12 g C m^{-2} $yr.^{-1}$ (Kaplan et al. 1978). And our preliminary measurements suggest that the export of methane from the marsh is small. A number of recent studies have documented the importance of sulfate reduction to the metabolism of benthic communities (Jorgensen and Fenchel 1974; Jorgensen 1977: Nedwell and Abram 1978). We report here a detailed investigation of sulfate reduction in the peat of the Great Sippewissett Salt Marsh on Cape Cod, Massachusetts.

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METHODS AND MATERIALS

Cores were taken at intervals of one to two months from a single homogeneous stand of dwarf Spartina alterniflora in the Great Sippewissett Salt Marsh on Cape Cod. On a few occasions, cores were also taken from a creekside stand of tall Spartina alterniflora or from a mixed stand of tall Spartina alterniflora, Spartina patens, and Distichlis spicata which had been heavily fertilized for 7 years. Sulfate reduction rates and sulfate concentrations were determined at every sampling time. Salinities, pyrite (FeS_2) concentrations, and concentrations of acid-volatile sulfides (presumed to be FeS) were determined only a few times during the year.

Determination of Sulfate Reduction:

Sulfate reduction rates were measured using a modification of the technique of Jorgensen (Jorgensen and Fenchel 1974; Jorgensen in press). Cores of approximately 40-cm length were taken with 4.7 cm diameter plastic tubes, plugged, and returned to the lab within 1 hour. Little change in Eh or pH was noticeable during this time period. Working under an argon atmosphere in a glove box, subsamples of approximately 10 cm volume were taken at 2-3 cm intervals over the depth of each extruded core and were placed in 20-ml glass vials. Each subsample was injected with 20 µl of carrier-free $Na_2S^{35}O_4$ solution (activity = 1 to 5 µCi). The syringe needle was slowly pulled out as the label was injected to minimize variation due to microscale heterogeneity. Tests using point-source injections gave similar results, but with much more variation. The vials were sealed, and samples incubated for 8-24 hours in the dark at temperatures approximating those at 10-cm depth under existing field conditions. Samples were then

either processed immediately or frozen for a few days before being processed. Results were comparable whether or not samples were frozen.

Samples to be processed were first placed in a reaction vessel similar to that described by Jorgensen and Fenchel (1974). This transfer was performed under argon in the glove box. De-oxygenated water was added, and soluble sulfides were stripped by an argon stream into a zinc hydroxide trap for an hour (Jorgensen and Fenchel 1974). To insure that the argon contained no oxygen traces, it was first passed through an oxygen-reducing catalyst (Jorgensen in press). The zinc hydroxide traps were prepared by mixing 6 ml of 2.6% zinc acetate with 1.5 ml of 6% NaOH. With two traps in series, only the first trap collected any radioactivity. Following stripping, the resulting ZnS was reacted with acidic N,N-dimethyl-p-phenylenediamine and ferric chloride (Gilboa-Garber 1971), thus stabilizing and solubilizing the sulfides as methylene blue. Subsamples (0.5 ml) from the traps were added to 20 ml of "Aquafluor" scintillation fluid (New England Nuclear) and counted. Corrections for quenching were made by use of the external-standard channels-ratio method (Wang, Willis, and Loveland 1975). Quenching was in general minimal, and a quench-correction curve was obtained by adding methylene blue to samples. The standard deviation of the counts was $\pm$ 0.2%.

After stripping the soluble sulfides for 1 hour, the trap was replaced with another, enough 6 N HCl was added to the reaction vessel to lower the pH below 1, and the acid-volatile sulfides were stripped by the argon stream for another 2 hours (Jorgensen and Fenchel 1974). The acid-volatile sulfide traps were then handled

in the same manner as the soluble-sulfide traps.

Following stripping, the contents of the reaction vessel were rapidly filtered using Whatman's no. 4 paper in a Buchner funnel. The filtrate was neutralized with NaOH, its volume measured, and a 0.5 ml subsample taken and counted using Aquafluor. A quench-correction curve was prepared by adding $FeCl_3$ to solutions. The radioactivity in this fraction was assumed to be associated with $S^{35}O_4^=$. Occasionally, a subsample of the filtrate was run on paper electrophoresis to determine if some of the radioactivity of the filtrate may have been associated with other sulfur compounds. A 0.1 N NaOH buffer was used with an applied voltage of 100 V cm^{-1}. Good separation of sulfate, sulfite, thiosulfate, and elemental sulfur should have been achieved (Roy and Trudinger 1970).

The sediment remaining on the filter was repeatedly washed with distilled water, then digested in boiling aqua regia (1:1 $HCl:HNO_3$) with a few drops of bromine solution added to free ^{35}S from pyrite (Howarth 1979). (This must be done with care in a fume hood since NO_2 and bromine vapors are given off). Digestion takes 2 to 3 hours. Samples were then evaporated to near dryness and were made up to 25 ml with distilled water. Subsamples were counted as before. Blanks were run to insure that $S^{35}O_4^=$ was not adsorbed to the filters.

After correcting for the full volume of each fraction, the total radioactivities in the soluble-sulfide pool, the acid-volatile sulfide pool, and the pyrite pool were summed and divided by the radioactivity in the sulfate pool. This yielded a turnover time for sulfate. This was corrected to a 24-hour incubation time and multiplied by the total amount of sulfate present in 1 cm^3 of sediment (sulfate concentration

multiplied by water content) to yield the sulfate reduction rate in moles $SO_4^=$ cm^{-3} day^{-1}.

Pore-water Chemical Determinations:

Pore waters were squeezed using a Reeburgh (1967) press from cores which were duplicates of those used for the $S^{35}O_4^=$ reduction measurements. Sulfate was determined by an indirect titration (Howarth 1978). Salinities were measured with a refractometer.

Iron-monosulfide and Pyrite Determinations:

Sulfides from acid-volatile sulfides (presumed to be FeS) were distilled off acidified sediments in an argon stream, trapped as zinc sulfide, and converted to methylene blue (Gilboa-Garber 1971), as in the $S^{35}O_4^=$ reduction technique (above). The methylene blue was assayed on a Beckman D. U. spectrophotometer.

The remaining sediment was then washed repeatedly with distilled water and was digested to oxidize pyrite to sulfate as for the $S^{35}O_4^=$ reduction method. After neutralizing, sulfate was measured as above (Howarth 1978). It was assumed that all sulfur freed in this digestion represented pyrite (Goldhaber and Kaplan 1974; Berner 1970).

RESULTS AND DISCUSSION

Depth Profiles for Sulfate Reduction Rates and their Relationship to Below-Ground *Spartina* Production:

Sulfate reduction rates per unit volume obtained for the top 20 cm of peat in the Great Sippewissett Marsh are higher than those that have been reported for other natural systems (see Table 1). Because of the greater depth over which rates of sulfate reduction occur in the marsh peat relative to other sediment systems, sulfate reduction per unit area is proportionally even higher than other published values. In addition to our $S^{35}O_4^=$ measurements, we independently assessed the sulfate reduction rate by sealing unhomogenized peat cores in jars for 1 week at room temperature and observing the depletion of sulfate. This method indicated a sulfate reduction of approximately 1.4 μmoles $SO_4^=$ cm^{-3} day^{-1}, in the range of values determined from the $S^{35}O_4^=$ method and approximately twice the rate determined by Martens and Berner (1977) when they sealed homogenized pan sediments from a Long Island Sound salt marsh in jars for several days (Table 1).

All of the sulfate reduction measurements made at the short *Spartina alterniflora* site during this study are shown in Figure 1 in order to illustrate the unusual depth profile. On any given day, variation over depth was great. Nonetheless, the collective data indicate a peak in sulfate reduction at depths between 4 and 18 cm. Sulfate reduction rates tended to be slightly lower in the top few centimeters than between 4 and 18 cm, and rates fell off sharply at depths below 22 cm. Such patterns are related to the distribution of roots, rhizomes, and "dead matter" in the peat. (See, for example, data on the nearby low-marsh control site of Valiela et al. 1976).

The growth and decomposition of the below-ground grass biomass provides a large annual input of organic carbon throughout the top 20 cm of the peat, and it is for this reason that sulfate reduction rates are so high over such a great depth. The pattern of sulfate reduction in most sub-tidal sediments is very different: sulfate reduction is low or absent in the relatively oxidized surface sediments containing oxygen or nitrate, peaks at a depth where oxygen and nitrate are lacking, and then falls off rapidly with depth. Such a pattern is a reflection of the supply of organic substrates from above the sediments. The pattern in the marsh peat is different because the organic substrates are being produced directly in the peat. Nedwell and Abram (1978) in a study of a British salt marsh found maximum sulfate reduction rates at the sediment surface with rates falling very rapidly with depth. However, they investigated only creek bottoms and pans covered with Oscillatoria mats, not peat containing grass roots as we have done.

Seasonal Trends in Sulfate Reduction:

Rates of sulfate reduction integrated over depth for each of the days that the short Spartina alterniflora site was sampled are shown in Figure 2. The sulfate reduction rates show a pronounced seasonal trend which is not entirely controlled by temperature. This is most easily seen by plotting sulfate reduction vs. sediment temperature at 10 cm depth for each month sampled (Figure 3A). The resulting plot is a distinctly non-linear trajectory with autumn rates higher and spring rates lower than would be expected on the basis of temperature alone. The high autumn rates are probably caused by the pulse of readily available substrate as the grass plants mature and

senesce starting in August. Sediment oxygen uptake, on the other hand, shows no such trend and is more strictly controlled by temperature (Figure 3B). Oxygen uptake data collected at the Sippewissett marsh in 1971-1972 by K. Smith and J. Teal is plotted vs. estimated sediment temperature. Temperatures were estimated to be the same as those measured during the sulfate reduction experiments, for the same time of year. The plot is linear with similar rates for spring and fall. Apparently oxygen uptake is largely a measure of metabolism just for the surface layer of the marsh peat whereas sulfate reduction is a measure of metabolism in the root zone. These zones appear to be poorly coupled; their micro-flora probably rely on different sources of organic matter. The surface aerobic microbial community receives a steady input of organic matter from the death of grass blades and surface algae and from the sedimentation of phytoplankton. The anoxic community in the peat receives its organic substrates from the death of roots and rhizomes and from the excretion of dissolved organics from living roots and rhizomes, processes which are related to the seasonal activities of the grass. Some of the oxygen uptake at the peat surface undoubtedly reflects the chemical or biological oxidation of reduced sulfur compounds which are diffusing up from deeper in the peat. Although it might be expected that this portion of the oxygen uptake would be tightly coupled to the rate of sulfate reduction, such is not necessarily the case. As discussed later, much of the reduced sulfur resulting from sulfate reduction is stored in the peat as pyrite during the fall, winter, and spring and is released during the summer. Thus, the oxidation of reduced sulfur compounds at the surface of the peat may not show the same seasonality as does sulfate reduction. Further, muc f the reduced sulfur is laterally transported from the

peat to creeks as soluble components in pore water and does not diffuse to the surface of the peat.

Sulfate Concentrations Within the Peat:

Since the salt marsh is intertidal, sulfate is rapidly and regularly resupplied to the peat by infiltration at high tide. Consequently, although sulfate in the peat is almost always depleted relative to chloride, sulfate concentrations remain high (usually 10-25 mM). Since the water infiltrating the peat is of highly variable salinity and of variable sulfate to chloride ratios, profiles of sulfate concentrations or sulfate:chloride ratios over depth yield little quantitative information. However, the extent of sulfate depletion at various depths can be calculated if it is assumed that at the time a water parcel entered the peat it had a sulfate to chloride ratio equivalent to that at the surface at the time the core was taken. The sulfate depletion is plotted vs. depth for three dates in Figure 4. We cannot calculate the sulfate depletion for other times of the year because adequate salinity and chlorinity data are lacking.

We can use the sulfate depletion data combined with the average sulfate reduction rate in the top 20 cm as determined from the radiotracer results to calculate the average infiltration rate (Howarth and Teal in prep.). For the three dates shown in Figure 4, infiltration was 2.9 to 3.3 cm per tide. Such rates are very reasonable in light of directly measured rates in the Sippewissett Marsh (R. Burke, personal communication). Further, during low tide at the same site the "water table" in the peat (as measured by digging a small well) is usually 2 to 4 cm below the surface of the peat, again suggesting an infiltration rate of approximately 3 cm per tide. The internal agreement

combined with the reasonableness of the calculated rates gives us confidence in our sulfate reduction data.

Our data also indicate that sulfate is not limiting to sulfate reduction in the active root zone of the peat, for the sulfate depletion curves are relatively smooth while the sulfate concentration varies greatly with depth. If the sulfate reduction rates were controlled by sulfate at the concentrations found in the peat, we would expect larger irregularities in the sulfate depletion curves corresponding to variations in the sulfate concentration. Further, the sulfate depletion curves for September and December start to level off at the same depth (around 20 cm), but the sulfate concentration at that depth was approximately 12.5 mM in September and approximately 17.7 mM in December. Thus, sulfate reduction in the peat seems to be controlled not by the $SO_4^=$ concentration but rather by substrate availability (energy) and temperature. Laboratory studies have indicated that sulfate reduction is independent of sulfate at concentrations greater than 2 to 10 mM (Goldhaber and Kaplan 1974). The rapid replacement of sulfate at depth in the peat via infiltration of tidal waters is one major prerequisite for the extraordinarily high sulfate reduction rates found there. Such rates could not occur in most sub-tidal sediments without the sulfate concentration quickly becoming limiting. In marshes with a slower rate of infiltration, sulfate depletion is more likely to limit sulfate reduction.

The End-Products of Sulfate Reduction:

In most marine sediments soluble sulfides (H_2S, HS^-) and iron monosulfide (FeS) are the only major short-term end-products of sulfate reduction. On the other hand, in the peat of local marshes,

pyrite (FeS_2) is a major end-product (Howarth 1979). In our radiotracer measurements of sulfate reduction, 70% or more of the reduced S^{35} was usually recovered in the pyrite pool. Hydrogen sulfide and FeS tended to be minor end-products. Although we know that most of the reduced S^{35} in the "pyrite pool" was actually contained in pyrite, we cannot be certain that some of the label (perhaps 5%) was not in fulvic acids or other such refractory organic compounds (Howarth 1979). Such label would still represent sulfate reduction, however, since sulfur is almost certainly introduced into such refractory organic matter as the sulfide (Nissenbaum and Kaplan 1972; Howarth 1979). That is, $S^{35}O_4^=$ must first be reduced to H_2S^{35} with the S^{35} then being incorporated into the refractory organics. For our routine measurement, therefore, we did not distinguish between pyritic sulfur and sulfur in fulvic acids or other refractory organic compounds. Periodic checks for other end-products from our $S^{35}O_4^=$ incubations revealed none.

Pyrite or refractory organic sulfur is abundant throughout the marsh peat and makes up from 1.8 to 9% of the peat by weight (0.3 to 1.5 mMoles FeS_2-S per g dry weight of sediment. In Figure 5, the pyrite concentration at various depths is plotted for 3 different dates. The pyrite concentration varies seasonally, increasing over the fall, winter, and spring and then decreasing over the summer. We tested the statistical significance of the increase from 19 January to 31 May using a two-way analysis of variance and found that the increase was significant at the 96% level ($F_{cal.}$, 1, 10 = 6.91). Since we had sampled different depths and had no replicates for the 26 September data, we did not test the significance of the increase from September to January, but the increase is clearly real. By integrating the curves in Figure 5, we can calculate that the top 25 cm of peat contains

approximately 23 moles FeS_2^- S m^{-2} on September 26, 33 moles FeS_2^- S m^{-2} on January 19, and 43 moles FeS_2^- S m^{-2} on May 31. From September to January the marsh peat accumulated approximately 10 moles S m^{-2}. Our $S^{35}O_4^=$ reduction measurements indicate that approximately 31 moles S m^{-2} was reduced during that period. From January to May, the peat accumulated approximately 10 moles S m^{-2}, while the $S^{35}O_4^=$ reduction measurements showed that approximately 12 moles S m^{-2} were reduced. And from May to September, the peat lost approximately 20 moles S m^{-2} while the $S^{35}O_4^=$ measurements showed that approximately 32 moles S m^{-2} were reduced. We interpret these results as showing that in the summer although much pyrite is being formed in the peat, even more is being oxidized by the roots of the Spartina grass or by bacteria associated with the roots. Autotrophic iron and sulfur bacteria such as Ferrobacillus ferrooxidans and Thiobacillus ferrooxidans are known to be able to catalyze the oxidation of pyrite, gaining energy from the reaction (Stumm-Zollinger 1972). Such bacteria may well be associated with the Spartina roots, the roots providing oxygen or other oxidants such as peroxides (see Appendix 2).

As discussed earlier, our profiles of sulfate depletion with depth for three times during the year (Figure 4) combined with our radio-tracer measurements of sulfate reduction strongly indicate that most of the reduced sulfur, including pyritic sulfur, is not being re-oxidized to sulfate within the peat. It seems likely that pyrite is instead being oxidized to an intermediately reduced, soluble sulfur compound such as thiosulfate. The thiosulfate (or other such compound) is then probably exported from the peat in pore water as it drains laterally from the peat into the creeks at low tide.

The oxidation of pyrite and other reduced sulfur compounds in the peat appears to be tied to the metabolism of the grasses (see Appendix 2). We hypothesize that during the summer, pyrite forms around older roots and is oxidized in other micro-zones associated with the metabolically more active tips of new roots. During the fall as the grasses senesce, the oxidation of pyrite in the peat greatly slows. Although some pyrite is still oxidized, a net accumulation occurs. In the winter and spring, essentially no oxidation of pyrite occurs, because the grasses are totally inactive, and pyrite continue to accumulate. However, the net accumulation is no greater in the spring than it was in the fall because the rate of sulfate reduction is so much less.

In most anoxic marine sediments where pyrite forms slowly from the gradual conversion of iron monosulfides, iron monosulfides are present in high concentrations which decrease with increasing depth. The pyrite concentrations increases proportionally as the iron monosulfide concentration decreases. In the peat at the Sippewissett marsh, pyrite does tend to increase with depth (Figure 5), and this increase is statistically significant at the 95% level ($F_{cal.}$, 4, 10 = 3.52 for two-way analysis of variance). However, pyrite is abundant even at the surface, and the increase with depth is small relative to seasonal changes in pyrite concentrations. Moreover, iron monosulfides are absent or present in only very small concentrations in the peat, usually much less than 5×10^{-7} moles FeS-S per g dry weight sediment. The very low FeS concentrations combined with the relatively small change in FeS_2 concentration with depth indicates that the FeS_2 is being formed rapidly and directly from ferrous ions and polysulfides rather than from a gradual conversion of FeS (Howarth 1979).

