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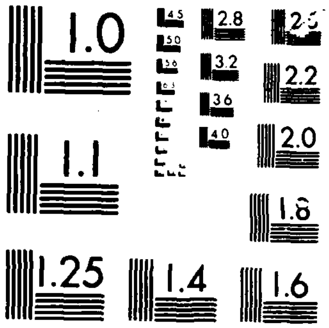
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EFFECTS OF WATER CHEMISTRY ON AQUATIC PLANTS: GROWTH AND PHOTOSYNTHESIS OF *MYRIOPHYLLUM SPICATUM* L.

by

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Growth of the submersed macrophyte, *Myriophyllum spicatum* L., was examined in nine solutions differing in major cation (Ca, Mg, Na, and K) and inorganic carbon C_T levels. Growth experiments were conducted under two conditions differing in the relative magnitudes of gas exchange and solution volume. Under conditions of limited solution volume, growth was adversely affected by the combination of low cation (particularly Ca) and low C_T levels. However, where solution volume was greater, there was no effect of cations. Irrespective of

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volume, growth was increased by increasing C_T from 3.5 to 10.5 mg/ℓ, but was unaffected by further increase in C_T to 21.0 mg/ℓ.

Photosynthetic uptake of C_T during growth reduced levels of C_T and acidity and increased pH. Alkalinity and Ca levels were also reduced in some solutions due to the precipitation of $CaCO_3$. Diurnal analyses indicated C_T was not appreciably replenished during the night; thus, the experimental systems continually lost C_T throughout the study.

Initial photosynthetic rates, measured in the same solutions, indicated a strong relationship between photosynthesis and solution C_T. Photosynthetic rates were adversely affected by the combination of low cation and low C_T levels. At favorable cation levels, photosynthetic C_T uptake rates followed Michaelis-Menten kinetics, with a half-saturation constant K_m of 25 mg $C_T \cdot ℓ^{-1}$. Photosynthetic C_T uptake was relatively unaffected by pH over the range 8.0 to 10.2.

Measurements of the exchange of CO_2 between the atmosphere and the solution during the photosynthesis experiments revealed the potential importance of the atmosphere as a significant source of carbon. As photosynthetic C_T uptake reduced the concentration of solution C_T, the rate of supply of atmospheric CO_2 rapidly increased to a near maximal value which was unaffected by the initial solution composition. Atmospheric CO_2 exchange thus moderates the influence of solution C_T.

Comparisons of growth with measured photosynthetic rates indicated that these measures of plant response to solution composition were not closely related. While photosynthetic C_T uptake was strongly affected by solution C_T, growth was less affected. In an attempt to resolve this difference, a carbon budget for growth of *M. spicatum* was constructed. The atmosphere was a major source of carbon in the growth experiments, particularly in the lower C_T solutions. While the influence of solution C_T on photosynthesis and growth diminishes over time (due to decreases in solution HCO_3^-), the rate of atmospheric CO_2 exchange increases to a maximal value independent of the initial level of C_T. Thus, as the duration of the experiment increases, the plant response (either photosynthesis or growth) becomes independent of the initial level of C_T. In the short-term photosynthesis experiments photosynthesis was strongly affected by solution C_T; however, in the longer term growth experiments, plant growth was relatively less affected by solution C_T.

In order to determine the potential importance of carbon as a factor limiting the growth of submersed aquatic plants, an additional experiment was conducted to examine the growth response of *M. spicatum* to the combined effects of varying solution C_T and atmospheric CO_2 supply. Augmenting the airstream CO_2 concentration resulted in increased growth in each of the solutions and also increased the responsiveness of growth to solution C_T. The increased supply of CO_2 prevented solution C_T levels from being depleted in spite of the increased photosynthetic carbon uptake associated with the increased growth. By maintaining higher levels of solution C_T, the enhanced CO_2 supply allowed the plants to photosynthesize at higher rates, thus resulting in an increase in growth in relation to solution C_T.

Growth and distribution of *M. spicatum* are unlikely to be limited by cation concentrations. While growth of this species may be limited by carbon availability, the degree of limitation will depend on the magnitude of the various sources of carbon supply and not on the level of C_T at any one time.

PREFACE

The studies reported herein were sponsored by the Department of the Army, Office of the Chief of Engineers (OCE) Directorate of Civil Works (DAEN-CW), through the US Army Corps of Engineers (CE) Aquatic Plant Control Research Program (APCRP). Funds were provided by DAEN-CW under Department of the Army Appropriation No. 96X3122 Construction General. Technical Monitor for OCE was Mr. E. Carl Brown. The APCRP is managed by the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss.

Principal investigator for these studies was Mr. R. Michael Smart, Environmental Laboratory (EL), WES. Experimental design, data analysis, and interpretation were provided by Mr. Smart and Dr. John W. Barko. Technical assistance was provided by Ms. M. Susan Hennington, Ms. Dwilette G. McFarland, and Ms. Ramona H. Warren. The report was prepared by Mr. Smart and Dr. Barko. This report was edited by Ms. Jamie W. Leach of the WES Publications and Graphic Arts Division (PGAD). The figures were prepared by Mr. George G. Tom of PGAD.

This investigation was performed under the supervision of Dr. John Harrison, Chief, EL; Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division; and Dr. Thomas L. Hart, Chief, Aquatic Processes and Effects Group. The APCRP was managed at WES by Mr. J. Lewis Decell.

Director of WES was COL Allen F. Grum, USA. Technical Director was Dr. Robert W. Whalin.

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CONTENTS

EFFECTS OF WATER CHEMISTRY ON AQUATIC PLANTS: GROWTH AND PHOTOSYNTHESIS OF *MYRIOPHYLLUM SPICATUM* L.

PART I: INTRODUCTION

Background

1. It has long been realized that the chemical composition of the water is of primary importance in affecting the distribution of aquatic plants (West 1905; Iversen 1929, cited in Hutchinson 1975; Moyle 1945). Various investigators have attempted to relate the species distributions of submersed macrophytes to generalized water chemistry parameters such as hardness, alkalinity, conductivity, pH, total dissolved solids, or salinity (see reviews by Moyle 1945; Hutchinson 1975). In this regard, *Myriophyllum spicatum* L. and the closely related *Myriophyllum exalbescens* Fern. have been characterized as typically occurring in waters that are hard (particularly with respect to Ca), moderate to high in alkalinity, neutral to high in pH, high in electrical conductivity, and eutrophic (Moyle 1945; Spence 1967; Hutchinson 1970; Seddon 1972; Kadono 1982). Many exceptions, however, have been noted to these general patterns of occurrence (Hutchinson 1970; Giesy and Tessier 1979; Kadono 1982), and it remains difficult to explain observed distributions on the basis of general water chemistry conditions.

2. As Hutchinson (1970) noted, many water chemistry parameters are interrelated, with pH, Ca concentration, bicarbonate concentration (or alkalinity), and total electrolyte content generally varying in a correlative manner. Although these parameters may act independently in affecting plant distribution, it is difficult to clearly separate their effects through examinations of species distributions in relation to water chemistry alone (Hutchinson 1970; Seddon 1972; Kadono 1982). Another difficulty is that sites differing in water chemistry are likely to differ in other environmental parameters (notably sediment composition) as well (Pearsall 1920; Moyle 1945). For these reasons it is necessary to determine the effects on growth of independently varying important water chemistry parameters under otherwise uniform environmental conditions.

3. Many previous studies on the effects of water chemistry on submersed

macrophyte growth have concentrated on the macronutrients N and P (Mulligan and Baranowski 1969; Ryan, Reimer, and Toth 1972; Mulligan, Baranowski, and Johnson 1976; Nichols and Keeney 1976; Best and Mantai 1978; Patterson and Brown 1979; Barko 1983). Results of these studies indicate that rooted submersed macrophytes are generally capable of acquiring both N and P by either root uptake from sediment or shoot uptake from the water. Consequently, the effects of N and P in the water column are dependent, in part, on levels of these in the sediment. Likewise, the role of solution K as a nutrient for submersed macrophyte growth has also been addressed (Barko 1982; Huebert and Gorham 1983), and there is some evidence that individual species may differ in their requirements for K in the water column. Other studies have considered the effects of salinity on submersed macrophyte growth and distribution (Bourn 1932; Haller, Sutton, and Barlowe 1974; Kadono 1982). These studies have indicated that *M. spicatum* is moderately tolerant of brackish waters.

4. Relatively little attention has been devoted to the effects on growth of other water chemistry parameters such as alkalinity, dissolved inorganic carbon C_T , or major cation concentrations. Huebert and Gorham (1983) demonstrated that *Potamogeton pectinatus* L. required Ca, Mg, and HCO_3^- in solution to achieve normal growth. Barko (1983) indicated that low levels of Ca and C_T in solution depressed growth of *M. spicatum*.

5. While little is known of the effects of C_T , alkalinity, and major cations on growth of submersed macrophytes, there is a considerable amount of information on the effects of these on photosynthesis. Among the many published reports on photosynthesis in relation to water chemistry, the most notable concerned with *M. spicatum* are Steemann Nielsen (1947), Adams, Guilizzoni, and Adams (1978), and Titus and Stone (1982). These authors indicate that *M. spicatum* can use HCO_3^- as a carbon source for photosynthesis with uptake following Michaelis-Menten kinetics. Steemann Nielsen (1947) demonstrated that cations, particularly Ca, stimulated photosynthesis in *M. spicatum*. A stimulatory effect of Ca was also observed by Stanley (1970). Photosynthetic uptake of HCO_3^- in *M. spicatum* has been shown to be little affected by pH over the range of 8.0 to 10.2 (Steemann Nielsen 1947); however, more recently Titus and Stone (1982) indicated that photosynthesis in this species declines over the range of 7 to 9. In view of the diurnally and seasonally occurring high pH values commonly observed in submersed macrophyte populations (McCracken et al. 1975; Van, Haller, and Bowes 1976; Halstead and

Tash 1982), the effects of pH on photosynthesis should be clearly resolved.

Objective and Scope

6. The objective of this report is to evaluate the effects of major cation and inorganic carbon levels on the growth and photosynthesis of *M. spicatum*. A secondary objective is to evaluate the relationship between growth and photosynthesis. If these two measures of plant response to water chemistry are closely related, it might be possible to predict potential growth of submersed macrophytes in a system based on photosynthetic responses measured in the laboratory.

PART II: GENERAL METHODOLOGY

7. Growth and photosynthesis experiments were conducted using a 3 by 3 factorial design with three levels of major cations (Na, K, Ca, Mg) at each of three levels of C_T. Major cation concentrations and C_T levels (Table 1) were based on analyses of bicarbonate lake waters (Hutchinson 1957) and were selected to cover a broad range in water composition. Concentrations in the high level solution were six times those in the low level solution, with the intermediate (mid) level solution being three times the concentration of the low level solution. Solutions were formulated by additions of reagent grade chemicals to deionized (reverse osmosis) water. Gaseous CO_2 was administered to solution prior to adding required amounts of $CaCO_3$ to achieve solubility (Smart and Barko 1985). Concentrations of Ca and C_T in the high level solution were near the upper limit for solutions of this type in equilibrium with atmospheric pCO_2.

8. The chemical composition of the experimental solutions was verified by chemical analysis. Initial and final solution samples were collected and analyzed for Na, K, Ca, and Mg by atomic absorption spectrophotometry. Electrical conductivity (25° C) was determined with a YSI Model 32 conductivity meter. Dissolved inorganic carbon was determined with a Beckman Model 915A total organic carbon analyzer. pH was measured with a Beckman Expandomatic IV expanded scale pH meter. Equilibrium calculations are based on equations provided in Stumm and Morgan (1981). Specific experimental details regarding the individual experiments will be presented in subsequent sections.

PART III: GROWTH EXPERIMENTS

Methods

9. Sediment used in the growth experiments was collected from Lake Washington in Washington State. This sediment was selected based on earlier investigations which indicated a high potential for supporting luxuriant submersed macrophyte growth (Barko and Smart 1981, 1983, in press). Physical and chemical characteristics of the sediment were similar to those provided earlier (Barko and Smart 1983, in press). Sediment was stored at room temperature under anaerobic conditions until needed. The sediment was thoroughly mixed with a rotary mixer and allocated to sediment containers a few days prior to experimentation.

10. Apical shoots, 15 cm in length, were taken from greenhouse cultures of *M. spicatum* which had been maintained at 25° C and at light levels similar to those to be used in the experiments. Four apices were randomly selected and allocated to each container. After planting, a 2-cm layer of washed silica sand was placed over the sediment to minimize physical exchanges with the overlying water. Plants were allowed to grow for 6 weeks. At termination of the experiments plant shoots were clipped at the sediment surface, bagged, and dried at 80° C in a forced draft oven to constant weight. Roots were washed over a 1-mm sieve to remove sediment and debris and dried as for shoots. Weights of root and shoot samples were recorded to the nearest milligram. Biomass accrual (final biomass minus initial biomass) was used as a measure of plant growth in all experiments. Root and shoot samples were ground in a Wiley mill to pass a 40 mesh sieve. Subsamples of the dried and ground material were combusted in a muffle furnace at 550° C to determine ash content. Additional subsamples were digested in H_2O_2 - H_2SO_4 (Allen et al. 1974) and analyzed for total Kjeldahl nitrogen (TKN) and total P on a Technicon Autoanalyzer. Major cations (Na, K, Ca, Mg) were determined by atomic absorption spectrophotometry. Data were subjected to one- and two-way analysis of variance procedures as appropriate (Statistical Analysis System, Cary, N. C.).

11. Experiments were conducted under two conditions differing in the relative magnitudes of atmospheric gas exchange (aeration) and solution volume in an attempt to evaluate the relative importance of the atmosphere and

solution as sources of inorganic carbon. Gas exchange rate coefficients K for the two experiments were evaluated by measuring 0_2 exchange (American Public Health Association (APHA) 1980) between the atmosphere and deoxygenated, deionized water under the appropriate experimental conditions. Dissolved 0_2 and temperature were measured with a YSI Model 57 dissolved oxygen meter. Mass transfer coefficients D/z and boundary layer thicknesses z were calculated according to Emerson (1975) and Goldman and Dennett (1983) using the diffusion coefficient D for 0_2 of 8.28×10^{-6} $m^2 \cdot hr^{-1}$ (Broecker 1974). Characteristics of the two experiments are provided in Table 2.

High D/z , low solution volume experiment

12. The high D/z , low solution volume experiment was conducted in 150-cm-tall by 15-cm-outside-diameter lucite columns in an environmental chamber. (See Barko and Smart 1980 for additional details.) Air temperature was maintained at 25° C providing water temperatures of 25° ± 1° C. Light was provided over a 14-hr photoperiod by a combination of Hg vapor and Na vapor lamps. Photosynthetically active radiation (PAR) levels measured at the midpoint of the experimental columns were about 350 μE $m^2 \cdot sec^{-1}$. Gas exchange and mixing were facilitated by administering humidified, compressed air at the bottom of the water column. After 2 weeks, and weekly thereafter, one half the volume of solution was removed and replaced with fresh solution.

Low D/z , high solution volume experiment

13. The low D/z , high solution volume experiment was conducted in 150- by 90- by 90-cm-deep, white fiberglass tanks in a greenhouse. Experimental solutions were randomly assigned to tanks in the greenhouse. Solutions were maintained at 25° ± 1° C with thermostatically controlled liquid circulators (Remcor Corp., Chicago, Ill.). Gas exchange and mixing were facilitated by administering humidified, compressed air through twin air lifts in each tank. Experimental tanks were fitted with lucite covers to prevent the entry of dust and other contaminants. Neutral density shade fabric was used to reduce solar irradiance to PAR levels of about 750 $\mu E \cdot m^{-2} \cdot sec^{-1}$, measured at midday.

Growth Responses

High D/z experiment

14. Weekly height measurements obtained during the high D/z , low solution volume experiment indicated no appreciable effect of cation treatments on plant shoot height. However, shoot height was appreciably affected by C_T level (Figure 1). Plants grown in the higher C_T solutions were significantly taller than those grown in the low C_T solution, and this difference in plant height increased throughout the study period.

15. Plant growth responses were analyzed by two-way ANOVA (analysis of variance); however, the occurrence of significant interaction terms (Appendix A, Tables A1, A2) indicated that the effects of cation and C_T treatments were not additive. These interactions preclude the use of the two-way ANOVAs for determining the significance of the main effects due to cation and C_T levels. Therefore, the experiment was re-analyzed as a series of six one-way ANOVAs to determine the effects of cations at each C_T level and the effects of C_T at each cation level (Appendix A, Tables A1, A2). From these analyses it was found that shoot, root, and total biomass accrual increased with increasing cation levels only under low C_T conditions. Total biomass accrual increased with increasing C_T at each of the cation levels (Figure 2). However, consideration of ash-free biomass indicates that the increase in shoot and total biomass accrual between the mid and high C_T levels was due to an increase in ash content of plants grown under the higher C_T and cation levels (Figure 2). Consideration of ash-free biomass thus provides a clearer interpretation of the effects of solution composition on submersed macrophyte growth.

16. Under these experimental conditions, growth of *M. spicatum* (as ash-free biomass) was responsive to an increase in C_T from 3.5 to 10.5 $mg \cdot \ell^{-1}$. Under similar conditions, Barko (1983) noted an increase in biomass (uncorrected for ash content) of the same species by increasing C_T from 2.5 to 5.5 $mg \cdot \ell^{-1}$ or increasing Ca from 7.5 to 20 $mg \cdot \ell^{-1}$. In the present investigation growth increases under low C_T conditions were associated with Ca increases from 10 to 30 $mg \cdot \ell^{-1}$; however, since major cations were increased collectively, it is not possible from these data to attribute the increased growth to Ca alone.

Specific cation experiments

17. In order to determine which cations were responsible for the increased growth under low C_T conditions, two additional experiments were conducted in which the levels of specific cations were varied independently. General experimental conditions were the same as in the previous high D/z experiment. The cation addition experiment used the low cation, low C_T solution as a control and independently added individual cations (Na, K, Ca, Mg) to achieve concentrations equivalent to those in the high cation, low C_T solution. Also included, for reference purposes, was a treatment in which all cations were increased to the high level and a treatment in which all cations were held at the low level with C_T increased to the high level. The cation reduction experiment used the high cation, low C_T solution as a control and independently omitted a portion of each cation (Na, K, Cd, Mg) to achieve the low level for each particular cation.

18. Independent additions of any of the major cations except Na resulted in an increase in total ash-free biomass accrual (Figure 3, graph A). Addition of all cations in combination was no more effective in increasing growth than additions of K, Mg, or Ca alone. However, addition of C_T was more effective in increasing growth than addition of cations. Plants grown in low cation, low C_T solution exhibited premature senescence after only a few weeks of growth. Addition of K slightly moderated this occurrence while additions of Na or Mg had no effect (Figure 3, graph B). Addition of Ca, either alone or in combination with other cations, completely prevented senescence, as did addition of C_T alone.

19. Reduction of K or Ca concentrations from the high level to the low level resulted in a slight reduction in plant growth under low C_T conditions (Figure 3, graph C). Reduction in the level of all major cations produced a similar effect. Senescence was not appreciable under high cation conditions; however, reduction in the level of Ca, either alone or in combination with other cations, resulted in a high degree of senescence (Figure 3, graph D).

20. These results indicate that increasing levels of K partially moderate the deleterious effects on growth of low Ca in combination with low C_T. However, K, alone or in combination with Na and Mg, cannot substitute for Ca in preventing premature senescence. The importance of Ca in the photosynthetic process of HCO_3^- utilization has been documented (Steemann Nielsen 1947; Lowenhaupt 1956; Stanley 1970; Lucas 1976; Lucas and Dainty 1977), and the

premature senescence noted here may be a symptom of the inability of *M. spicatum* to efficiently utilize bicarbonate under conditions of low C_T and low Ca. However, the substantial increase in growth due to C_T addition under low Ca conditions suggests that HCO_3^- utilization is not impaired under low Ca conditions. Calcium may also be involved in maintaining membrane integrity (Poovaiah and Leopold 1976) or in preventing the toxicity of other ions (Huebert and Gorham 1983); however, these possible explanations for the stimulatory effect of added Ca under low C_T , low Ca conditions do not explain enhanced growth due to increased C_T under low Ca conditions.

Low D/z experiment

21. Plant growth responses in the low D/z , high solution volume experiment were analyzed by both two-way and one-way ANOVA procedures (Appendix A, Tables A3, A4). As in the high D/z experiment, the C_T and cation interactions were significant. From the one-way ANOVAs it was found that both shoot and total biomass accrual increased with increasing cation levels only under higher C_T conditions. Thus, the stimulatory effect of increasing cation levels under low C_T conditions noted in the high D/z experiment was not observed in the low D/z experiment. Total biomass accrual increased with increasing C_T at each of the cation levels (Figure 4); however, the increase in total biomass between the mid and high C_T solutions was entirely due to an increase in the ash content of plants grown under higher levels of cations. Ash-free biomass accrual, which is a better measure of plant response, was relatively unaffected by cation levels and increased with increasing C_T only up to the mid level.

