

# Calcite in the metaphyton and sediments of the experimental wetland basins, 1999

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## Introduction

One of the most important functions of wetlands is their role as nutrient sinks (Mitsch and Gosselink, 1993). Their high productivity, low flow, and coupled anoxic and oxidized zones make them ideal for removal of nutrients such as nitrogen and phosphorus. Phosphorus (P) is a particularly important nutrient in freshwater ecosystems. It is often the limiting nutrient in freshwater ecosystems, and when present in excess can cause eutrophication of water bodies (Wetzel, 1983). Wetlands can act as sinks for phosphorus, thus reducing the nutrient loading of downstream water bodies. Constructed wetlands use the capability of these ecosystems to remove nutrients as a final polishing step in wastewater treatment (Kadlec and Knight, 1996).

Wetlands remove phosphorus from the water column through a variety of processes: sedimentation of particulate matter, which often contains phosphorus, direct uptake from the water column by plankton, periphyton, submersed aquatics and metaphyton, and in some cases, sorption with minerals precipitating in the water column (Scinto, 1997). Ecosystem metabolism is central to the direct uptake and sorption processes. In productive wetlands, aquatic primary production increases pH levels during the day, which may be enough to cause calcium carbonate, or calcite, to precipitate in wetlands fed by hardwater sources. While sedimentation and direct uptake of phosphorus have been studied in detail in wetlands, sorption with calcite remains a relatively unstudied mechanism for phosphorus immobilization.

This study examined calcite precipitation and associated phosphorus removal at the Olentangy River Wetland Research Park. An earlier article (Liptak and Mitsch, 1999) details the changes in water quality which occurred in the wetland basins. This article presents the results of the metaphyton biomass and sediment sampling.

## Methods

### *Site description*

This study was carried out at the Olentangy River Wetland Research Park (ORWRP), a 12-ha wetland research facility located on the campus of The Ohio State University, Columbus, Ohio; 83° 1' 81" W longitude, 40° 1' 59" N latitude.

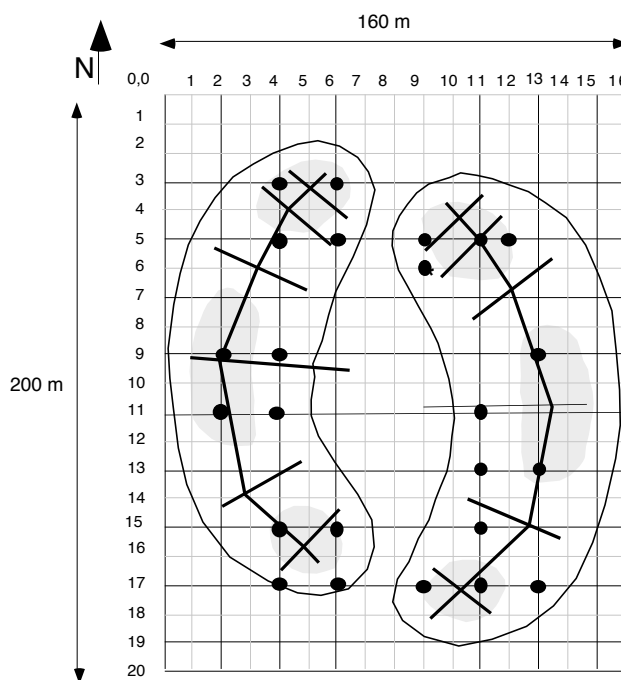


Figure 1. Locations of soil (•) samples in the two experimental marshes for this study (1996-99) with a permanent 10 m x 10 m grid system established in 1993. Prior to flooding (1993), soil samples were collected at grid intersections within the wetland basins (Nairn, 1996). In 1995, soil samples were collected at every other intersection (the intersection of dark lines within the basins)(Nairn, 1996).

### *Metaphyton analyses*

#### Field sampling

Algal biomass was sampled in the wetland basins at the height of the growing season (August 28, 1998) and early in the growing season (May 27, 1999). Sampling was done using a plastic 20-L bucket (inner diameter = 26.7 cm) with no bottom. The bucket was placed in an area with 100 percent algal cover and sunk into the sediment. All of the algae within the bucket was harvested by hand, placed into plastic bags, and transported on ice to the laboratory.

## Laboratory analyses

Upon transport to the laboratory, samples were washed in distilled water to remove any sediment, then placed into aluminum weighing dishes and dried in a drying oven at 105° C. After drying, samples were weighed for estimation of total dry biomass. Samples were ground in a Wiley mill before any further analysis.

Ground algae samples were analyzed for calcite and dolomite using an acid reaction method (Dreimanis, 1962). Dried sample was weighed into a reaction vessel, which was attached to a manometer, and 20 mL of 6M HCl with FeCl<sub>3</sub> was added to the sample. The temperature, air pressure, and volume of CO<sub>2</sub> evolved were measured at 30 sec and at 40 min using the manometer to determine the amount of calcite and dolomite in the algae, respectively.

The dried biomass samples were so fine that after weighing, they had to be wetted with distilled water prior to the addition of the 6M HCl to prevent them from floating on the acid and not all reacting at once. Since the time of reaction is the method of determining whether the carbonate is calcite or dolomite, results for the algae samples are presented in calcium carbonate equivalents. The carbonates associated with the algal samples reacted immediately upon acidification, and so the major proportion of carbonates associated with the algal mat was assumed to be calcite and not dolomite, which has a much slower reaction time. Sample values were checked by adding known amounts of ground CaCO<sub>3</sub> to algal samples, and these values were within 11% of calculated values. Subsamples were also placed into weighing jars and dried at 105° C overnight to determine the moisture content of the samples.

The remaining ground samples were then sent to the Service Testing and Research (STAR) Lab at the agricultural experiment station of The Ohio State University in Wooster for major elements analysis (P, K, Ca, Mg, Al, B, Cu, Fe, Mn, Mo, Na, S, Zn) using inductively coupled plasma (ICP) emission spectroscopy (APHA, 1989 Method 3120).

Microscopy and elemental analysis were conducted on algal samples at the Electron Microscopy and Microanalysis Lab, The Ohio State University, using a JEOL 820 scanning electron microscope. Oven-dried algal samples were affixed to carbon stubs using double-sided carbon tape. Copper tape was then affixed to the stub to prevent the sample from accumulating an electrical charge during examination. Samples were then coated with elemental C and examined in the microscope. Unground algal filaments were used for photomicrographs and elemental analysis, and ground samples were used for elemental analysis when whole samples were not available.

X-ray diffraction analysis was performed on some of the ground algal samples to verify the crystal form of the calcium carbonate found by scanning electron microscopy and acidification. Powder mounts of samples were prepared by backloading into aluminum holders. Samples were scanned in the range of 2–80° 2θ with a Philips XRG-3100 generator and a Philips Electronics PW 1316/90 wide range

goniometer equipped with a curved graphite monochromator and theta compensating slit. Step-scan data were collected with Databox Communication Software (Materials Data, Inc.)

## *Sediment analyses*

### Field sampling

Sediment cores were taken from the wetlands on May 5, 1999 to determine whether the wetland sediments had accumulated calcite or dolomite over the five years during which they had been flooded. Samples were taken at 12 locations in each basin (Figure 1), giving two core samples each for the deep and shallow areas in the inflow, middle, and outflow subbasins of each wetland at specific locations on a permanent 10 m x 10 m. When possible, samples were taken at points corresponding to previous soil samples taken in 1995 and 1993 (Nairn 1996), so that the current samples could be compared with the previous ones. Samples were taken 30 cm west of the selected grid locations whenever possible, using a 3.8 cm inner diameter PVC pipe, which was pushed into the sediment approximately 16 cm and then removed. The pipes were sealed with duct tape and transported back to the laboratory, where they were stored frozen until analysis.

To determine whether calcite or dolomite crystals were being imported into the wetlands through suspended sediment from the Olentangy River, water samples were collected from the river inflow pipe of Wetland 1 during the non-growing season (November 1999- January 2000) and during the growing season (August 2000). On each sampling date, 60L of water was collected from the inflow pipe, flocculated with MgCl<sub>2</sub>, the supernatant liquid poured off and the sample decanted into successively smaller containers until the suspended sediment could be dried at 105° C and analyzed for carbonates in the same method as the sediments cores.