Nedwell and Abram (1978) in their studies of pan sediments in a British salt marsh found much higher concentrations of FeS than occur in Sippewissett. In their sediments, the FeS concentration decreased and the FeS_2 concentration increased with depth. Such findings are reminiscent of typical anoxic sediments and suggest that the FeS_2 was forming relatively slowly in that pan from the gradual conversion of FeS, and not rapidly as is the case in our marsh peat. Thus, Nedwell and Abram (1978) may have correctly estimated sulfate reduction even though they ignored FeS_2 as a possible end-product in their $S^{35}O_4^=$ measurements.

Sulfides are toxic to many microorganisms and plants. We have preliminary evidence suggesting that sulfides at relatively low concentrations, 0.20 mM, can kill Spartina alterniflora plants growing hydroponically. Although sulfate reducing bacteria have been shown to be relatively insensitive to sulfides (Miller 1950), the fermentative bacteria and other bacteria which provide substrates for the sulfate reducing bacteria may be more easily poisoned.

Thus, if sulfides accumulated, the Spartina grasses could be killed or their growth inhibited or the sulfate-mediated respiration system of the peat (sulfate-reducing bacteria and associated fermentative bacteria) could be inhibited. The sulfate-mediated respiration system is essential in providing nutrients, particularly nitrogen, to the grass plants through remineralization. So any inhibition of this system would slow plant growth through nutrient limitation.

But sulfide concentrations in marsh peat tend to remain relatively low, generally less than 30 μM , and most of the reduced sulfur formed during sulfate reduction is stored for the short term in the marsh peat as non-toxic, relatively inert pyrite. Iron-monosulfides are a much less suitable storage product because 1) only half as much sulfur

can be stored per unit available iron and a lack of iron may limit the amount of sulfur which can be stored, and 2) iron monosulfides are much more soluble than pyrite and hence will act to "buffer" soluble sulfide concentrations at a much higher concentration. Somewhat paradoxically, pyrite forms much more rapidly when iron-monosulfides are undersaturated, and it is for this reason that pyrite forms so rapidly in salt-marsh peat (Howarth 1979). The undersaturation of iron-monosulfides in marsh peat results both from the low pH, generally 5.0-6.5, and from the relatively low concentration of total soluble sulfides. The Spartina grasses probably act to create both of these conditions, the low pH resulting from the excretion of organic acids by the plant roots and the relatively low concentration of total soluble sulfides resulting from the grasses partially oxidizing some of the sulfides. Thus, it seems likely that the grasses structure the peat environment so as to create favorable conditions for the formation of pyrite, and this in turn allows higher rates of plant production and sulfate reduction than if HS^- and FeS were the major end products of sulfate reduction.

The Role of Sulfate Reduction in Remineralizing Organic Carbon:

Integrating the sulfate reduction rates over time yields an annual rate of approximately 75 moles $SO_4^=$ m^{-2} $yr.^{-1}$. Since on average two moles of organic carbon are oxidized for every mole of sulfate reduced (Jorgensen 1977), the sulfate reducing bacteria and associated microorganisms in the marsh peat annually respire 1,800 g C m^{-2} $yr.^{-1}$. The assumption of two moles carbon respired per mole of sulfate reduced is not strictly true for some substrates which the sulfate reducing bacteria can use if these bacteria are considered alone in isolation from the ecosystem. For example, sulfate reducing bacteria can oxidize

methane according to the following equation:

$$CH_4 + SO_4^{=} + 2H^{+} = CO_2 + H_2S + 2H_2O,$$

where only one mole carbon is respired for each mole of sulfate reduced. However, in a natural sediment, other bacteria must be forming the methane which the sulfate reducers are respiring. These methanogenic bacteria are forming methane according to an equation such as:

$$CH_3COOH = CH_4 + CO_2$$

Thus, considering the methanogenic bacteria and sulfate reducers together in the ecosystem, the net affect is:

$$CH_3COOH + SO_4^{=} + 2H^{+} = 2CO_2 + H_2S + 2H_2O,$$

where two moles of carbon are oxidized for every mole of sulfate reduced.

Similarly, although sulfate reducing bacteria are known to use only a few specific low-molecular weight substrates, other fermentative heterotrophic bacteria can provide these substrates through degradation of other organic compounds. Tezuka (1966) has demonstrated such a commensalism between sulfate reducing bacteria and other heterotrophic bacteria in mixed cultures. In such a relationship, the fermentative bacteria provide substrate for the sulfate reducers, and the sulfate reducers consume the organic end-products produced by the fermenters. This increases the free energy available to the fermenters in further fermentations. The net effect is a microbial community including sulfate reducers which can degrade a wide variety of organic compounds using sulfate as the final electron acceptor. This community oxidizes to CO_2 two moles of organic carbon for every mole of sulfate reduced.

We do not yet have enough data to construct a complete carbon budget for the marsh peat. Some reduced sulfur compounds are probably oxidized by chemosynthetic bacteria within the peat and at the surface of the peat with a concomitant production of new organic carbon. The extent of carbon production by such pathways in the peat is unknown. Nonetheless, we can compare sulfate reduction in the peat with respiration using other terminal electron acceptors and with net primary production. (Production of carbon through chemosynthesis in a salt marsh is not primary production since it is based on energy originally fixed in plant photosynthesis).

Net primary production for a nearby, similar stand of Spartina alterniflora in the same marsh has been estimated as 1,880 g C m^{-2} $yr.^{-1}$ (Valiela et al. 1976). Most of this production, 1680 g C, is the below-ground production of roots and rhizomes. Only 200 g C m^{-2} $yr.^{-1}$ are produced above ground. Valiela et al. (1976) state that their estimate for below-ground production is an underestimate since it does not correct for loss of dead underground plant parts due to decomposition between the time of minimum dead standing crop in June and the maximum in October. They may also have underestimated production because they did not include root exudates in their productivity estimates; Spartina may excrete significant quantities of root exudates. Nevertheless, it is clear that sulfate-reducing respiration is of the same order of magnitude as net primary production.

Oxygen uptake by marsh muds represents both oxygen respiratory activity and the oxidation of reduced inorganic compounds, whether purely chemical or chemo-synthetic (biological). In a salt marsh, oxygen respiration is certainly less than oxygen uptake, perhaps much less. Measurements of Smith and Teal (Figure 6) indicate an annual

oxygen uptake of 6.5 moles O_2 m^{-2} at the Great Sippewissett marsh. These measurements were made *in situ* at high tide. Consequently, they may underestimate yearly oxygen uptake if oxygen uptake is higher at low tide when the marsh surface is exposed to air. However, Teal and Kanwisher (1961) found no significant differences in oxygen uptake by cores from a Georgia marsh whether measured in air or in water. The measurements at Sippewissett showed a summertime uptake of 0.018 to 0.03 moles O_2 m^{-2} day^{-1}. Summertime uptake in Georgia marshes is approximately 0.056 moles O_2 m^{-2} day^{-1} (Teal and Kanwisher 1961) and in Louisiana marshes is approximately 0.058 to 0.12 moles O_2 m^{-2} day^{-1} (Day et al. 1973). We would expect oxygen uptake to be higher in those southern marshes, so we have confidence that the measured uptake of 6.5 moles O_2 m^{-2} $yr.^{-1}$ for Sippewissett is reasonable. Oxygen also enters the peat due to the infiltration of oxygen-rich water at high tide. Assuming an infiltration rate of 2.9 cm $tide^{-1}$ (calculated earlier in this paper) with water containing 6 ml O_2 $liter^{-1}$, infiltration supplies an additional 5.7 moles O_2 m^{-2} $yr.^{-1}$ to the peat. Therefore, we estimate total oxygen consumption by the marsh peat as approximately 12.2 moles O_2 m^{-2} $yr.^{-1}$. If all of this consumption represented oxygen respiration (which it does not since some of the oxygen is consumed in oxidizing reduced inorganic compounds), it would account for the oxidation of approximately 150 g C m^{-2} $yr.^{-1}$. Thus, sulfate-mediated respiration in the peat oxidizes perhaps some 12 times more organic matter than does oxygen-mediated respiration. We have not included *Spartina* respiration in the estimate of oxygen respiration (Teal and Kanwisher 1961) since our estimate for primary production is net, not gross plant production.

Forms of anaerobic respiration other than sulfate reduction are

much less important in carbon turnover in the peat. Dissimilatory nitrate reduction (denitrification) has been measured as 0.8 to 1.5 (average for the whole marsh of 0.95) moles NO_3^- m^{-2} $yr.^{-1}$ in the Spartina peat at Sippewissett (Kaplan et al. 1978), indicating that only 12 g C m^{-2} $yr.^{-1}$ are oxidized through denitrification. Rates of methanogenesis have not been measured at Sippewissett, but we have data indicating a methane export from the peat of 0.7 moles CH_4 m^{-2} $yr.^{-1}$, or 8 g C m^{-2} $yr.^{-1}$. Iron (III) compounds can potentially act as electron acceptors in bacterial respiration (Woolfolk and Whiteley 1968 as cited in Blackburn and Fenchel in press). However, many sediments in which sulfate has been depleted still contain iron (III) compounds, so it seems likely that sulfate is reduced before iron (III) (Bostrom 1967). As sulfate is always abundant in the pore waters of the upper 20 cm of the peat at Sippewissett, it is unlikely that iron reduction is important in the marsh carbon cycle. Iron reduction in the marsh ecosystem is probably chemical and not biological.

Eutrophication and Sulfate Reduction:

A number of authors have suggested that cultural eutrophication of coastal ecosystems might tend to increase sulfate reduction rates and thereby might increase "natural" fluxes of reduced sulfur compounds to the atmosphere (Deevey 1974; Hitchcock and Wechsler 1972). We were able to test the first part of this hypothesis--that eutrophication increases rates of sulfate reduction--as it applies to a salt marsh by comparing rates from our regular short Spartina alterniflora site with rates from a site which had been treated with commercial 10-6-4 fertilizer at a rate of 25 g m^{-2} $week^{-1}$ for 7 years. We also compared the rates from the regular site with rates from a creekside

site. All three sites are in close proximity. The fertilized site was originally characterized by a homogeneous stand of short Spartina alterniflora and appeared to be very similar to our regular sampling site. The fertilization had caused the grass composition to change to a mixed stand of Spartina alterniflora, Spartina patens, and Distichlis spicata by the time of our sulfate reduction measurements in 1977. Primary production was greater as a result of the fertilization, and we believe that the fertilization treatment is a good mimic of severe nutrient loading. The creekside site was a stand of tall Spartina alterniflora, a stand which was naturally more productive than our regular site. Sulfate reduction measurements were made in the fertilized site in July and September of 1977 at the same time as the regular site was sampled. Measurements in the creekside sites were made in August and October of 1977, again at the same time as the sampling for the regular site.

No significant differences in sulfate reduction per unit surface area of marsh could be detected between the creekside site and the regular site. However, sulfate reduction per unit volume of peat was slightly less in the creekside site that in the regular site. Significant rates of reduction occurred at greater depths (25-30 cm) in the creekside peat than at the regular site (20-25 cm). The variation in rates with depth was also considerably greater at the creekside site, perhaps reflecting the patchy distribution of fiddler crab burrows and less even root distribution at that site. Fiddler crab burrows were absent from the regular sampling site, and roots were more closely packed and evenly spaced.

Sulfate reduction rates in the fertilized site appeared to be slightly le- than at the regular sampling site whether the rates were

expressed per unit surface area of marsh or per unit volume of peat. However, such apparent differences were not statistically significant. Nonetheless, it is clear that fertilization and eutrophication did not increase sulfate reduction in the marsh. Such a result is not as paradoxical as it might at first seem since the sulfate reducing bacteria and associated anaerobic microbiota are limited by the availability of energy (substrate) and not nutrients (Blackburn and Fenchel 1978). Although fertilization increases total plant production, it has no major effect on the below-ground production. At high levels, fertilization may actually decrease below-ground production (Valiela et al. 1976). Sediment oxygen consumption is significantly increased by fertilization (Figure 6). Again, these findings demonstrate the importance of the above-ground production for the metabolism of the sediment-surface microbiota and the decoupling of this surface community metabolism from that of the anoxic community below.

Although increased levels of sulfate reduction are not linked with fertilization and cultural eutrophication in salt marsh peat, sulfate reduction rates are clearly linked with organic matter supply. Thus, it seems likely that cultural eutrophication of planktonic coastal ecosystems would cause higher rates of sulfate reduction in the bottom sediments.

Energy Flow in the Salt Marsh Ecosystem:

In an oxidizing environment in which all metabolism is aerobic, energy flow is proportional to and is largely mediated by organic carbon cycling. That is, once energy is fixed as plant biomass in primary production, all subsequent energy flows in a totally oxic ecosystem are flows of organic carbon. Consequently, energy flow is often expressed either in true energy units (i.e., KJoules or

Kcal m^{-2} day^{-1}) or by weight of organic matter or organic carbon (g^{C} m^{-2} day^{-1}), and it is usually assumed that 42 KJ (10 Kcal) are approximately equivalent to 1 g carbon. This assumption is based on the energy released and potentially available to an organism when average organic matter is respired. For example, when glucose is respired, the energy released is approximately the standard free energy of the reaction:

$$Glucose_{(aqueous)} + 6O_2 = 6CO_{2(aqueous)} + 6H_2O$$

which is -2.83 MJ (mole glucose)$^{-1}$ or -39.3 KJ (g carbon) $^{-1}$. (We should actually calculate the free energy rather than the standard free energy, but to calculate the free energy, the concentrations of products and reactants must be known. For most conditions under which organisms aerobically respire glucose, the free energy is probably not greatly different from the standard free energy).

For an ecosystem such as a salt marsh which is partially anoxic and in which a significant percent of the metabolism is anaerobic, carbon cycling is no longer proportional to energy flow and a carbon budget is not an adequate representation of energy flow. When bacteria respire glucose using sulfate rather than oxygen as the terminal electron acceptor, the reaction can be written thus:

$$Glucose_{(aqueous)} + 3SO_4^{=} + 6H^{+} = 6CO_{2(aqueous)} + 3H_2S + 6H_2O$$

which has a standard free energy of -688 KJ (mole)$^{-1}$. Under the conditions found in the Sippewissett salt marsh, the actual free energy is approximately -607 KJ (mole glucose)$^{-1}$ or -8 KJ (g carbon)$^{-1}$ (calculated assuming $(CO_2) = 1.4 \times 10^{-4}$ M, $(H_2S) = 3 \times 10^{-5}$ M, pH = 6.0, $(SO_4^{=}) = 2.1 \times 10^{-2}$ M, and (glucose) = 10^{-10} M; the calculation is relatively insensitive to the assumed glucose concentration). Clearly,

the energy available per unit of organic carbon respired is much less under these anoxic conditions than under the oxic conditions, and an energy flow involving the respiration of 1 g C is approximately 8 KJ (2 Kcal) rather than 42 KJ (10 Kcal). However, the reduced end-products of sulfate reduction "store" the difference in energy. When these end products (H_2S, etc.) move to an oxic environment, they can be oxidized with a net release of energy:

$$HS^- + 2O_2 = SO_4^= + H^+$$

The standard free energy of this reaction is -755 KJ (mole) $^{-1}$. Assuming that the oxidation occurs under conditions where $(O_2) = 2.6 \times 10^{-4}$ M, $(HS^-) = 3 \times 10^{-6}$ M, $(SO_4^=) = 2.1 \times 10^{-2}$ M, and pH = 8, the actual free energy would be -736 KJ (mole).$^{-1}$ Since 2 moles organic C were originally respired to produce one mole of sulfide, the oxidation of the sulfide releases 368 KJ per mole of organic C originally respired, or 30.7 KJ per g C originally respired. The energy of this and other such oxidations can potentially support chemo-autotrophic bacteria, in which case much of the energy "stored" in the reduced inorganic compounds is converted back to organic biomass. Thus, energy flows resulting from the movement and oxidation of reduced inorganic compounds must be included in ecosystem energy flow, and such flows can be quite large. For sulfate reduction, more energy is potentially available from the reoxidation of the reduced end products than is actually available from the sulfate reduction itself.

Energy flow involving inorganic reduced substances in the salt marsh ecosystem may be very large, involving perhaps 54.4 MJ m^{-2} $yr.^{-1}$ as reduced sulfur compounds alone (Howarth and Teal in prep.). Such a flow is equivalent to 70% of net primary production and seems likely

to have profound effects on the structure and function of the ecosystem. Perhaps as much as 18 MJ m^{-2} $yr.^{-1}$ in energy is exported from the marsh peat to the creeks as reduced inorganic sulfur compounds (Howarth and Teal in prep.). Such export is an important mechanism for taking the energy of the extremely high below-ground production and moving it to an area where it can enter food chains of potential significance to man. The export of energy from the peat as inorganic chemical compounds may be more than twice as large as the above-ground production.

Conclusions

The high rate of sulfate reduction in the Great Sippewissett Marsh results in part because the system is intertidal and in part from the activity and production of Spartina alterniflora. Since the peat is intertidal, resupply of sulfate in infiltrating water is high. The high below-ground production of Spartina provides a large input of organic substrates every year over a considerable depth. And Spartina structures the peat so as to keep sulfides low; thus sulfides do not inhibit Spartina growth or sulfate reduction and related fermentative processes. Consequently, sulfate reduction rates in the marsh peat are much higher than in other natural systems. Sulfate reduction and the related fermentative processes in turn supply nutrients to Spartina.

Over the short term, much of the reduced sulfur is stored in the marsh peat as pyrite. Pyrite must be considered when measuring $^{35}SO_4^=$ reduction in a salt marsh. On an annual basis, however, most of this pyrite appears to be oxidized. Although some of the pyrite is oxidized to sulfate, most probably is only partially oxidized to thiosulfate or some similar intermediate. Water which is partially depleted in sulfate and enriched in reduced sulfur compounds moves laterally

from the peat to creeks and is replaced with water from above at high tides. Such exchanges are important in marsh functioning and should be considered in addition to gas exchange across the surface in studies of energy flow in marsh ecosystems.