Experimental comparison

22. The effect of cations on growth of *M. spicatum* at low C_T in the high D/z experiments was not observed in the low D/z experiment. Likewise, there was no evidence of premature senescence in the low D/z experiment. These differences in results were likely due to the large differences in solution volume between the two experiments (Table 2). In the low solution volume experiment (high D/z), levels of both Ca and C_T may have been depleted due to the demands of a relatively high mass of plant tissue in relation to the relatively low volume of solution. This depletion may have been particularly acute in the low Ca, low C_T solution. While the weekly replacement of one half of the solution volume in the high D/z experiment should have ensured a steady supply of these elements, it is possible that

levels were occasionally depleted. However, analysis of the solutions at the end of the experiments (data not presented) indicated that, while C_T levels were depleted in the low C_T solutions in the high D/z experiment, Ca levels were not. The depletion of C_T in the low C_T solutions was less severe in the high solution volume (low D/z) experiment. Thus, plants growing in the low C_T solutions in the high D/z experiment may have been subject to more stressful conditions with regard to low levels of C_T . The possible implications of this stress will be considered in a subsequent section.

23. As in the high D/z experiment, biomass accrual was significantly higher in the mid and high level C_T treatments than in the low level C_T treatment. In both experiments growth of *M. spicatum* was proportional to solution C_T only up to a level of 10.5 $mg \cdot \ell^{-1}$.

Diurnal Metabolism

24. After 5 weeks of growth in each experiment, water samples were taken every 2 hr during the photoperiod and at the end of the following dark period. These samples were analyzed for C_T and pH as described earlier to provide estimates of diurnal uptake, precipitation, and recharge of the carbonate system. In addition, samples of the aerating gas were collected at midday to determine the efficiency of CO_2 exchange between the airstream and the experimental solutions.

High D/z experiment

25. Diurnal changes in pH and C_T in the high D/z , low solution volume experiment are presented in Figure 5. Maximal pH levels were higher in the mid and high level C_T solutions than in the low C_T solution (Figure 5). The high pH levels reported here (10 to 10.8) are similar to those measured in some natural submersed macrophyte populations (Van, Haller, and Bowes 1976; Halstead and Tash 1982). The attainment of these high values is somewhat surprising considering the high gas exchange coefficient measured in this experiment (Table 2). Another significant feature of these diurnal pH curves is that pH decreases during the dark phase were slight except under low C_T conditions. This indicates that, in spite of the high gas exchange rate, equilibration of the carbonate system was not attained during the night. High cation solutions generally exhibited a greater decrease in pH during the dark phase suggesting that cations may be involved in the regeneration of the

carbonate system. Examination of diurnal C_T levels (Figure 5) indicates that this is only partially correct. Regeneration of the carbonate system was greater in the higher C_T, higher alkalinity solutions. All solutions exhibited increased pH and decreased C_T relative to initial conditions in spite of the weekly replacement of half of the solution.

26. Diurnal changes in alkalinity and acidity, calculated from pH and C_T, are presented in Figure 6. Alkalinity was reduced relative to initial conditions, particularly in the solutions containing higher levels of C_T and cations, indicating probable precipitation of $CaCO_3$ during the experiment (Appendix B). However, alkalinity changed little over the diurnal cycle, indicating that diurnal changes in C_T were largely due to photosynthetic uptake of H_2CO_3 or HCO_3^-. Photosynthetic C_T uptake and regeneration of C_T are reflected in the rather large diurnal changes in acidity (Figure 6).

27. Measured CO_2 concentrations at midday in the effluent airstreams from each of the experimental solutions varied only slightly among the different solutions. The transfer of airstream CO_2 in this experiment was operating at near the maximum rate, and 86 to 95 percent of the airstream CO_2 was removed during passage through the solutions. Thus, in spite of the relatively minor decreases in C_T during the day, photosynthetic carbon uptake was operating at a considerable rate. The rate of carbon uptake was roughly equivalent to the influx of atmospheric CO_2.

Low D/z experiment

28. Diurnal changes in pH and C_T in the low D/z, high solution volume experiment are presented in Figure 7. Maximum pH values (8.8 to 9.5) were less than those attained in the high D/z experiment, due in part to the lesser depletion of C_T in the low D/z experiment. As in the high D/z experiment, equilibration of the carbonate system did not occur during the dark phase, and levels of C_T increased only slightly in the low C_T solutions and not at all in the higher C_T solutions. All solutions lost C_T over the 24-hr period.

29. Alkalinity (Figure 8) was reduced relative to initial conditions and decreased over the 24-hr period suggesting that changes in C_T over the diurnal period were partly due to precipitation of $CaCO_3$. Changes in acidity (Figure 8) were similar in magnitude to those in alkalinity, indicating that photosynthetic uptake of H_2CO_3 or HCO_3^- and precipitation of $CaCO_3$ were roughly proportional.

30. Measured CO_2 concentrations at midday in the effluent airstreams from each of the experimental solutions varied only slightly, ranging from 140 × 200 $\mu\ell \cdot \ell^{-1}$. Thus, in contrast with the high D/z experiment where approximately 90 percent of airstream CO_2 was removed during passage through the solutions, airstream CO_2 supply in the low D/z experiment was operating at only ≈ 50-percent efficiency. The difference in the efficiency of CO_2 transfer from the atmosphere into solution was partly due to the differences in the degree of C_T depletion between the two experiments. In the high D/z experiment, C_T depletion was considerably greater than in the low D/z experiment.

31. Results of the diurnal metabolism studies indicate that, in spite of the high mass transfer coefficients and high efficiencies of airstream CO_2 exchange, atmospheric CO_2 supply was inadequate for overnight attainment of equilibrium in the carbonate system. Moreover, C_T levels were considerably reduced relative to the initial conditions, particularly in the high D/z experiment. Reductions in C_T were due to both photosynthetic C_T uptake and $CaCO_3$ precipitation. Precipitation of $CaCO_3$ was greater in the solutions containing higher levels of C_T and Ca. As a result of these depletions in C_T , photosynthetic carbon uptake was increasingly dependent on the transfer of atmospheric CO_2 into solution. These observations suggest that growth of *M. spicatum* in these experiments may have been limited by the availability or supply of carbon.

Growth Limitation

32. If biomass accrual was limited by carbon, it is difficult to explain the lack of a growth response in either experiment to increasing concentrations of C_T between the mid (10.5 $mg \cdot \ell^{-1}$) and high (21.0 $mg \cdot \ell^{-1}$) levels. One possible explanation is that photosynthesis (and growth) was impaired by the higher pH levels attained in the higher C_T solutions. However, pH levels were similar in the mid and high level C_T solutions, and in the low D/z experiment there was little difference in pH between the low C_T solutions and some of the higher C_T solutions. In addition these pH levels were considerably lower than those in the high D/z experiment, yet biomass accrual exhibited the same pattern in both experiments.

33. Another possible explanation for the lack of a growth response to

increasing C_T from 10.5 to 21.0 mg·ℓ^{-1} is that growth was limited by some other factor. Space and light limitations can be ruled out on the basis of the lack of visible crowding relative to that observed in prior experiments and in dense cultures grown under similar conditions (Smart and Barko 1985).

34. Nutrient limitation is unlikely on the fertile Lake Washington sediment used in this investigation. Tissue levels (on an ash-free dry weight basis) of P and K in plants grown in the low D/z experiment (see Appendix A, Table A5) were above critical (limiting) concentrations established for *M. spicatum* (Gerloff 1975). Although tissue N levels of plants grown under mid and high C_T conditions were within the critical concentration range, we have twice failed to demonstrate N limitation of plant growth on the sediment used in this investigation (Barko and Smart 1983, and unpublished data). Limitation of growth on this sediment by other sediment nutrients is also unlikely (Smart and Barko 1985, Barko and Smart in press).

PART IV: PHOTOSYNTHESIS EXPERIMENTS

Methods

35. Photosynthetic carbon uptake was measured in each of the experimental solutions using an apparatus that allowed for measuring changes in atmospheric CO_2 from an open system airstream as well as changes in solution C_T (Figure 9). This configuration was chosen because it was felt that while aquatic systems are generally considered open with respect to exchange of gases, the environment of littoral submersed macrophyte populations is relatively closed with respect to water exchange over short time periods.

36. The photosynthetic apparatus consisted of a 1,000-ml volumetric hydrometer cylinder fitted with a four-hole rubber stopper. Solution was recirculated at 1 $\ell \cdot min^{-1}$ with a peristaltic tubing pump by withdrawal from the bottom of the cylinder and delivery to the top of the cylinder. An in-line sample port consisted of a polypropylene "Y" connector fitted with a rubber serum bottle stopper. Samples for C_T analysis were obtained with 1-cc disposable syringes. Compressed atmospheric air, from an outside source, was humidified by passing through a water column, and delivered to a 40-ℓ carboy for mixing and damping short-term changes in atmospheric CO_2 concentration. Air was then pumped at 1 $\ell \cdot min^{-1}$ through a diffuser positioned at the bottom of the cylinder and exited through the top of the cylinder. The effluent airstream then passed through a 500-ml mixing reservoir, fitted with a two-hole rubber stopper containing a sample port fitted with another rubber serum bottle stopper. Gas samples were taken from the influent and effluent airstreams with 3-cc disposable syringes. Photosynthesis cylinders were contained in a water bath to maintain temperature at 25° ± 0.5° C. Three cylinders were operated concurrently by using multichannel pumps and by staggering sample periods by 1 min. Samples were collected every 4 min during the initial 24 min and every 8 min thereafter until changes in C_T and airstream CO_2 were minimal (96 to 120 min). Additional water samples were taken at the beginning and end of the experiments for analysis of Ca to determine the magnitude of $CaCO_3$ precipitation. These analyses indicated that precipitation of $CaCO_3$ had occurred in some of the solutions containing higher levels of C_T and Ca, resulting in overestimates of photosynthesis in these solutions. The onset of $CaCO_3$ precipitation was determined by calculating $CaCO_3$ ion activity

products in relation to measured changes in C_T . Photosynthetic rates were determined over the change in C_T occurring prior to the onset of $CaCO_3$ precipitation. Photosynthetic rates uncorrected for $CaCO_3$ precipitation are referred to here as "apparent" photosynthetic rates.

Initial Photosynthesis

37. Apparent initial photosynthetic C_T uptake rates were calculated from the slope of C_T over time during the initial, linear portion of the relationship. These rates were unaffected by $CaCO_3$ precipitation except in the high Ca, high C_T solution (Table 3). Initial photosynthetic rates in the remaining solutions increased with increasing C_T at all cation levels.

38. Photosynthetic C_T uptake was also affected by cation levels. Under low C_T conditions photosynthetic C_T uptake was greatly diminished at lower cation levels. This result is consistent with the effect of cations on growth of *M. spicatum* in the high D/z and specific cation experiments described earlier. Steemann-Nielsen (1947) likewise demonstrated increased photosynthesis of *M. spicatum* due to cation additions to low C_T solutions. Stanley (1970) also demonstrated that additions of Ca increased photosynthesis in the same species. These results, in conjunction with the results of the high D/z and specific cation experiments, suggest that Ca is involved in photosynthetic uptake at HCO_3^-. The nature of this involvement remains unclear, however, as the requirement for Ca decreases with an increase in C_T . Possible explanations are that Ca is required for the transport of HCO_3^- across the plasmalemma under conditions of severe HCO_3^- depletion (Lucas and Dainty 1977) or that the precipitation of $CaCO_3$ on the leaf surfaces ameliorates the stress of high pH and excessive CO_3^{--} concentrations at sites of OH^- efflux (Raven 1970).

39. Kinetics of photosynthetic C_T uptake in the experimental solutions are provided in Figure 10. Kinetic constants were determined by linear transformation (Hanes-Woolf plot of S/v vs. S) of initial photosynthetic rate (v) in relation to solution C_T (S) according to Segel (1976). Solutions in which $CaCO_3$ precipitated or which contained unfavorably low cation concentrations were excluded from the analysis. Transformed data were linear (r^2 = 0.98) and provided a half-saturation constant K_m of 24.5 mg $C_T \cdot \ell^{-1}$. This estimate of K_m is within the range of published estimates for *M. spicatum*

(Adams, Guilizzoni, and Adams 1978; Titus and Stone 1982). However, our estimate of V_{max} of 20.9 mg $C_T \cdot g^{-1} \cdot hr^{-1}$ is over twice those of Adams, Guilizzoni, and Adams (1978). This difference may reflect the differences in physiological state between plants obtained from laboratory cultures, which were essentially free of senescent tissue, epiphytes, adherent sediment, and carbonate encrustation (Smart and Barko 1985), and those obtained directly from the field. Direct comparison of kinetic constants is also hampered by differences in methodology (Titus and Stone 1982) and will not be further attempted here.

Nonlinear Photosynthetic Rate Dynamics

40. The concentration of C_T decreased exponentially with time over the course of the photosynthesis experiment (Figure 11). Curves were fit to these data using an iterative, nonlinear, least-squares estimation technique (Statistical Analysis System, Cary, N. C.). The curve-fitting equation used was:

$$C_T = ae^{-bt} + c \qquad (1)$$

where

a = an empirical constant
b = the photosynthetic rate coefficient
t = time
c = the asymptotic limit of C_T

Taking the first derivative with respect to t gives the first-order photosynthetic rate as a function of C_T :

$$P = b(C_T - c) \qquad (2)$$

where P = photosynthetic C_T uptake rate for values of C_T between the initial concentration and the asymptotic limit of C_T uptake. The advantage of this method of analysis is that it provides continuous estimates of the rate of photosynthesis over the change in C_T between the initial condition (maximum rate) and the limit of C_T depletion (rate = 0).

41. The influence of $CaCO_3$ precipitation on the measured C_T uptake rates was evaluated as stated in the previous section on initial linear photosynthesis. Since the nonlinear analysis employed a longer measurement period, these rates were more likely to be affected by precipitation.

42. Apparent photosynthetic C_T uptake rates are expressed in Table 4 as the product of a rate coefficient (corrected for system volume and plant mass) and the difference between C_T and c . Calculated values of pH at the C_T uptake limit are also shown except where $CaCO_3$ precipitation occurred. In these cases changes in alkalinity precluded the calculation of final pH levels. Precipitation of $CaCO_3$ significantly affected the measured nonlinear rates of C_T uptake in the high C_T solutions containing mid and high levels of cations. These rates will not be considered further. Although precipitation also occurred in the mid C_T , high cation solution, the precipitation did not occur until near the end of the measurement period and was readily discernible by an abrupt change in the slope of C_T versus time. The nonlinear rate of C_T uptake in this solution was calculated from the initial portion of the curve, prior to the onset of precipitation.

43. Photosynthetic rates calculated for the initial C_T concentrations (Table 4) generally compare favorably with rates determined from the initial linear portion of the C_T concentration curve (Table 3). Linear rates were generally somewhat less than initial rates determined by nonlinear analysis due to the decrease in photosynthetic rate over the time interval required in the former analysis. However, this slight difference did not affect estimated kinetic constants. The kinetics curve fit to the initial nonlinear photosynthetic C_T uptake rates (Figure 12) is virtually identical ($K_m = 25.8$, $V_{max} = 23.4$) to that fit to the initial linear phase photosynthetic rates ($K_m = 24.5$, $V_{max} = 20.9$).

44. The increase in solution pH due to H_2CO_3 or HCO_3^- uptake is proportional to the quantity of C_T taken up (assuming precipitation does not occur), and this relationship has been used to measure photosynthesis (Allen and Spence 1981). For this reason final pH levels attained in the higher C_T , higher alkalinity solutions were higher than those in the lower C_T , lower alkalinity solutions (Table 4). Estimated C_T uptake limits (roughly analogous to C_T compensation points) increased with increasing C_T and alkalinity, except where precipitation occurred (Table 4). This increase in C_T compensation point undoubtedly reflects the increasing proportion of C_T

in the form of CO_3^{--} (which cannot be used in photosynthesis) in the higher alkalinity solutions at high pH. The utility of experimentally determined C_T compensation points for comparative purposes is thus severely limited, as the C_T compensation point is as much dependent on the properties of the solution (alkalinity, pH, and C_T) as on the photosynthetic capacity of the plant.

Photosynthetic C_T uptake in relation to C_T and pH

45. As photosynthetic C_T uptake proceeds, the level of C_T decreases and pH increases. Since photosynthetic C_T uptake rates are first order with respect to C_T , the rate of C_T uptake also decreases. Examination of photosynthetic C_T uptake under the changing conditions of C_T and pH should provide information on the relative importance of these parameters in affecting photosynthetic rates.

46. The divergence of photosynthetic C_T uptake rates from the kinetics curve (Figure 12) might be considered as an indication of a pH effect on photosynthesis. For example, C_T uptake at 10 mg $C_T \cdot \ell^{-1}$ in the mid level solution (initially 10.5 mg $C_T \cdot \ell^{-1}$) was 6.5 $mg \cdot g^{-1} \cdot hr^{-1}$ at a pH of 8.8 compared with the rate of 2.4 $mg \cdot g^{-1} \cdot hr^{-1}$ in the high level solution (initially 21.0 mg $C_T \cdot \ell^{-1}$) at a pH of 10.6. However, this difference in photosynthetic rate is largely due to the difference in HCO_3^- concentration between the two solutions--9.7 mg $HCO_3^- \text{-} C \cdot \ell^{-1}$ in the mid level solution and 3.5 mg $HCO_3^- \text{-} C \cdot \ell^{-1}$ in the high level solution.

47. Replotting the photosynthetic rate data against HCO_3^--C (Figure 13, graph A) rather than C_T indicates that photosynthetic C_T uptake rates in each of the solutions are similar at equal HCO_3^- concentrations, in spite of large differences in pH (Figure 13, graph B). In addition, photosynthetic C_T uptake in individual solutions approximates the kinetics curve down to a HCO_3^--C concentration of ≈4 $mg \cdot \ell^{-1}$. In neither the mid nor the high level solutions is there an appreciable difference in the slopes of the photosynthesis vs. HCO_3^- lines below a pH of ≈10.2, indicating that photosynthetic HCO_3^- uptake in *M. spicatum* was unaffected over the pH range 8.0 to 10.2. This observation is in agreement with the earlier findings of Steemann Nielsen (1947) who indicated that photosynthetic HCO_3^- uptake in the same species was relatively unaffected over the same pH range. In contrast, the recent work of Titus and Stone (1982) indicates that photosynthesis in *M. spicatum* is greatly

reduced with increasing pH over the range 7 to 9. One possible reason for this discrepancy is that Titus and Stone used buffered solutions to obtain different pH levels. Recent work, reviewed by Lucas (1983), has repeatedly demonstrated inhibitory effects of buffers on HCO_3^- assimilation (Lucas 1977; Prins, Snel, and Zanstra 1982).

48. It is interesting that photosynthetic C_T uptake in each of the solutions approaches zero at a HCO_3^--C concentration of ≈ 1.5 mg·ℓ$^{-1}$ (Figure 13, graph A). This observation may be indicative of a HCO_3^--C compensation point in *M. spicatum* of 1.5 mg·ℓ$^{-1}$.

Effects of atmospheric CO_2 exchange

49. Plots of open system gas phase CO_2 concentration against time (not presented) were roughly similar in shape to those for C_T. Exponential equations were fit to these data as described earlier. Photosynthetic CO_2 uptake rates were determined by calculating the product of the flow rate and the difference in influent and effluent airstream CO_2 concentrations.

50. Concurrent with the decrease in C_T due to photosynthetic C_T uptake, the equilibrium between the solution and the atmosphere is disturbed, resulting in a gradient in pCO_2 between the atmosphere and solution. Atmospheric CO_2 exchange thus increases with time while both C_T and C_T uptake decrease (Figure 14). Atmospheric CO_2 exchange rates rapidly reached similar maximal values in all solutions, averaging 5.0 ± 0.2 mg C·g^{-1}·hr^{-1}. The exchange of CO_2 was thus not affected by the initial solution composition. Atmospheric CO_2 exchange was a significant source of carbon in these experiments, supplying 20 to 80 percent of maximal carbon demands. As C_T declines to the asymptotic limit, a steady-state is approached with uptake of C_T being balanced by CO_2 exchange from the atmosphere (Figure 14). At the asymptotic limit of C_T declines, the atmosphere is effectively supplying all of the carbon required for photosynthesis.