## Laboratory analyses

Core samples were extruded from the PVC pipe and split lengthwise into two halves. One half of each core was used for analysis; the other half was stored frozen until a later date, when it was dried at 105° C, stored in a plastic bag, and placed in an archive for possible future use. The sediment at the wetlands consisted of two main layers: an upper layer of very soft muck, approximately 8 cm deep, and a lower clay layer. For the purposes of the analysis, the sample was split into these two layers.

The color of each sediment layer was determined using a Munsell Soil Color Chart (Kollmorgen Instruments Corporation, Baltimore, MD). A 5-mL subsample was taken of each layer, placed into tared crucibles, and the wet weight was determined. Subsamples were dried at 105° C for five days, cooled in a desiccator, and weighed to determine the dry weight. Samples were then ashed in a muffle furnace for 24 h at 550° C, cooled in a desiccator, and weighed to determine the ash weight. From these measurements, the bulk density, percent water, and percent

organic matter were estimated.

Dried sediment samples from 1999 (five years post-flooding), 1995 (one year post-flooding) and 1993 (pre-flooding) were ground to pass through a 1 mm sieve and analyzed for percent calcite and dolomite by acidification (Dreimanis, 1962). Dried sample was weighed into a reaction vessel, which was attached to a manometer, and 20 mL of 6M HCl with FeCl<sub>3</sub> was added to the sample. Temperature, air pressure, and volume of CO<sub>2</sub> evolved was measured at 30 sec and 40 min using the manometer to determine the amount of calcite and dolomite in the sediment, respectively. Subsamples were also placed into weighing jars and dried at 105° C overnight to determine the moisture content of the samples.

Ground sediment samples were analyzed at the STAR Lab in Wooster for pH, exchangeable P, cation exchange capacity, exchangeable Ca, Mg, and K, and 13 major elements (P, K, Ca, Mg, Al, B, Cu, Fe, Mn, Mo, Na, S, Zn) using inductively coupled plasma (ICP) emission spectroscopy (APHA, 1989 Method 3120).

Ground sediment samples from the inflow and outflow sites of the two basins were examined using scanning electron microscopy and microanalysis in the same manner as the algal samples. Sediment cores from the middle of the two basins were fixed in epoxy and then sliced lengthwise and coated with elemental C to provide 2 continuous core samples for examination along the depth profile. The form of calcium carbonate found in the sediment samples was confirmed using X-ray diffraction

of ground sediment samples.

## Statistical analyses

Because of the different methods used to collect the data, different statistical methods were used to analyze the data.

Because the sediment samples were taken at the same locations over a period of time, but each location could be considered independent of the others, sediment data were compared using paired t-tests ( $\alpha=0.05$ ). Algae samples were compared using a general linear model, as the number of samples varied between sampling dates.

## Results

### *Metaphyton*

Metaphyton biomass was highest in the middle subbasins for both sampling dates (Table 1). Estimates of total biomass showed that the middle subbasins had higher total algal biomass than inflow and outflow sites. This is due to the larger area covered by metaphyton in the middle subbasins, as the middle subbasins usually did not have the highest biomass values per unit area. Wetland 1 had more total algal biomass than did Wetland 2; this corresponds with field observations of the two wetlands. Nutrient levels in the middle subbasins are higher than in the outflow subbasins; however, nutrient levels were not significantly correlated with algal biomass, indicating that another factor, perhaps light, may be limiting the growth of algae.

Table 1. Algal biomass and percent cover in deepwater areas of experimental wetlands at Olentangy River Wetland Research Park, 1998-99. C= *Cladophora*, R=*Rhizoclonium*, H=*Hydrodictyon*, S=*Spirogyra*, NP= no macroalgae present. W1 = Wetland 1 (planted), W2 = Wetland 2 (naturally colonizing).

Date	Wetland	Location	Major algal spp.	Dry algal biomass g m <sup>-2</sup>	Area covered by algal mat m <sup>2</sup>	Total biomass kg
Aug 27, 1998	1	Inflow	NP	0	0	0
Aug 27, 1998	1	Middle	C, R, H, S	62 ± 7 (5)	1130	69.6
Aug 27, 1998	1	Outflow	C, R	70 ± 10 (6)	580	40.6
			W1 Total	1710	110.2	
Aug 27, 1998	2	Inflow	H	13 ± 2 (5)	120	1.6
Aug 27, 1998	2	Middle	C, R, H	58 ± 11 (5)	890	51.9
Aug 27, 1999	2	Outflow	R, C	19 ± 5 (5)	46	0.9
			W2 Total	1056	54.4	
May 24, 1999	1	Inflow	H	70 ± 18 (2)	580	40.6
May 24, 1999	1	Middle	H, C	78 ± 10 (2)	1870	144.9
May 24, 1999	1	Outflow	C	136 ± 11(2)	48	6.6
			W1 Total	2498	192.1	
May 24, 1999	2	Inflow	S, H	70 (1)	370	25.8
May 24, 1999	2	Middle	H	33 ± 3 (2)	1090	36.1
May 24, 1999	2	Outflow	H, S	23 ± 3 (2)	550	12.5
			W2 Total	2010	74.4	

Metaphyton showed significant amounts of calcium carbonates attached to their filaments, except for *Spirogyra*, which showed no evidence of any carbonates associated with its biomass. 1998 end-of-season samples showed a total calcium carbonate content of 30.5 kg in Wetland 1 and 16.1 kg in Wetland 2 (Table 2). Spring samples in 1999 showed higher amounts of calcium carbonates in Wetland 1 than in Wetland 2 (52.2 kg and 6.8 kg, respectively). This corresponds to observations that Wetland 2 developed algal cover at a slower rate than Wetland 1.

Scanning electron microscopy showed that the calcium carbonate materials associated with the algal mat were attached to and surrounding individual algal filaments (Figures 2 - 5). X-ray diffraction analysis confirmed the presence of crystalline calcite in the algal samples. Elemental analysis indicated the presence of small amounts of P, at an approximate molar ratio of  $10.2 \pm 1.2$  mmol P/mol Ca. This becomes a mass ratio of  $3.15 \pm 0.39$  mg P/g  $\text{CaCO}_3$ . Using these ratios, the carbonates in the metaphyton mat contained approximately 200 g P in the algae in summer 1998 and 185 g P in spring 1999 (Table 2). This is equivalent to 47 percent of the total P contained in the metaphyton mat in 1998 and 32 percent of the 1999 total. Therefore, P sorption with calcium carbonates is a significant sink for P, in some subbasins immobilizing more P in carbonates than is present in the algal biomass itself (Table 2).

### Sediments

Having shown that phosphorus is associated with calcium carbonate in the metaphyton mats, the sediments were then examined to see whether any carbonates from the algal mat were deposited into the sediments for more long-term storage.

### Sediment analyses

Two layers of sediments in the experimental wetlands could be easily distinguished, and were treated separately in analyses. The upper layer (generally the first 8 cm) was visibly different than the underlying layer. It was very dark and gelatinous, while the lower layer appeared similar to the parent upland soils at the ORWRP. Because of this, more physical and chemical changes were expected to occur in the upper layer than the lower sediment layer. The upper layer's bulk density was significantly lower, and percent organic matter significantly higher, than the lower layer (Table 3). The relative isolation of the lower sediments is shown by the fact that percent organic matter in the lower sediments remained unchanged from 1993 to 1999. The upper sediments were darker and had more variable soil color than did the lower sediments.

Calcite and calcium carbonate equivalent (CCE) concentrations in the sediments were not significantly different between basins for 1993, 1995, or 1999 samples;

Table 2. P associated with algal biomass and with calcium carbonate in deepwater areas of experimental wetland basins at the Olentangy River Wetland Research Park, 1998-99.