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Table 1. A comparison of some published rates of sulfate reduction

Location and Conditions	$SO_4^=$ Reduction Rate (μmoles cm^{-3} day^{-1})	Measurement Technique	Reference
Laboratory model sediment system	0.055 - 0.135	$S^{35}O_4^=$	Jorgensen and Fenchel 1974
Danish fjord surface sediments during summer	0.3	$S^{35}O_4^=$	Jorgensen 1977
Barents Sea surface sediments	0.5 - 1.4	$S^{35}O_4^=$	Ivanov 1968 as reported in Jorgensen and Fenchel 1974
Pan and creek sediments in a British salt marsh	0.1 - 0.5	$S^{35}O_4^=$	Nedwell and Abram 1978
Pan sediments in a Long Island Sound salt marsh	0.77	Sulfate depletion in sealed jars over several days	Martens and Berner 1977
Peat from Great Sippewissett Marsh during summer	0.25 - 6.0	$S^{35}O_4^=$	Present study
Peat from Great Sippewissett Marsh during summer	1.4	Sulfate depletion in sealed jars over 1 week	Present study

FIGURE 1.

All sulfate reduction measurements made at the short *Spartina alterniflora* site plotted vs. depth to illustrate the unusual depth profile.

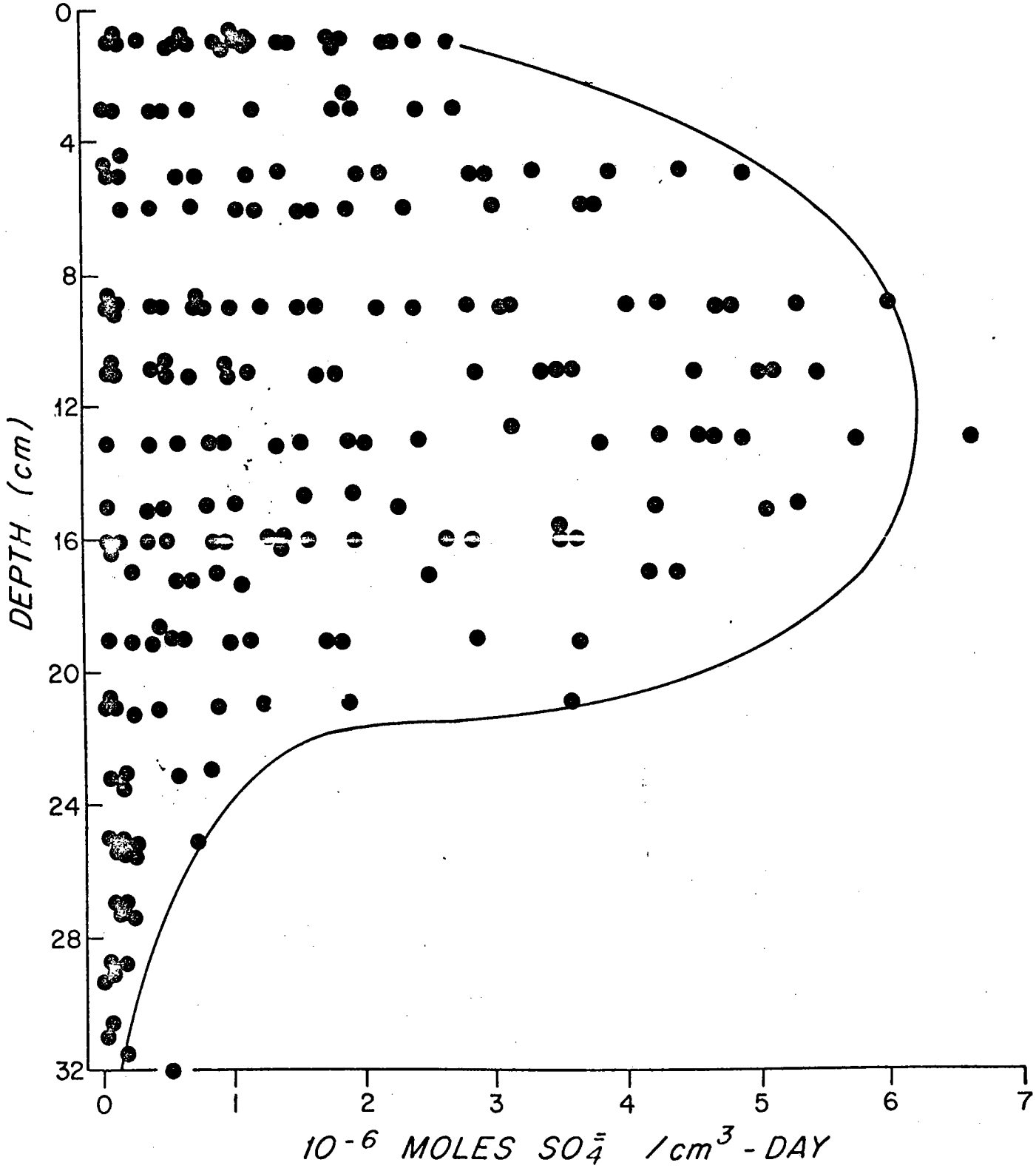
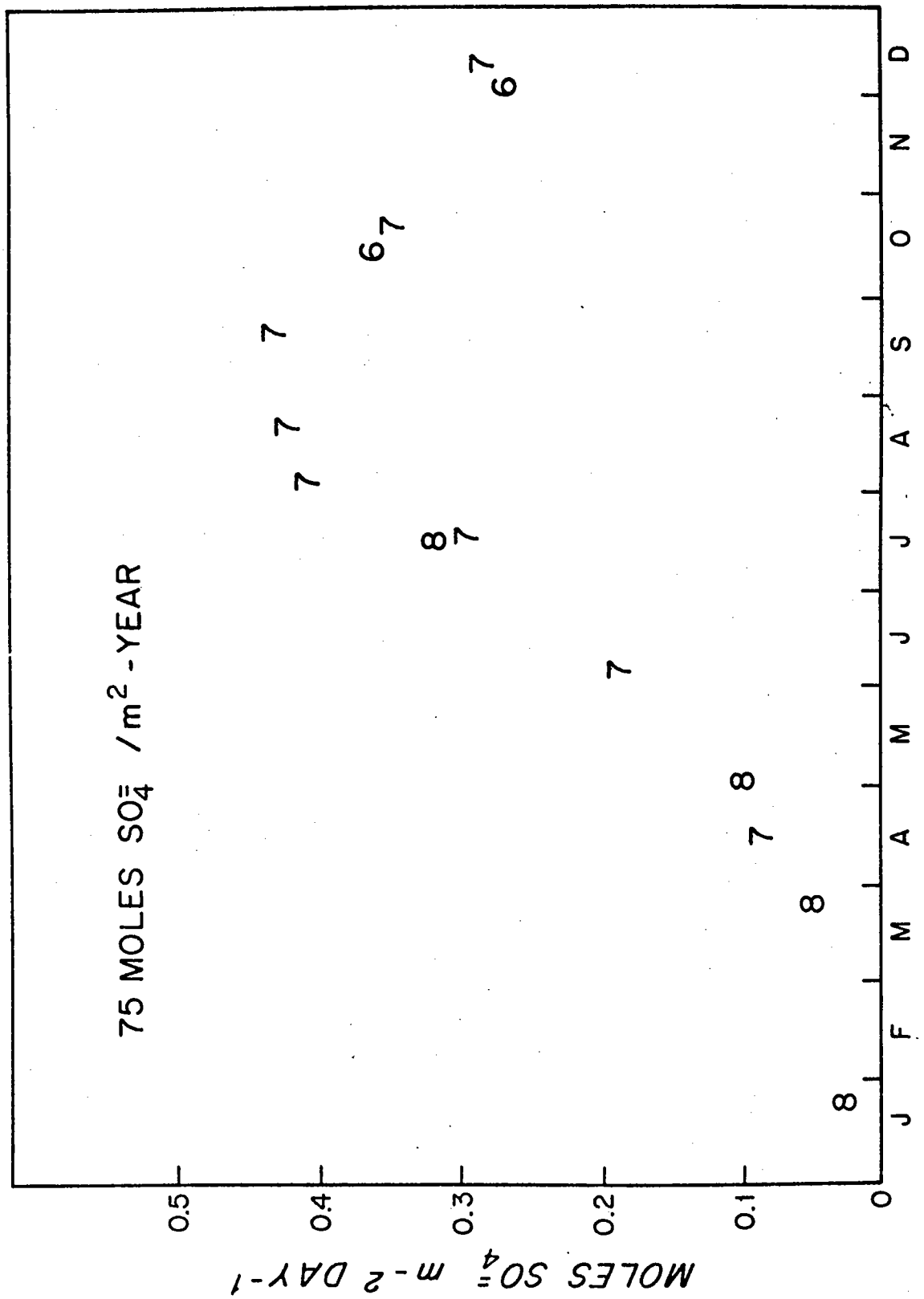

0
4
8
12
16
20
24
28
32
DEPTH (cm)
0
1
2
3
4
5
6
7
10⁻⁶ MOLES SO₄⁼ /cm³ - DAY

FIGURE 2.

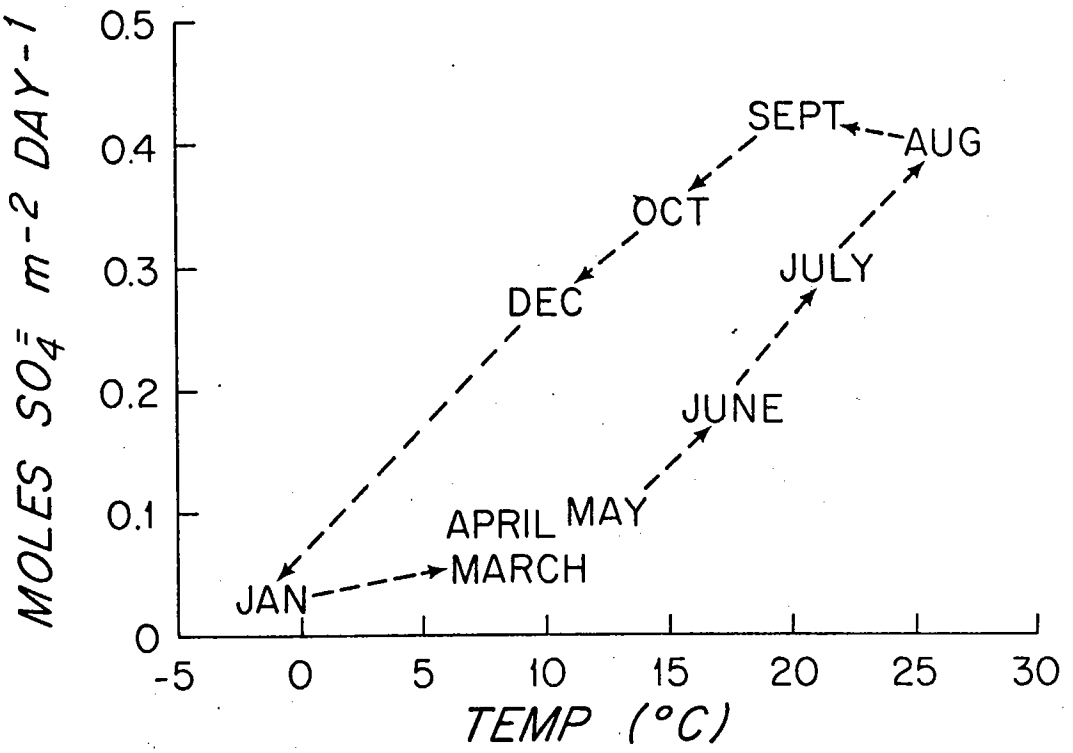
Rates of sulfate reduction at the short Spartina alterniflora site integrated over depth and plotted vs. the time of year. Measurements were made during 1976, 1977, and 1978. The plotted numbers indicate in which year a particular set of measurements were made.



DATA COLLECTED FROM 1976 THROUGH 1978

FIGURE 3.

In part A (top) rates of sulfate reduction at the short Spartina alterniflora site integrated over depth are plotted vs. the sediment temperature. In part B (bottom) rates of sediment oxygen uptake (K. Smith and J. Teal, unpublished) are plotted vs. temperature. Measurements were made in stands of grass similar to the site in which sulfate reduction was monitored. For both parts, A and B, the months are plotted to illustrate the seasonality of the measurements. See text.


MOLES $SO_4^=$ m^{-2} DAY^{-1}
0.5
0.4
0.3
0.2
0.1
0
SEPT
AUG
OCT
JULY
DEC
JUNE
APRIL
MAY
MARCH
JAN
-5
0
5
10
15
20
25
30
TEMP (°C)

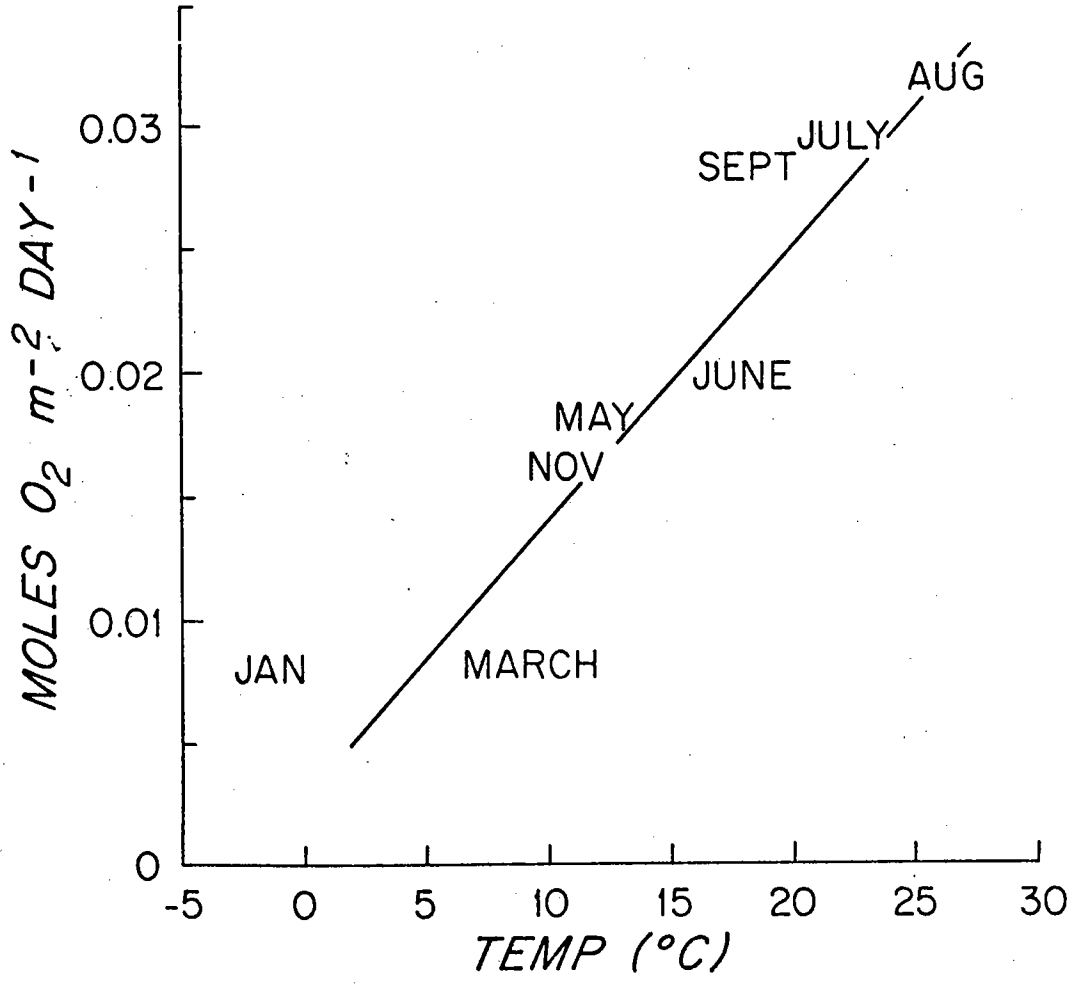

MOLES O_2 m^{-2} DAY^{-1}
0.03
0.02
0.01
0
AUG
JULY
SEPT
JUNE
MAY
NOV
JAN
MARCH
-5
0
5
10
15
20
25
30
TEMP (°C)

FIGURE 4.

Extent of sulfate depletion plotted vs. depth in peat. Sulfate depletion is calculated relative to the amount of sulfate estimated to be in a water parcel at the time it entered the peat. See text. Data are plotted for 3 dates: 26 September, 1977 (x), 6 December 1977 (o), and 19 January, 1978 (Δ).