51. The exchange of CO_2 across the air/water interface is a function of the mass transfer coefficient and the gradient in pCO_2. The gradient in pCO_2 across the air/water interface rapidly approaches a maximum as solution C_T is depleted, regardless of the initial C_T level (Figure 15). This explains the relative similarities in atmospheric CO_2 exchange rates measured at steady-state in the different solutions.

52. Although photosynthetic C_T uptake rates were proportional to

solution C_T, the uptake of atmospheric carbon was unaffected by solution C_T. Thus, consideration of uptake from only the liquid or only the gas phase could result in very different interpretations of the effects of solution on photosynthesis. Also, since atmospheric CO_2 exchange was relatively unaffected by the initial solution composition, consideration of atmospheric exchange in photosynthesis measurements lessens the effects of solution composition on the total (air + water) photosynthetic rate.

Kinetics including atmospheric CO_2 exchange

53. Considering the importance of the atmospheric CO_2 exchange component on the total rate of photosynthesis, it is of interest to examine the kinetics of the overall photosynthetic process. In attempting to describe the kinetics of total photosynthetic carbon uptake in relation to solution C_T, it is not possible to use initial rates because the initial atmospheric CO_2 exchange rate is zero and total photosynthesis equals solution C_T uptake. Therefore, the maximum total carbon uptake rates were used to determine kinetic constants. The resulting kinetics curve (Figure 16) provided a good fit to the maximum rate data. The K_m value of 8.5 mg $C \cdot \ell^{-1}$ determined from this analysis is quite different from the K_m values based on solution C_T uptake alone. This difference indicates the large effect on K_m of considering atmospheric CO_2 exchange in addition to direct C_T uptake from solution. Quite different conclusions regarding the importance of C_T might be reached depending on the analysis employed.

PART V: GROWTH IN RELATION TO PHOTOSYNTHESIS

54. The nearly linear response of photosynthetic C_T uptake to solution C_T contrasts with the slight responses in growth. Plotting growth of *M. spicatum* (as ash-free biomass accrual) in the two experiments against initial photosynthetic C_T uptake in the same solutions (Figure 17, graph A) suggests that growth was not strongly related to photosynthesis. Integration of photosynthetic rate over the change in solution C_T measured in the low D/z experiment (Figure 17, graph B) does not improve the relationship between growth and photosynthesis. Inclusion of atmospheric CO_2 exchange in the integrated photosynthetic rate (Figure 17, graph C) likewise does not improve the relationship due to the similarity in atmospheric CO_2 exchange rates among the different solutions used in the photosynthesis experiment.

Carbon Sources Used in Growth

55. To determine the relative importance of the atmosphere and solution as sources of carbon, a carbon budget was constructed for growth in the low D/z experiment (Figure 18). Total carbon uptake during growth was the sum of plant organic carbon (0.465 × ash-free dry weight) and plant inorganic carbon ($CaCO_3$ encrustation). Plant inorganic carbon was determined by measuring weight loss of ashed plant samples (550° C) after reheating to 1,100° C for 4 hr to convert $CaCO_3$ to CaO. This procedure was verified by analyzing tissues for Ca and also by direct measurement of total carbon in ashed plant samples (LECO Carbon Determinator, Model WR 12). The quantity of carbon taken up from solution was the difference between the molar change in solution C_T and the molar change in solution Ca which was used as a measure of $CaCO_3$ precipitation. Precipitation of $CaCO_3$ was further partitioned between $CaCO_3$ encrustation (measured) and precipitation in the tanks (by subtraction). The remainder of the total carbon uptake was considered to have been supplied by atmospheric CO_2 exchange. Measurement of heterotrophic respiration of the water column was neglected. Microbial respiration should be minor in these experimental solutions, which lack N, P, and organic carbon (Smart and Barko 1985). An estimate of sediment respiration as a source of inorganic carbon was based on measurements of CO_2 release from Lake Washington sediments (Kuivila and Murray 1984). This estimate was considerably less than 1 g and

was not included in the figure. Direct uptake of CO_2 by roots of *M. spicatum* has likewise been shown to be insignificant (Loczy, Carignan, and Planas 1983) and was not included.

56. Much of the carbon used for growth in these experiments was apparently derived from the atmosphere. This is not surprising considering the large gradients in pCO_2 across the air/water interface and the high mass transfer coefficient measured here. The effect of initial solution composition, in particular C_T and alkalinity, on atmospheric CO_2 exchange was slight as in the photosynthesis experiments. In the high C_T, high cation solution, atmospheric CO_2 supply was diminished, probably due to the lessening of the gradient in pCO_2 by $CaCO_3$ precipitation and the consequent decrease in alkalinity and pH of this solution.

57. The quantity of carbon derived directly from solution C_T increased with increasing C_T and to a lesser extent with increasing solution cations. The latter effect is due to the increase in $CaCO_3$ precipitation with increasing solution Ca and C_T. Although carbon derived from solution increased with increasing C_T between 10.5 and 21.0 $mg \cdot \ell^{-1}$, this increased uptake from solution was offset by decreased CO_2 supply from the atmosphere. The magnitude of atmospheric CO_2 exchange is dependent on the gradient in pCO_2 across the air/water interface. As demonstrated earlier (Figure 15), the gradient in pCO_2 rapidly approaches a maximum as C_T uptake proceeds. In low C_T solutions, CO_2 exchange reaches a maximum after a relatively small depletion of C_T. In higher C_T solutions, maximal CO_2 exchange requires a greater depletion of C_T. Moreover, in situations where $CaCO_3$ precipitation occurs, the gradient in pCO_2 is diminished, and atmospheric CO_2 exchange may be reduced. For these reasons the importance of atmospheric CO_2 exchange in the carbon budget is not easily predicted from the initial solution composition.

58. The inability to relate growth of *M. spicatum* in these experiments to photosynthetic rates measured in the same solutions is due to an inability to assess changes in the role of atmospheric CO_2 exchange during the changing water chemistry conditions occurring in the growth experiments. However, photosynthetic C_T uptake rates, integrated over the change in C_T occurring in the low D/z experiment, were linearly related to the quantity of C_T supplied by solution during growth (Figure 19). Thus, while photosynthetic measurements may be useful for assessing the uptake of solution C_T, they may not be good predictors of plant growth in natural systems.

59. Many of the environmental parameters known to affect the relative availability of atmospheric and solution carbon were controlled in these experiments. These parameters include temperature, mass transfer coefficients, mixing, and water exchange. In addition, other sources of carbon were minimized. These sources include water column respiration, sediment respiration, diffusion of HCO_3^- from the sediment, and dissolution of sedimentary carbonates. In view of the much greater complexity of natural systems, the use of photosynthetic rates as a diagnostic tool for assessing the submersed macrophyte growth potential of natural systems may be unwarranted without a better understanding of the importance of these other sources of carbon.

Carbon Limitation

60. The growth responses of *M. spicatum* in these experiments suggest that growth was limited by the availability of carbon at the low level of C_T ($3.5\ mg \cdot \ell^{-1}$) but not at the mid or high levels. In order to assess the possible limitation of growth by carbon, an additional experiment was conducted in the lucite columns in the growth chamber. In this experiment the same three levels of C_T were examined and each solution was balanced with respect to cations. For example, the low C_T solution had the low level of cations and the high C_T solution had the high level of cations (see Table 1). Two sets of columns were used with one set receiving ambient CO_2 aeration ($\approx 350\ \mu\ell \cdot \ell^{-1}$) and the other receiving about 3.5 × ambient CO_2 ($\approx 1,200\ \mu\ell \cdot \ell^{-1}$). The high CO_2 aeration was accomplished by metering compressed CO_2 into the metered airstream. Other experimental details were as described earlier.

61. Augmenting the airstream CO_2 concentration produced a dramatic increase in growth of *M. spicatum* in all solutions (Figure 20). In addition to the increased growth in each solution, a difference in growth between the mid and high level C_T solutions was also noted, suggesting that addition of CO_2 increased the availability of C_T under high C_T conditions. This increased availability of C_T was due to the lessened C_T depletion in all solutions and decreased $CaCO_3$ precipitation in high Ca, high C_T solutions (data not presented).

62. In these short-term (6 weeks) growth experiments, biomass accrual is directly proportional to growth rate and can be used to examine the

kinetics of growth in relation to C_T. Figure 21 is a plot of biomass accrual under ambient and elevated CO_2 conditions during the high D/z experiment along with Michaelis-Menten kinetics curves calculated for these data. The half-saturation constant for growth under ambient CO_2 ($K_m = 2.1$ mg $C_T \cdot \ell^{-1}$) is quite different than that presented earlier for photosynthesis. The half-saturation constant for growth in the low D/z experiment ($K_m = 1.9$, data not shown) is essentially identical to the K_m for growth in the high D/z experiment. Augmenting the CO_2 supply during growth changed both K_m and V_{max} (Figure 21). Interestingly, the 3.5-fold increase in airstream CO_2 produced a 3.4-fold increase in V_{max}.

63. These changes in kinetic constants indicate that the growth response of *M. spicatum* to solution C_T is highly dependent on the characteristics of both solution composition and atmospheric CO_2 exchange. Photosynthesis experiments involving only the direct uptake of C_T generally produce high values of K_m (Figure 10). Consideration of atmospheric exchange during photosynthesis results in a considerably lower K_m (Figure 16). Growth experiments conducted under conditions favoring depletion of solution C_T and the exchange of CO_2 between the atmosphere and solution produce even lower estimates of K_m (Figure 21). Enhancing the replenishment of solution C_T by augmenting the airstream CO_2 concentration produced an intermediate value of K_m.

64. These results indicate that growth of *M. spicatum* was limited by availability of carbon under all experimental conditions employed here. The lack of a growth response to increasing C_T above the mid level (10.5 $mg \cdot \ell^{-1}$) under ambient CO_2 conditions may have been due to both the magnitude of, and the relative similarity in, the transfer of atmospheric CO_2 into solution (Figure 18). The differences in the kinetics of growth and photosynthesis in these experiments arise from differences in the two measurement parameters. In the photosynthesis experiments, both C_T uptake and atmospheric CO_2 exchange varied in relation to changes in solution C_T. However, in the growth experiments, biomass accrual integrated the changes in photosynthesis occurring over the course of the experiment. In the growth experiments photosynthetic C_T uptake lowered the concentration of C_T in solution. The decrease in C_T resulted in a corresponding decrease in photosynthetic C_T uptake rate. Atmospheric CO_2 exchange increased in response to C_T depletion (Figure 15), but was unable to maintain the initial C_T levels. In both

growth experiments, measured CO_2 concentrations in the effluent airstreams varied only slightly among the different solutions. The transfer of airstream CO_2 in these experiments was presumably operating at near the maximum possible rates for each of the experimental systems. Approximately 50 and 90 percent of the airstream CO_2 was removed during passage through the solutions in the low and high D/z experiments, respectively. Thus, as C_T depletion continued, the systems approached steady-states, with C_T uptake from solution being balanced by atmospheric CO_2 exchange. For sufficiently long experimental periods a similar steady-state will be attained for all starting conditions, and growth would be independent of the initial C_T conditions. In the growth experiments conducted under ambient atmospheric CO_2 conditions, the mid and high C_T solutions had achieved equivalence with respect to their ability to support photosynthesis through the combined processes of C_T supply and atmospheric CO_2 exchange. Under elevated atmospheric CO_2 conditions, CO_2 exchange was sufficient to maintain the solutions at higher levels of C_T. Therefore, steady-state conditions were not approached, and differences in growth among the different solutions were observed. In the experiments reported here, the influence of C_T on growth was therefore dependent on the rate of supply of atmospheric CO_2.

PART VI: GENERAL DISCUSSION

Cation Effects

65. The influence of specific cations on the growth and photosynthesis of *M. spicatum* seems to be limited primarily to the involvement of Ca in the photosynthetic process. As noted earlier, the deleterious effects of low Ca are only observed under low C_T, high pH conditions. Under conditions of limited solution volume (i.e. photosynthesis experiments), C_T depletion rapidly becomes acute, and the deleterious effects of low Ca are pronounced. As solution volume increases (i.e. growth experiments), C_T depletion is less severe, and the requirement for Ca seems to decrease. Increasing either C_T or the airstream CO_2 concentration alleviates the effects of low Ca by reducing the severity of C_T depletion. These observations suggest that Ca may be involved in alleviating stress due to the occurrence of low levels of HCO_3^- in combination with relatively high levels of pH and CO_3. The CO_3 has been shown to be a competitive inhibitor of HCO_3 uptake (Lucas 1977), and Ca may moderate this inhibition by precipitation of CO_3 at sites of OH efflux.

66. Although Ca is clearly required for photosynthesis and growth, these results indicate that low levels of Ca (or other cations) are unlikely to limit the growth or distribution of *M. spicatum*. The establishment of *M. spicatum* in ponds of extremely low Ca (Giesy and Tessier 1979) substantiates this finding. Earlier evidence that Ca may affect the growth or distribution of this species (Hutchinson 1970; Barko 1983) should be reevaluated in light of these findings. Observed patterns of the distribution of *M. spicatum* in relation to Ca levels may reflect the natural correlation between Ca and C_T, rather than an environmental requirement for high Ca levels.

Carbon Effects

67. The influence of C_T on the growth and photosynthesis of *M. spicatum* is more difficult to quantify. Photosynthetic C_T uptake from solution is strongly related to solution C_T concentration. However, the influence of C_T on growth is more dependent on the ability of the system to maintain levels of C_T than on the actual concentration of C_T in solution. Thus, the effect of C_T on the growth of submersed macrophytes cannot be

determined a priori, as it will more likely depend on the rate of replenishment of the C_T pool than on the actual quantity of C_T. Unlike the experiments reported here, in which the atmosphere was the major source of carbon, in natural plant populations it is likely that sediment respiration, water column respiration, and atmospheric exchange are all significant sources of carbon input to the C_T pool.

68. The importance of C_T in affecting the growth and distribution of *M. spicatum* in nature involves all components of the carbon budget. Systems containing low levels of C_T are likely to also exhibit low rates of carbon regeneration (respiration), and are unlikely to support excessive growth of submersed macrophytes. Systems chronically supporting luxuriant growth of submersed aquatic plants are likely to exhibit high rates of both water column and sediment respiration, resulting in a high rate of supply of carbon to the C_T pool. In systems which have not previously been exposed to submersed macrophytes, the potential influence of the C_T level on macrophyte growth potential will depend on the intrinsic capacity of carbon regeneration. If the carbon-regenerating capacity of the system is low, the development of invasive submersed macrophytes (e.g., *M. spicatum*) may be short lived. It is of interest in this regard that the decline of *M. spicatum* in Lake Wingra, Wis., coincided with a 31-percent reduction in C_T levels in the lake (Titus and Stone 1982).

PART VII: CONCLUSIONS AND RECOMMENDATIONS

69. Based on this research and a review of the technical literature on the effects of water chemistry on *M. spicatum*, the following conclusions and recommendations are made:

<u>a</u>. Under conditions of limited Ca and C_T availability, growth and photosynthesis of *M. spicatum* can be adversely affected. While K can partially substitute for Ca, neither Na nor Mg are effective substitutes. Additions of carbon as either C_T or CO_2 lessen the requirement for Ca. The requirement for Ca seems to involve the total quantity available rather than the concentration in solution. In spite of these findings, levels of the major cations in most surface waters should be sufficient to support nuisance growths of *M. spicatum*. Observed patterns of the distribution of *M. spicatum* in relation to Ca levels may reflect the natural correlation between Ca and C_T, rather than an environmental requirement for high Ca levels.

<u>b</u>. While photosynthetic C_T uptake is strongly affected by solution composition, exchange of atmospheric CO_2 is relatively unaffected. Atmospheric CO_2 exchange therefore moderates the influence of solution C_T. Additional studies on photosynthesis of submersed aquatic plants should consider the interrelationship between uptake of solution C_T and exchange of atmospheric CO_2 into solution.

<u>c</u>. Photosynthetic C_T uptake is dependent on the concentration of HCO_3^- and, at equivalent HCO_3^- levels, is relatively unaffected by pH over the range 8.0 to 10.2. Additional studies on the effects of solution C_T and pH on photosynthesis of submersed aquatic plants should be conducted in unbuffered solutions, allowing pH levels to be controlled naturally by changes in solution C_T. This method, in addition to being more natural, will allow study of the combined effects of changes in C_T and pH on the photosynthetic process.

<u>d</u>. Photosynthesis and growth of *M. spicatum* can result in considerable changes in water chemistry conditions, and levels of C_T in solution can be rapidly depleted through the combined processes of photosynthetic C_T uptake and $CaCO_3$ precipitation. Continuous addition of gaseous CO_2 can be effective in moderating C_T depletion and $CaCO_3$ precipitation, thus maintaining high levels of C_T in solution. Elevated CO_2 levels can thereby enhance the growth response of *M. spicatum* to solution C_T. Regeneration of carbon through ecosystem respiration seems to be required to maintain C_T levels adequate to support luxuriant growth of *M. spicatum*. Therefore, the carbon-regenerating capacity, rather than the level of C_T in solution, will limit the submersed macrophyte production potential of the system. Additional studies of the effects of water chemistry on the growth of submersed aquatic plants should

consider the major fluxes of inorganic carbon and their relation ship to the maintenance of the C_T pool.

e. The relationship between photosynthesis and growth is complicated by growth-related changes in solution composition as well as by changes in the rate of exchange of atmospheric CO_2. The utility of photosynthesis measurements as indicators of plant growth potential cannot be realized without a better understanding of the roles of atmospheric CO_2 exchange and carbon regeneration in maintaining the level of C_T in solution.

f. Carbon limitation of *M. spicatum* growth is likely in lentic systems exhibiting low rates of carbon regeneration. The prevalence of carbon limitation of submersed macrophyte growth should be evaluated in a variety of natural systems.

g. The responses of other species of submersed aquatic plants to water chemistry conditions should be evaluated and compared with results obtained for *M. spicatum*. These comparative evaluations may suggest mechanisms to explain observed patterns of growth and distribution.

h. Although water chemistry conditions (specifically those affecting carbon availability) may set limits on the growth potential of submersed macrophyte populations, it is not readily apparent that increased understanding of these limits will lead to efficacious aquatic plant management techniques. Although levels of C_T could be reduced in localized areas through chemical precipitation, this technique may be thwarted by redissolution of $CaCO_3$ by respiratory CO_2. Field investigations of the feasibility of $CaCO_3$ precipitation as a means of reducing submersed macrophyte growth might be initiated on a small scale. If successful, this technique might provide an inexpensive and environmentally acceptable alternative to the use of herbicides in certain situations.

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Table 1

Composition and Characterization of Experimental Solutions

Treatment Levels		Chemical Composition, mg·ℓ$^{-1}$							Conductance μS·cm^{-1} (25° C)	pH	Ionic Strength mM	Alkalinity meg·ℓ
C_T	Cation	Ca	Mg	Na	K	C_T	Cl	SO_4				
Low	Low	10	1.7	2.8	1.0	3.5	7	14	100	7.5	1.3	0.29
Low	Mid	30	5.0	8.4	3.1	3.5	32	57	276	7.5	3.9	0.29
Low	High	60	10.1	16.9	6.1	3.5	69	120	505	7.5	7.9	0.29
Mid	Low	10	1.7	8.0	1.0	10.5	2	4	110	8.0	1.4	0.88
Mid	Mid	30	5.0	8.4	3.1	10.5	22	41	260	8.0	3.7	0.88
Mid	High	60	10.1	16.9	6.1	10.5	58	107	490	8.0	7.7	0.88
High	Low	10	1.7	28.1	1.0	21.0	2	4	175	8.3	2.2	1.75
High	Mid	30	5.0	8.4	3.1	21.0	9	17	230	8.3	3.5	1.75
High	High	60	10.1	16.9	6.1	21.0	44	83	490	8.3	7.5	1.75

Table 2

Characteristics of Experimental Environments

Characteristic	Units	Experiment Low D/z	 High D/z
Environment		Greenhouse	Growth chamber
Experimental system		Tanks	Columns
Surface area	m^2	1.35	0.013
Volume	m^3	1.15	0.015
Gas exchange coefficient, K	hr^{-1}	0.46	3.56
Mass transfer coefficient, D/z	$m \cdot hr^{-1}$	0.39	4.03
Boundary layer thickness, z	μ	21	2
Maximum O_2 transfer rate	$mg \cdot \ell^{-1} \cdot hr^{-1}$	3.7	28.6
Solution volume	$\ell \cdot plant^{-1}$	190	15

Table 3

Apparent Initial (Linear Phase) Photosynthetic C_T Uptake Rates ($mg \cdot g^{-1} \cdot hr^{-1}$) of *M. spicatum* in the Experimental Solutions

Cation Level	C_T Level Low	 Mid	 High
Low	0.96	3.80	9.70
Mid	0.84	6.56	9.38
High	2.50	6.31	16.4*

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* Measured rate includes $CaCO_3$ precipitation.