Date	Wetland	Location	Total P in		P in			% of total P as $\text{CaCO}_3$ -P	
			Algal biomass kg	$\text{CaCO}_3$ g/g dry biomass	$\text{CaCO}_3$ kg	P in $\text{CaCO}_3$ g	<sup>a</sup> algal mat g		algal biomass g
Aug 27, 1998	1	Inflow	0.0	0.00	0.0	0.0	0.0	0.0	
Aug 27, 1998	1	Middle	69.6	0.25	17.6	75	190	115	40
Aug 27, 1998	1	Outflow	40.6	0.32	12.9	55	60	5	91
		W1 Total	110.2	0.28	30.5	130	250	120	52
Aug 27, 1998	2	Inflow	1.6	0.00	0.0	0.1	12	12	1
Aug 27, 1998	2	Middle	51.9	0.31	15.9	68	158	90	43
Aug 27, 1998	2	Outflow	0.9	0.16	0.1	0.6	2.2	1.6	27
		W2 Total	54.4	0.30	16.1	69	172	103	40
		W1 and W2	164.6	0.28	46.6	199	422	223	47
May 24, 1999	1	Inflow	40.6	0.19	7.9	25	238	214	10
May 24, 1999	1	Middle	144.9	0.28	41.2	130	189	59	69
May 24, 1999	1	Outflow	6.6	0.47	3.1	10	5	0	
		W1 Total	192.1	0.27	52.2	164	432	268	38
May 24, 1999	2	Inflow	8.9	0.00	0	0	37	37	0
May 24, 1999	2	Middle	36.1	0.11	4.1	13	105	93	12
May 24, 1999	2	Outflow	12.5	0.22	2.7	9	13	4	66
		W2 Total	57.5	0.12	6.8	21	155	134	14
		W1 and W2	249.6	0.24	59.0	185	588	402	47

a) Average P/ $\text{CaCO}_3$  ratio =  $3.15 \pm 0.39$  mg P/g  $\text{CaCO}_3$

Table 3. Bulk density and percent organic matter of sediments in the experimental wetlands at the Olentangy River Wetland Research Park. Different letters indicate significant difference ( $p < 0.05$ ) between years. \* indicates significant difference ( $p < 0.05$ ) between upper (0-8 cm) and lower (8-16 cm) sediment layer in the same year. Data from 1993 and 1995 were taken from a previous study of the experimental wetland basins (Nairn, 1996).

Year	Bulk density, $\text{g cm}^{-3}$		Organic matter, %	
	0-8 cm	8-16 cm	0-8 cm	8-16 cm
1993	$1.31 \pm 0.037$ (24)a*	$1.19 \pm 0.020$ (24)a	$5.4 \pm 0.10$ (24)a	$5.5 \pm 0.07$ (24)a
1995	$0.69 \pm 0.028$ (24)b*	$0.99 \pm 0.033$ (24)b	$6.3 \pm 0.22$ (24)b*	$5.5 \pm 0.12$ (24)a
1999	$0.96 \pm 0.043$ (24)c*	$1.43 \pm 0.016$ (24)c	$9.4 \pm 0.62$ (24)c*	$5.5 \pm 0.11$ (24)a

Table 4. Accumulation rates for calcium carbonate minerals (calcite, dolomite, and calcium carbonate equivalent (CCE)) precipitated in the experimental wetland basins at the Olentangy River Wetland Research Park, 1994-1999. \* denotes accumulation rates significantly different from zero ( $\alpha = 0.05$ ).

Site	Calcite $\text{g m}^{-2} \text{ yr}^{-1}$	Dolomite $\text{g m}^{-2} \text{ yr}^{-1}$	CCE $\text{g-eq CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$
0-8 cm			
Inflow	$76.2 \pm 18.9$ (6)*	$48.2 \pm 10.0$ (6)*	$128.6 \pm 28.1$ (6)*
Middle	$97.0 \pm 25.9$ (8)*	$24.7 \pm 4.1$ (8)*	$123.8 \pm 29.4$ (8)*
Outflow	$30.4 \pm 14.4$ (8)	$9.6 \pm 5.0$ (8)	$40.8 \pm 18.5$ (8)
8-16 cm			
Inflow	$17.0 \pm 5.9$ (7)*	$12.2 \pm 6.4$ (7)	$30.3 \pm 10.9$ (7)*
Middle	$6.4 \pm 3.9$ (9)	$4.6 \pm 2.0$ (9)	$11.4 \pm 4.5$ (9)*
Outflow	$2.8 \pm 4.3$ (9)	$-4.9 \pm 2.2$ (9)	$-2.5 \pm 5.1$ (9)

Table 5. Calcite precipitation and deposition rates in wetlands and hardwater lakes.

Study site	$\text{CaCO}_3$ deposition rate $\text{g CaCO}_3 \text{ m}^{-2} \text{ d}^{-1}$	Author
experimental wetlands, OH	1.2	this study
Lake La Cruz, Spain	2.23	Rodrigo et al., 1993
Pyramid Lake, NV	4.2	Galat and Jacobson, 1985
Lake Constance, Germany	0.69 - 1.04	Rossknecht, 1980
Lawrence Lake, MI	1.22	Otsuki and Wetzel, 1974
laboratory algal cultures	avg = 1.05, max=1.63	Yates, 1996
Lake Constance, Germany	1.24	Stabel, 1989
Lake Wallensee, Germany	1.29 - 3.22	Jager and Rohrs, 1990
Lake Sempach, Switzerland	1 - 14	Weilenmann et al., 1989
Lake Zurich, Switzerland	1 - 6	Weilenmann et al., 1989
Fayetteville Green Lake, NY	1.45	Brunskill, 1969
Green Lake, Jamesville NY	4.85	Effler et al., 1981
Lake Attersee, Austria	0.68	Schroder et al., 1983

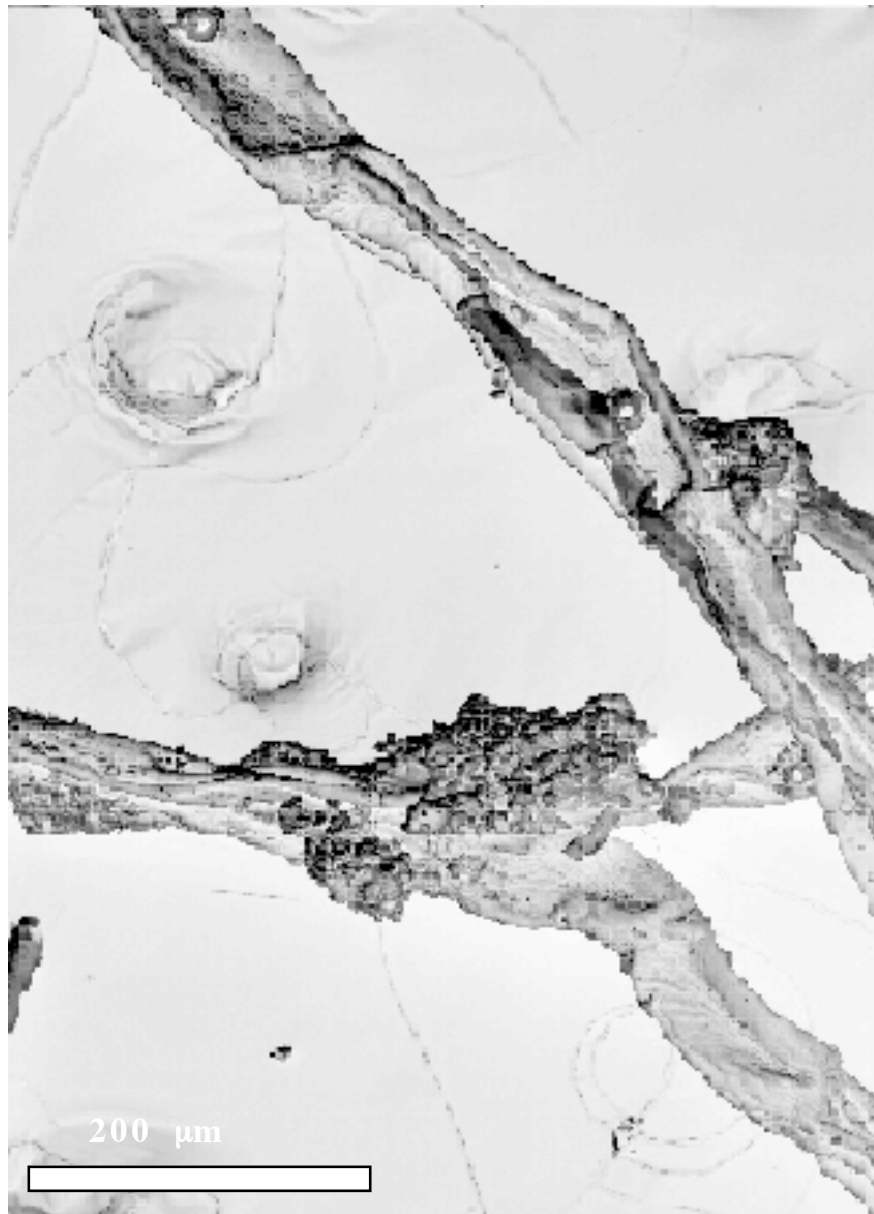


Figure 2. Scanning electron micrograph of *Cladophora* filament encrusted with calcium carbonate. Sample was taken in mesocosm 46 on September 8, 1998.