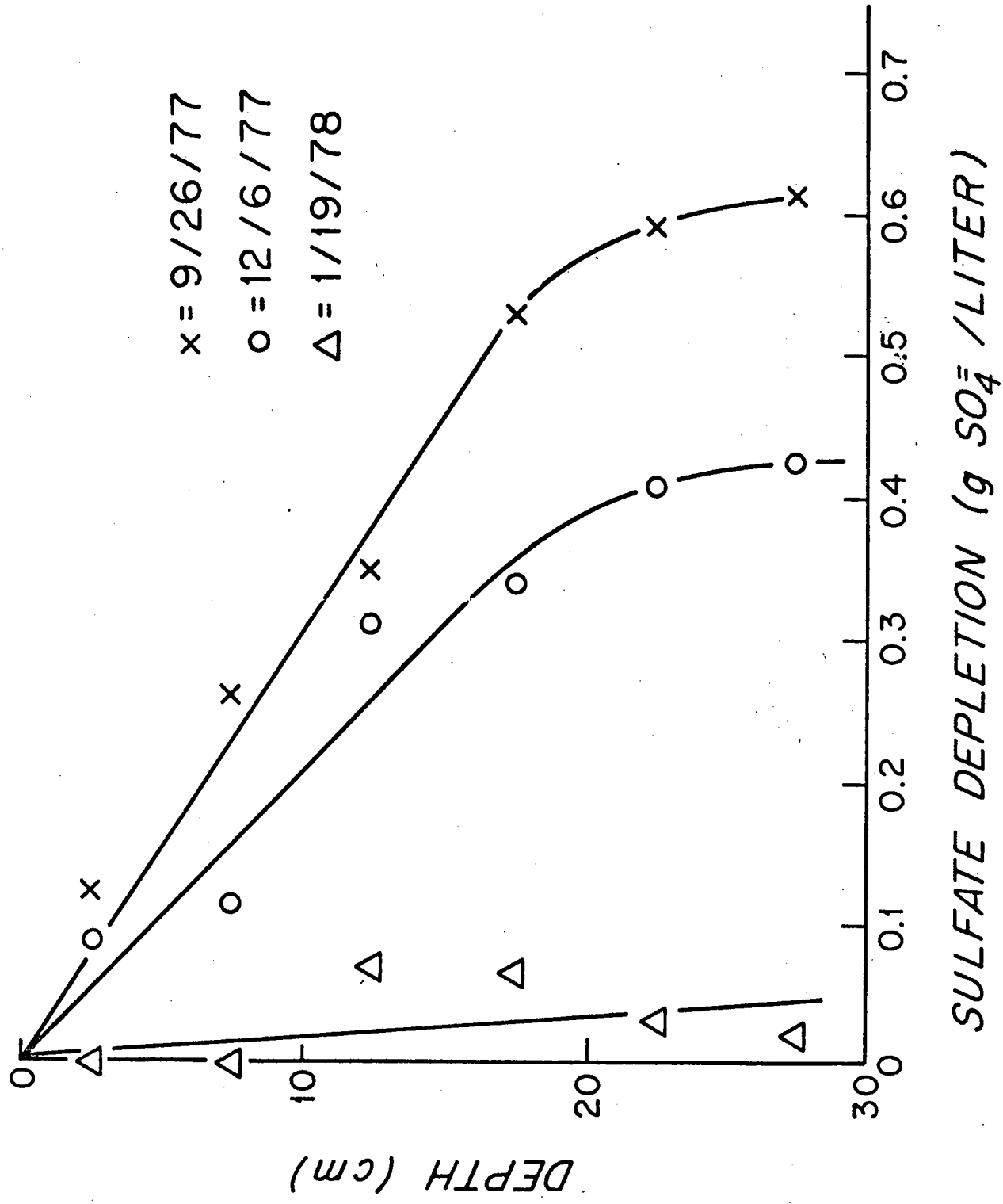

x = 9/26/77
o = 12/6/77
Δ = 1/19/78
DEPTH (cm)
0
10
20
30
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
SULFATE DEPLETION (g $SO_4^=$ /LITER)

FIGURE 5.

Pyrite concentrations plotted vs. depth in peat for 3 dates: 26 September 1977 (o), 19 January, 1978 (x), and 31 May, 1978 (Δ). Standard errors are plotted for the 31 May and 19 January data except when the standard error is less than the size of the data point. No replicates were run for the 26 September data.

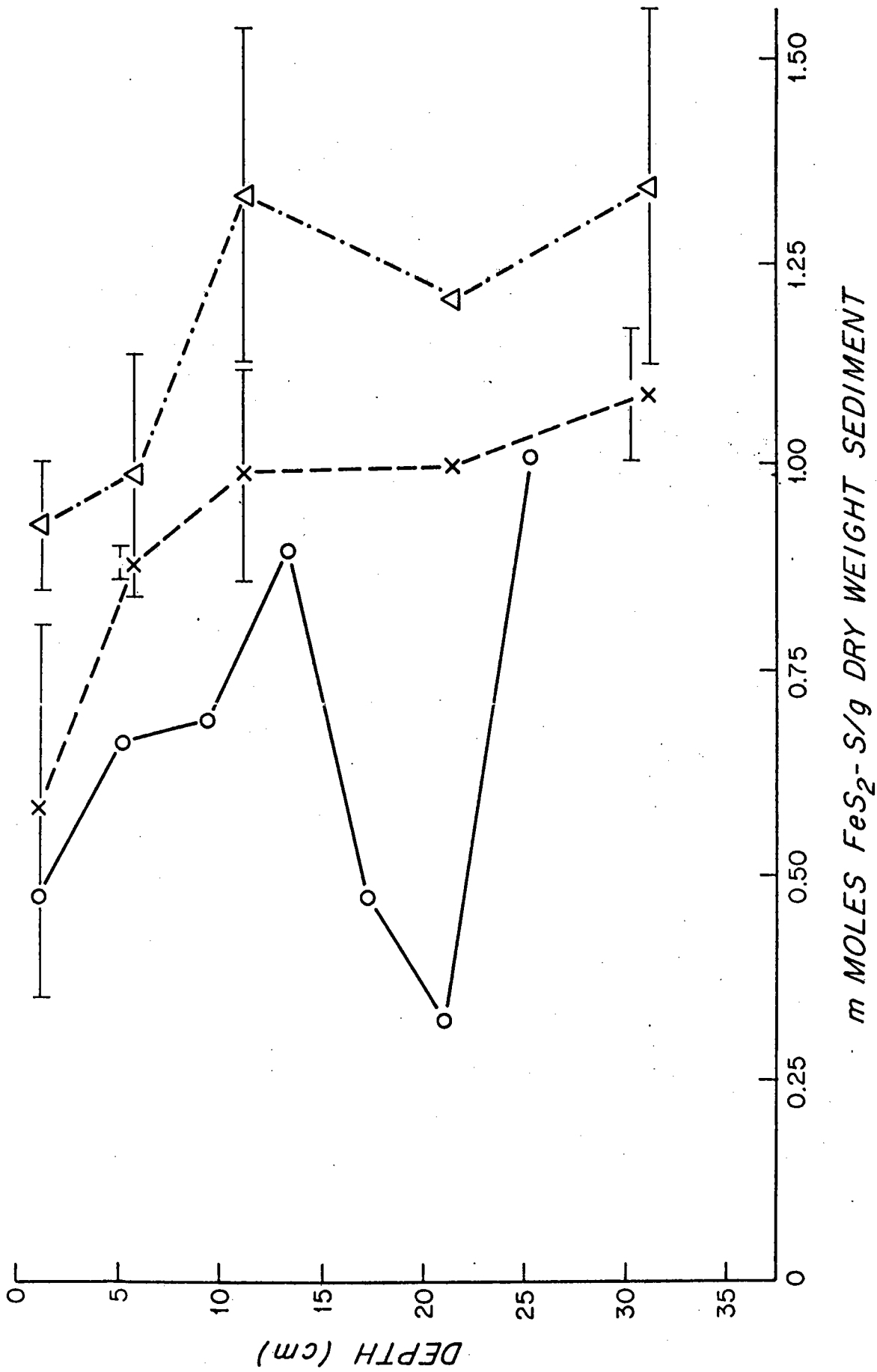
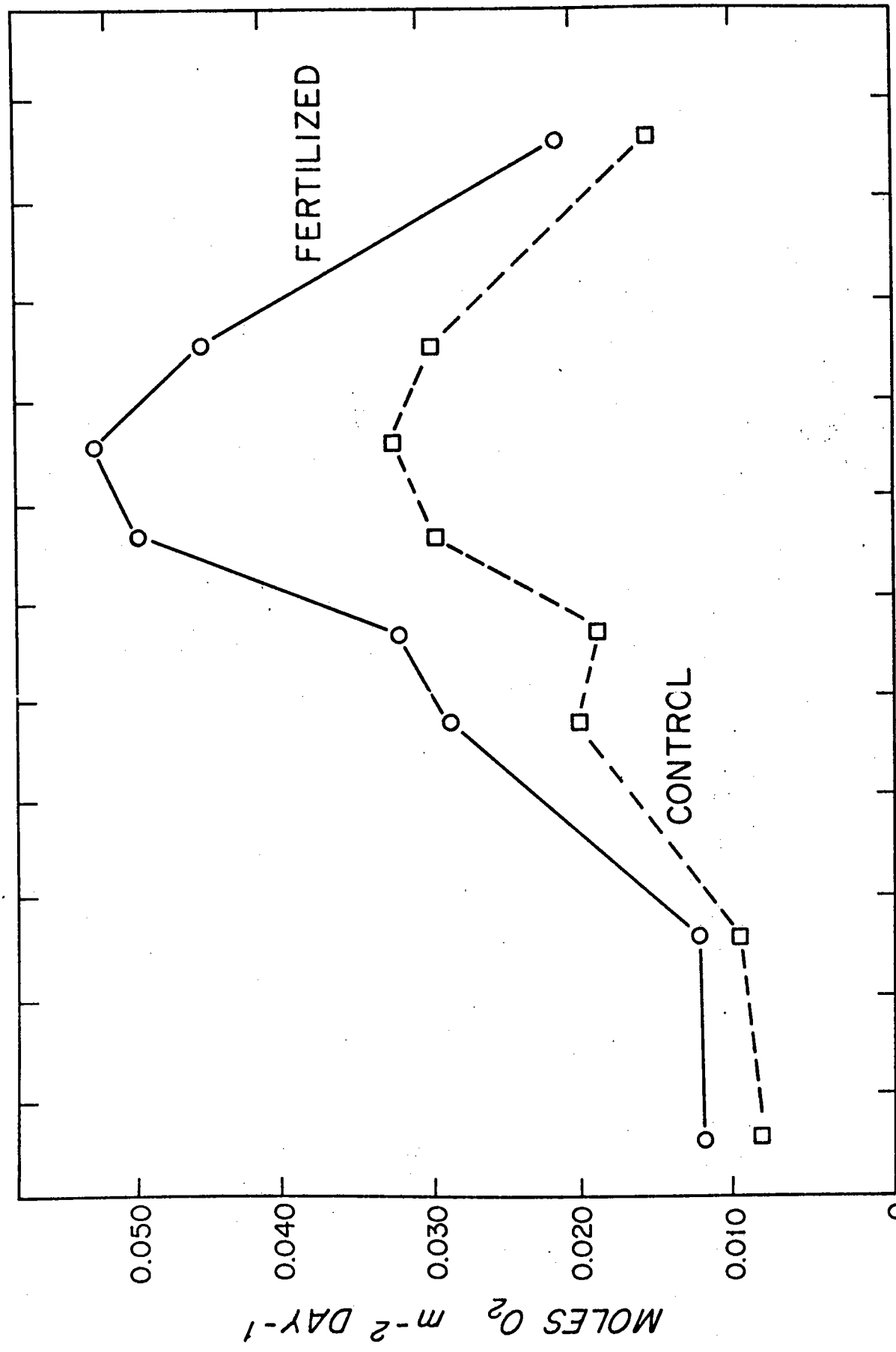

DEPTH (cm)
0
5
10
15
20
25
30
35
0
0.25
0.50
0.75
1.00
1.25
1.50
m MOLES FeS_2- S/g DRY WEIGHT SEDIMENT

FIGURE 6.

Rates of sediment oxygen uptake plotted vs. time of year for two sites of Spartina alterniflora, one fertilized and one not (K. Smith and J. Teal, unpublished). The fertilized site received 50.4 g m^{-2} of fertilizer made from secondary treated sludge from Chicago. It contained 10% N, 6% P_2O_5, and 4% K_2O. Measurements were made at high tide in situ while the marsh peat was covered with water. Stirred respirometers were used. The grass was cut before making measurements. Measurements took 0.5 to 2 hours. Data are from 1971 and 1972.


FERTILIZED
CONTRCL
J F M A M J J A S O N D
0
0.010
0.020
0.030
0.040
0.050
MOLES O_2 m^{-2} DAY^{-1}

CHAPTER 4

ENERGY FLOW IN A SALT MARSH ECOSYSTEM: THE ROLE OF REDUCED INORGANIC SULFUR COMPOUNDS

ENERGY FLOW IN A SALT MARSH ECOSYSTEM: THE ROLE OF REDUCED INORGANIC SULFUR COMPOUNDS[1]

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ABSTRACT

Model calculations show the importance of reduced inorganic sulfur compounds in the energy flow of a New England salt marsh. The export of energy from the peat as reduced inorganic sulfur compounds is perhaps twice the net above-ground production by Spartina alterniflora.

In an oxidizing environment where all metabolism is aerobic, energy flow is proportional to and is largely mediated by organic carbon cycling. That is, once energy is fixed as plant biomass in primary production, nearly all subsequent energy flows in a totally oxic ecosystem are flows of organic carbon. Under such conditions the respiration of 1 g organic carbon-C will yield approximately 42 KJ (10 Kcal).[1]

Recent studies have documented the importance of anaerobic processes in a variety of aquatic ecosystems (Jorgensen and Fenchel 1974, Jorgensen 1977, Rich and Wetzel 1978, Howarth and Teal in prep.). Sulfate reduction is of particular importance in the degradation of organic matter in a salt marsh, degrading perhaps twelve times more organic matter than do oxygen respiration and denitrification combined (Howarth 1979, Howarth and Teal in prep.). The energy yield is significantly less for anaerobic sulfate reduction than for aerobic respiration. For example, under the conditions found in the Great Sippewissett Salt Marsh, the respiration of 1 g organic C by the sulfate-reducing microbial community yields 11 KJ (2.6 Kcal), only one fourth of the energy available from oxygen-mediated respiration (see Table 1 and explanations which follow). Under anaerobic conditions, the rest of the energy (the difference between the anaerobic and the aerobic yield) is stored as reduced inorganic sulfur compounds such as H_2S. When these reduced inorganic compounds are subsequently oxidized, energy is released. This energy can be tapped by a variety of organisms (Roy and Trudinger 1970, Kuznetsov 1970).

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[1] We use KJoules (KJ) rather than Kcal as the predominate energy unit in this paper, but for clarity we present some comparisons with which ecologists are familiar in both Kcal and KJ. One Kcal = 4.184 KJ. Energy flow is best presented in units of power (watts) rather than energy (KJ). One watt = 1 Joule second^{-1}, so 1 watt m^{-2} (annual mean) = 31.5 MJ m^{-2} yr.$^{-1}$.

Energy flows involving such reduced inorganic compounds can be quite large and must be included in the energy budget of any ecosystem which contains anoxic portions. The importance of such energy flows in a New England salt marsh, the Great Sippewissett Marsh, is examined in this paper.

Methods

Our study is confined to areas of short Spartina alterniflora in the Sippewissett marsh. Such areas make up over 9 hectares, approximately 40% of the vegetated area of the entire marsh. We did most of our sampling in an area where the hydrology of the peat is well defined (Figure 1). The peat is flooded from above on almost every tide and drains very slowly from the "bottom" into a creek approximately 10-15 meters away. Fresh water wells up and also moves out to the creek, but little or no mixing of this fresh water up into the most biologically active portions of the peat (the upper 30 cm or so) occurs. The water flow in the peat is simply modeled as a series of water parcels, one added on each tide, which slowly move down to the zone of fresh water mixing and then move laterally to the creek. From data presented in Table 2 for 19 January, we have calculated an infiltration rate of 5.8 cm per day or 2.9 cm per tide.

Data are available on rates of primary production by Spartina alterniflora and by the surface micro-algae and photosynthetic bacteria, on total oxygen consumption and animal respiration, on rates of sulfate reduction, and on concentrations of sulfate and sulfides in the peat. Using these data and our hydrologic model described above, we have constructed a simple model of energy flow in the marsh ecosystem to demon-

strate the role of reduced inorganic compounds. The model is limited to energy flow involving reduced sulfur and carbon compounds. We recognize that other elements are also involved in energy flow, but their importance in the marsh is dwarfed by carbon and sulfur. For example, respiration through denitrification is less than 3 mW m^{-2} (calculated from data in Kaplan et al. in press) and export of energy from the peat as ammonium is less than 13 mW m^{-2}. We have ignored many other transfers which we feel to be relatively unimportant. For instance, direct feeding on Spartina by insects is ignored because it involves less than 10% of the above-ground production.[2]

Energy flows are calculated using reaction free energies (Table 1) and are calculated relative to a completely oxidized state. Reduced carbon compounds (organics) and reduced sulfur compounds "carry" energy while CO_2 and $SO_4^=$ do not. We have defined three standard conditions under which reactions may occur corresponding to 1) the anoxic portion of the peat, 2) the rhizosphere surrounding the Spartina roots, and 3) the oxic zones of the peat surface and creeks. The actual free energies for these conditions are calculated and used rather than the standard free energies of reactions. The use of standard free energies is conceptually incorrect (Martens and Berner 1977) and can lead to significant errors under some circumstances.

We define the marsh ecosystem as the association of Spartina and the biologically active zone of the peat (the top 30 cm), specifically excluding creeks, embayments and mudflats. This definition is consistent with that of Teal (1962) and Day et al. (1973) but is more narrow in scope

[2]Personal communication from S. Vince, School of Natural Sciences, University of Michigan, September, 1978.

than that used by some other workers (Valiela et al. 1978, Woodwell et al. in press). We include the microflora of the Spartina rhizosphere as part of the Spartina. The completed energy flow model is shown in Figure 2.

Many estimates in the energy flow model are subject to error, and we and others are working to improve many of the measurements and estimates. Nonetheless, the conclusion that reduced inorganic sulfur compounds are a critical component in the marsh energy flow is inescapable.

Primary Production

Valiela et al. (1976) estimated the net primary production of Spartina alterniflora in areas of short Spartina in the Great Sippewissett Marsh as approximately 3.9 kg (dry weight) organic matter m^{-2} $yr.^{-1}$; almost 90% of this is in below-ground production of roots and rhizomes. This may be an underestimate because it does not correct for loss of dead underground plant parts due to decomposition between the times of minimum and maximum dead standing crops (Valiela et al. 1976) and because it does not include root exudates. The grass is 48% carbon by weight, and assuming that the energy content of this organic matter is 502 KJ per mole C (Table 1), we calculate that the net primary production of the grasses is 2.5 watts (annual mean) m^{-2}. Using the data of Van Raalte et al. (1976), we estimate that net production by micro-algae and photosynthetic bacteria on the marsh surface under the grass canopy is approximately 350 g C m^{-2} $yr.^{-1}$, or an annual mean of 46 mW m^{-2}.