Table 4

Apparent Photosynthetic C_T Uptake Rate Coefficients, and Photosynthetic C_T Uptake of *M. spicatum* in the Experiment Solutions

Solution Composition			Coefficient	Limit		
Carbon Level	Cation Level	C_T $mg \cdot \ell^{-1}$	b $\ell \cdot g^{-1} \cdot hr^{-1}$	c $mg \cdot \ell^{-1}$	pH Limit	Initial Rate $mg \cdot g^{-1} \cdot hr^{-1}$
Low	Low	3.5	1.63	2.64	9.4	1.40
Low	Mid	3.5	2.00	2.57	9.5	1.86
Low	High	3.5	2.23	2.19	9.7	2.92
Mid	Low	10.5	1.08	6.50	10.1	4.32
Mid	Mid	10.5	1.07	4.15	10.5	6.79
Mid	High	10.5	0.61	0.00	*	6.41
High	Low	21.0	0.75	6.72	10.9	10.7
High	Mid	21.0	0.65	0.00	*	13.6**
High	High	21.0	1.21	0.56	*	24.7**

Note: The pH Limit for C_T uptake is calculated from the asymptotic limit of photosynthetic C_T uptake (c) where $CaCO_3$ precipitation did not occur. Initial photosynthetic C_T uptake rates were calculated from these values and Equation 2.

* Indicates precipitation of $CaCO_3$.

** Indicates that the calculated rate is overestimated due to $CaCO_3$ precipitation.

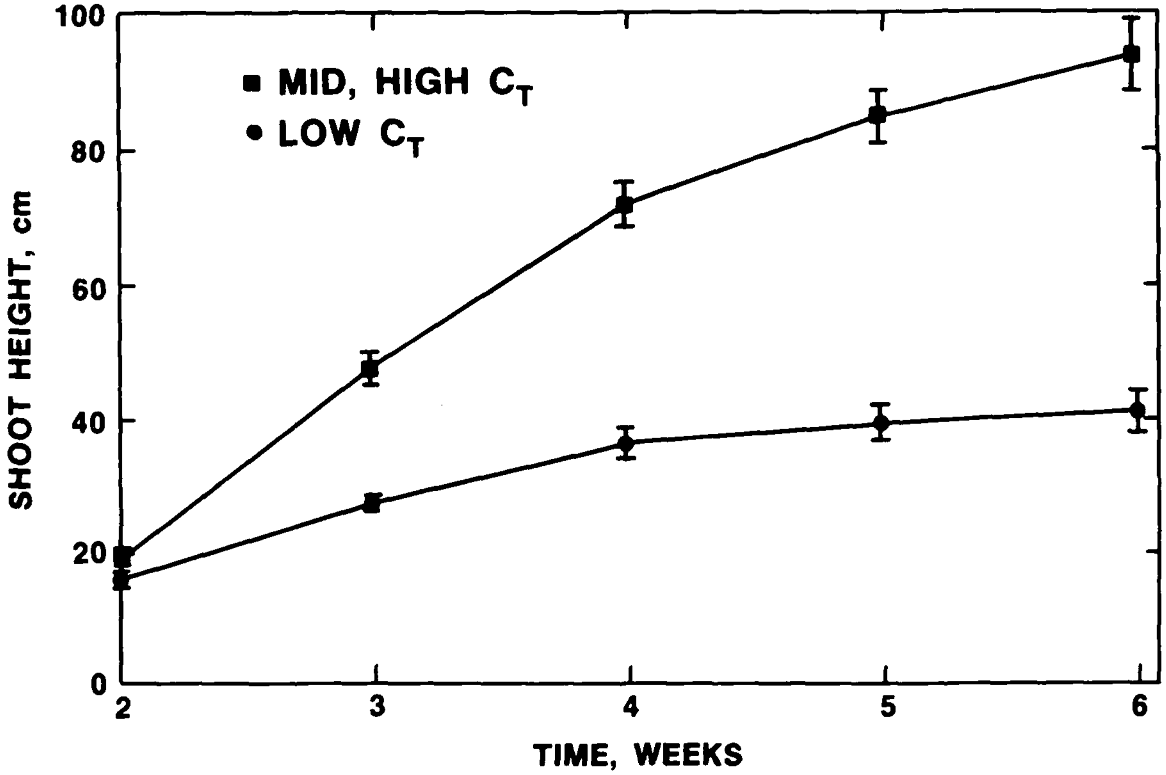

Figure 1. Shoot height of *M. spicatum* during the high D/z experiment. Data are pooled across cation levels within C_T levels. High and mid level C_T treatments are similarly pooled

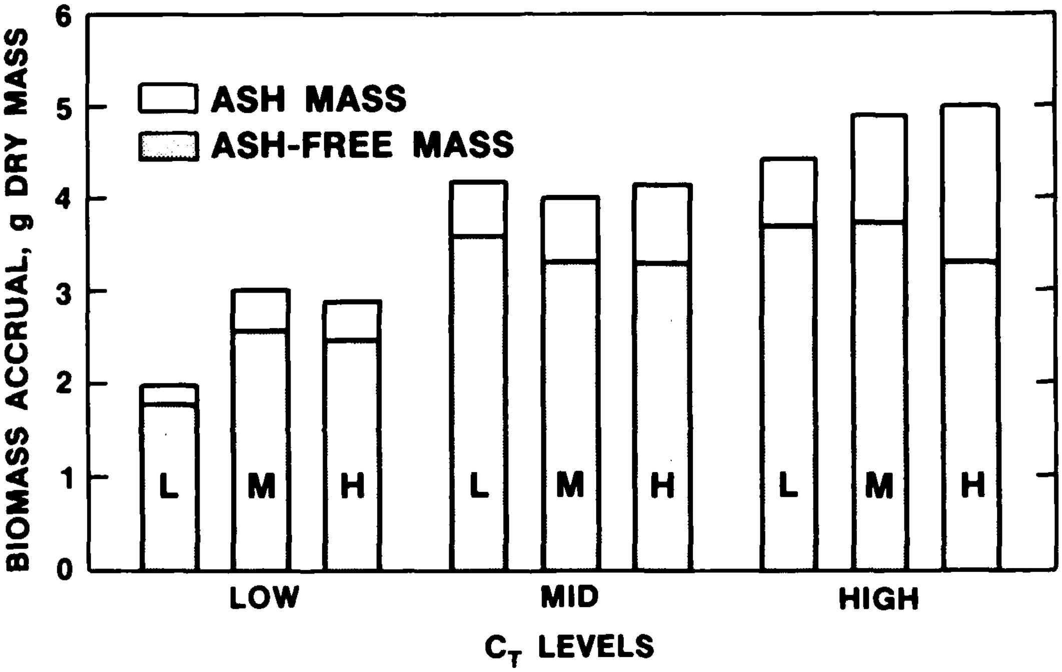

Figure 2. Total biomass and total ash-free biomass accrual of *M. spicatum* in relation to solution cation and C_T levels in the high D/z experiment. Cation levels are indicated within bars: L = low, M = mid, H = high

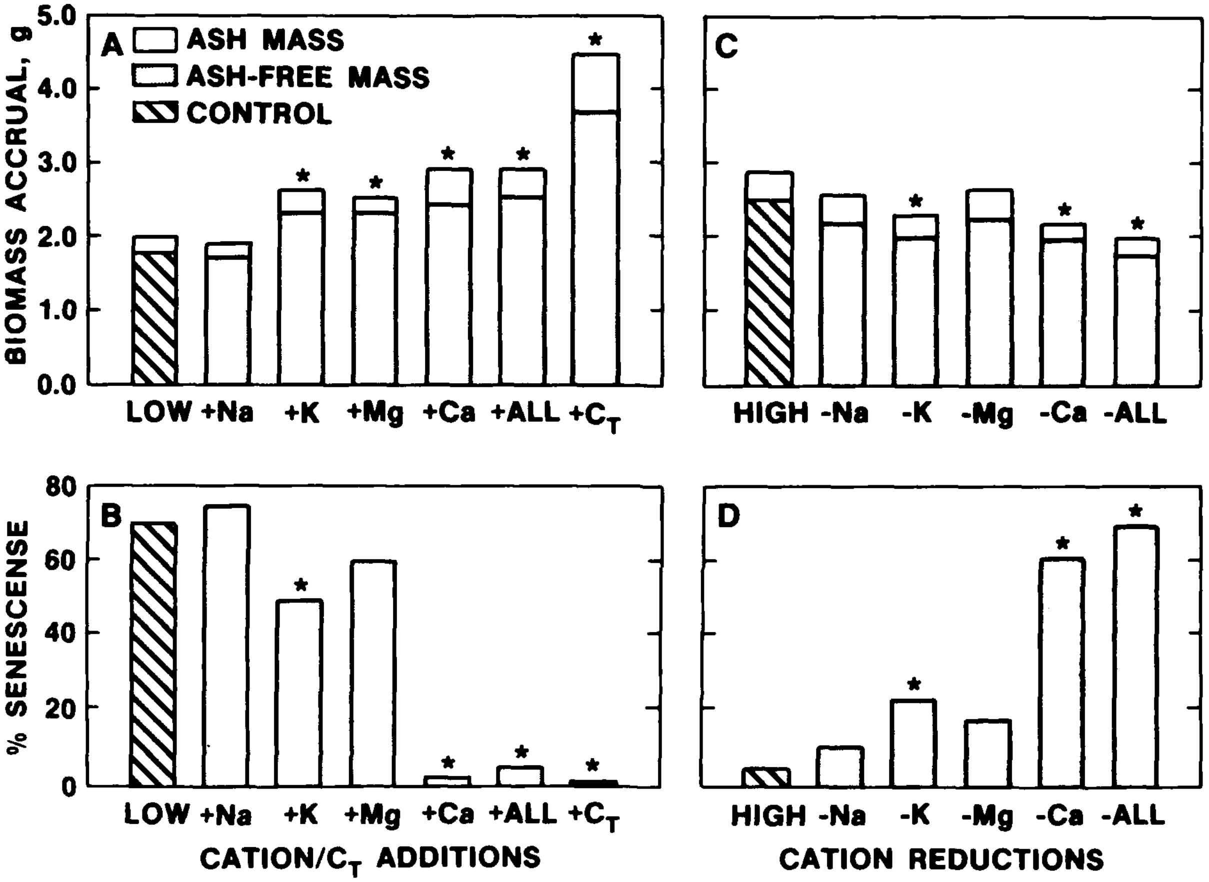

Figure 3. Total biomass and total ash-free biomass accrual (graphs A and C) and percent senescence (graphs B and D) of *M. spicatum* in relation to specific cation additions and reductions in the specific cation experiment. Cation additions increased the level of cation from the low (control) level to the high level. Cation reductions reduced the cation from the high (control) level to the low level. Asterisks denote a significant difference from the control treatment at $\alpha < 0.05$, as determined by Dunnett's procedure

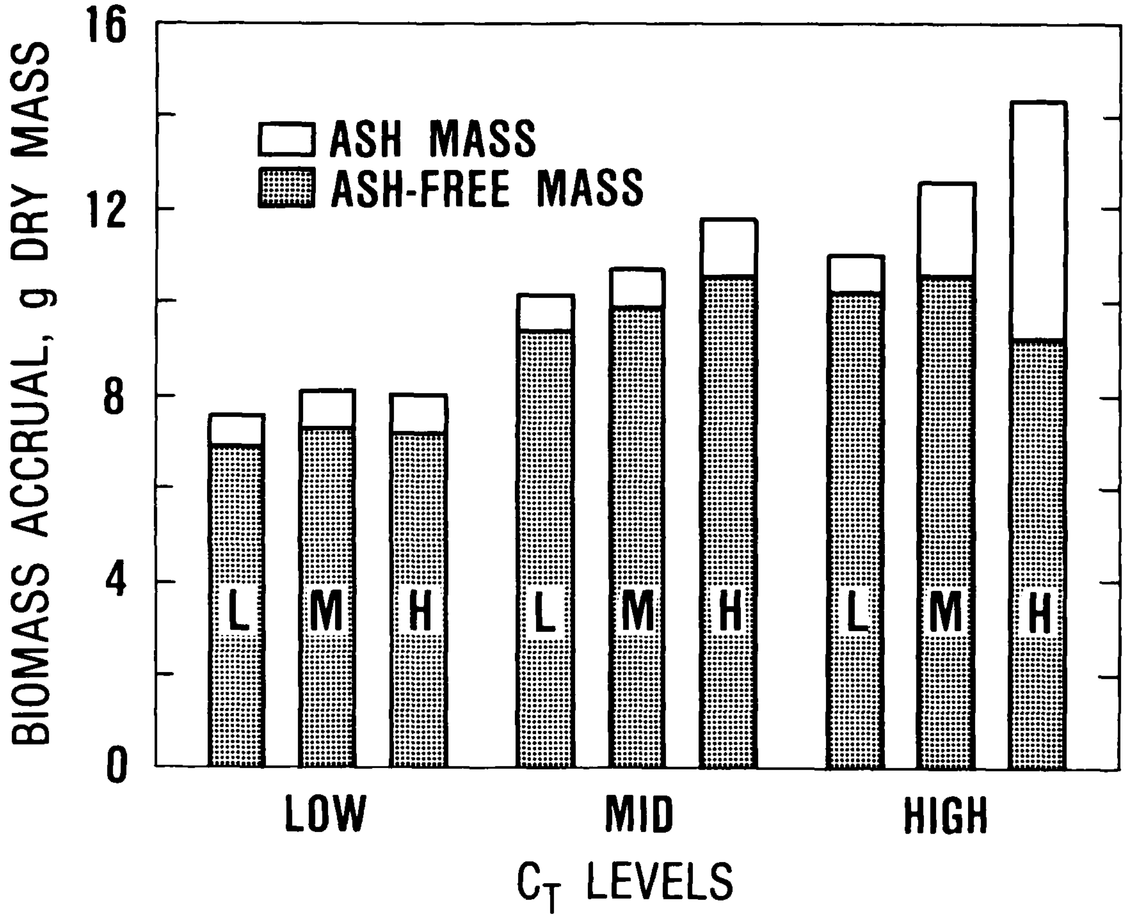

Figure 4. Total biomass and total ash-free biomass accrual of *M. spicatum* in the low D/z experiment. Cation levels are indicated within bars: L = low, M = mid, H = high

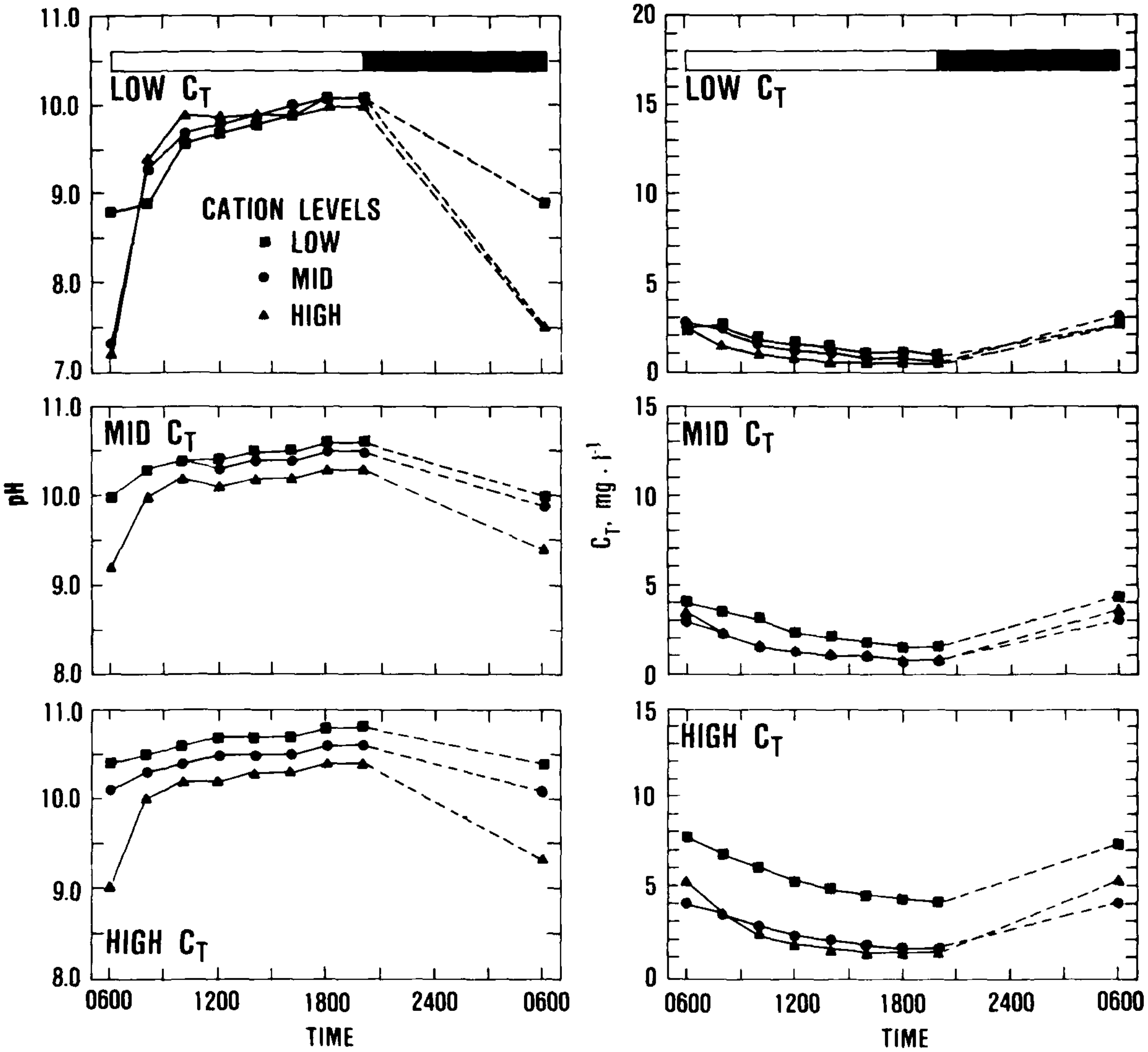

Figure 5. Diurnal changes in pH and C_T during the fifth week of the high D/z experiment. Horizontal bars indicate light and dark portions of the experimental period

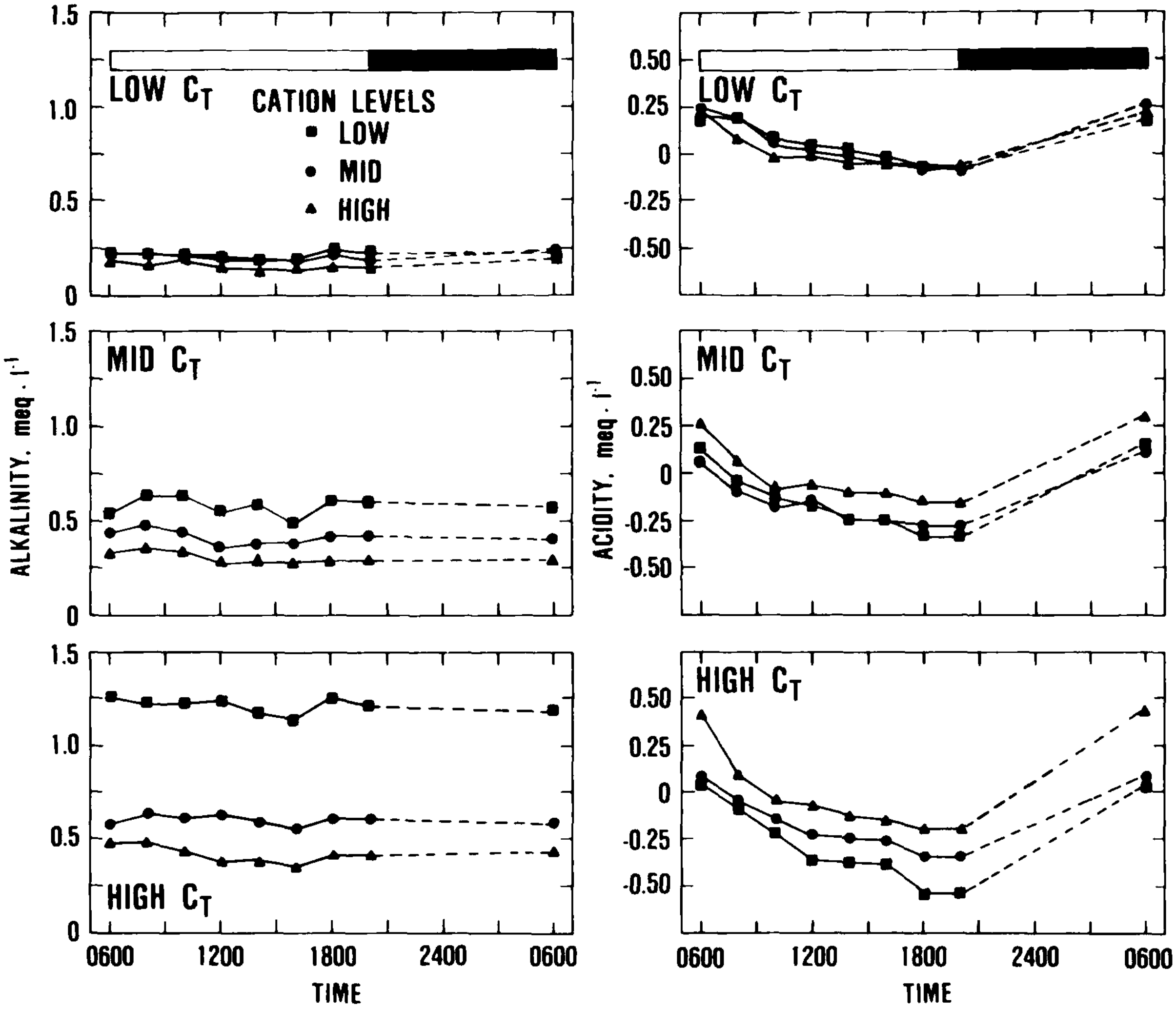

Figure 6. Diurnal changes in alkalinity and acidity during the fifth week of the high D/z experiment. Data were calculated from measured pH and C_T values. Horizontal bars indicate light and dark portions of the experimental period