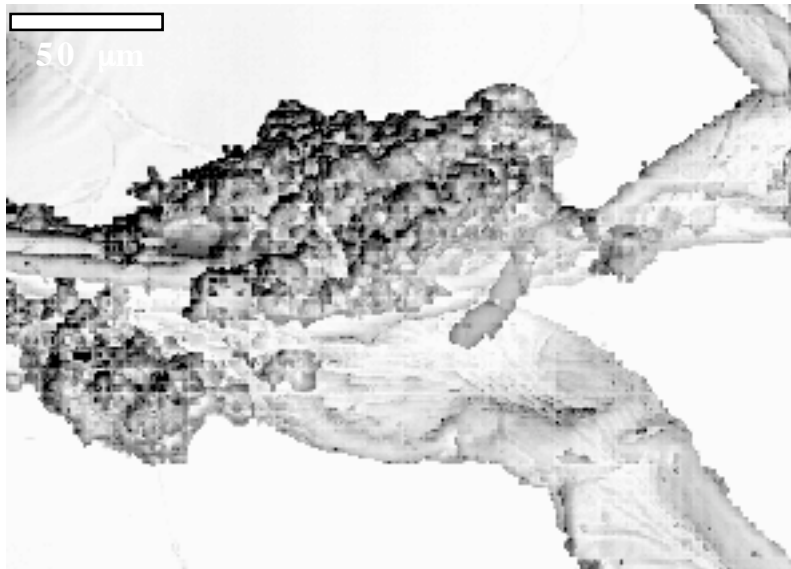


Figure 3. Detail of *Cladophora* filament encrusted with calcium carbonate. Sample was taken in mesocosm 46 on September 8, 1998.

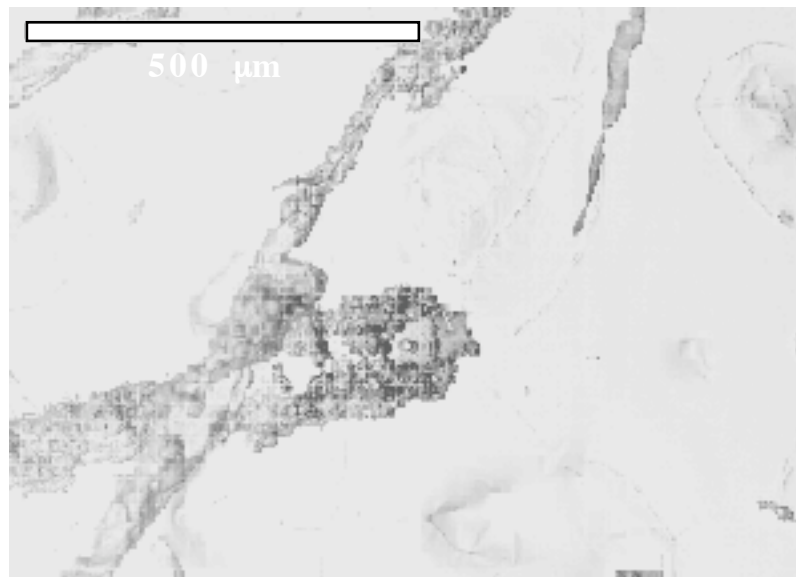


Figure 4. Calcium carbonate in algal sample taken in mesocosm 46 on September 8, 1998. This calcite formed around an algal filament, which afterwards decayed, leaving only the calcite.

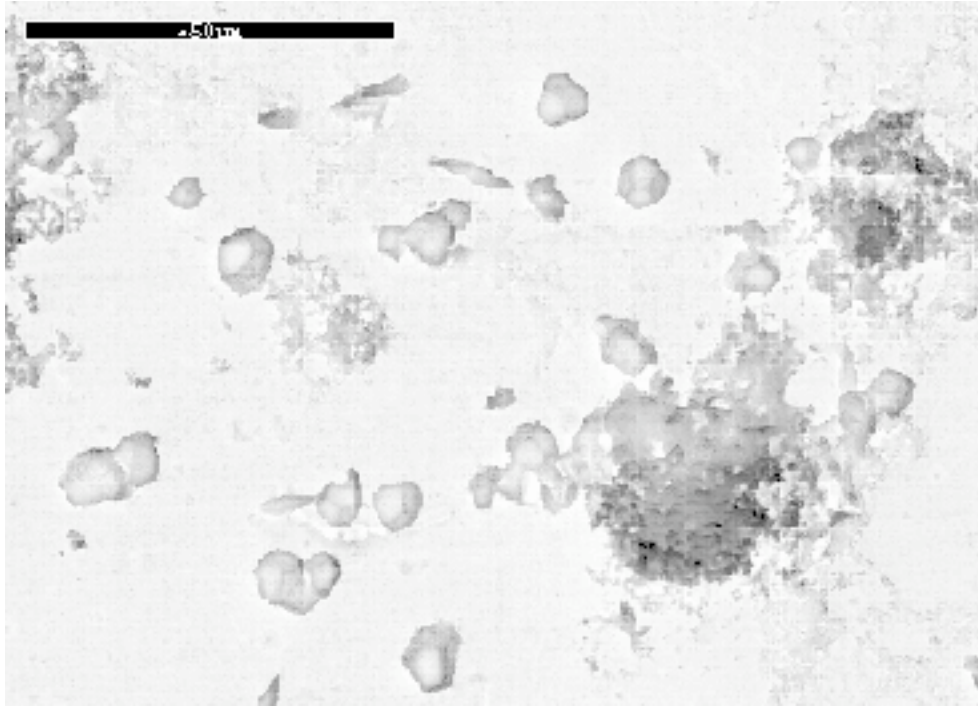


Figure 5. Calcite in sediment samples taken in the experimental wetland basins at the Olentangy River Wetland Research Park in 1998. Both the well-defined crystals and the finer-grained precipitate are calcium carbonate. Algal filaments were found to be associated with only the fine-grained form, not the larger crystals.