Sulfate Reduction

Sulfate in the marsh peat is reduced at a rate of 75 moles m^{-2} $yr.^{-1}$

(Howarth 1979, Howarth and Teal in prep.). Sulfate-reducing bacteria themselves can oxidize only a limited range of organic substrates, but fermenting bacteria can degrade a much wider range of substrates. Their metabolic products provide sulfate reducers with substrates. By oxidizing these fermentation end products, the sulfate reducers allow the fermentations to continue at optimum rates. Tezuka (1966) has demonstrated such a commensalism between sulfate-reducing bacteria and other heterotrophic bacteria in laboratory cultures. The net effect is a bacterial community, the sulfate-reducing microbial community, which oxidizes 2 moles of organic carbon for every mole of sulfate reduced (Howarth and Teal in prep.). This community in our marsh oxidizes 150 moles organic C m^{-2} $yr.^{-1}$. This represents an energy flow of 2.39 watts m^{-2} since the energy content of 150 moles of organic carbon (relative to complete oxidation) is 75.3 MJ (Table 1). The amount of energy potentially available to the organisms of the sulfate-reducing microbial community each year is only 132 KJ per mole of organic C, or a total of 19.8 MJ m^{-2} (Table 1). Assuming that these anaerobic bacteria can convert 50% of this energy flow to biomass[3], their productivity is 310 mW m^{-2}. The other 310 mW is dissipated as heat, the metabolic cost. The bacteria production itself becomes a part of the organic detritus. The difference in the amount of energy potentially available from sulfate-mediated respiration and that potentially available from oxygen-mediated respiration is stored as reduced sulfur compounds. Thus energy is transferred to inorganic sulfur compounds at a rate of 1.77 watts m^{-2}.

[3]Approximately 0.118 g (dry weight) of bacteria are produced for every Kcal of energy "removed" by them from the culture media whether the bacteria are growing aerobically or anaerobically (Payne, 1970). This represents an energy conversion efficiency of approximately 50% (Table 1 .

Oxygen Consumption

Total oxygen consumption by the peat in the Great Sippewissett Marsh has been measured as 6.5 moles m^{-2} $yr.^{-1}$ (Smith and Teal as reported in Howarth and Teal in prep.). These measurements probably underestimate total oxygen consumption by the amount of oxygen delivered in infiltrating water on flood tides, approximately 5.7 moles O_2 m^{-2} $yr.^{-1}$.[4] We therefore estimate total oxygen consumption as 12.2 moles O_2 m^{-2} $yr.^{-1}$. Smith and Teal (unpublished) also measured animal respiration by observing oxygen uptake after poisoning the water in their bell jars with 50 mg $liter^{-1}$ of streptomycin-penicillin and subtracting the residual oxygen uptake which continued after formalin poisoning. Their estimate of 3.8 moles O_2 m^{-2} $yr.^{-1}$ includes fungal respiration and some bacterial respiration in addition to animal respiration. Nevertheless, the estimate is about half of that found in a Georgia marsh (Teal and Kanwisher 1961) which seems reasonable for our colder climate. We used this uncorrected measure for animal respiration, and we assumed that fecal production is approximately 15% greater than respiration, which is consistent with estimates for energy flow in snails, mussels, and crabs in a Louisiana marsh (Day et al. 1973).

For our model we have assumed that oxygen-respiring heterotrophic microbes at the marsh surface process approximately 60% of the above-ground production, or 160 mW m^{-2}. This assumption is little more than a guess,

[4] The infiltrating water was assumed to have an oxygen content of 2.7×10^{-4} M. An infiltration rate of 5.8 cm day^{-1} was calculated from the 19 January data in Table 2. We have evidence that the surface of the peat becomes somewhat more oxidized as oxygen-rich waters percolate down at high tide, indicating that infiltrating waters, in addition to diffusion across the surface of the peat are an important mechanism for delivering oxygen.

but in any case does not affect our conclusion concerning the role of inorganic reduced sulfur compounds in marsh energy flow. If true, and assuming a 50% energy conversion efficiency (see footnote 3), the production of these heterotrophs is 80 mW m^{-2} and their respiratory energy dissipation another 80 mW m^{-2}. Their respiration would consume 5.1 moles O_2 m^{-2} $yr.^{-1}$. We estimate oxygen consumption by chemoautotrophs at the peat surface as 3.3 moles O_2 m^{-2} $yr.^{-1}$ by subtracting animal respiration and aerobic micro-heterotrophic respiration from total community oxygen uptake. Using this oxygen consumption estimate and Table 1, we calculate that chemoautotrophs at the marsh surface process 40 mW m^{-2} of reduced inorganic compounds. Assuming a 25% energy conversion efficiency, they produce 10 mW m^{-2} as organic biomass. This assumed efficiency is typical of "young" cultures of chemoautotrophs (Kuznetsov 1970) and therefore seems reasonable for chemoautotrophs in a marsh where they are probably heavily grazed. These microbial activities at the marsh surface are rough estimates, and future work should try to refine them. However, they are of the correct order of magnitude. For example, if there were no chemoautotrophy and all microbial oxygen consumption was by heterotrophs, hetrotrophs would process a maximum of 260 mW m^{-2} organic detritus or about 60% more than our estimate. If there were no heterotrophic activity at the marsh surface and all microbial oxygen consumption was by chemoautrotrophs, they would process a maximum of 100 mW m^{-2} of reduced inorganic compounds. This is 2.5 times larger than our estimate above, but is still a very small proportion of the total energy flow involving reduced inorganic sulfur compounds. The dark CO_2 fixation data reported by Van Raalte et al. (1974) can be used to give the

same maximum estimate for chemoautotrophic production (assuming the same seasonality as for oxygen consumption and assuming no algal or heterotrophic activity).

Fate of Reduced Sulfur

Over the short term most of the reduced sulfur formed by sulfate reduction ends up as pyrite (FeS_2) with lesser amounts of H_2S being formed (Howarth 1979, Howarth and Teal in prep.). However, little of the pyrite is permanently buried in the peat. Assuming a sedimentation rate of 2 mm $yr.^{-1}$, only 0.47 moles FeS_2-S m^{-2} $yr.^{-1}$ are permanently buried. This represents a rate of energy burial of approximately 10 mW m^{-2}. The rest of the pyrite is oxidized (Howarth and Teal in prep.). There is a net oxidation of pyrite during the summer when Spartina is most active and a net build-up during the rest of the year (Howarth and Teal in prep.). We hypothesize that pyrite is oxidized by new, extending Spartina roots (Howarth and Teal in prep., Appendix 2). Oxygen in infiltrating water is probably consumed near the surface of the peat and cannot oxidize pyrite at depth.

When pyrite is oxidized, the products are probably thiosulfate ($S_2O_3^=$) (or a polythionate of similar energy content) and sulfate. We have not been able to detect sulfite ($SO_3^=$) in marsh pore waters, and we would not expect that sulfite would ever be abundant there because it would quickly react with elemental sulfur (which is always present) and be chemically reduced to thiosulfate (Volkov and Ostroumov 1957, Roy and Trudinger 1970). We can estimate the rate at which pyrite and other reduced organic compounds are oxidized to sulfate by comparing the actual extent of sulfate depletion (Howarth and Teal in prep.) with the predicted depletion based on rates of sulfate reduction assuming no reoxidation

(Table 2). By January the peat was frozen and the Spartina completely inactive, so we are reasonably certain that no reoxidation was occuring in the root zone. If we assume that the September rate of reoxidation represents the annual mean rate, the reoxidation rate is 24 moles $SO_4^=$ m^{-2} $yr.^{-1}$. This probably overestimates reoxidation to sulfate since little or no reoxidation occurs in the winter and spring (Howarth and Teal in prep.).

The average H_2S concentration in the peat at 25-30 cm depth is approximately 3×10^{-5} M. Using our hydrologic model, we calculate an H_2S export of 0.64 moles m^{-2} $yr.^{-1}$, representing an energy export of 15 mW m^{-2}. Our preliminary data suggest that export to the atmosphere is much smaller and we are not including such export in the model.

Sulfur which is reduced in sulfate reduction is, on an annual basis, 1) sedimented as pyrite, 2) exported as H_2S, 3) oxidized at the surface of the peat, 4) reoxidized to sulfate in the grass rhizosphere, or 5) oxidized to thiosulfate (or other intermediately reduced sulfur compound of similar energy content) and exported laterally from the peat to creeks in pore water. We calculate then that "thiosulfate" export from the peat amounts to 47 moles $S_2O_3^=$-S m^{-2} $yr.^{-1}$. This represents an energy export of 560 mW m^{-2}. Adding the energy export as H_2S, we get a total energy export as inorganic sulfur compounds of 570 mW m^{-2} (22% of the total net primary production and more than twice the above-ground net production). Energy export as reduced sulfur compounds may be larger than 570 mW m^{-2}, since we probably have overestimated the reoxidation of reduced sulfur compounds to sulfate in the rhizosphere by using the September rate as an annual mean. Even if we have greatly underestimated the rate of chemoautotrophy at the marsh surface, we know that the error is less than 60 mW m^{-2} (see earlier discussion), and reduced

inorganic sulfur export is still greater than 500 mW m^{-2}. Also, the argument is not changed if the oxidation of reduced sulfur of the marsh surface is chemical and not chemoautotrophic.

Within the peat, 24 moles H_2S m^{-2} $yr.^{-1}$ are oxidized to sulfate and 50 moles H_2S m^{-2} $yr.^{-1}$ are oxidized to thiosulfate. Combined, these oxidations represent an energy flow of approximately 1.08 watts m^{-2}. We do not know how much of this energy is used and converted to organic biomass by Spartina and the microbes of the rhizosphere.

Organic Sedimentation and Export

Assuming a sedimentation rate of 2 mm $yr.^{-1}$, 9.4 moles organic C are permanently buried each year per m^2. This represents an energy burial of 150 mW m^{-2} (6% of total net primary production). By difference, the export of organic matter as both particulate and dissolved organic carbon represents an energy export of 180 mW m^{-2} (7% of total net primary production). This estimate is subject to error due to difficulties in estimating underground plant production, chemoautotrophic production in the rhizosphere and at the peat surface, and organic degradation by oxygen-respiring heterotrophs.

Conclusions

The complete energy flow model is shown in Figure 2 and is summarized in Table 3. The estimates for energy export and for energy flow within the peat will be refined by future work, but the qualitative pattern should remain unchanged since the model is relatively insensitive to the likely errors. Most of the energy export is as reduced inorganic sulfur compounds. Such energy is in a form more labile and easily used

than many organic compounds such as humic acids and lignin. If the energy available from the oxidation of the reduced inorganic sulfur compounds were used with 25% efficiency by chemoautrtrophs in the nearby creeks and estuaries, it would support a bacterial production equivalent to more than half of the above-ground production by Spartina alterniflora. Most of the oxidation of these reduced sulfur compounds is probably biological since our evidence indicates that the rate is too high to be solely chemical. The 25% energy efficiency is typical of "young" cultures of chemoautotrophs (Kuznetsov 1970) and so is reasonable for heavily grazed bacteria. This bacterial production would likely be available as an easily assimilable food for coastal food webs. Thus the export of reduced inorganic sulfur compounds from the peat is a mechanism for the transfer of energy from the very large below-ground grass production to the coastal food webs.

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TABLE 1. CALCULATION OF FREE ENERGIES USED IN ENERGY FLOW MODEL.

Reaction	Location of Reaction	Standard Free Energy (KJoules)[1]	Actual Free Energy (KJoules)[2]
$"CH_2O" + O_2 = CO_2 + H_2O$ [3.]	oxic zone[4.]	-493	-502
$"CH_2O" + 1/2SO_4^{=} + H^+ = 1/2H_2S + CO_2 + H_2O$ [3.]	anoxic zone[5.]	-136	-132
$H_2S + 2O_2 = SO_4^{=} + 2H^+$	oxic zone[4.]	-714	-728
$H_2S + 2O_2 = SO_4^{=} + 2H^+$	rhizosphere[6.]	-714	-704
$H_2S + O_2 = 1/2\ S_2O_3^{=} + 1/2H_2O + H^+$	rhizosphere[6.]	-357	-341
$1/2S_2O_3^{=} + O_2 + 1/2H_2O = SO_4^{=} + H^+$	oxic zone[4.]	-357	-375

Continued on next page

TABLE 1. (Continued)

1. The standard free energy is the free energy under conditions where all reactants and products have an activity of one.
2. The actual free energy (ΔF) of the reaction $xA + yB = mC + nD$ is given by $\Delta F = \Delta F^\circ + RT \ln \frac{(C)^m(D)^n}{(A)^x(b)^y}$, where ΔF° is the standard free energy of the reaction, R is the gas constant, and T is the absolute temperature.
3. "CH_2O" is used to represent typical organic matter in the marsh. We have arbitrarily assigned it a standard free energy of formation of -130 KJoules, intermediate between that of glucose and that of cellulose. Its activity is taken as one.
4. The following activities have been assumed for the oxic zone: $(SO_4^=) = 2.1 \times 10^{-2}$, $(CO_2) = 1 \times 10^{-5}$, $(H_2S) = 1.5 \times 10^{-6}$, $(O_2) = 2 \times 10^{-4}$, $(S_2O_3^=) = 2 \times 10^{-4}$, pH = 7.0.
5. The following activities have been assumed for the anoxic zone: $(SO_4^=) = 2 \times 10^{-2}$, $(CO_2) = 1.4 \times 10^{-4}$, $(H_2S) = 3 \times 10^{-5}$, pH = 6.0.
6. The following activities have been assumed for the rhizosphere: $(SO_4^=) = 2 \times 10^{-2}$, $(H_2S) = 1 \times 10^{-5}$, $(O_2) = 6 \times 10^{-6}$, $(S_2O_3^=) = 1.5 \times 10^{-3}$, pH = 6.0.

TABLE 2. CALCULATION OF SULFATE REOXIDATION.

	26 Sept.1977	6 Dec.1977	19 Jan. 1978
$SO_4^=$ depletion at 30 cm depth (moles liter^{-1})	6.35×10^{-3}	4.43×10^{-3}	5.21×10^{-4}
Average $SO_4^=$ reduction rate in top 30 cm (moles liter^{-1} day^{-1})	1.44×10^{-3}	8.96×10^{-4}	1.0×10^{-4}
Calculated $SO_4^=$ depletion if no reoxidation (moles liter^{-1})[1].	7.50×10^{-3}	4.67×10^{-3}	5.21×10^{-4}
Rate of reoxidation of reduced sulfur to $SO_4^=$ (moles m^{-2} day^{-1})	6.6×10^{-2}	1.4×10^{-2}	0

1. Calculated using an infiltration rate of 5.76 cm day^{-1} (which was calculated from 19 Jan. data assuming no reoxidation at that time).

TABLE 3. SUMMARY OF MARSH ENERGETICS.

	watts m^{-2}	Percent of net primary production
Net primary production - Total	2.55	
-Above-ground *Spartina*	0.27	
-Below-ground *Spartina*	2.23	
-"Algal"	0.05	
Total energy dissipation by consumers	0.48	19%
Non-biological energy dissipation plus energy recycled to primary producers	1.12	44%
Energy export as reduced sulfur compounds	0.57	22% (211% of above-ground grass production)
Energy export as organic carbon	0.18	7%
Total energy export	0.75	29%
Burial of energy	0.16	6%

FIGURE 1.

Hydrologic model of the peat at the experimental site. The peat is completely flooded on nearly every high tide. At low tide, pore waters drain laterally to the creek. Dashed horizontal lines indicate the estimated movement of a water parcel during one tidal cycle. See text.