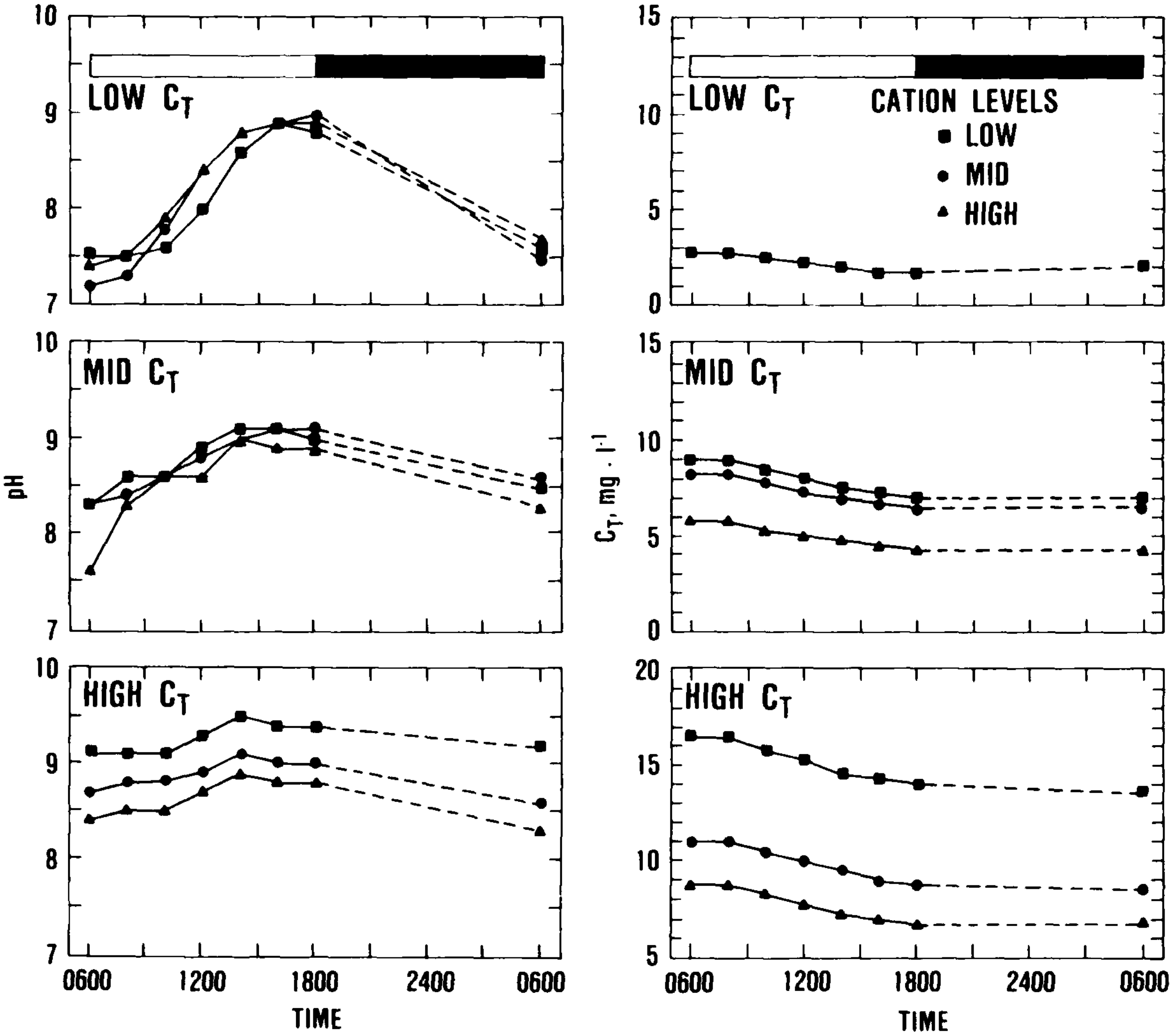

Figure 7. Diurnal changes in pH and C_T during the fifth week of the low D/z experiment. Horizontal bars indicate light and dark portions of the experimental period

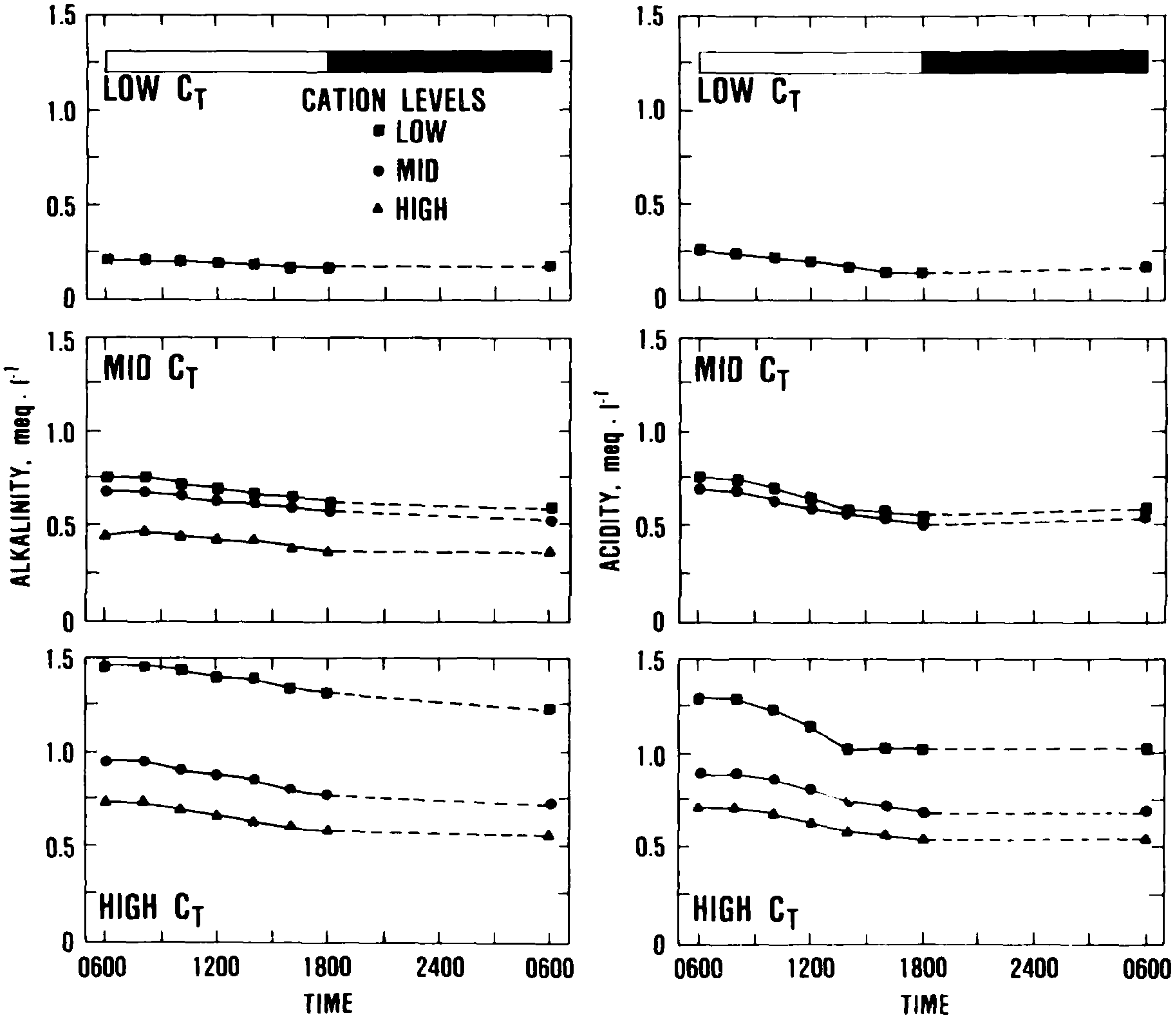

Figure 8. Diurnal changes in alkalinity and acidity during the fifth week of the low D/z experiment. Data were calculated from measured pH and C_T values. Horizontal bars indicate light and dark portions of the experimental period

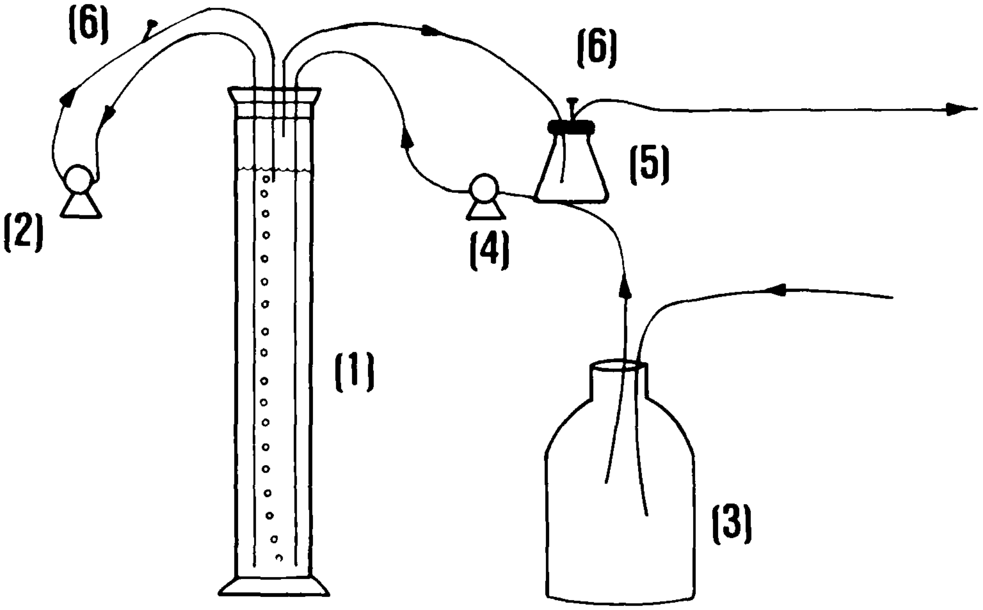

Figure 9. Diagrammatic representation of the experimental apparatus used for concurrent measurement of gas phase CO_2 concentrations and liquid phase C_T concentrations during photosynthesis by *M. spicatum*. (1) 1-ℓ glass hydrometer cylinder fitted with neoprene stopper; (2) peristaltic tubing pump for water circulation; (3) 40-ℓ mixing vessel for damping changes in influent compressed airstream; (4) peristaltic tubing pump for air flow; (5) 1-ℓ mixing vessel; (6) sampling ports fitted with rubber serum bottle stoppers. Arrows show direction of liquid and gas flow in the system

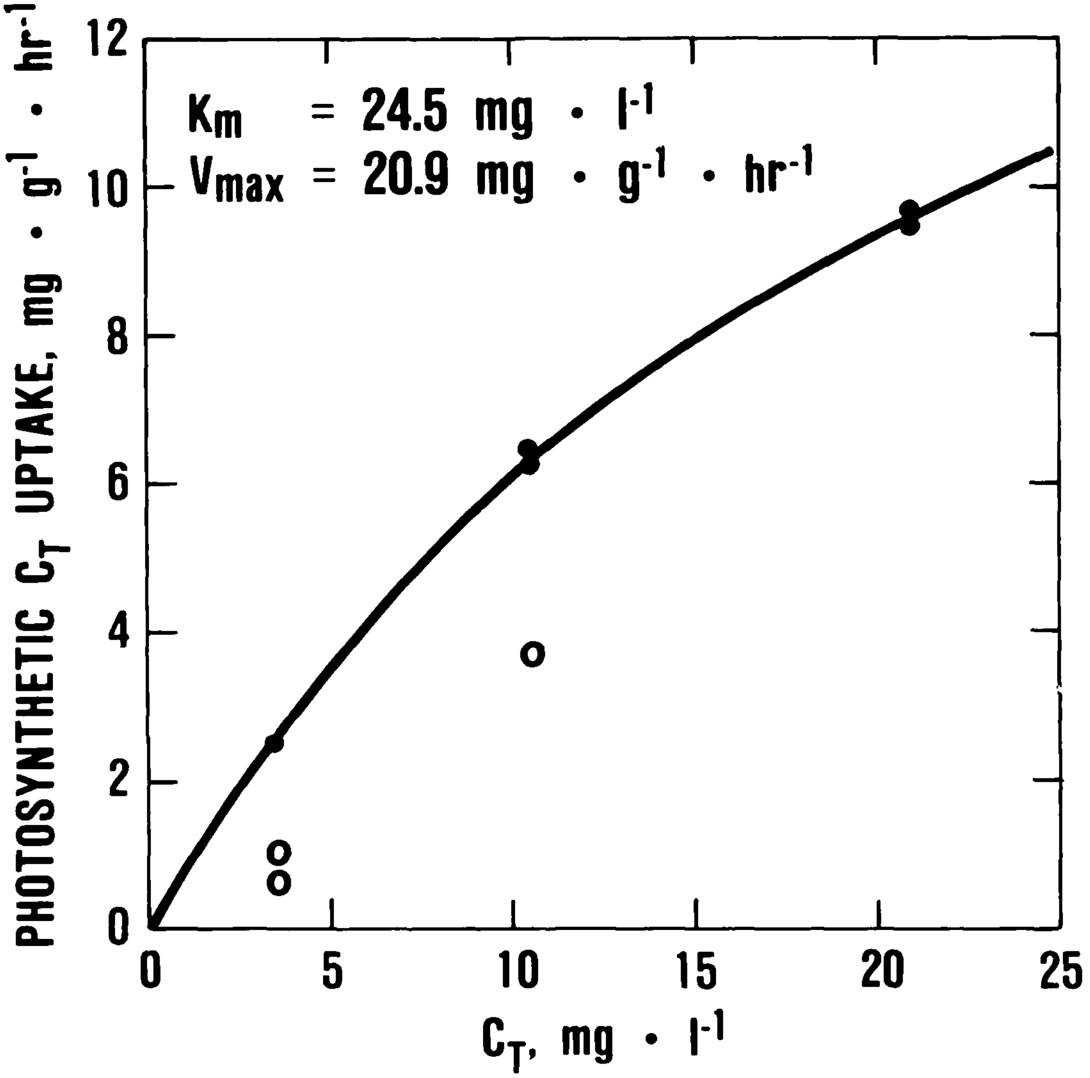

Figure 10. Relationship between initial (linear phase) photosynthetic C_T uptake by *M. spicatum* and C_T in the photosynthesis experiment. The kinetics curve was obtained by linear transformation of initial rate data in relation to solution C_T at each of the experimental C_T levels. Solutions containing unfavorable cation levels (o) were excluded from the analysis. The measured photosynthetic C_T uptake rate in the high C_T , high cation solution (not shown) was affected by $CaCO_3$ precipitation and was likewise excluded. K_m = half-saturation constant; V_{max} = maximal photosynthetic rate

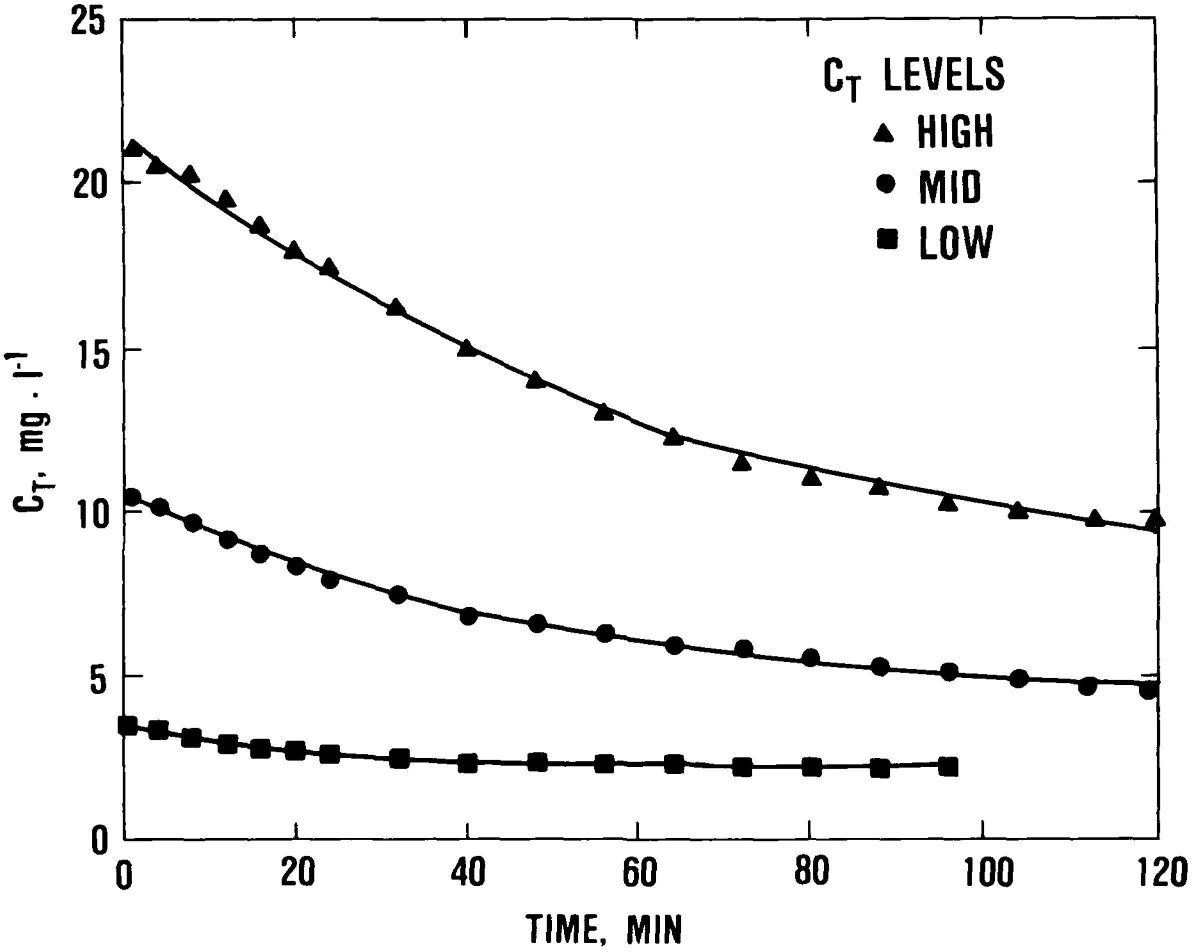

Figure 11. Representative curves showing temporal changes in C_T during the nonlinear photosynthesis experiment. Curve fitting was accomplished with a nonlinear least-squares estimation technique