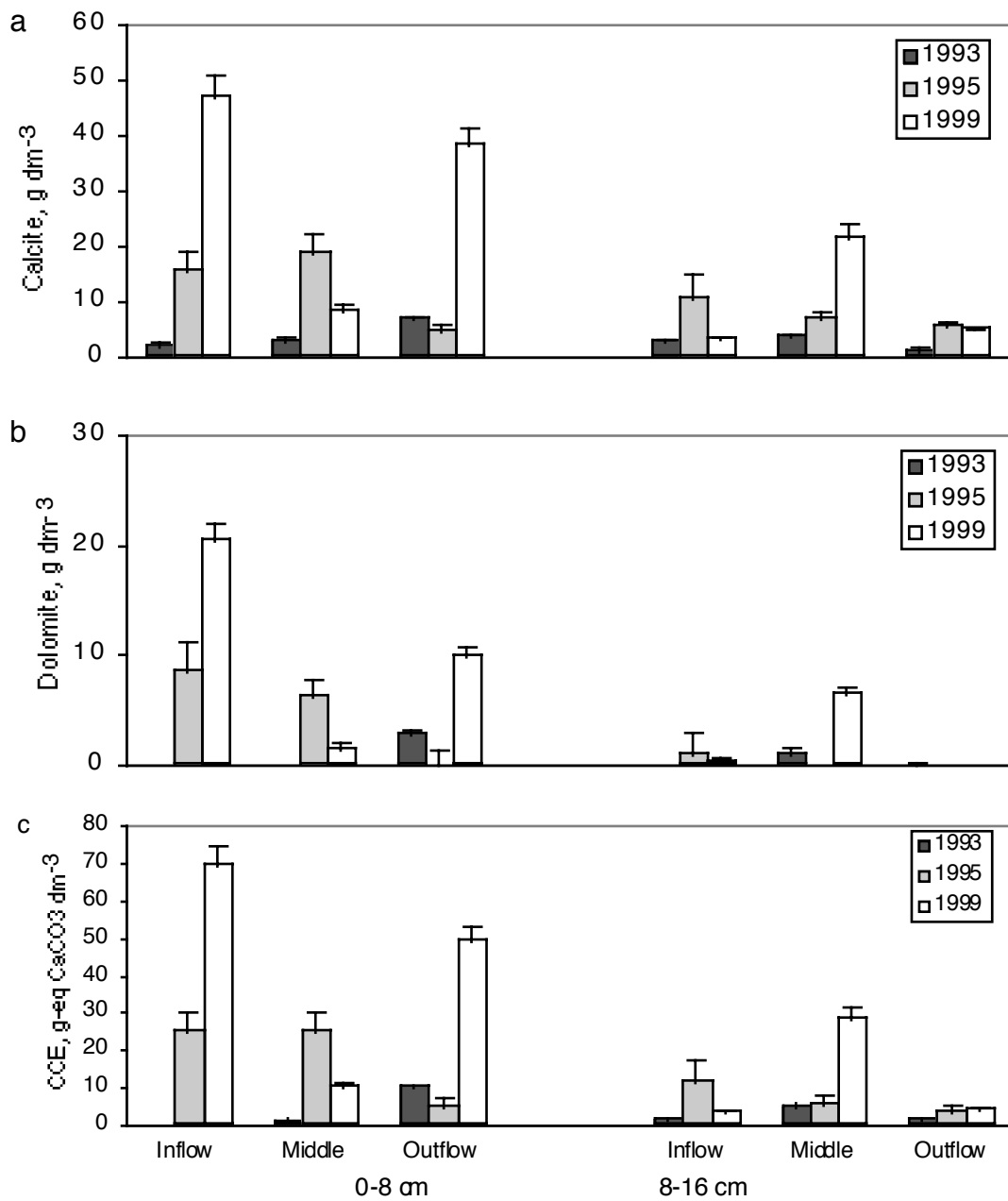


Figure 6. Calcite, dolomite, and calcium carbonate equivalent in the sediments of the experimental basins at the Olentangy River Wetland Research Park in 1993, 1995 and 1999. Archived samples from a previous study (Nairn, 1996) were used for the calcium carbonate analysis of 1993 and 1995 samples.

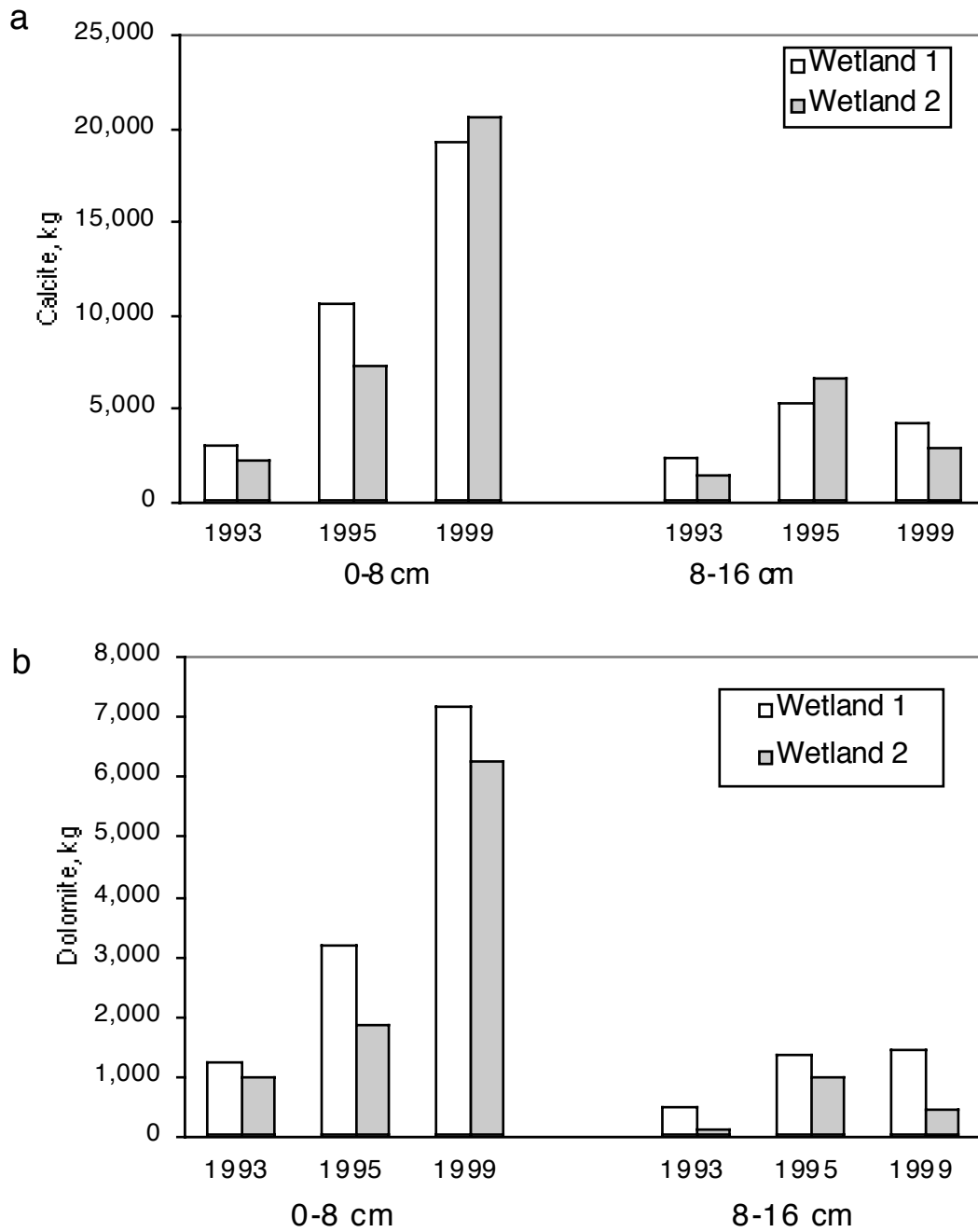


Figure 7. Calculated totals of calcite and dolomite contained in the sediments of the experimental wetland basins at the Olentangy River Wetland Research Park in 1993, 1995 and 1999.