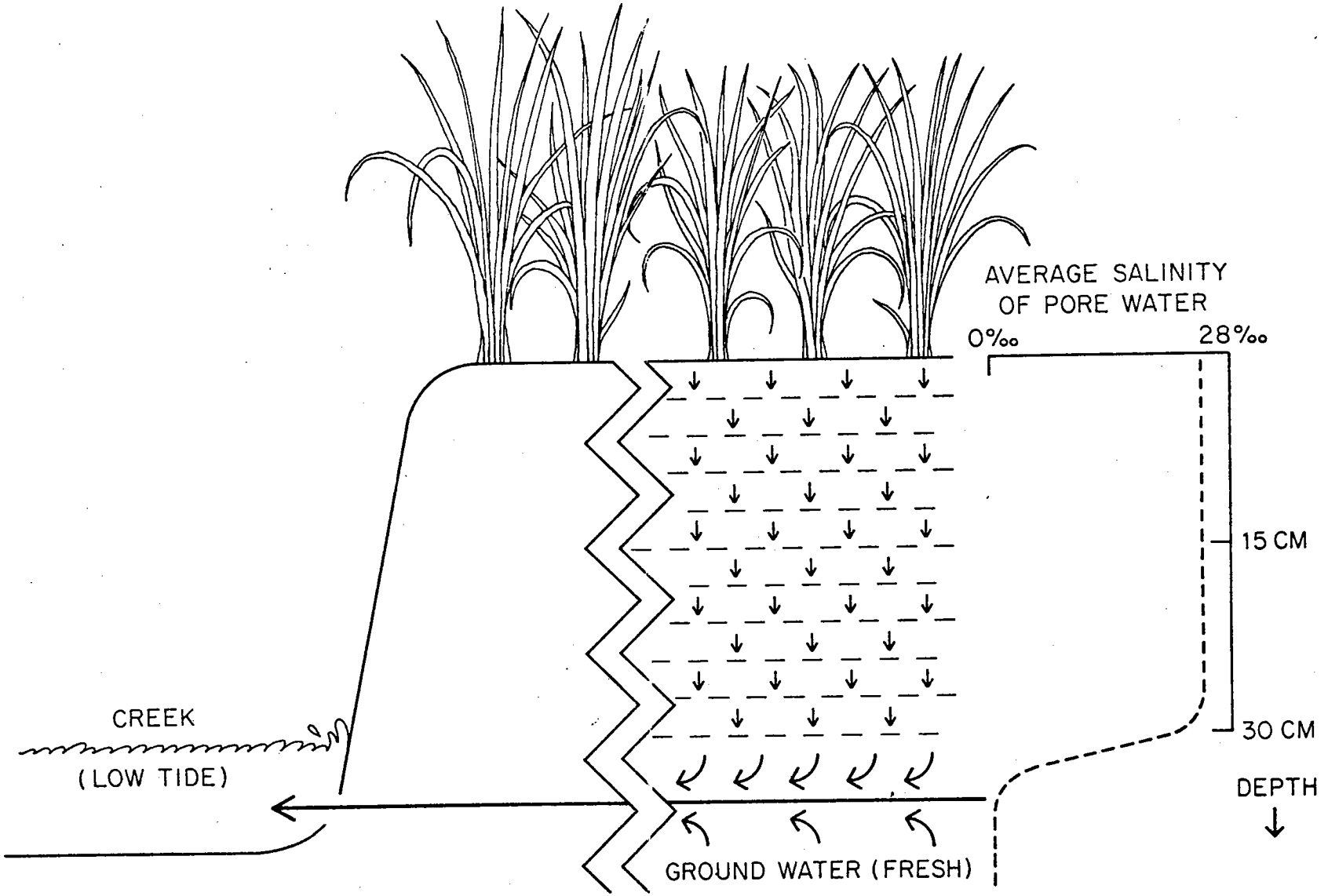
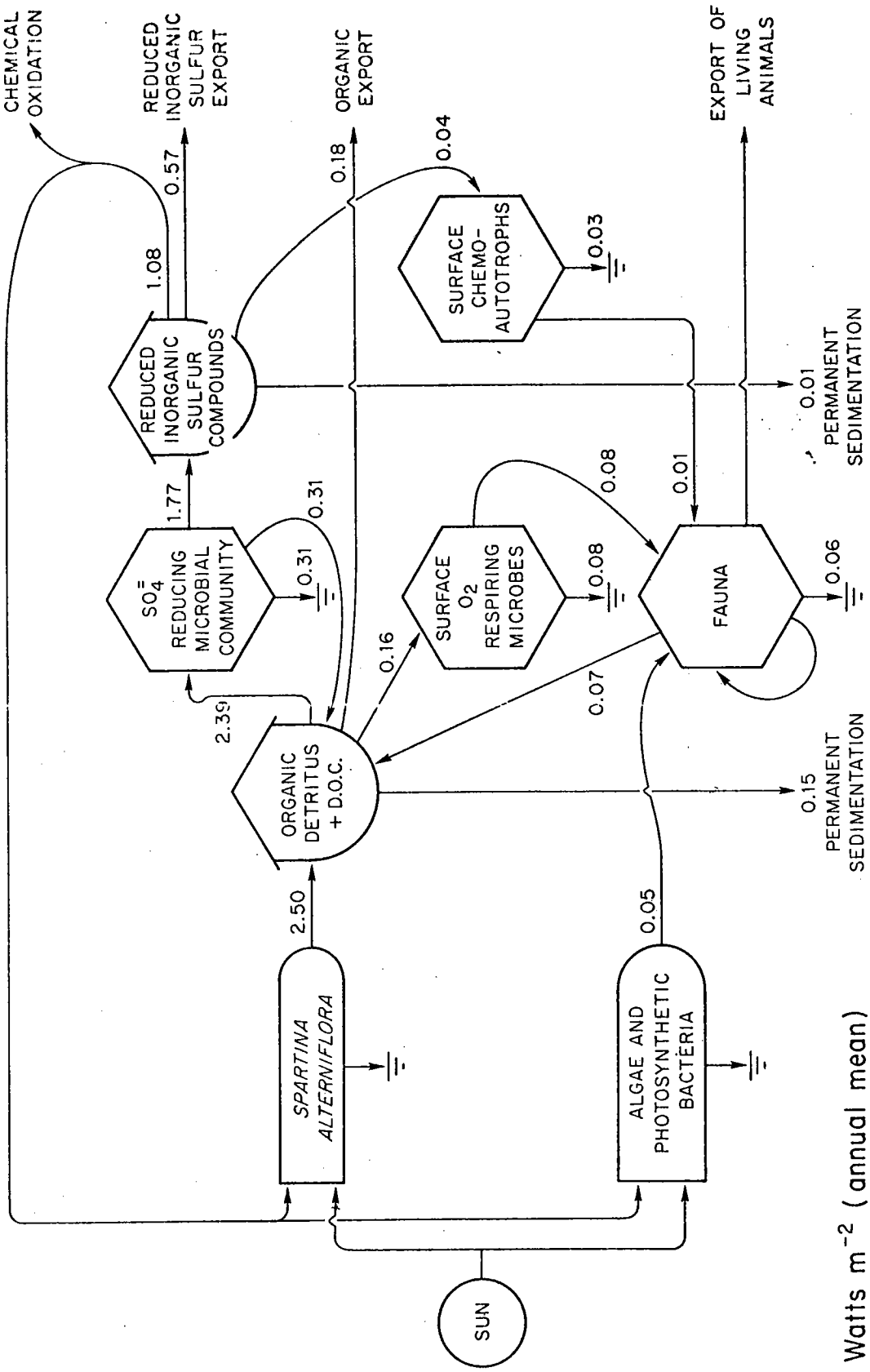

AVERAGE SALINITY
OF PORE WATER
0‰
28‰
15 CM
30 CM
DEPTH
CREEK
(LOW TIDE)
GROUND WATER (FRESH)

FIGURE 2.

Energy flow model for the salt marsh ecosystem. Symbols are after Odum (1971). Units are watts (annual mean) m^{-2}. Some pathways represent flows of organic carbon; others are flows of reduced inorganic sulfur compounds. See text.


CHEMICAL OXIDATION
REDUCED INORGANIC SULFUR EXPORT
ORGANIC EXPORT
EXPORT OF LIVING ANIMALS
0.57
1.08
0.18
0.04
REDUCED INORGANIC SULFUR COMPOUNDS
SURFACE CHEMO-AUTOTROPHS
0.03
1.77
0.31
0.31
$SO_4^=$ REDUCING MICROBIAL COMMUNITY
SURFACE O_2 RESPIRING MICROBES
0.08
0.08
0.01
FAUNA
0.06
0.01 PERMANENT SEDIMENTATION
0.16
2.39
0.07
ORGANIC DETRITUS + D.O.C.
0.15 PERMANENT SEDIMENTATION
2.50
0.05
SPARTINA ALTERNIFLORA
ALGAE AND PHOTOSYNTHETIC BACTERIA
SUN
Watts m^{-2} (annual mean)

APPENDIX 1:

A RAPID AND PRECISE METHOD FOR DETERMINING SULFATE IN SEAWATER, ESTUARINE WATERS, AND SEDIMENT PORE WATERS[1]

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1. Howarth, R. W. (1978)
Limnol. Oceanogr. 23: 1066-1069

ABSTRACT

Sulfate can be rapidly and accurately measured by means of an indirect titration. Barium sulfate is precipitated in acid EDTA solution, the precipitate filtered and dissolved in an excess of EDTA at high pH, and the excess EDTA titrated with $MgCl_2$. Interferences from chloride, iron, or phosphate are negligible. Sulfides may interfere, but there is a procedure to remove this interference. The method determines the sulfate concentration in 1.0 ml of seawater with a standard deviation consistently <0.5% of the mean determination of three replicates.

Sulfate reduction is an important diagenetic process in anoxic marine and estuarine sediments, but techniques for sulfate determination are subject to several interferences likely to be particularly severe in estuarine and sediment pore waters. The method presented here is reliable under the highly diverse chemical conditions in salt marsh waters: salinity from 8-30% and variable concentrations of metals, phosphates, reduced sulfur anions, and organic matter. It works with pore water volumes as small as 1.0 ml, is rapid, precise, accurate, inexpensive, and suitable for use in the field.

The barium sulfate gravimetric technique, generally used in seawater when high accuracy is desired (Morris and Riley 1966) is subject to serious coprecipitation interferences, particularly from iron and phosphates (Haddock 1962), which may be troublesome in estuarine or sediment pore waters. Further, the gravimetric approach is slow. The direct titration of Macchi et al. (1969) suffers from a chloride interference, requiring precise standardization at each salinity. Although this method is useful for open-ocean seawater, the disadvantage of constant restandardization in an estuary is obvious. Difference chromatography is capable of highly accurate sulfate determinations (Sayles and Mangelsdorf 1977) but requires expensive apparatus and may suffer from the interference of some reduced sulfur anions (my unpublished observations; F. Sayles pers. comm.).

The method presented here involves precipitation of barium sulfate in acid EDTA solution, dissolution of the precipitate in excess of EDTA at high pH, and titration of the excess EDTA with $MgCl_2$ using eriochrome black-T as the indicator. Successful application of this method to the determination of sulfate in fertilizers (Campbell et al. 1974), urine

(Lewis 1962), and organic materials (Belcher et al. 1958) suggests no problems for its use under the conditions found in estuaries and sediment pore waters.

Unless otherwise stated, all chemicals used were analytical reagent grade. All distilled water was double distilled in a glass still.

Ammonium hydroxide -- 29.4% NH_3, specific gravity of 0.900.

Barium chloride solution, 10% -- dissolve 117 g $BaCl_2 \cdot 2H_2O$ in double-distilled water and make up to 1 liter.

EDTA solution, 0.0100 M -- prepared by Resources Services, Inc., for Arthur H. Thomas, Co.

Magnesium chloride titrant, 0.025M -- dissolve 5.08 g $MgCl_2 \cdot 6H_2O$ in double-distilled water and make up to 1 liter.

HCl, 0.4M -- dilute 33 ml concd HCl to 1 liter with double-distilled water.

HCl, 0.05 M -- dilute 4 ml concd HCl to 1 liter with double-distilled water.

Buffer solution, pH 10 -- dissolve 7 g NH_4Cl and 57.0 ml NH_4OH (sp gr of 0.900) in about 25 ml of double-distilled water. Dilute to 100 ml with double-distilled water.

Indicator Solution -- dissolve 0.40 g of eriochrome black-T (also called solochrome black-T) in 30 ml of triethanolamine and 10 ml of absolute ethanol.

One milliliter of sample is placed in a 50-ml Erlenmeyer flask, 3 ml of 0.4 M HCl and 4 ml of 0.0100 M EDTA are added, and the mixture boiled gently for 2 min to speed the chelation of any metals present in the sample. Ten milliliters of 0.05 M HCl are added, and the flask is allowed to cool for a few minutes. Then 5 ml of 10% $BaCl_2$ is added and the fla left at room temperature for another 20 min to allow the

$BaSO_4$ to precipitate; excessively long standing time (overnight) will make subsequent dissolution of the $BaSO_4$ difficult, resulting in an underestimate of sulfate. The mixture is then filtered through a small 0.45-μ Millipore filter (diameter of 1.8cm) to remove the solubilized interfering ions. Because of the massive addition of Ba^{2+}, and the acid conditions, the EDTA does not interfere with the precipitation. And since barium-EDTA complexes are somewhat unstable under acid conditions (West 1969), swamping the solution with barium does not result in the release of interfering cations from their EDTA complexes. The flask is carefully rinsed into the filter apparatus three times with double-distilled water, and the filter and precipitate are washed with 10 ml of 0.05 M HCl and 20.0 ml of double-distilled water. Additional washing may result in some loss of $BaSO_4$. Then the filter and precipitate are transferred back to the original 50-ml flask. The rinsing and the size of the glassware used may be important due to $BaSO_4$ sorption to glass. Exactly 5.00 ml of 0.0100 M EDTA are added to the precipitate, then 4.0 ml of NH_4OH solution (sp gr = 0.900) to drive the pH up as an aid in dissolving the $BaSO_4$. To speed dissolution of the $BaSO_4$ precipitate, I heat the flask to about 90°C for 15 min while stirring occasionally. After the flask has cooled to room temperature, 0.5 ml of the pH 10 buffer solution is added and the mixture titrated with standard $MgCl_2$ solution, using a drop of eriochrome black-T solution as the indicator. A 2.0 ml microburette (Gilmont) is used for the titration. The end point is indicated by a well developed red/pink color which forms as Mg^{2+} binds to the indicator dye; in the absence of Mg^{2+}, the dye is purple/blue.

The technique was standardized with Copenhagen Standard Seawater (collected 27 July 1974; chlorinity = 19.3675‰). The sulfate concentra-

tion of the standard was calculated as 2.777 g·liter^{-1} (24°C) by assuming a sulfate:chloride ratio of 0.1400 (Morris and Riley 1966) and a density of 1.024 kg·liter^{-1} (Pickard 1963). A standard curve prepared by serial dilution of the standard seawater was linear down to zero sulfate concentration (r^2 = 1.00 with 17 observations at four concentrations); the standard deviation of the sulfate concentration estimator on volume of titrant added was 0.005. Thus, the method works well over a wide range of sulfate concentrations.

When the $MgCl_2$ titrant was standardized in pH 10 buffer and this determination used to calculate sulfate concentrations, the resulting values were consistently 2.8% lower than values determined from the direct standardization with Standard Seawater. This discrepancy probably reflects $BaSO_4$ adsorption to glassware. It should not be a serious problem so long as sulfate standards are used.

A possible phosphate interference was tested by adding phosphate to make sulfate and phosphate about equimolar (adding 1 ml of 0.028 M PO_4^{3-} to 1 ml of Standard Seawater). A possible iron interference was tested in the same manner. In addition, a few grains of the insoluble salt $Fe_3(PO_4)_2$ were added to Standard Seawater samples. The method is remarkably free from interferences (Table 1). Even the addition of solid $Fe_3(PO_4)_2$ resulted in no major errors. By precipitating the $BaSO_4$ in the presence of acidified EDTA, iron is solubilized and coprecipitation is prevented. The gravimetric determination of sulfate would clearly incur serious errors under the severe conditions tested.

I also determined the sulfate concentration of pore water samples taken from two different locations in a coastal salt marsh. The pore waters were pressed from shallow surface cores with a Reeburgh press.

The chlorinity, as determined with a refractometer, was 12.7‰ at one site and 14.9‰ at the other. Each 1-ml sample was then enriched with 1 ml of 1.550 g·liter^{-1} sulfate and 30‰ NaCl; the chloride was added incidentally to the experiment since it was in the sulfate standard. Chloride should not interfere with the method, as is indicated by the large amounts of HCl and $BaCl_2$ used in the assay. This experiment shows that the method works well under field conditions (Table 2).

Kwiecinski (1965) also used an indirect titration involving barium precipitation to determine sulfate in seawater, a method superficially similar to the one described here. However, Kwiecinski's method makes no attempt to eliminate interferences from cations present in the sample, and such interferences must be determined with a separate titration. This greatly increases the time necessary for the assay and lowers the precision. Further, Kwiecinski titrated the excess Ba^{2+} not precipitated as $BaSO_4$. In my method excess Ba^{2+} and interfering ions are washed away, the $BaSO_4$ is dissolved and a known excess of EDTA is added, and this amount of EDTA is determined with $MgCl_2$ titration. The single titration increases the precision. The titration of an excess of EDTA with $MgCl_2$, rather than an excess of Ba^{2+} with EDTA, causes the eriochrome black-T indicator end point to be much sharper and easier to determine (Belcher et al. 1954). Further, the direct titration of barium with EDTA is subject to error due to the precipitation of $BaCO_3$, but this problem is avoided by adding the excess of EDTA and black-titrating with $MgCl_2$ (West 1969).

The standing time I used in the barium sulfate precipitation is quite brief but is adequate under these acid conditions (Belcher et al. 1954). This short standing time speeds the assay and aids in dissolu-

tion of $BaSO_4$ (Campbell et al. 1974). The acid conditions also prevent precipitation of barium orthophosphate.

Soluble sulfides present in a pore water sample may be oxidized to sulfate during the assay, sometimes introducing considerable error. For example, the sulfide concentration measured at 25 cm in one core was 48 mg $S^{2-} \cdot liter^{-1}$. The sulfate method outlined here indicated a sulfate concentration of 0.150 $g \cdot liter^{-1}$ in this sample, of which as much as 0.144 may have been due to the oxidation of sulfides. Such interferences could be minimized by precipitating the sulfides with zinc acetate and removing the ZnS by Millipore filtration immediately after the pore waters are pressed from the core.

The variance in the method probably could be substantially reduced by scaling up to larger sample volumes, if this seemed desirable. For pore water samples, however, the small amount of sample required for analysis is a definite advantage. By this method a dozen samples can be assayed for sulfate in just a few hours, yielding excellent results free from most interferences.

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TABLE 1. Determination of Interference from PO_4^{3-}, $FeCl_3$, and $Fe_3(PO_4)_2$.

Copenhagen Seawater+	No. replicates	SO_4^{2-} ($g \cdot liter^{-1}$)	SD	Error ($g \cdot liter^{-1}$,%)*
1 ml of 0.028 M PO_4^{3-}	6	2.775	0.009	-0.002, 0.074%
1 ml of 0.028 M $FeCl_3$	4	2.780	0.010	+0.003, 0.11%
A few grains of $Fe_3(PO_4)_2$	3	2.780	0.0113	+0.003, 0.11%

*Error determined by comparison with calculated sulfate concentration of 2.777 $g \cdot liter^{-1}$(24.0°C). See text.

TABLE 2. Recovery of Sulfate Added to Salt Marsh Pore Water Samples.

Salt Marsh Pore Water	No. replicates	Mean SO_4^{2-} ($g \cdot liter^{-1}$)	SD	Error ($g \cdot liter^{-1}$, %)
Sample 1 (Cl^- = 12.7‰)	3	1.823	0.010	-
No. 1 + 1.0 ml of 1.550 $g \cdot liter^{-1}$ sulfate in 30‰ NaCl (calc sulfate = 3.373 $g \cdot liter^{-1}$)	3	3.368	0.009	-0.005, 0.15%
Sample 2 (Cl^- = 14.9‰)	3	2.178	0.002	-
No. 2 + 1.0 ml of 1.550 $g \cdot liter^{-1}$ sulfate in 30‰ NaCl (calc sulfate = 3.728 $g \cdot liter^{-1}$)	3	3.735	0.007	+0.007, 0.18%

APPENDIX 2:

THE ABILITY OF SPARTINA ALTERNIFLORA TO MAINTAIN AN OXIDIZED RHIZOSPHERE:

Spartina alterniflora maintains a relatively oxidized peat around its roots with fairly low concentrations of soluble sulfides despite the high rates of sulfate reduction. Although some oxygen diffuses down Spartina shoots to the roots and rhizomes, in a waterlogged soil all of this oxygen is used to support the respiratory needs of the plant. Probably, little or no oxygen escapes the roots and rhizomes to the peat. Thus, the peat is being oxidized in other ways. The same is also true of rice; for rice oxygen diffusion per se accounts for only 11% of the oxidizing activity of the roots (Armstrong, 1975, and references therein).