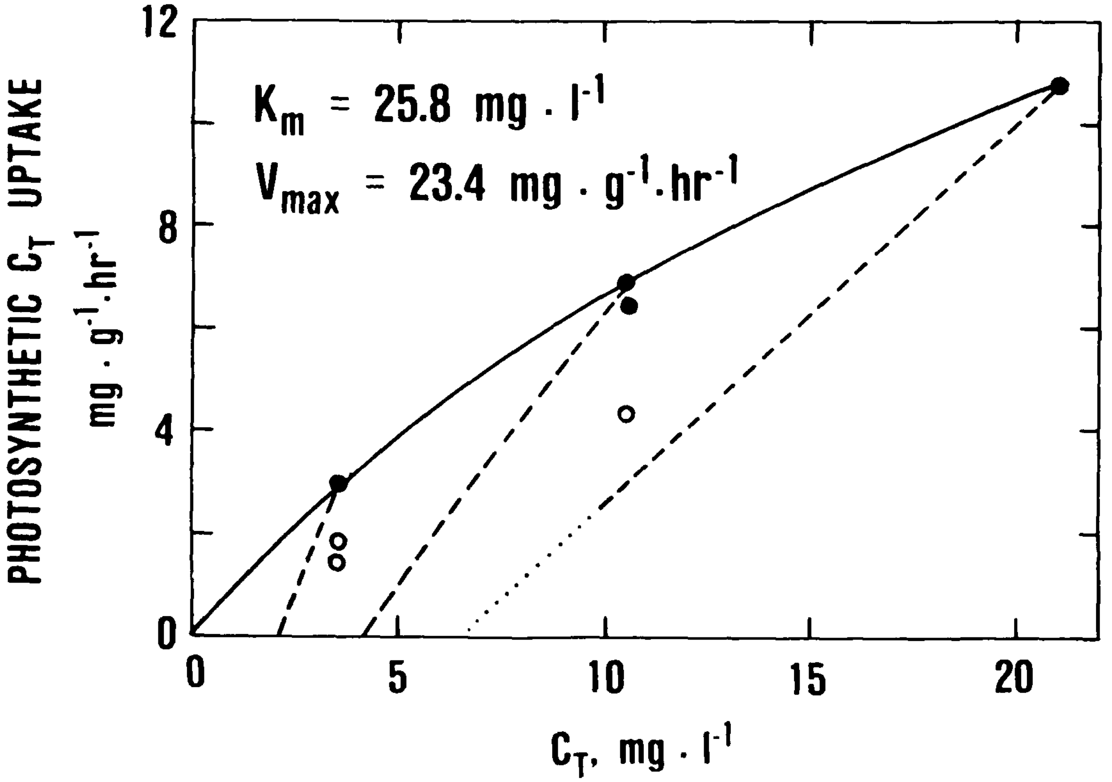

Figure 12. Relationship between photosynthetic C_T uptake by *M. spicatum* and solution C_T. The kinetics curve (solid line) was obtained by linear transformation of initial rate data in relation to solution C_T at each of the experimental C_T levels. Rates obtained in solutions containing unfavorably low cation levels (o) were excluded from the analysis. Photosynthetic C_T uptake rates which were affected by $CaCO_3$ precipitation (not shown) were also excluded. Photosynthetic C_T uptake rates in each solution (dashed lines) decreased linearly as C_T decreased. The dotted portion of the line was extrapolated

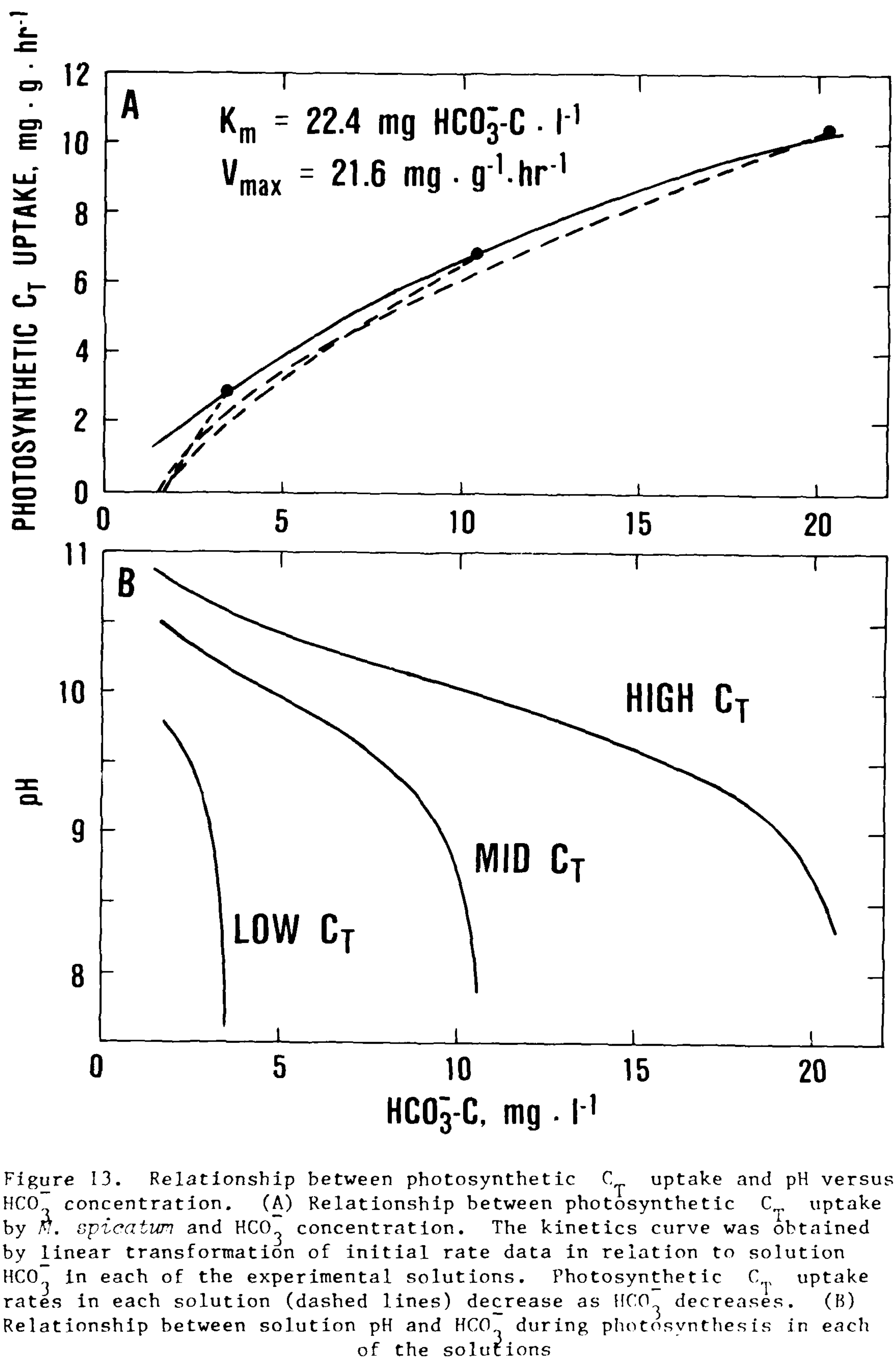

Figure 13. Relationship between photosynthetic C_T uptake and pH versus HCO_3^- concentration. (A) Relationship between photosynthetic C_T uptake by *M. spicatum* and HCO_3^- concentration. The kinetics curve was obtained by linear transformation of initial rate data in relation to solution HCO_3^- in each of the experimental solutions. Photosynthetic C_T uptake rates in each solution (dashed lines) decrease as HCO_3^- decreases. (B) Relationship between solution pH and HCO_3^- during photosynthesis in each of the solutions

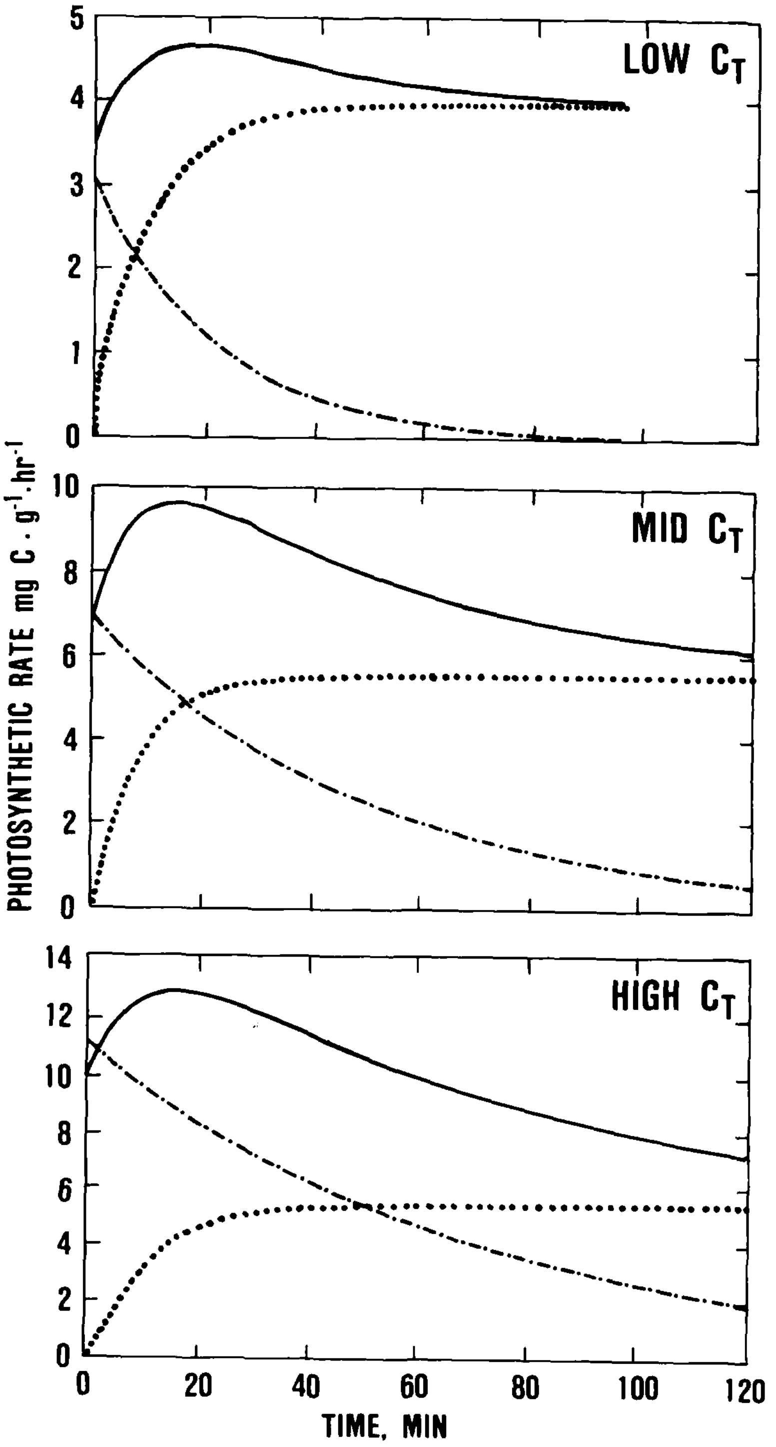

Figure 14. Temporal changes in rates of photosynthetic C_T uptake (dashed line), atmospheric CO_2 exchange (dotted line), and total photosynthetic carbon uptake (solid line) by *M. spicatum* in solutions containing low, mid, and high levels of C_T

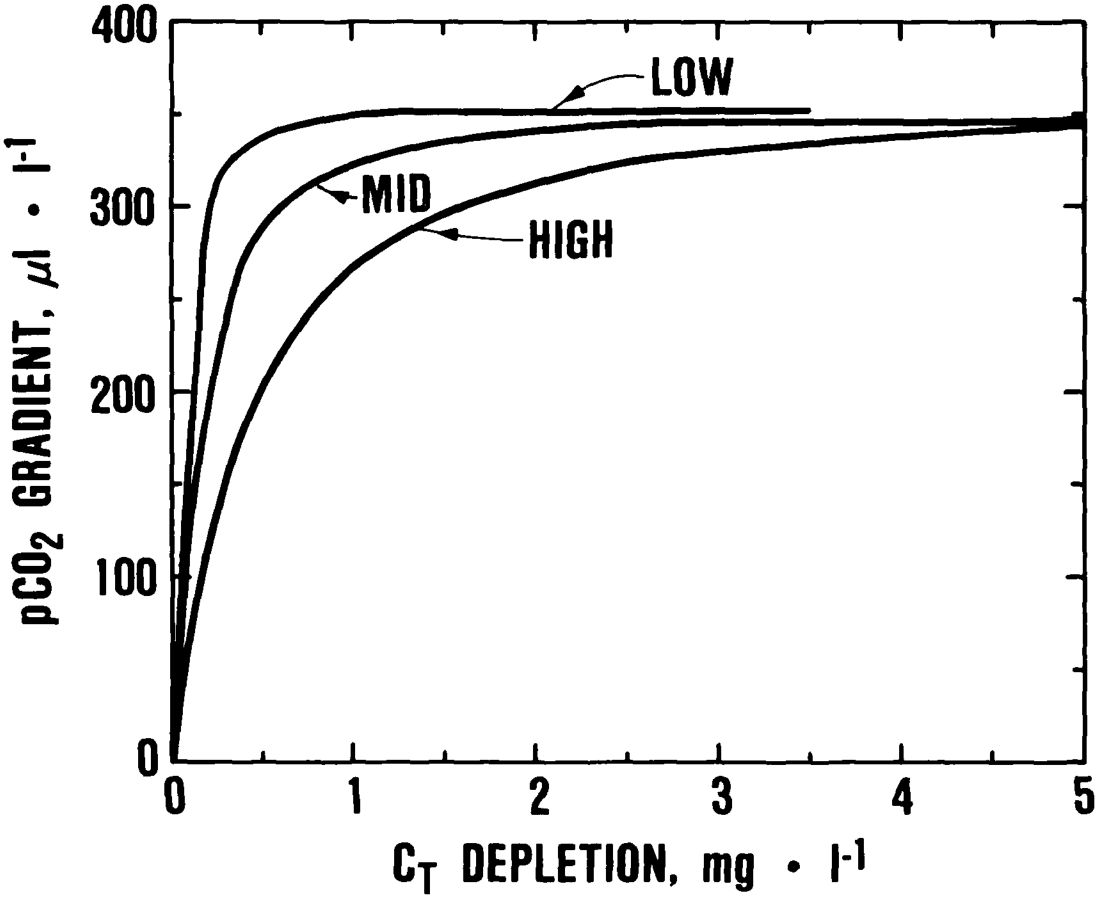

Figure 15. Relationship between the gradient in pCO_2 across the air/water interface and the depletion of C_T from solutions of different C_T levels. Curves are calculated from carbonate equilibria for each of the solutions. Alkalinity was held constant at the initial level

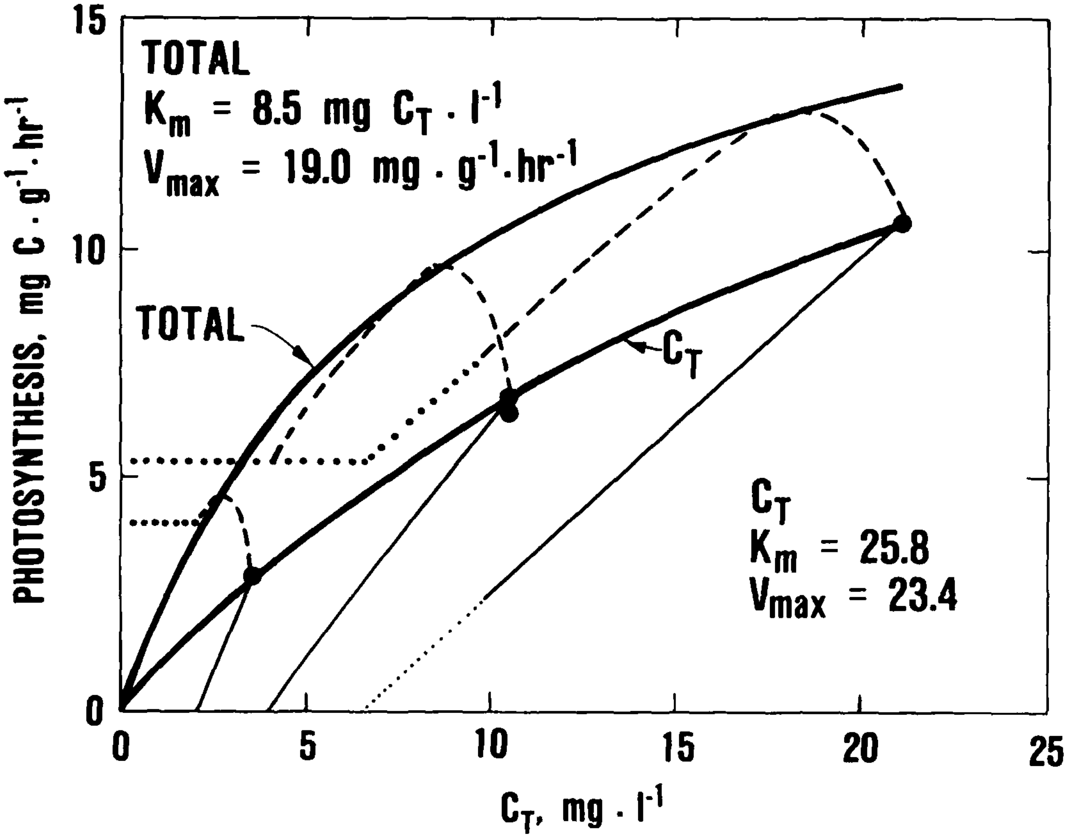

Figure 16. Relationship between total photosynthesis by *M. spicatum* and solution C_T. Photosynthetic C_T uptake rates and C_T uptake kinetics are shown for reference. Total photosynthesis (dashed lines) is the sum of C_T decline and atmospheric CO_2 exchange. Dotted portions of the lines are extrapolated. The horizontal portions of the total photosynthesis curves are equivalent to the steady-state maximum rate of atmospheric CO_2 exchange ($\Delta C_T = 0$). The kinetics curve for total photosynthesis (bold line) was calculated from linear transformation of maximal rate data in relation to solution C_T at each of the experimental C_T levels

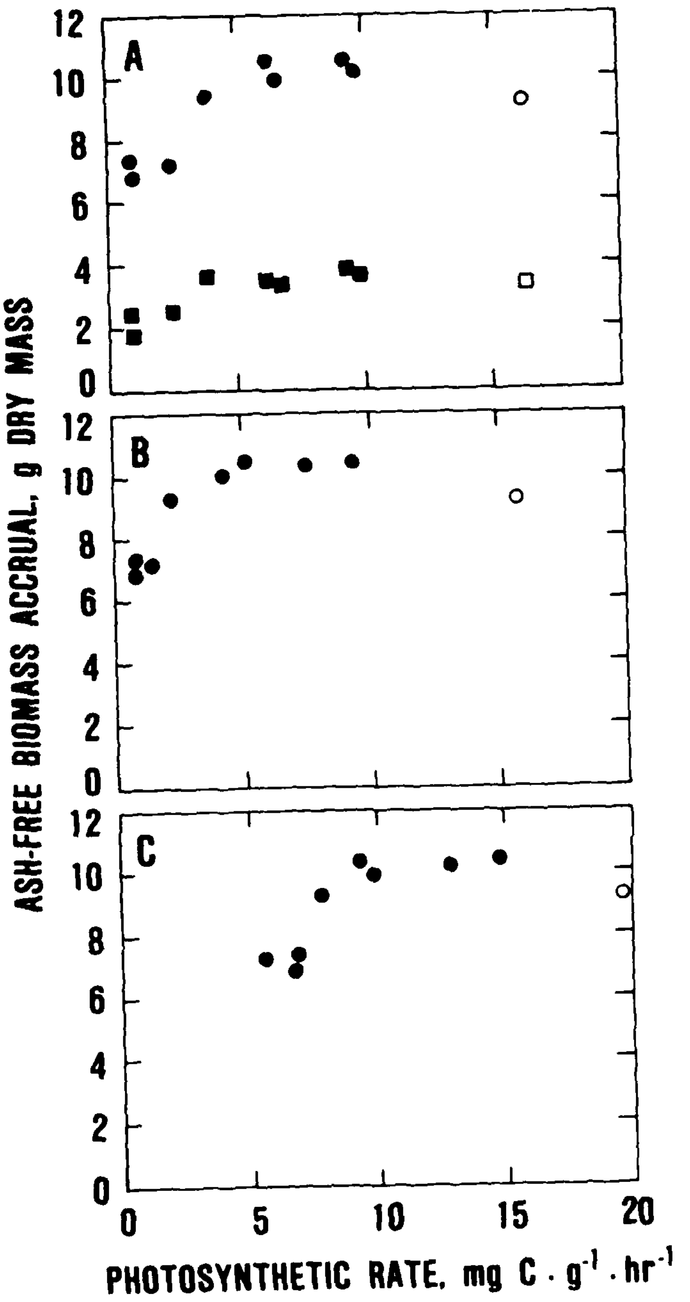

Figure 17. Relationship between growth (total ash-free biomass accrual) in the low D/z (●) and high D/z (■) experiments and several measures of photosynthesis of *M. spicatum*: (A) growth in relation to initial linear photosynthetic C_T uptake rates, (B) growth in relation to integrated apparent photosynthetic C_T uptake rates, and (C) growth in relation to integrated total apparent photosynthetic rates. Open points (○,□) indicate photosynthetic rates affected by $CaCO_3$ precipitation. Integrated photosynthetic rates were obtained by integration of photosynthetic rate equations over the change in solution C_T measured during the low D/z growth experiment