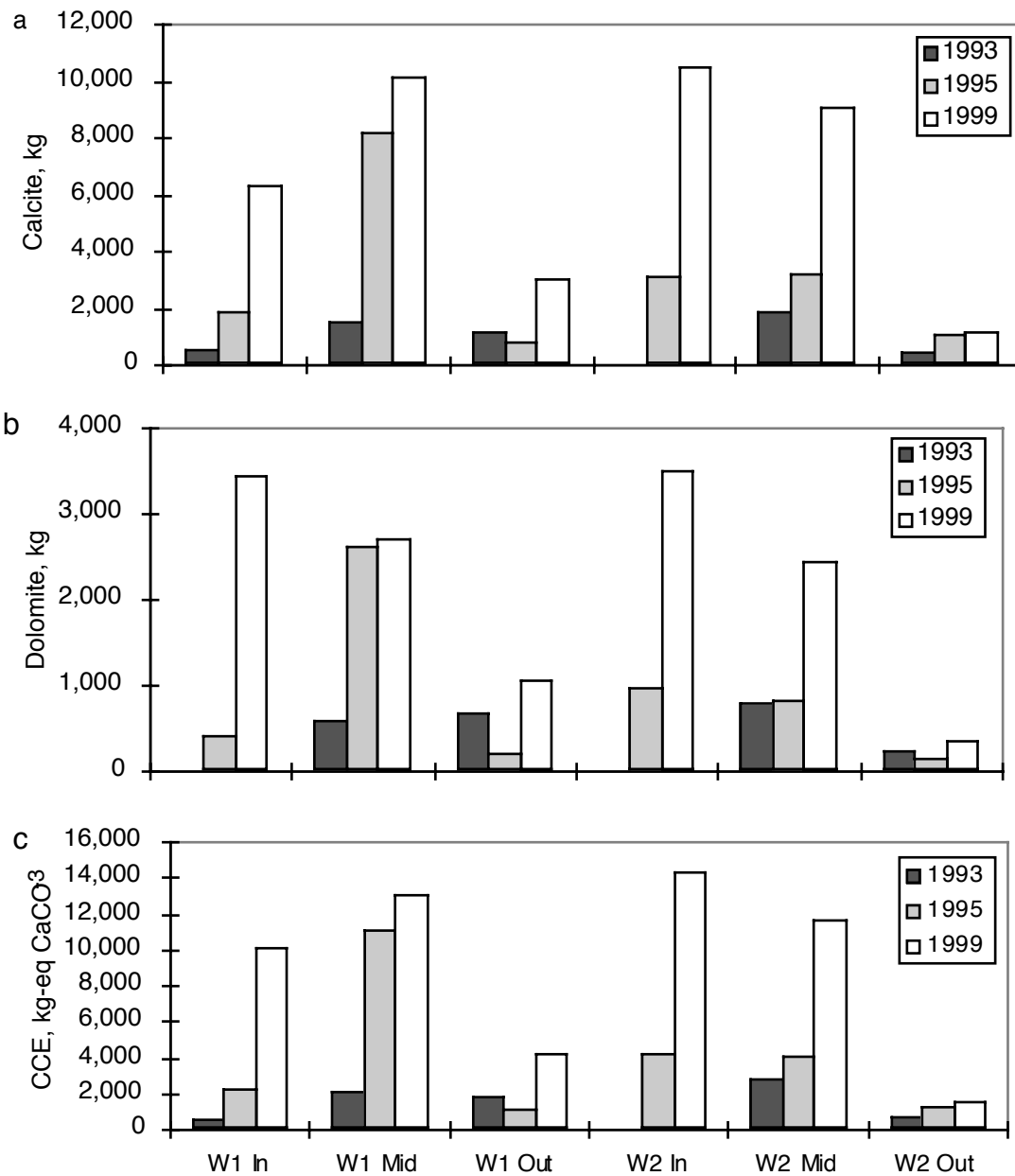


Figure 8. Calculated totals of calcium carbonate minerals in the sediments of the Olentangy River Wetland Research Park prior to flooding (1993), 17 months post-flooding (1995), and 5 years post-flooding (1999).

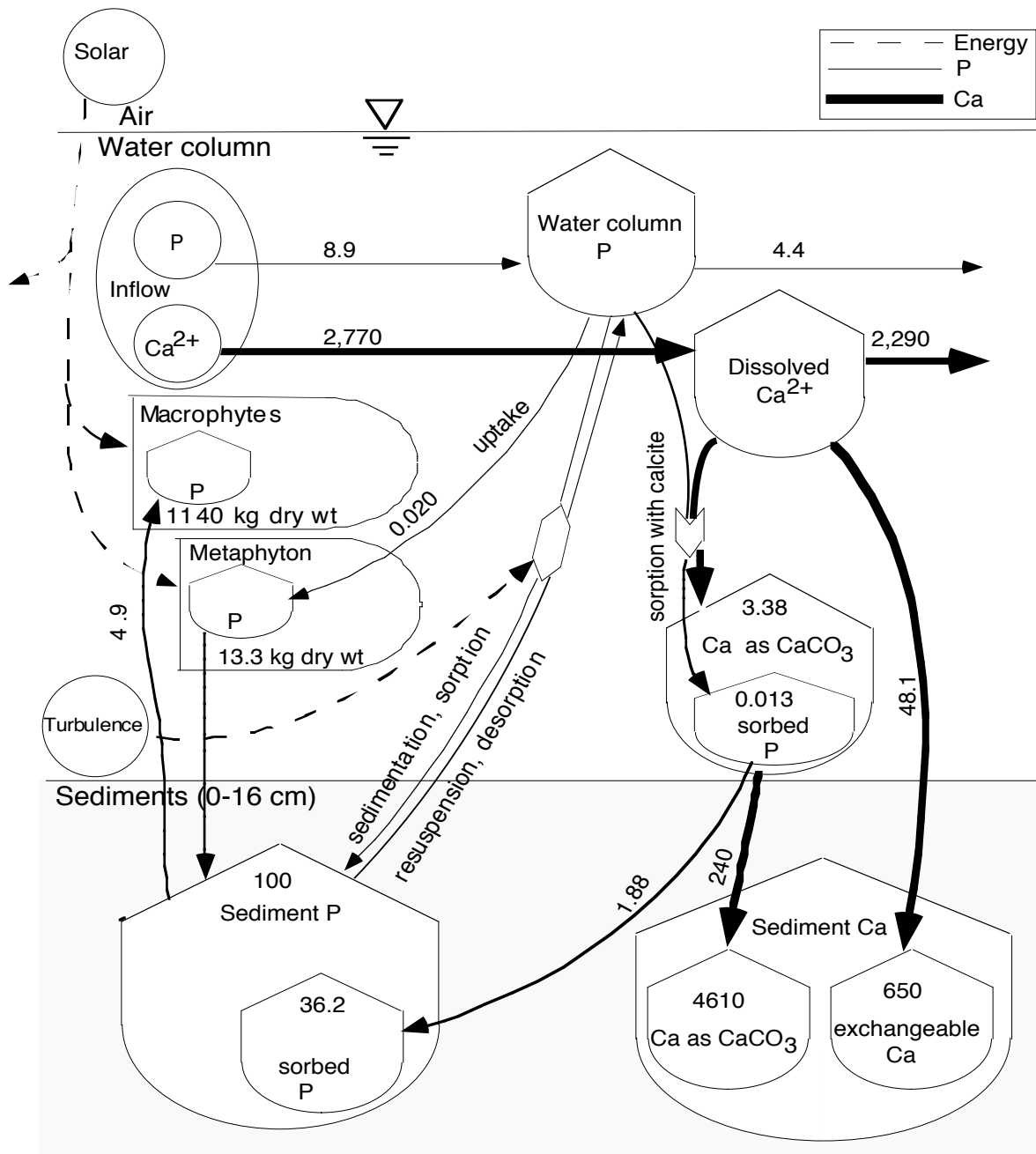


Figure 9. Diagram of the flows of calcium and phosphorus in an average experimental wetland basin in 1998. Flows are in g m<sup>-2</sup> yr<sup>-1</sup>, and storages are in g m<sup>-2</sup>.

dolomite was significantly higher in Wetland 1 in 1995, but otherwise showed no significant differences between basins (Figure 6). The upper and lower sediment layers were not significantly different in 1993 except for calcite in Wetland 1. In 1995, Wetland 1 showed significantly higher calcite, dolomite and CCE in the upper sediment layer than Wetland 1, while Wetland 2 showed significantly higher dolomite in the upper sediment layer. In 1999, calcite, dolomite and CCE were significantly higher in the upper sediment layer than the lower sediment layer (Figure 6).

Comparing between years, calcite, dolomite and CCE in the sediments increased significantly from 1993 to 1995 and from 1995 to 1999 in the surface sediments at the inflow and middle subbasins. At the outflow, dolomite and CCE increased from 1995 to 1999, but no other differences in the surface sediments were significant. The lower sediment layer showed significant increases in calcite and CCE at the inflow subbasin from 1995 to 1999. The lower sediment layer at the middle subbasin showed a significant increase in CCE from 1995 to 1999 and an increase in calcite and CCE from 1993 to 1995. The lower layer at the outflow showed no significant changes in carbonates (Figure 7).

Calcite, dolomite, and total carbonate accumulation rates were calculated from the change in concentration of carbonates in the sediments from the initial samples in 1993 to the 1999 samples (Table 4). The amount of carbonates in the sediments was assumed to be constant from 1993 to initial flooding in March 1994. The inflow and middle subbasins had higher accumulation rates than the outflow subbasins. Carbonate accumulation was higher in the upper sediment layer than in the lower sediment layer.

The average calcite concentration in the suspended sediments entering the wetlands during the non-growing season was  $0.013 \pm 0.069$  percent by mass ( $n=6$ ), while dolomite averaged  $10.4 \pm 7.9$  percent by mass ( $n=6$ ). During the growing season, calcite concentrations were  $1.06 \pm 0.681$  percent by mass ( $n=3$ ), while dolomite averaged  $2.32 \pm 0.576$  percent by mass ( $n=3$ ). Calcite concentrations were not significantly different from zero during the growing and non-growing seasons. Therefore, it is unlikely that the calcite found in the wetland sediments is imported from the river. However, dolomite concentrations were significantly different from zero in the growing season samples, indicating that the dolomite in the sediment could be imported from the Olentangy River.

The total mass of calcite and dolomite in the sediment of each wetland basin was calculated for 1993, 1995, and 1999. While these calculated totals cannot be compared statistically, both calcite and dolomite increased with time. From 1993 to 1999, over 16,000 kg of calcite was deposited into the surface sediments of Wetland 1, and over 18,000 into the surface sediments of Wetland 2 (Figure 7). Calcite in the lower clay layer of sediments increased as well, increasing by over 1800 kg in Wetland 1 and over 1400 kg in Wetland 2. Dolomite showed similar patterns, increasing by over 5000 kg in the surface sediments in both basins. Dolomite

in the lower clay layer of sediments increased by over 900 kg in Wetland 1 and over 300 kg in Wetland 2.