For both rice and Spartina plants, sulfide oxidation is greater in the light than in the dark. The actual mechanism of oxidation is unknown and several possibilities exist. Sulfides are toxic and the plants may expend energy to oxidize them. This could be done for example by excreting organic oxidants which would then either chemically oxidize sulfides or be used by chemosynthetic bacteria living symbiotically on the plant roots to oxidize the sulfides. Or the sulfides could be oxidized by hydrogen peroxide excreted as a respiratory by-product of the plant roots. This might actually save the plant some energy since fewer catalases would have to be synthesized. Spartina may be able to heterotrophically oxidize sulfides and other reduced sulfur compounds, as can a wide variety of heterotrophic organisms; it may even get energy from such oxidations. Or Spartina may possibly use hydrogen sulfide as an electron donor in its photosynthesis, thereby gaining an energy advantage. Each of these possi-

bilities is explored below.

Evidence for the Oxidation of Reduced Sulfur:

Although sulfate reduction rates are high throughout the active root zone of Spartina (the upper 20-25 cm), soluble sulfide concentrations tend to be low, often less than 1×10^{-7} M. At greater depths in the peat or in creek bottoms, soluble sulfide concentrations are as high as $3\text{-}6 \times 10^{-4}$ M. So the active root zone is both the zone of high sulfide production and of low soluble sulfide concentrations. We find that sulfide concentrations in the peat are higher at night than during the day. Further, there appears to be a net oxidation of pyrite in the root zone (Chapter 3). It is clear, then, that Spartina, like many other wetland grasses (Armstrong, 1975) is capable of maintaining its root zone in a fairly oxidized state.

Oxygen Diffusion in Spartina Shoots:

Teal and Kanwisher (1966) have demonstrated that oxygen can diffuse down Spartina shoots into the roots and rhizomes. Such diffusion is entirely in the gas phase. When the roots are in gas,as they were in Teal and Kanwisher's (1966) work, the resulting fluxes of oxygen to the roots can be much larger than the respiratory needs of the plant. Consequently, such a diffusion of oxygen to the roots and rhizomes has usually been assumed to be the oxidizing source for the peat. This now seems unlikely, at least for highly waterlogged marshes.

We have repeated the experiments of Teal and Kanwisher (1966). Spartina alterniflora plants (either grown hydroponically or freshly excised from the mud at School Street marsh) were placed with their shoots in air but their roots and rhizomes in a closed tube or flask. The gas around the roots and rhizomes was displaced with nitrogen, and the appear-

ance of oxygen was monitored over time by gas chromatography. The results were comparable to those of Teal and Kanwisher (1966). Oxygen quickly built up in the root zone. However, if the roots and rhizomes were kept in water rather than in a gas phase, the results were quite different. Root respiration was as great as or greater than the diffusion of oxygen to the root zone. If at the beginning of the experment, oxygen was stripped from solution with a nitrogen stream, the resulting low level of oxygen was maintained. If we started with oxygen saturation, the root and rhizome respiration quickly consumed it until a very low level of oxygen was reached. The actual respiration rates of the roots and rhizomes were comparable whether they were in water or a gas phase, so these experiments indicate that oxygen diffusion is much less with the roots in water. Many of the marsh soils are completely waterlogged at all times, so the diffusion of oxygen down Spartina shoots to the soil may be much less than has previously been thought.

Armstrong (1975, and references therein) has demonstrated that the diffusion of oxygen per se accounts for only 11% of the oxidizing activity of rice roots. The percentage for Spartina alterniflora is unknown.

Sulfide Toxicity and the Role of Light in Sulfide Oxidation:

Vámos and Köves (1972) have demonstrated that hydrogen sulfide is toxic to rice plants but that this toxicity can be counteracted by light. Apparently, the ability of rice plants to oxidize sulfides in their root zones is greater in the light than in the dark. The source of oxidizing power is unknown, but Vámos and Köves (1972) suggest that it may be hydrogen peroxide produced by the glycolic acid pathway or as a by-product of some other respiratory process. Another possibility is the production of peracids in the leaves which are then transported to the root zone. Such

peracids can form from ·OH free radicals which are known to be formed during photosynthesis (Vâmos and Köves, 1972). Our data suggest that Spartina root respiration is greater when the above-ground portions of the plants are in the light than when they are in the dark. The day/night difference in sulfide concentrations in the marsh peat may therefore be related to respiratory process of the sort suggested by Vâmos and Köves (1972) for rice plants.

Goodman and Williams (1961) have demonstrated that the "die-back" of Spartina Townsendii can be mimicked in the laboratory by adding sulfides to the culture media. They conclude that the die-back is definitely the result of toxic effect by sulfides or related inorganic ions such as phosphite. The oxygen contents of rhizomes in "die-back soils" were essentially the same as those from healthy soils. Further, die-back plants show no mineral deficiencies or unbalances, and fertilization of die-back soils did not prevent or effect the die-back. Goodman and Williams (1961) conclude therefore that there is no oxygen limitation in the die-back Spartina plants. This further suggests that sulfide oxidation does not primarily involve molecular oxygen.

Symbiotic Micro-organisms and the Oxidation of Sulfides:

Joshi and Hollis (1977) have demonstrated that although rice plants alone can oxidize sulfides around their roots, such oxidations are greater when the chemosynthetic bacterium Beggiatoa is living symbiotically with the roots. Beggiatoa is also associated with the roots of Spartina alterniflora and may well serve to oxidize sulfides and protect Spartina as it does rice. Other chemosynthetic bacteria likely play a similar role. The roots presumably supply an oxidant to the bacteria, but the oxidant is unknown. In light of our findings reported here and the findings of

Armstrong (1975 and references therein), the oxidant is unlikely to be oxygen. It could be hydrogen peroxide or a peracid as suggested by Vámos and Köves (1972). From a thermodynamic standpoint, the oxidant could also be a common plant acid such as oxalate or glycolate, as the following equations illustrate:

For oxalate,

$$H_2S + 2C_2HO_4^- = SO_4^= + C_2H_4 + 2CO_2 \qquad \Delta F^\circ = -60.2KJ$$

$$2H_2S + 2C_2HO_4^- = S_2O_3^= + C_2H_4 + 2CO_2 + H_2O \qquad \Delta F^\circ = -60.3KJ$$

$$FeS_2 + 3C_2HO_4^- + 3H^+ = S_2O_3^= + Fe^{++} + C_2H_4 + 4CO_2 + H_2O \qquad \Delta F^\circ = -96.4KJ$$

For glycolate,

$$H_2S + 2C_2H_3O_3^- = SO_4^= + CO_2 + C_2H_4 + CH_4 \qquad \Delta F^\circ = -38.8KJ$$

$$2H_2S + 2C_2H_3O_3^- = S_2O_3^= + CO_2 + C_2H_4 + CH_4 + H_2O \qquad \Delta F^\circ = -39.1KJ$$

Ethanol could be an alternative end-product to ethylene for any of the above reactions. The resulting standard free energies would be 10.7 KJ more positive (less favorable). Both ethylene and ethanol are common products of anaerobic plant metabolism (Armstrong, 1975). We have evidence that ethylene production in marsh peat can be fairly high.

Soybean root nodules subjected to waterlogging have enhanced evolution of CO_2 and ethanol (Sprent and Gallacher, 1976). This may reflect the metabolism of organic oxidants such as glycolate and oxalate in order to maintain an oxidized rhizosphere.

Bacteria capable of living off of the free energy of such reactions have not been isolated, but probably no one has tried to do so. Vámos

and Köves (1972) found that about 5% of the organic acids found in rice roots was glycolic acid, while oxalic acid was somewhat less abundant. Shading the plants resulted in an absolute decrease in the amount of these acids in the roots. Mac Rae and Castro (1967) have demonstrated that rice plants which are relatively resistant to sulfide toxicity excrete more carbohydrates from their roots than do non-resistant plants. They did not look for the excretion of organic acids. Nonetheless, these data suggest that microbial oxidation of sulfides using organic acids supplied by root exudates is at least plausible.

Heterotrophic Oxidation of Reduced Sulfur Compounds:

A wide variety of bacteria and fungi are known to be able to oxidize various reduced sulfur compounds (Roy and Trudinger, 1970). The mechanisms are almost completely unknown, and for the most part it is not known if the organisms involved get any energy from the oxidations they catalyze. However, at least one "heterotrophic" marine pseudomonad can use the energy of thiosulfate oxidations as a sole or supplemental energy source (Tuttle and Jannasch, 1977). Mitochondria from pea internodes, oat seedlings, and pea roots can oxidize sulfite to sulfate. It would therefore not be surprising if the roots and rhizomes of Spartina can oxidize reduced sulfur compounds in a non-photosynthetic manner, possibly using the resulting energy.

Photosynthetic Oxidation of Reduced Sulfur Compounds by Spartina:

The use of sulfide as an electron donor in bacterial photosynthesis is well known. According to Knobloch (1966-a, 1966-b, 1969) sulfide can also serve as a secondary electron donor in the photosynthesis of a number of algae and at least some flowering plants such as Lemna and

Spirodela. Knobloch (personal communication, 12/27/75) believes that Spartina probably uses sulfides in a similar manner. However, the transport of sulfides to the photosynthetic site would seem to be a problem.

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APPENDIX 3:

Sulfate Reduction, Methanogenesis, and Methane Oxidation:

The degradation of organic matter in anoxic environments can proceed using either sulfate ("sulfate reduction") or carbon dioxide ("methanogenesis") as a terminal electron acceptor for a microbial community. Sulfate reducing bacteria and methanogenic bacteria can compete for substrates. For instance, both can use acetate as a substrate (Blackburn and Fenchel, in press). Based on standard free energies, it is generally thought that sulfate reducers obtain a greater energy yield per unit of substrate oxidized and that therefore sulfate reducers can out-compete methanogenic bacteria. It is assumed that methane formation occurs only when sulfate is very low or absent. However, methane is frequently found at low concentrations coexisting with sulfate in anoxic sediments, and sulfate reducing bacteria can oxidize methane. Examination of actual free energies rather than standard free energies yields some new insights to the relationships among sulfate reduction, methanogenesis, and methane oxidation.

The reaction for the oxidation of methane by sulfate reducing bacteria can be written as follows:

$$CH_4 + SO_4^{=} + 2H^+ = CO_2 + H_2S + 2H_2O \quad \Delta F^o = -95.1 \text{ KJ}. \qquad (1)$$

The actual free energy is given by:

$$\Delta F = -95.1 \text{ KJ} + RT \ln \frac{(H_2S)(CO_2)}{(H^+)^2(SO_4^{=})(CH_4)}, \qquad (2)$$

where R is the gas constant and T the absolute temperature.

At equilibrium, $\Delta F = 0$, so the equilibrium condition is given by:

$$\frac{(H_2S)(CO_2)}{(H^+)^2(SO_4^=)(CH_4)} = 5 \times 10^{16}. \qquad (3)$$

This equilibrium condition, although derived from the oxidation of methane by sulfate reducers, also describes the relationship between methanogenic bacteria and sulfate reducers when both types of bacteria are using the same substrate. Therefore, it follows from equation 2 that the relationship between sulfate reduction and methanogenesis depends not merely on the sulfate concentration, but also on the pH and the concentrations of sulfides, carbon dioxide, and methane. Low sulfate and methane concentrations and high pH's and sulfide and carbon dioxide concentrations will all tend to favor methanogenesis. Interestingly, the relationship is more sensitive to changes in the pH than to changes in the sulfate concentration.

Under equilibrium conditions (equation 3), sulfate reducing bacteria get exactly the same amount of energy per unit of substrate oxidized (assuming the same substrate is used; for instance, acetate) as do methanogenic bacteria. Neither type of bacteria would out compete the other. This is true regardless of how high or low the sulfate concentration is. Methanogenesis would tend to displace the equilibrium so as to favor sulfate reduction, and sulfate reduction would tend to displace the equilibrium so as to favor methanogenesis. The oxidation of methane by sulfate reducing bacteria would tend to maintain equilibrium conditions. However, the range of substrates available to sulfate reducers and to methanogens can be quite different, so if equilibrium conditions were to exist between sulfate and methane, it would probably be controlled

by methane oxidation by sulfate reducers.

It is unlikely that the equilibrium conditions described by equation 3 exist in natural sediments. For equilibrium to exist, it must be assumed that bacteria can live off the free energy of an exothermic reaction (i.e. oxidation of methane using sulfate as an electron acceptor) no matter how little the free energy yield of that reaction. As equilibrium conditions are approached, the free energy yield of a reaction will decrease and it probably becomes more difficult for a bacterium to use that reaction for its livelihood. It seems likely that there is some critical free energy value less than zero above which a bacterium cannot get useful energy from a reaction.

Such a critical free energy value for the oxidation of methane by sulfate reducers can be roughly estimated from the data of Martens and Berner (1977). For the variety of cores and depths they sampled, the following approximate condition holds:

$$\frac{(HS^-)(HCO_3^-)}{(SO_4^=)(CH_4)} = 30. \qquad (4)$$

Therefore,

$$RT \ln \frac{(HS^-)(HCO_3^-)}{(SO_4^=)(CH_4)} = 8.4KJ. \qquad (5)$$

Equation 1 can be rewritten as:

$$CH_4 + SO_4^= = HCO_3^- + HS^- + H_2O \qquad \Delta F^\circ = -18.9KJ, \qquad (6)$$

for which the free energy is given as:

$$\Delta F = -18.9\ KJ + RT \ln \frac{(HS^-)(HCO_3^-)}{(SO_4^=)(CH_4)}. \qquad (7)$$

Combining equations 5 and 7 allows an estimate of the critical free energy value for the oxidation of methane by sulfate reducers as approximately -10.5 KJ. I suggest that bacteria would be unable to carry out this reaction if they got any less energy from it. This critical free energy value for the oxidation of methane by sulfate reducers would then control the relative abundances of sulfides, bicarbonate, sulfate, and methane in the samples of martens and Berner (1977).

For organisms to make use of the energy released in an exothermic reaction, it must be coupled to an energy storage or transfer process such as phosphorylation (the conversion of ADP to ATP) or the reduction of NAD^+ to NADH. Consequently, it might be assumed that the energy required for phosphorylation or the reduction of NAD^+ or other such reactions would set a lower limit for the useful free energy change of a reaction which supports an organism (the critical free energy value). Sulfate-reducing bacteria used ATP as an energy storage product (Roy and Trudinger, 1970), so it might be assumed that the free energy of phosphorylation would describe the critical free energy value for the oxidation of methane by sulfate reducers. The free energy of phosphorylation depends upon internal concentrations of products and reactants and is poorly known, but the standard free energy is approximately 37 KJ $mole^{-1}$ (Burton, 1955), almost 3 times higher than the hypothesized critical free energy value. However, Peck (1966 as reported in Roy and Trudinger) has demonstrated that the ratio of phosphorylation to H_2 consumption by sulfate-reducing bacteria using H_2 as a sole substrate is 0.18. Sulfate reducers use 4 moles of H_2 to reduce one mole of $SO_4^=$, so Peck's results suggest that 1.4 moles of sulfate are reduced per mole of ATP formed. If the ratio of ATP formed per mole of sulfate reduced is less than one when bacteria

are using an optimum substrate such as H_2, there is no reason to believe that the ratio cannot be very much less than one and still have the bacteria grow. That is, the free energy of phosphorylation probably does not control the critical free energy value for the oxidation of methane by sulfate reducers. In support of this, Kiefer (personal communication, 1977) has found that some bacteria which live off reactions with low free energy yields (i.e. nitrifiers) have elaborate means for coupling the reaction of several substrate molecules with the formation of one molecule of ATP or other storage product. If my estimate for the critical free energy value for methane oxidation by sulfate reducers (-10.5 KJ) is correct, it suggests that the bacteria can couple the oxidation of 3 molecules of methane with the formation of 1 molecule of ATP.

The critical free energy value of approximately -10.5 KJ estimated for reaction 6 is just as valid for reaction 1 since reactions 1 and 6 are equivalent. Therefore, we can predict the following relationship to generally hold for anoxic sediments:

$$-10.5 \text{ KJ} = -95.1 \text{ KJ} + RT \ln \frac{(H_2S)(CO_2)}{(H^+)^2(SO_4^=)(CH_4)} \quad (8)$$

Therefore,

$$\frac{(H_2S)(CO_2)}{(H^+)^2(SO_4^=)(CH_4)} = 7.5 \times 10^{14}. \quad (9)$$

This equation (9) is a restatement of equation (4) and can also be written in the following manner:

$$\frac{(HS^-)(CO_2)}{(H^+)(SO_4^=)(CH_4)} = 7.6 \times 10^{7}. \quad (10)$$

It is difficult to test these relationships with published data; simultaneous measurements of all of the necessary parameters are very rare. Nonetheless, these relationships seem very reasonable, and I could find no published data which contradicts them. Assuming reasonable estimates for pH and sulfides, Whelan's (1974) measurements of methane, carbon dioxide, and sulfate in the interstitial waters of sediments from various Louisiana marshes fit the patterns suggested.

For the Great Sippewissett Marsh, (CH_4) is predicted from equation 8 as 8×10^{-12}, assuming that $(H_2S) = 1 \times 10^{-6}$, $(CO_2) = 1.2 \times 10^{-4}$, $(SO_4^{=}) = 2 \times 10^{-2}$, and pH = 6.0. This estimate is reasonable given the rather low export of methane from the marsh (approximately 0.5% of net primary production).

In other salt marshes, the pH of the peat can be significantly higher or lower than at Sippewissett. At Sapelo Island, Georgia, the pH can be as high as 8.5 - 9.0, and occasionally 10 (Pomeroy, 1959). Assuming pH = 9.0, $(SO_4^{=}) = 2 \times 10^{-2}$, $(HS^{-}) = 1 \times 10^{-6}$, and $(CO_2) = 1.2 \times 10^{-4}$, (CH_4) is predicted from equation 10 as 8×10^{-8}, four orders of magnitude higher than at Sippewissett. The lower sulfate concentrations in the peat at Sapelo (personal communication from W. Wiebe to J. Hobbie), if considered, would further increase this estimate. However, the effect of higher pH is much greater than the effect of sulfate depletion. The data of King and Pomeroy (talk delivered in June, 1976, at the annual meeting of the American Society of Limnology and Oceanography in Savannah, Georgia) on high fluxes of methane from the Sapelo Island marshes to the atmosphere (5-10% of net primary production) suggest that the methane concentration in the peat there actually is much higher than at the Sippewissett Marsh.