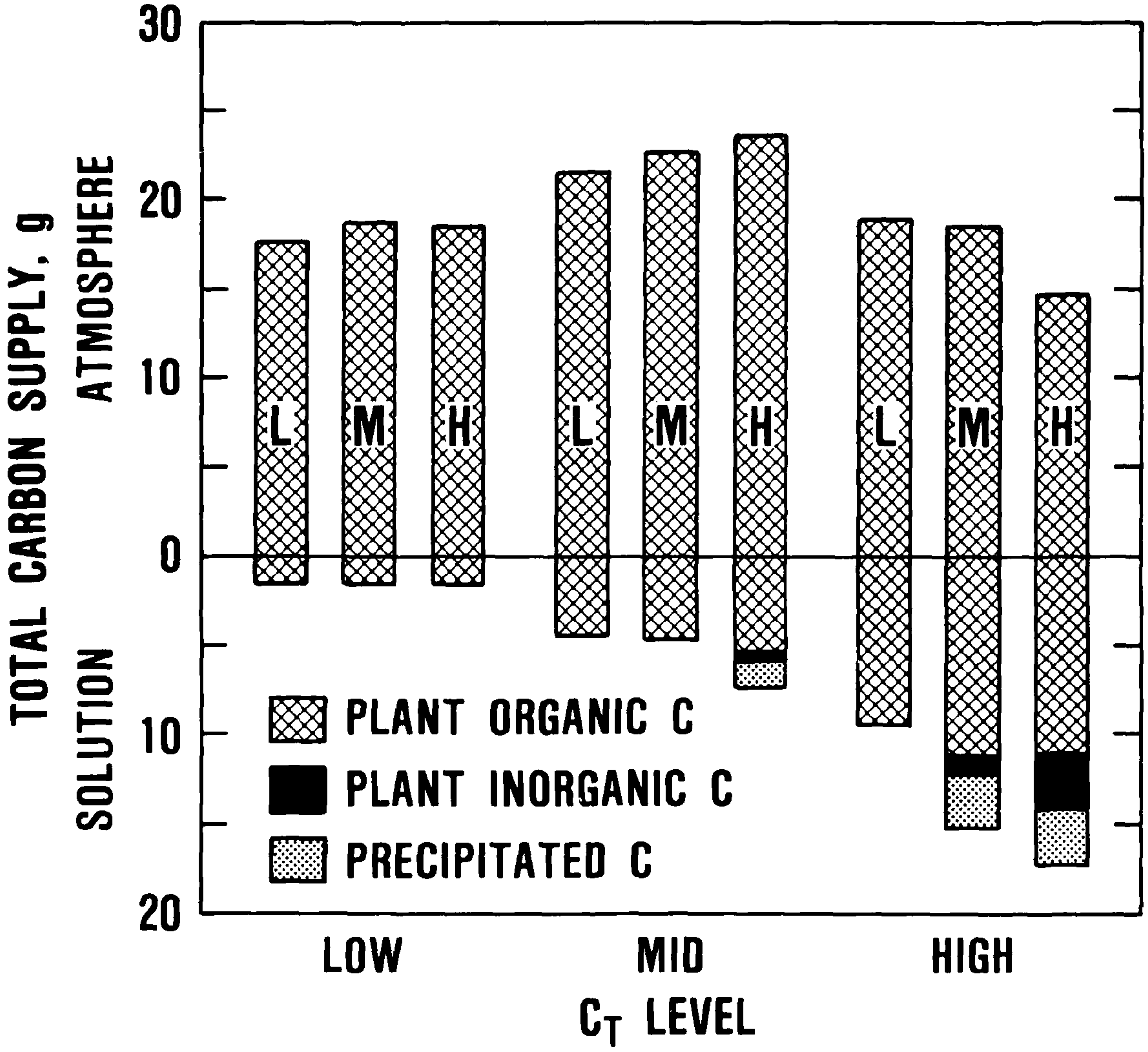

Figure 18. Sources of carbon used during growth (cross-hatched bars) of *M. spicatum* in the low D/z experiment. Solution cation levels are indicated within bars: L = low, M = mid, and H = high

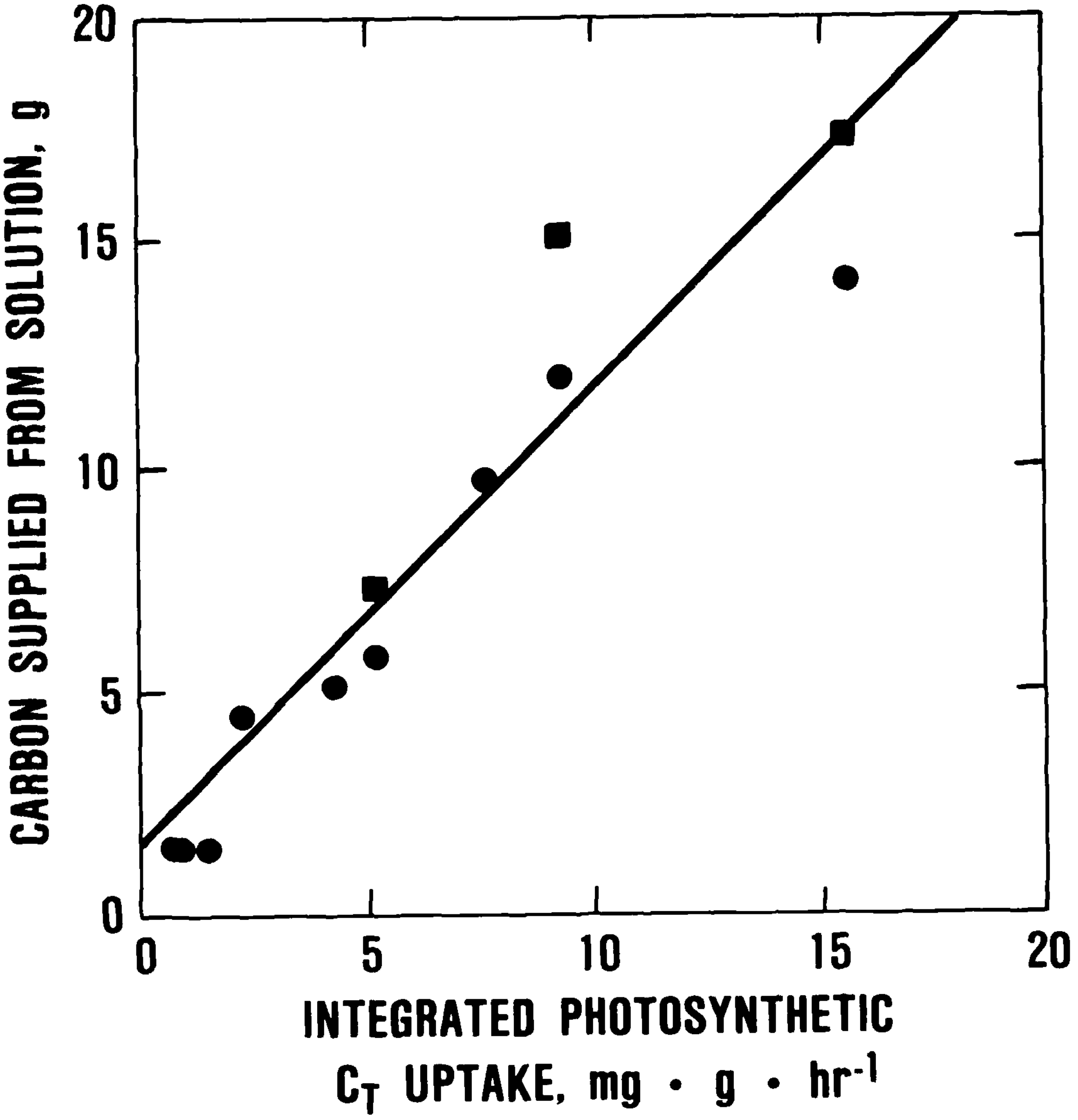

Figure 19. Relationship between the quantity of carbon supplied from solution during growth and integrated apparent photosynthetic C_T uptake rates. Photosynthetic C_T uptake rates were integrated over the measured changes in C_T occurring during the growth experiment. Carbon supplied from solution includes the quantities of C_T removed from solution and either incorporated into plant organic matter or precipitated on the plant tissues (●). Alternate values (■) also include C_T removed from solution by precipitation of $CaCO_3$ from the water column

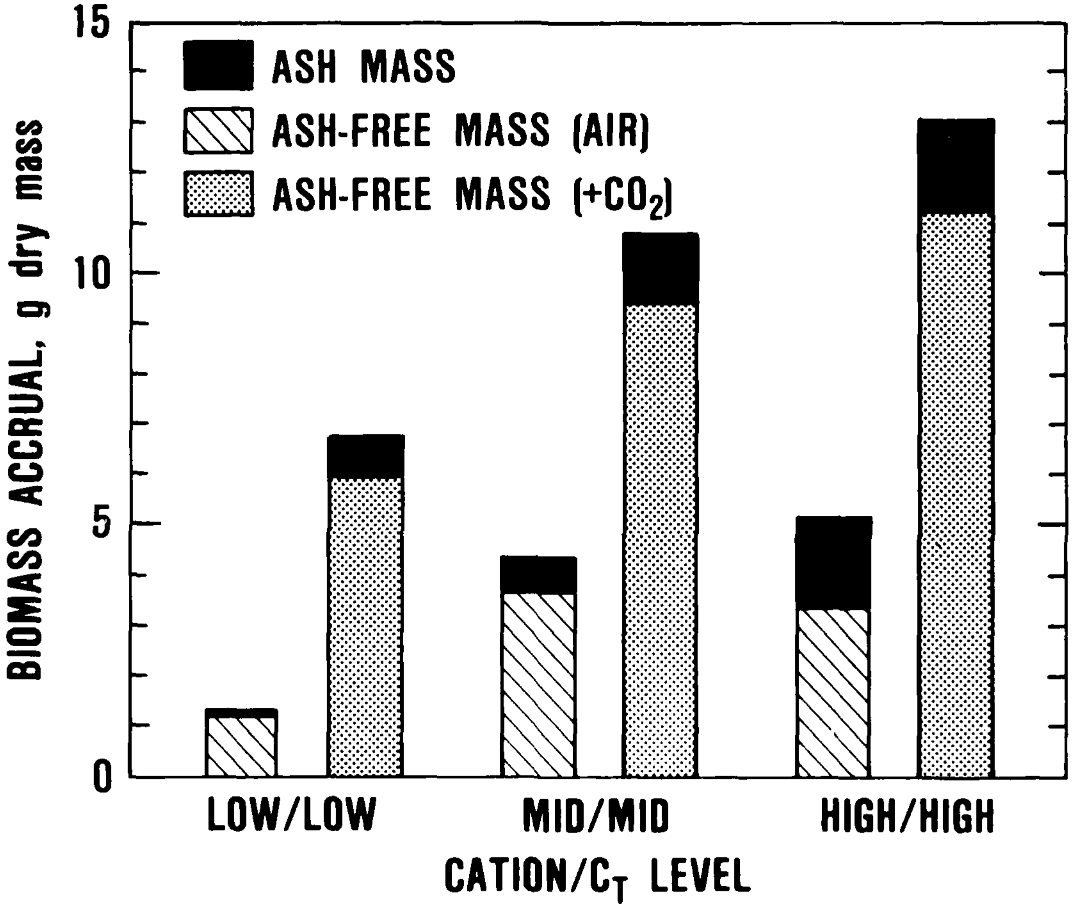

Figure 20. Total biomass and total ash-free biomass accrual of *M. spicatum* in relation to solution composition and atmospheric CO_2 supply

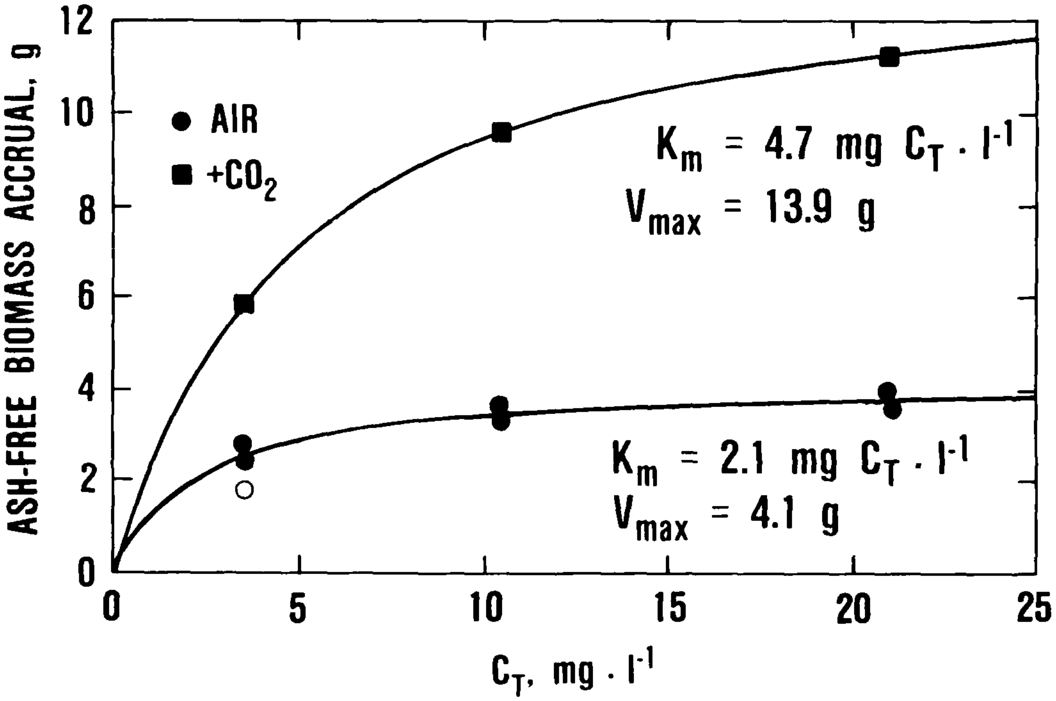

Figure 21. Kinetics of growth of *M. spicatum* in relation to solution C_T in the high D/z experiment under ambient and elevated airstream CO_2 conditions. Kinetics were determined by linear transformation of ash-free biomass accrual values in relation to solution C_T

APPENDIX A: STATISTICAL COMPARISON OF MEAN VALUES FOR GROWTH AND NUTRIENT CONCENTRATIONS IN RELATION TO SOLUTION CATION AND INORGANIC CARBON C_T LEVELS IN THE LOW AND HIGH D/z EXPERIMENTS

Table A1

Treatment Means and Statistical Comparisons of Plant Growth Parameters (g Dry Weight) Among Treatments in the High D/z Experiment

Cation Level	C_T Level Low	 Mid	 High	Pooled
Shoot biomass				
Low	1.79Aa	3.74Ba	3.90Ba	3.14a
Mid	2.71Ab	3.42Ba	4.30Cab	3.48ab
High	2.52Ab	3.66Ba	4.56Cb	3.58b
Pooled	2.34A	3.61B	4.25C	
Root biomass				
Low	0.21Aa	0.47Ba	0.53Bab	0.40a
Mid	0.31Ab	0.54Ba	0.62Ba	0.49b
High	0.36Ab	0.49Ba	0.46ABb	0.44ab
Pooled	0.29A	0.50B	0.54B	*
Total biomass				
Low	2.00Aa	4.20Ba	4.43Ba	3.54a
Mid	3.02Ab	3.97Ba	4.92Ca	3.97b
High	2.89Ab	4.15Ba	5.02Ca	4.02b
Pooled	2.64A	4.11B	4.79C	

Note: Individual treatment means (n = 4) followed by different letters (upper case for C_T levels, lower case for cation levels) are significantly different ($\alpha \leq 0.05$) as determined by Duncan's Multiple Range Test applied to individual one-way analysis of variance (ANOVA). Main effects (from two-way ANOVAs) of C_T and cation treatments are similarly indicated for values pooled over cation levels and C_T levels, respectively.

* Denotes a significant ($\alpha \leq 0.05$) C_T × cation interaction.

Table A2

Treatment Means and Statistical Comparisons of Plant Growth Parameters (g Ash-Free Dry Weight) Among Treatments in the High D/z Experiment

	C_T Level			
Cation Level	Low	Mid	High	Pooled
Ash-free shoot biomass				
Low	1.62Aa	3.22Ba	3.27Ba	2.71a
Mid	2.33Ab	3.88Ba	3.27Ba	2.83a
High	2.19Ab	3.06Ba	2.95Ba	2.73a
Pooled	2.05A	3.06B	3.16B	*
Ash-free root biomass				
Low	0.16Aa	0.37Ba	0.42Bab	0.32a
Mid	0.25Ab	0.43Ba	0.49Bb	0.39a
High	0.29Ab	0.38Ba	0.36ABa	0.35a
Pooled	0.23A	0.39B	0.43B	*
Ash-free total biomass				
Low	1.78Aa	3.59Ba	3.70Ba	3.02a
Mid	2.58Ab	3.31Ba	3.76Ba	3.21a
High	2.48Ab	3.45Ba	3.31Ba	3.08a
Pooled	2.28A	3.45B	3.59B	*

Note: Individual treatment means ($n = 4$) followed by different letters (upper case for C_T levels, lower case for cation levels) are significantly different ($\alpha \leq 0.05$) as determined by Duncan's Multiple Range Test applied to individual one-way ANOVAs. Main effects (from two-way ANOVAs) of C_T and cation treatments are similarly indicated for values pooled over cation levels and C_T levels, respectively.

* Denotes a significant C_T × cation interaction.

Table A3

Treatment Means and Statistical Comparisons of Plant Growth Parameters (g Dry Weight) Among Treatments in the Low D/z Experiment

	C_T Level			
Cation Level	Low	Mid	High	Pooled
Shoot biomass				
Low	6.65Aa	9.00Ba	9.80Ba	8.48a
Mid	7.08Aa	9.62Ba	11.48Cb	9.39b
High	6.96Aa	10.56Bb	13.29Cc	10.27c
Pooled	6.90A	9.73B	11.52C	**
Root biomass				
Low	0.95Aa	1.10Ba	1.21Ba	1.08a
Mid	1.04Aa	1.10Aa	0.98Ab	1.04a
High	1.03Aa	1.18Bb	1.04ABb	1.08a
Pooled	1.01A	1.13B	1.08AB	*
Total biomass				
Low	7.60Aa	10.10Ba	11.00Ca	9.51a
Mid	8.11Aa	10.72Ba	12.46Cb	10.43b
High	7.99Aa	11.74Bb	14.33Cc	11.35c
Pooled	7.90A	10.85B	12.60C	**

Note: Individual treatment means (n = 6) followed by different letters (upper case for C_T levels, lower case for cation levels) are significantly different ($\alpha \le 0.05$) as determined by Duncan's Multiple Range Test applied to individual one-way ANOVAs. Main effects (from two-way ANOVAs) of C_T and cation treatments are similarly indicated for values pooled over cation levels and C_T levels, respectively.

* Denotes a significant ($\alpha \le 0.05$) C_T × cation interaction.

** Denotes a significant ($\alpha \le 0.01$) C_T × cation interaction.

Table A4

Treatment Means and Statistical Comparisons of Plant Growth Parameters (g Ash-Free Dry Weight) Among Treatments in the Low D/z Experiment

Cation Level	C_T Level Low	 Mid	 High	 Pooled
Ash-free shoot biomass				
Low	6.11Aa	8.49Ba	9.23Cb	7.94a
Mid	6.51Aa	9.03Bab	9.71Bb	8.42a
High	6.37Aa	9.58Cb	8.33Ba	8.10a
Pooled	6.33A	9.03B	9.09B	**
Ash-free root biomass				
Low	0.76Aa	0.87Ba	0.96Bb	0.87a
Mid	0.83ABa	0.88Ba	0.78Aa	0.83a
High	0.82Aa	0.94Aa	0.83Aa	0.86a
Pooled	0.80A	0.90B	0.86AB	*
Ash-free total biomass				
Low	6.87Aa	9.36Ba	10.19Cb	8.81a
Mid	7.33Aa	9.91Bab	10.49Cb	9.25a
High	7.19Aa	10.52Cb	9.16Ba	8.96a
Pooled	7.13A	9.93B	9.95B	**

Note: Individual treatment means (n = 6) followed by different letters (upper case for C_T levels, lower case for cation levels) are significantly different ($\alpha \leq 0.05$) as determined by Duncan's Multiple Range Test applied to individual one-way ANOVAs. Main effects (from two-way ANOVAs) of C_T and cation treatments are similarly indicated for values pooled over cation levels and C_T levels, respectively.