The spatial pattern of carbonate deposition shows that the inflow and middle subbasins deposit more calcite and dolomite than the outflow subbasins (Figure 8). The levels of calcite and dolomite in the inflow areas are puzzling, because there was very little macroalgal biomass and low GPP in the inflow subbasins during the years of the study. This precipitation could be the result of decreased calcite solubility due to the warming of the water in the first subbasin or other physical factors, or to production of macrophytes such as *Potamogeton natans*, which are common in the inflow areas. Plants of the genus *Potamogeton* have been shown to precipitate calcite on the surfaces of their leaves (Prins et al., 1980).

Scanning electron microscopy showed calcite inclusions in the upper sediments. However, the concentrations of P in the calcite could not be determined due to interference effects of the surrounding sediment matrix. Assuming that the calcite in the sediment is completely due to algal-induced precipitation, and that the ratio of P to calcite remained similar, 57 kg of P were removed from 1993 to 1999 through sorption with calcite deposited into the sediments of Wetland 1, and 62 kg of P were retained in Wetland 2.

## Discussion

A comparison of the rate of calcite deposition into the sediments of the full-scale wetlands to those for eutrophic hardwater lakes shows a remarkable similarity in deposition rates (Table 5). The study site's estimated calcite deposition rate of  $1.2 \text{ g CaCO}_3 \text{ m}^{-2} \text{ d}^{-1}$  is similar to rates reported by Stabel (1989) and Otsuki and Wetzel (1974) for eutrophic hardwater lakes ( $1.24$  and  $1.22 \text{ g CaCO}_3 \text{ m}^{-2} \text{ d}^{-1}$ , respectively), although precipitation rates as high as  $14 \text{ g CaCO}_3 \text{ m}^{-2} \text{ d}^{-1}$  have been reported in an Austrian eutrophic hardwater lake (Weilenmann et al. 1989). The similarity in calcite precipitation rates between hardwater wetlands and eutrophic hardwater lakes suggests that even though different algal genera are involved, the calcite precipitation process is similar in both these ecosystems. Calcite precipitation may be a more widespread result of photosynthesis in productive hardwater wetlands than is currently known, and may play a significant role in P immobilization. Calcite precipitation may play a role in immobilization of other chemical constituents in wetlands as well, such as dissolved organic carbon and metals.

Concentrations of P on the surface of the calcite crystals in the hardwater experimental wetlands were  $7.88 \text{ mg P/g Ca}$ , higher than those reported for eutrophic hardwater lakes (Table 6). This could be due to higher P concentrations in the water column of the research wetlands than in the lakes. Wu and Mitsch (1998) found average Ca concentrations of  $137 \text{ mg Ca/g dry weight}$  in the study site, and P concentrations in the metaphyton mat of  $0.5 - 4 \text{ mg P/g dry weight}$ . Assuming that 50% of the P found in the metaphyton mat was coprecipitated with calcite, calculated P/Ca ratios ranged

from to 1.8 - 14.6 mg P/g Ca. No seasonal difference was found in the P/Ca ratio for this study; however, other researchers have found seasonal differences in this ratio due to changes in water column P concentrations (Jager and Rohrs, 1990; Rossknecht, 1980). Kleiner (1988) found P concentrations of 3.44 mg P/g Ca in Lake Constance, Germany, while House and Donaldson (1986) found a range of 0.4–4 mg P/g Ca, with P coprecipitation increasing as P concentrations in the water column increased.

### *Ca and P flow through the experimental wetlands*

An ecosystem diagram for the study site is shown in Figure 9, detailing the flow of Ca and P through the ecosystem, and the relative role of calcite precipitation upon P retention. The diagram is for a 1 m<sup>-2</sup> area in an average experimental wetland basin at the study site in 1998. In this study, an average wetland basin retained 4.5 g P m<sup>-2</sup> yr, or 49% of the total P entering it. This is consistent with the results of Mitsch et al. (1995), who found P retention rates in constructed Illinois wetlands ranging from 0.4-2.9 g P m<sup>-2</sup> yr, and higher than the sustainable P removal rate of 1 g P m<sup>-2</sup> yr for natural wetlands found by Richardson and Qian (1999).

Over 1850 kg of calcium carbonates are deposited into the sediments of each basin yearly (240 g m<sup>-2</sup> yr). Assuming that the carbonates in the sediment are entirely from algal-induced precipitation, 1.88 g P m<sup>-2</sup> are deposited to the sediments through sorption and sedimentation of calcium carbonates. This is equal to 42% of the total P or 85% of the soluble reactive P retained in the wetlands, a significant percentage for a previously unstudied wetland pathway.

In lakes, sorption/coprecipitation with calcite is also significant as a mechanism for P removal. Shafer and Armstrong (1994) found that deposition of calcite-P accounted for 12-15% of the annual flux of P to the sediments in Lake Michigan. Danen-Louwerse et al. (1995) calculated that coprecipitation accounted for 50-85% of the total P removal over three years in the eutrophic Lake Veluwe. In the mesotrophic Lake Constance, 35% of the total P removal from the epilimnion occurred via coprecipitation (Kleiner, 1988). Jager and Rohrs (1990) found that 25% of the P removal from the water column in a eutrophic German lake occurred by coprecipitation. These similar results show that when calcite precipitation occurs in productive aquatic systems, sorption with calcite can be a significant sink for phosphorus. Because productive wetlands often have high P concentrations in the water column, and because of their compressed eutrophic zone and high primary productivity which can cause the high pH values necessary for calcite precipitation, eutrophic hardwater wetlands could remove large quantities of phosphorus through sorption with calcite.

Algal systems show promise for nutrient removal (Craggs et al., 1996), and are being tested as a possible final polishing step for removing P to the low levels necessary for

the Everglades Nutrient Removal Project (R.L. Knight, pers.comm.). The possibility of P removal through both direct uptake and sorption with calcite, as well as the short generation time and rapid growth of algal species makes algal removal of phosphorus an important emerging ecological engineering technique.

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APPENDIX A. CALCIUM CARBONATES IN WETLAND SEDIMENTS

Pre-flooding samples were taken in September 1993 (Nairn, 1996). Post-flooding samples were taken in September 1995 (Nairn, 1996) and May 1999. All samples were analyzed for carbonates in 1999. X,Y coordinates refer to permanent 10 m x 10 m grid set up in 1993 (See Figure 1). CCE = Calcium carbonate equivalent.

Sampling Date	x,y	Depth cm	Calcite %	Dolomite %	CCE %	Bulk density g dry wt/mL	Calcite mg/mL	Dolomite mg/mL	CCE mg/mL
1993	2,9	0-8	0.9	0.8	1.8	0.98	9.1	7.4	17.2
1993	2,9	0-8	0.7	0.8	1.5	0.98	6.6	7.8	15.0
1993	2,9	8-16	0.2	0.0	0.3	1.25	2.7	0.6	3.3
1993	2,11	0-8	0.9	0.4	1.3	1.45	13.4	5.5	19.4
1993	2,11	8-16	0.5	0.0	0.5	1.21	5.9	0.0	5.8
1993	4,3	0-8	0.7	-0.2	0.4	1.45	9.5	-2.9	6.3
1993	4,3	8-16	1.0	-0.2	0.9	1.25	12.9	-2.0	10.8
1993	4,3	8-16	0.2	0.3	0.5	1.25	2.2	3.3	5.8
1993	4,5	0-8	0.4	0.0	0.4	1.28	4.5	0.1	4.6
1993	4,5	8-16	0.7	-0.2	0.5	1.04	7.6	-2.5	5.0
1993	4,9	8-16	0.8	0.1	0.9	1.01	8.0	0.9	9.0
1993	4,9	0-8	0.7	-0.2	0.5	1.15	8.1	-2.5	5.4
1993	4,11	8-16	0.2	-0.6	-0.4	1.24	2.3	-6.8	-5.1
1993	4,15	0-8	0.9	0.4	1.3	1.18	11.0	4.5	15.8
1993	4,15	8-16	-0.3	-0.5	-0.8	1.24	-3.2	-6.7	-10.5
1993	4,17	8-16	-0.2	0.6	0.4	1.12	-2.4	6.8	5.0
1993	4,17	0-8	0.7	0.7	1.4	1.56	10.2	11.3	22.5
1993	6,3	8-16	-0.1	0.0	-0.1	1.39	-2.0	0.5	-1.5
1993	6,3	0-8	-0.1	-0.2	-0.4	1.25	-1.3	-2.9	-4.5