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APPENDIX 4:

Reduced Phosphorus Compounds in Sediments:

Whether or not phosphine (PH_3) and iron phosphides (FeP, Fe_2P) are found in natural sediments and soils is still an open question. Lüning and Brohm (1933) reported phosphine in polluted springs. Barrenscheen and Beckh-Widmanstetter (1923) and Rudakov (1927) reported the existence of bacteria which could reduce phosphate to phosphine. Elementary chemistry texts (i.e. Partington, 1949) have stated that phosphine is formed in nature from the putrefaction of proteins and by the bacterial reduction of phosphate. Liebert (1927, as cited in Burford and Bremner, 1972) failed to detect microbial reduction of phosphate and argued that the process cannot occur for thermodynamic reasons. Tsubota (1959, as cited in Burford and Bremner, 1972) on the other hand found phosphine evolution in a paddy soil from the reduction of phosphate. He cited redox-potential data as evidence that phosphate reduction to phosphine cannot be excluded on thermodynamic grounds. Bohn (1976) similarly states that the reduction of phosphate to phosphine is thermodynamically possible. He gives the "boundary" as $Eh = 0.06 - 0.059\ pH - 0.0074 \log (PH_3/H_3PO_4)$. Skinner (1968) was unable to find phosphate-reducing bacteria, and Burford and Bremner (1972) were unable to detect phosphine in waterlogged soils. However, Iverson (1968) reported the formation of iron phosphide (Fe_2P) in cultures of sulfate-reducing bacteria in a laboratory study. He postulated that the sulfate-reducing bacteria reduced phosphate to phosphine which then reacted with ferrous ions to form the iron phosphide. The iron phosphide was nonmagnetic and gave no x-ray diffraction pattern until it was heated to 1232°C, at which it was easily recognizable. Mossbauer studies showed that the material was iron phosphide before heating, also, but it was colloidal.

Experimental:

I have been unable to detect by gas chromatography any phosphine evolution from cores of marsh mud and peat incubated anaerobically in the lab. Additions of phosphate and/or lactate to cores did not result in any phosphine evolution. I have also placed gas permeable Teflon bags full of silver nitrate solution in situ in marsh peat and muds for periods of up to two weeks. Phosphine precipitates silver and a yellow intermediate compound from silver nitrate solution (Partington, 1949):

$$PH_3 + 6\ AgNO_3 = Ag_3P,\ 3AgNO_3 + 3HNO_3$$
$$Ag_3P,\ 3AgNO_3 + 3H_2O = 6Ag + 3HNO_3 + H_3PO_3.$$

In laboratory studies, bubbling acetylene (which containes traces of phosphine) through silver nitrate solutions resulted in phosphate in solution (after aerating the solutions overnight) which could be assayed. However, the solutions in situ in the marsh gained no measurable phosphate. Their only recognizeable precipitate was black silver sulfide.

Samples of marsh mud and peat given to C. C. Woo for x-ray diffraction analysis had no iron phosphide pattern. However, it is possible that the samples contained colloidal iron phosphides of the sort reported by Iverson (1968) in his bacterial cultures.

Calculations:

Soluble phosphates:

Bohn's (1976) thermodynamic argument for the reduction of phosphate to phosphine appears to me to be in error. If his equation (See page 122) were true, it would correspond to the reaction:

$$H_3PO_4 + 4H_2 = PH_3 + 4H_2O \qquad \Delta F^\circ = +46.4\ KJ.$$

I calculate ΔF° = + 192.9 KJ, making the reaction much less likely. Given a hydrogen activity of unity, the reaction is favorable only if the ratio of phosphine to phosphoric acid is less than 1.3×10^{-34}. If the hydrogen activity is less, the ratio must be even less. Consequently, phosphine formation from soluble phosphate is very unlikely. Consideration of other soluble phosphates ($H_2PO_4^-$, HPO_4^{-2}, PO_4^{-3}, $CaHPO_4$, etc.) does not improve the picture.

Aluminum phosphates:

However, the case with some solid phosphates is different. Consider the possible biological reduction of $AlPO_4$:

$$AlPO_4 + 4H_2 = Al(OH)_3 + PH_3 + H_2O \qquad \Delta F^{\circ} = + 11.5 \text{ KJ.}$$

The equilibrium condition then is $-2.03 = \log ((PH_3)/(H_2)^4)$. Given a hydrogen activity of unity, the reaction could occur exothermically producing phosphine activities of up to 9.3×10^{-3}. At pH = 6, $AlPO_4$ may be the most abundant solid phosphate in sediments (see p. 522 of Stumm and Morgan, 1970), so this reaction may be important in reducing sediments. Were phosphine to form through this mechanism, it would probably quickly be transformed to an iron phosphide:

$$2Fe^{++} + PH_3 + 1/2H_2 = Fe_2P + 4H^+ \qquad \Delta F^{\circ} = + 15.5 \text{ KJ}$$

$$Fe^{++} + PH_3 = FeP + 2H^+ + 1/2H_2 \qquad \Delta F^{\circ} = - 35.8 \text{ KJ}$$

For this calculation, I estimated the standard free energies of formation for the iron phosphides by assuming that they are 14.6 KJ more positive than are the standard heats of formation, as is true for Fe_2N and Fe_4N and as is very roughly so for pyrite. Under sediment conditions where $AlPO_4$ is being reduced, the formation of either of the iron phos-

phides is likely to be exothermic. Since these reactions drain off phosphine, they make the bacterial reduction of $AlPO_4$ more favorable. Which iron phosphide is thermodynamically preferred depends on the activities of Fe^{++} and H_2 and on the pH:

$$FeP + Fe^{++} + H_2 = Fe_2P + 2H^+ \qquad \Delta F^\circ = +51.5 \text{ KJ.}$$

At pH = 7, $(Fe^{++}) = 1 \times 10^{-3}$, and $(H_2) = 1$, Fe_2P is more stable. These conditions are probably similar to those of Iverson's (1968) bacterial cultures, possible explaining why he found Fe_2P and not FeP. At pH = 7, $(Fe^{++}) \leq 1 \times 10^{-5}$, and $(H_2) \leq 1$, FeP is more stable. These conditions are more likely for most sediments.

Assuming a pH = 6, $(Fe^{++}) = 1 \times 10^{-5}$, and assuming that PH_3 is rapidly converted to FeP, the overall reaction is:

$$AlPO_4 + 7/2H_2 + Fe^{++} = Al(OH_3) + FeP + 2H^+ + H_2O \qquad \Delta F^\circ = -24.3 \text{ KJ.}$$

The reaction is exothermic so long as (H_2) is greater than 6.1×10^{-4}. For comparison, the reduction of sulfate:

$$3/2\ SO_4^{=} + 6H_2 + 3\ H^+ = 3/2\ H_2S + 6H_2O \qquad \Delta F^\circ = -83.9 \text{ KJ.}$$

is exothermic if (H_2) is greater than 1.1×10^{-8} if $(SO_4^{=}) = 2 \times 10^{-2}$, $(H_2S) = 4 \times 10^{-5}$, and pH = 6. Thus, sulfate reduction is energetically preferred over $AlPO_4$ reduction under these conditions, and we would not expect sulfate reducing bacteria to reduce $AlPO_4$ in the presence of sulfate.

Iron phosphates:

The reduction of iron orthophosphate to iron phosphide is energetically much more favorable than the reduction of aluminum phosphates:

$$FePO_4 \cdot 2H_2O + 4H_2 = FeP + 6H_2O \qquad F^o = -359 \text{ KJ.}$$

This reaction is exothermic so long as (H_2) is greater than 1.8×10^{-16}. Thus, unlike aluminum phosphate, the reduction of iron orthophosphate is energetically preferred over sulfate reduction (see discussion and assumptions above). Iverson (1968) found that his pure cultures of sulfate reducing bacteria preferentially reduced phosphates over sulfates (as shown by the formation of iron phosphide). This may have been true because the bacteria were reducing iron orthophosphate which had formed in the culture medium. Iverson's (1968) explanation that the bacteria were reducing soluble phosphates which then reacted with ferrous ions is unlikely, as discussed earlier.

If bacteria can reduce iron orthophosphate, they are likely to do so in preference to sulfate, and iron phosphides should be found in anoxic sediments wherever there is an iron orthophosphate source. However, such phosphides may be colloidal (as in Iverson's (1968) cultures) and are therefore avoiding detection by x-ray diffraction. New techniques of phosphide detection should be developed for natural sediments.

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APPENDIX 5:

The Relationship Between % Organic Content and Sediment Oxygen Uptake:

There appears to be a rather strong inverse correlation between the percent organic content of a salt marsh sediment and its rate of oxygen consumption. In the accompanying figure 1 , percent organic content is plotted vs. the rate of oxygen uptake for data from Sapelo Island, Georgia,taken from Teal and Kanwisher (1961). The numbers refer to their stations. Station 3 clearly stands out, but for the other six stations oxygen consumption is higher when the organic content is lower.

Data reported by Duff and Teal (1965) for two marshes in Nova Scotia also fit this general trend. Oxygen consumption is roughly twice as high in a marsh with a low root content in Kingsport as in a marsh with a very high root content in West Lawrencetown.

The reason for such a relationship is not clear. The above-ground production tends to be higher in soils with a low organic content and a low root content, and the higher oxygen uptake in these soils may just reflect the higher above-ground production. As shown in Chapter 3, oxygen uptake in a marsh appears to be decoupled from below-ground production and related more strongly to above-ground processes. We have data which indicates that soils with a lower organic content tend to be less porous. They therefore offer more resistance to infiltrating waters at high tide and tend to be much drier. The higher oxygen uptake may just reflect a faster rate of oxygen diffusion in the drier, less organic soils.

The peat at the Great Sippewissett Marsh has an organic content of 40-75%, much higher than the marshes at Sapelo Island. If the relationship indicated by the accompanying figure 1 holds for such a high

organic content, we would expect oxygen uptake at the Great Sippewissett Marsh to be much lower even if we do not consider the effect of temperature on oxygen uptake.

Acknowledgments:

The porosity measurements were made with Brian Howes and Dale Goehringer.

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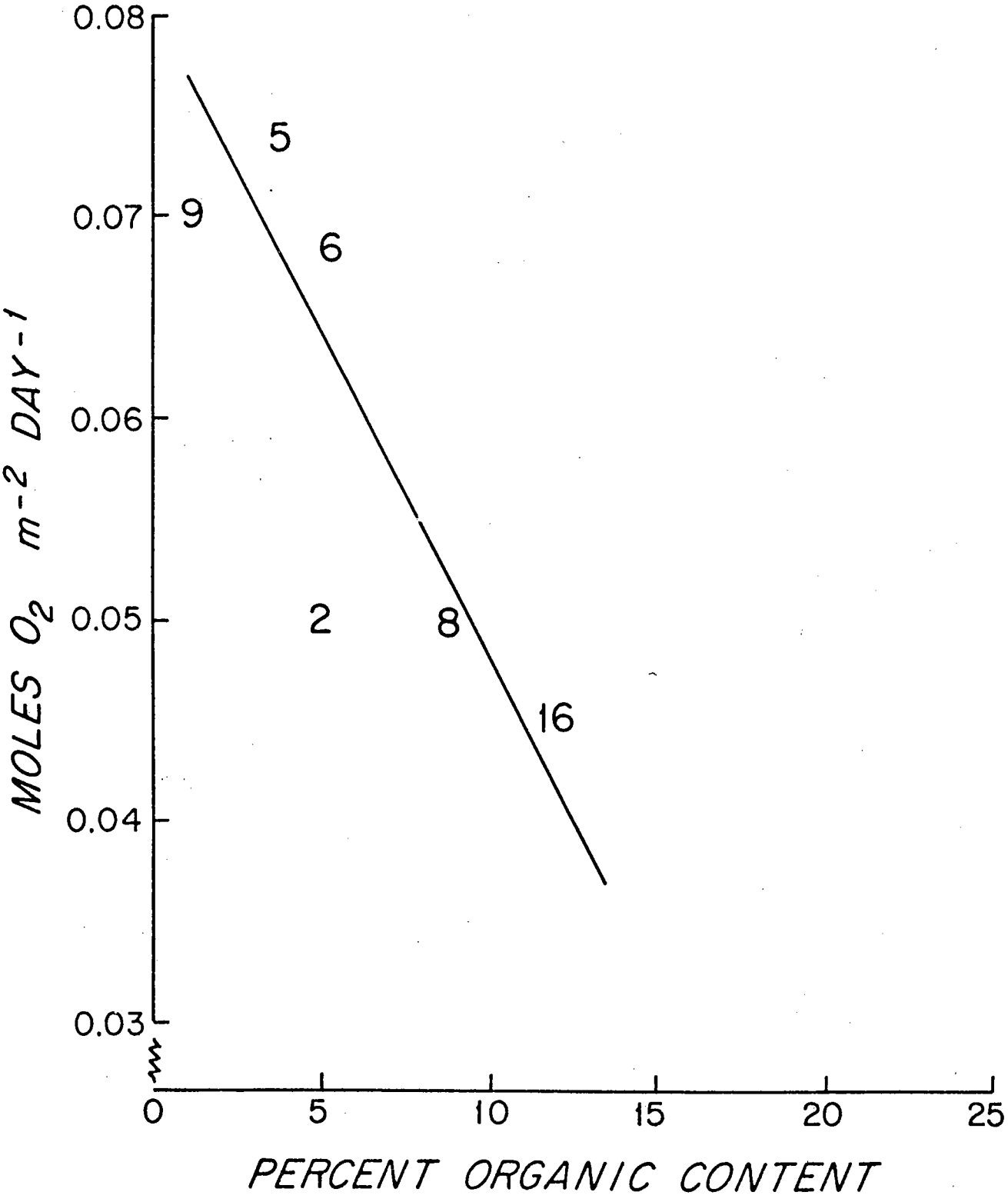
FIGURE 1.

Oxygen uptake by cores plotted vs. sediment organic content. Data is taken from Teal and Kanwisher (1961). Data for their creek bottom site is deleted.

3


0.08
0.07
0.06
0.05
0.04
0.03
MOLES O_2 m^{-2} DAY^{-1}
9
5
6
2
8
16
0
5
10
15
20
25
PERCENT ORGANIC CONTENT

APPENDIX 6:

THE INTERFERENCE OF REDUCED SULFUR COMPOUNDS IN THE WINKLER DETERMINATION OF O_2:

The Winkler determination of oxygen is based on the production of 1 mole $MnO(OH)_2$ from the reaction of 1/2 mole O_2 with $Mn(OH)_2$ under alkaline conditions and the subsequent oxidation of 3 moles iodide (I^-) to 1.5 moles iodine (I_2) by the one mole of $MnO(OH)_2$ under acidic conditions. The resulting iodine is titrated with a thiosulfate solution, using starch as an end-point indicator. The reaction is:

$$I_2 + 2S_2O_3^{=} = S_4O_6^{=} + 2I^- \qquad \text{(Treadwell and Hall, 1955)},$$

and so 2 moles of thiosulfate are needed for each mole of oxygen originally present in the sample. From this it is obvious that any thiosulfate which is originally present in the sample will interfere in the oxygen determination, giving a falsely low value.

Other reduced sulfur compounds and many other reduced ions will interfere as well. For example, hydrogen sulfide and sulfur dioxide will interfere as shown by the following equations:

$$H_2S + I_2 = 2\,HI + S^\circ$$

$$SO_2 + 2H_2O + I_2 = 4H^+ + 2I^- + SO_4^{=} \qquad \text{(Treadwell and Hall, 1955)}.$$

Consequently, great care should be taken in using the Winkler technique to measure oxygen in any waters where reduced substances may be abundant. An approximate correction can be made by adding a standard iodine solution to a replicate water sample and titrating it as for

the oxygen sample. However, this correction is only approximate because the pH changes of the Winkler technique can change the speciation of reduced sulfur compounds, and the iodine per mole of sulfur varies from one species to another.

I have found that when using the Winkler technique (uncorrected) to measure oxygen in waters overlying a marsh, 50% errors (as determined from an independently standardized oxygen electrode) are common. In some cases the Winkler measurement will indicate zero oxygen while the oxygen electrode will indicate concentrations as high as 2.5 ml $liter^{-1}$. Consequently, oxygen uptake measurements using an uncorrected Winkler technique or made using oxygen electrodes which have been standardized by uncorrected Winklers are suspect.

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BIOGRAPHICAL SKETCH

I was born in the Boston Naval Shipyard Hospital on February 11, 1952. My family moved to New Hampshire when I was 3, and I attended grades 1 through high school in the Oyster River Cooperative School System in Durham, New Hampshire. I developed an interest in research science while a pre-college summer student working with Charity Waymouth at the Jackson Lab in Bar Harbor, Maine, in 1969, and later with Robert Dunlop at the University of New Hampshire during my last year in high school. At about the same time, I developed a strong interest in environmental matters. I was an organizer for Earth Day in New Hampshire in 1970. I attended Amherst College from 1970 to 1974, when I graduated magna cum laude with an A.B. in Biology. While at Amherst, I worked with Lincoln Brower and Stuart Fisher, and through this contact, I became interested in ecology as a science. In the summer of 1972, I worked with Stephen Kessell and Stephen Carpenter as a research assistant in Glacier National Park, Montana. In the summer of 1973, I was a summer student with George Woodwell and his group at the Brookhaven National Lab. I entered the Joint Program in 1974 and have been working since then with John Teal. My professional interests concern: the structure and function of ecosystems, particularly salt marshes; marine pollution and resource management; and energy use.

Academic Honors:

Regional Winner, Ford Future Scientists of America, 1966

Class Valedictorian, Oyster River High School, 1970

Alfred P. Sloan Scholar, 1970-1974

Oscar Schotte Prize for Excellence in Biology, 1974

Amherst College Memorial Fellowship, 1974

Sigma Xi, associate membership, 1974

Teaching Experience:

Teaching Assistant for courses in Development Biology (1972, 1973, and 1974). Ecology (1972), and Aquatic Ecosystems (1973) at Amherst College.

Instructor (with Nick Staresinic and Larry Brand) for a course in oceanography in the M.I.T. High School Studies Program (1975).

Other Professional Interests:

I have reviewed (for the Executive Office of Environmental Affairs of the Commonwealth of Massachusetts) environmental impact statements on proposals for disposal of Boston's sewage sludge (1975) and on a proposed regional "resource recovery" plant to be located in Haverhill (1976). I helped prepare testimony for a suit by the Conservation Law Foundation and other plaintiffs which obtained an injunction against oil development on Georges Bank (1977 and 1978). I am scientific advisor to the Committee for the Preservation of Georges Bank and to Friends of the Earth.

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