* Denotes a significant ($\alpha \leq 0.05$) C_T × cation interaction.

** Denotes a significant ($\alpha \leq 0.01$) C_T × cation interaction.

Table A5

Treatment Means and Statistical Comparisons of Plant Shoot Nutrient Concentrations ($mg \cdot g^{-1}$ Ash-Free Dry Weight) Among Treatments in the Low D/z Experiment

	C_T Level			
Cation Level	Low	Mid	High	Pooled
Shoot N				
Low	11.3Aa	7.4Ba	6.1Ca	8.3a
Mid	10.2Ab	6.7Ba	5.9Ba	7.6a
High	10.3Aab	5.7Cb	6.8Bb	7.6a
Pooled	10.6A	6.6B	6.3B	
Shoot P				
Low	4.95Aa	3.76Ba	3.34Cab	4.01a
Mid	4.84Aa	3.46Ba	3.25Ba	3.85a
High	4.86Aa	3.53Ba	3.59Bb	4.00a
Pooled	4.88A	3.58B	3.39B	
Shoot K				
Low	20.0Aa	11.7Ba	11.4Ba	14.3a
Mid	19.2Aa	14.0Bb	13.0Bc	15.4a
High	10.5Aa	12.5Cab	15.8Bb	16.3a
Pooled	19.9A	12.7B	13.4B	

Note: Individual treatment means (n = 2) followed by different letters (upper case for C_T levels, lower case for cation levels) are significantly different ($\alpha \leq 0.05$) as determined by Duncan's Multiple Range Test applied to individual one-way ANOVAs. Main effects due to C_T and cation treatments are similarly indicated for values pooled over cation levels and C_T levels, respectively.

APPENDIX B: PHOTOSYNTHETIC $CaCO_3$ PRECIPITATION

1. In order to obtain photosynthetic rates of plants under conditions resulting in $CaCO_3$ precipitation, the authors elected to repeat the photosynthesis experiments with modifications to include measurement of Ca and pH in addition to inorganic carbon C_T. At each sampling interval an additional 10-mℓ water sample was collected in a disposable syringe. This sample was split and pH and Ca were determined as described earlier. In order to minimize the effect of the larger sample volume requirements, an additional vessel containing the experimental solution was included in the recirculation loop of the experimental apparatus. This served the dual purpose of increasing the system volume and slowing the rate of change of water chemistry parameters. Total sample volume withdrawn during the period was <5 percent of the system volume. This slight change in volume did not require correction.

2. Results obtained in the high C_T, low cation solution are presented in Figure B1, graphs A, B, C, and D. The C_T declined almost linearly over the measurement period, while Ca remained virtually constant at 9.5 ± 0.1 $mg \cdot \ell^{-1}$ (0.24 mM). Concurrent with the reduction in C_T, pH increased to 10.0 by the end of the measurement period. The ion activity product $[Ca^{++}][CO_3^{--}]$ increased from a level near saturation to over ten times the solubility product. In spite of this supersaturation, precipitation of $CaCO_3$ did not occur. Alkalinity, calculated from C_T and pH, remained virtually constant ($\bar{x} = 1.74 \pm 0.01$ S.E.). Acidity, also calculated from C_T and pH, declined almost linearly over the measurement period in a manner similar to that for C_T.

3. Photosynthetic C_T uptake of H_2CO_3 or HCO_3^-, in the absence of associated $CaCO_3$ precipitation, results in a decline in acidity while alkalinity remains constant--the change in acidity equaling 2 meq/mM change in C_T. In the high C_T, low cation solution, precipitation of $CaCO_3$ did not occur, and alkalinity (calculated from C_T and pH) remained constant (Figure B1, graph D). Thus, in short-term experiments such as these, changes in alkalinity due to cation uptake can be neglected.

4. Results obtained in the high cation, high C_T solution are presented in graphs E, F, G, and H of Figure B1. The C_T declined linearly over the first 60 min, then declined exponentially over the remainder of the measurement period. Calcium remained constant at 59.4 ± 0.2 $mg \cdot \ell^{-1}$ (1.48 mM) during the first 60 min, then declined exponentially coincident with the exponential decrease in C_T. Both pH and the ion activity product $[Ca^{++}]$ $[CO_3^{--}]$ increased during the first 60 min, then declined as $CaCO_3$ precipitated.

Alkalinity, calculated from C_T and pH, declined in a manner similar to that of Ca. Acidity declined almost linearly over the measurement period. By calculating the quantity of C_T precipitated as $CaCO_3$ and adding this to the measured value of C_T, the decline in C_T due to photosynthesis can be plotted (Figure B1, graph E). This correction for precipitation of $CaCO_3$ results in a nearly linear decrease in C_T due to photosynthetic C_T uptake in contrast with the exponential decrease in C_T due to the combined effects of photosynthesis and $CaCO_3$ precipitation.

5. Precipitation of $CaCO_3$, in the absence of photosynthetic C_T uptake, results in a decline in alkalinity while acidity remains constant--the change in alkalinity equaling 2 meq/mM change in C_T. In the high C_T, high cation solution, precipitation of $CaCO_3$ resulted in changes in alkalinity (Figure B1, graphs E and F). In this experiment alkalinity could be calculated from C_T and pH as well as from changes in Ca. Linear regression of the two measures of alkalinity resulted in a slope of 1.00^2, an intercept of 0.01^1, and an r^2 of 0.98. The close agreement between the two indicates that the change in alkalinity was entirely attributable to precipitation of $CaCO_3$.

6. Since acidity is unaffected by $CaCO_3$ precipitation, and alkalinity is unaffected by photosynthetic C_T uptake, these parameters should be useful in determining the individual rates of each process, even when both are occurring simultaneously. One obvious advantage of this approach is that it does not require analysis of solution Ca, only C_T and pH.

7. Plots of the change in acidity against the change in C_T due to photosynthesis should result in a slope of 2 meq acidity/mM C_T. Data obtained in the high C_T, low cation solution, where $CaCO_3$ precipitation did not occur, are plotted in Figure B2. These data are in close agreement with the theoretical slope ($m = 1.9$, $r^2 = 0.996$), indicating that acidity can be used as a measure of photosynthetic C_T uptake under conditions where $CaCO_3$ does not precipitate.

8. Plots of the change in alkalinity against the change in C_T or Ca due to $CaCO_3$ precipitation should likewise result in a slope of 2 meq/mM change C_T or Ca. Data obtained from the high C_T, high cation solution, where $CaCO_3$ precipitation did occur, are plotted in Figure B3, graph A. The close agreement between the calculated slope (2.0) and the theoretical slope indicates that alkalinity (calculated from C_T and pH) is an accurate measure

of $CaCO_3$ precipitation, even during photosynthetic C_T uptake. Similarly, linear regression of acidity against the change in C_T due to photosynthesis ($\Delta C_T - \Delta Ca$) results in a slope of 1.9, closely approximating the theoretical slope (Figure B, graph B). These results indicate that the parameters alkalinity and acidity can be used to differentiate the effects of $CaCO_3$ precipitation and photosynthetic C_T uptake on measured changes in C_T and pH. This method allows for the simultaneous determination of rates of photosynthesis and $CaCO_3$ precipitation under conditions where photosynthetic C_T uptake results in the precipitation of $CaCO_3$, a fairly common occurrence in hard waters.

9. The similarity of the slopes of alkalinity and acidity during photosynthesis in the high cation, high C_T solution (Figure B1, graph H) indicates that both the rate and quantity of $CaCO_3$ precipitation are similar to those for photosynthetic C_T uptake. These data are plotted in Figure B4. Photosynthetic C_T uptake initially exceeds $CaCO_3$ precipitation as can also be seen in Figure B1. However, as photosynthesis continues, the rate of precipitation increases and the two processes appear to be closely coupled. The slope calculated from linear regression ($r^2 = 0.998$) is 0.96 mM C_T precipitated per mM C_T assimilated in photosynthesis. This slope is in close agreement with the ratio of 0.90 calculated for this solution from carbonate equilibria and the solubility product for $CaCO_3$. It should be noted that the aberrant data points to the lower left in Figure B4 correspond to the period prior to the occurrence of steady-state conditions with respect to $CaCO_3$ precipitation in relation to photosynthetic C_T uptake (Figure B1, graphs G and H). These data would thus not be expected to fit equilibrium calculations. The principles applied here might be used to predict the relationship between $CaCO_3$ precipitation and photosynthetic C_T uptake in solutions of various alkalinity, C_T, and Ca^{++} levels.

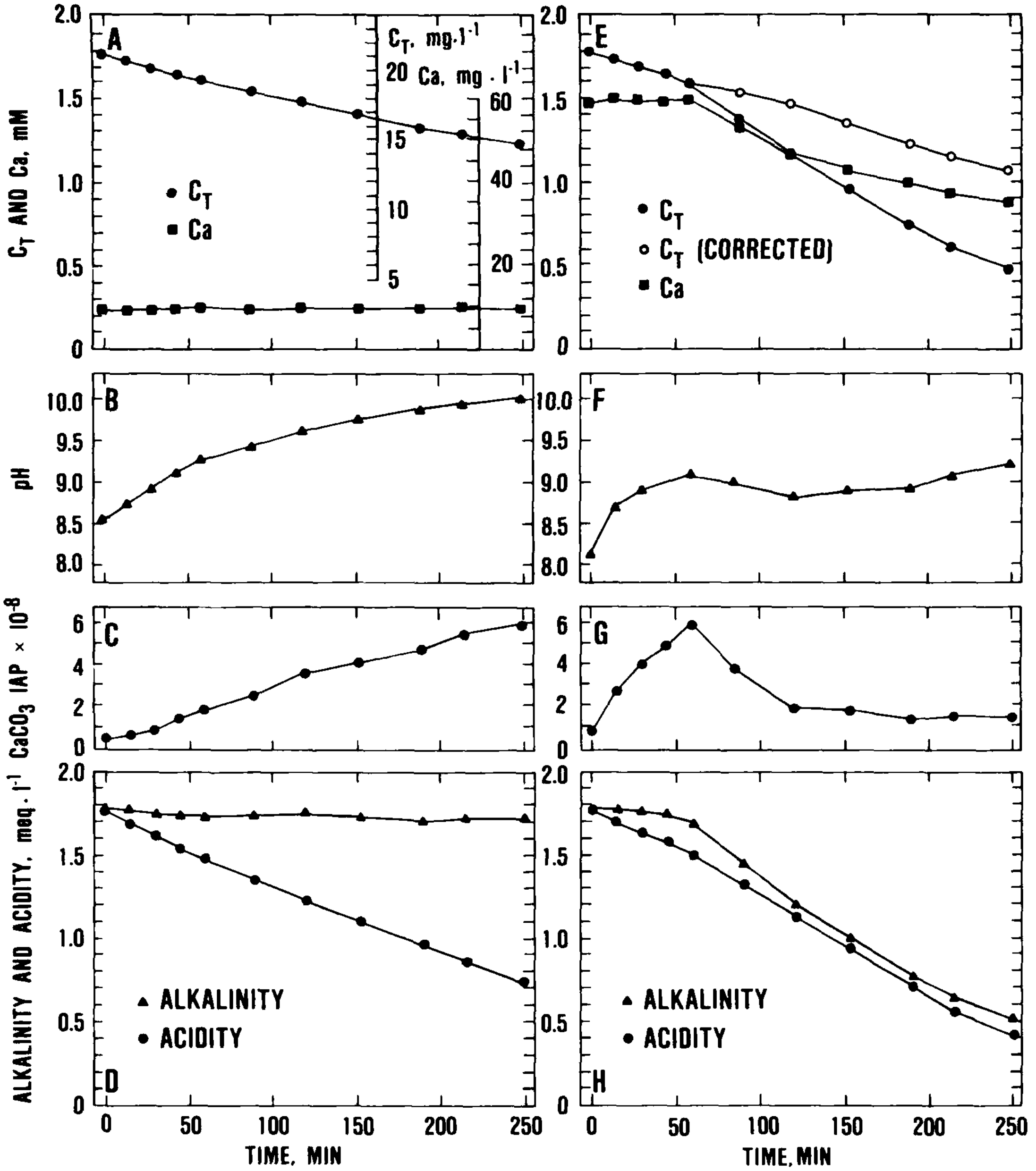

Figure B1. Temporal changes in C_T, Ca, pH, $CaCO_3$ ion activity product (IAP), alkalinity, and acidity during photosynthesis of *M. spicatum* in solutions containing the high level of C_T and the low (graphs A, B, C, D) or high (graphs E, F, G, H) level of cations. Corrected C_T values (graph E) are the sum of measured C_T plus the quantity of C_T precipitated as $CaCO_3$. Dual scales (mg·ℓ$^{-1}$ and mM) are provided for C_T and Ca for stoichiometric comparison

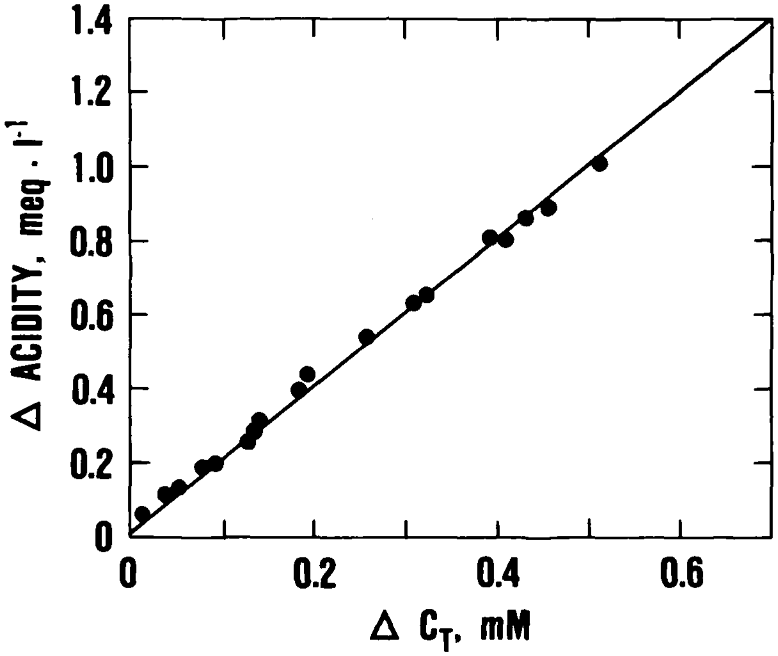

Figure B2. Relationship between Δ acidity and ΔC_T during photosynthesis of *M. spicatum* in the high C_T, low cation solution. The line represents the theoretical stoichiometric relationship

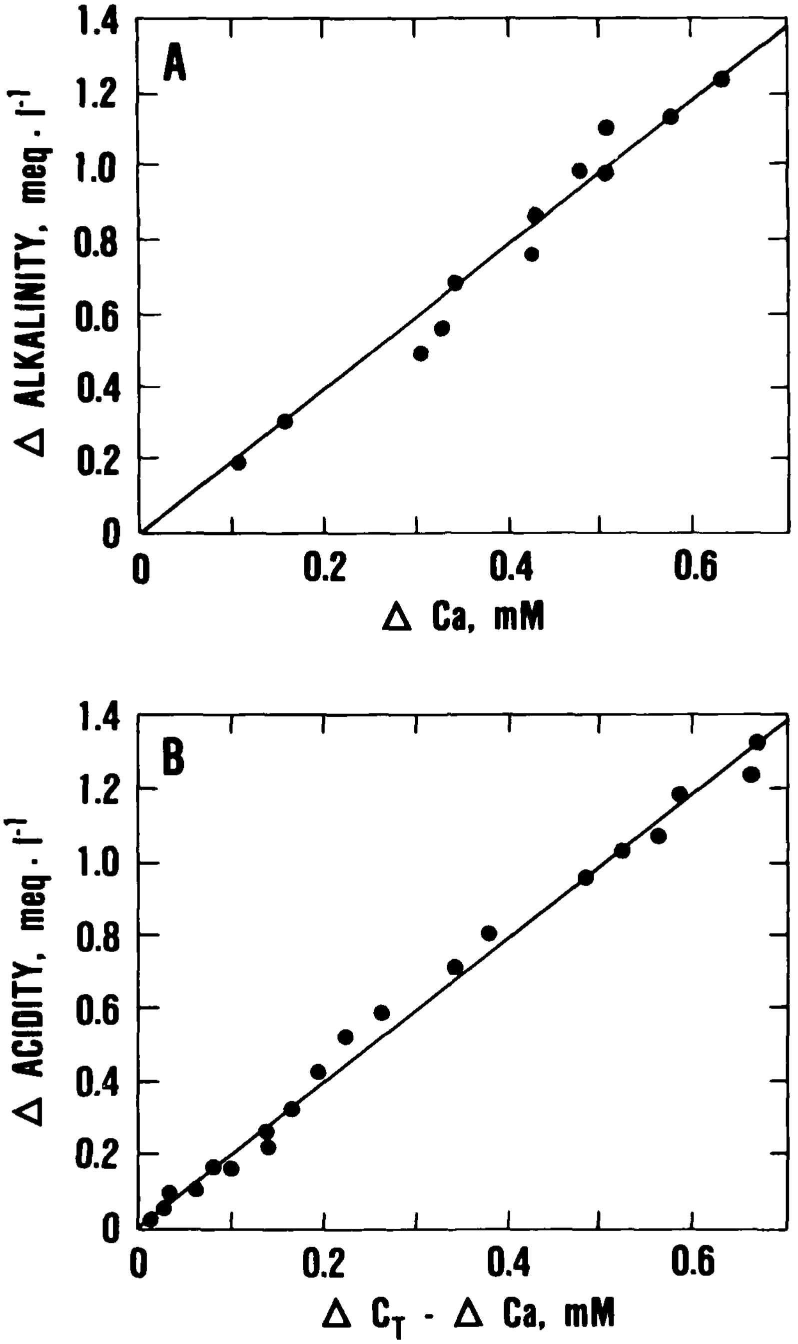

Figure B3. Alkalinity and acidity vs. ΔC_T and $\Delta C_T - \Delta Ca$. (A) Relationship between Δ alkalinity and ΔCa during photosynthetic $CaCO_3$ precipitation in the high C_T, high cation solution. The line represents the theoretical stoichiometric relationship. (B) Relationship between Δ acidity and $\Delta C_T - \Delta Ca$ (ΔC_T corrected for $CaCO_3$ precipitation) during photosynthesis of *M. spicatum* in the high C_T, high cation solution. The line represents the theoretical stoichiometric relationship

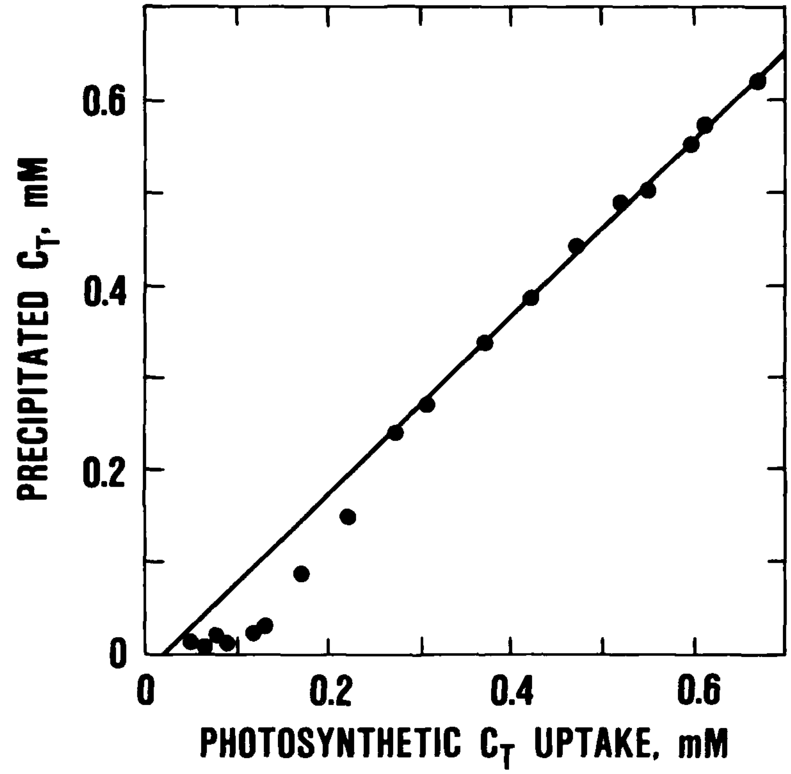

Figure B4. Relationship between $CaCO_3$ precipitation and photosynthetic C_T uptake during photosynthesis of *M. spicatum* in the high C_T, high cation solution. The regression line was calculated from the data obtained during steady-state conditions

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