Sampling Date	x,y	Depth cm	Calcite %	Dolomite %	CCE %	Bulk density g dry wt/mL	Calcite mg/mL	Dolomite mg/mL	CCE mg/mL
1993	6,5	0-8	-0.2	-0.6	-0.8	1.48	-3.1	-8.5	-12.3
1993	6,5	8-16	-0.3	-0.7	-1.1	1.30	-3.4	-9.5	-13.7
1993	6,15	0-8	0.0	-0.3	-0.3	1.11	0.4	-3.4	-3.4
1993	6,15	8-16	0.5	0.0	0.5	1.34	6.9	0.0	6.9
1993	6,15	8-16	-0.2	0.0	-0.2	1.34	-2.8	0.7	-2.1
1993	6,17	8-16	0.1	-0.1	0.0	1.15	1.4	-1.6	-0.3
1993	6,17	8-16	-0.2	0.2	0.0	1.15	-2.4	2.6	0.4
1993	6,17	0-8	0.3	-0.2	0.1	1.53	5.2	-3.7	1.2
1993	9,5	8-16	-0.1	-0.2	-0.3	1.14	-1.0	-2.5	-3.7
1993	9,17	0-8	-0.1	-0.2	-0.4	1.41	-1.5	-3.2	-5.0
1993	11,5	0-8	-0.1	-0.9	-1.1	1.25	-0.8	-11.5	-13.3
1993	11,11	0-8	0.7	-0.2	0.4	1.62	10.7	-3.3	7.2
1993	11,11	8-16	0.5	-0.4	0.1	1.06	5.2	-3.8	1.0
1993	11,13	0-8	0.3	0.4	0.7	1.15	3.1	4.7	8.2
1993	11,13	8-16	-0.1	-0.2	-0.3	1.15	-1.6	-1.8	-3.6
1993	11,15	0-8	0.3	-0.2	0.0	1.03	3.0	-2.3	0.5
1993	11,15	8-16	0.8	0.0	0.8	1.28	9.7	0.0	9.6
1993	11,17	8-16	0.3	-0.2	0.0	1.05	2.9	-2.3	0.4
1993	11,17	0-8	-0.2	0.2	0.0	1.33	-2.1	2.3	0.5
1993	13,9	0-8	0.2	-0.2	0.0	1.39	3.4	-2.8	0.4
1993	13,9	8-16	0.1	-0.1	0.0	1.23	1.1	-1.4	-0.4
1993	13,13	8-16	-0.1	0.2	0.1	1.12	-0.9	2.1	1.4
1993	13,13	0-8	0.0	0.4	0.5	1.19	0.4	4.7	5.6
1993	13,17	0-8	0.2	0.2	0.4	1.11	2.2	2.5	4.9
1993	13,17	8-16	0.0	0.0	0.0	1.22	0.0	0.0	0.0
1995	2,9	0-8	2.7	1.8	4.7	0.780	21.1	14.3	36.7
1995	2,9	8-16	1.0	0.0	1.0	0.780	7.6	0.2	7.8
1995	2,11	0-8	1.5	0.9	2.5	0.760	11.2	7.1	18.9
1995	2,11	8-16	1.0	0.5	1.6	1.130	11.4	5.9	17.9
1995	4,3	0-8	3.1	3.3	6.7	0.470	14.3	15.6	31.3
1995	4,3	8-16	-0.1	-0.1	-0.2	1.130	-1.7	-0.7	-2.4
1995	4,3	0-8	2.7	4.0	7.1	0.470	12.7	19.0	33.3
1995	4,5	8-16	1.2	-0.2	0.9	1.080	12.5	-2.4	9.8
1995	4,9	0-8	6.8	2.1	9.1	0.530	36.1	11.3	48.4
1995	4,9	8-16	0.9	-0.1	0.8	1.030	9.2	-0.6	8.6
1995	4,11	0-8	3.6	0.7	4.3	0.740	26.5	4.8	31.7
1995	4,11	8-16	-0.1	-0.4	-0.5	1.020	-1.3	-3.9	-5.5
1995	4,15	0-8	0.7	0.6	1.3	0.890	6.1	5.1	11.6
1995	4,5	0-8	3.6	1.3	5.0	0.820	29.4	10.9	41.3
1995	4,15	8-16	0.2	-0.3	-0.1	1.020	2.3	-3.0	-1.0
1995	4,17	0-8	1.1	0.1	1.2	0.760	8.4	0.8	9.2
1995	4,17	8-16	0.7	0.1	0.9	1.010	7.2	1.3	8.6
1995	6,3	0-8	0.4	0.0	0.4	0.790	3.0	0.3	3.4
1995	6,3	8-16	0.3	0.2	0.5	1.060	2.9	2.0	5.1
1995	6,5	0-8	1.3	0.2	1.5	0.780	10.2	1.3	11.6
1995	6,5	8-16	0.6	-0.2	0.4	1.2	7.5	-2.6	4.6
1995	6,15	0-8	0.9	-0.3	0.6	0.660	5.7	-1.7	3.9
1995	6,15	8-16	1.0	-0.5	0.4	0.980	9.6	-4.9	4.3
1995	6,17	0-8	-0.2	-0.5	-0.8	0.870	-1.7	-4.8	-6.9
1995	6,17	8-16	0.4	0.2	0.5	1.120	4.1	1.9	6.1
1995	9,5	0-8	1.6	0.5	2.1	0.620	9.9	3.1	13.3
1995	9,17	0-8	0.7	0.0	0.7	0.750	5.1	0.2	5.4
1995	9,17	8-16	0.9	-0.3	0.6	0.910	8.3	-2.4	5.7
1995	11,5	0-8	9.6	3.0	12.8	0.300	28.9	8.9	38.5
1995	11,5	8-16	4.2	1.5	5.9	0.710	29.8	11.0	41.7
1995	11,11	0-8	0.4	0.3	0.7	0.680	2.9	1.8	4.9
1995	11,11	8-16	1.0	-0.2	0.8	0.950	9.5	-2.2	7.1
1995	11,13	0-8	1.2	0.3	1.5	0.660	7.7	1.7	9.6



Sampling Date	x,y	Depth cm	Calcite %	Dolomite %	CCE %	Bulk density g dry wt/mL	Calcite mg/mL	Dolomite mg/mL	CCE mg/mL
1995	11,13	8-16	1.0	-0.3	0.7	0.670	6.9	-1.9	4.8
1995	11,15	8-16	0.7	-0.6	0.0	1.020	7.3	-6.5	0.3
1995	11,17	8-16	0.1	-0.5	-0.4	0.910	0.8	-4.3	-3.8
1995	13,9	0-8	3.2	0.6	3.9	0.740	23.7	4.6	28.7
1995	13,9	8-16	0.6	-0.2	0.4	0.870	5.3	-1.9	3.3
1995	13,13	0-8	3.1	0.6	3.8	0.600	18.7	3.6	22.6
1995	13,13	8-16	0.5	-0.5	-0.1	1.290	6.2	-6.7	-1.2
1995	13,17	0-8	0.9	0.1	1.0	0.770	7.0	0.9	8.0
1995	13,17	8-16	0.5	0.2	0.7	0.720	3.3	1.4	4.9
1995	9,5	8-16	1.2	-0.2	0.9	1.060	12.5	-2.5	9.8
1995	11,17	0-8	0.7	0.1	0.7	0.640	4.2	0.5	4.7
1999	2,9	0-8	7.7	1.8	9.6	1.465	112.1	26.4	140.9
1999	2,9	8-16	0.4	0.5	0.9	0.847	3.1	3.8	7.3
1999	2,11	0-8	4.6	1.5	6.2	0.976	44.5	14.9	60.7
1999	2,11	8-16	0.3	0.5	0.9	1.437	4.7	7.1	12.4
1999	4,3	0-8	6.5	4.9	11.9	0.686	44.6	33.9	81.4
1999	4,3	8-16	1.8	1.4	3.4	1.285	23.7	17.9	43.1
1999	4,5	0-8	4.2	1.7	6.1	0.942	39.4	16.2	57.0
1999	4,5	0-8	3.8	2.5	6.5	1.481	56.5	36.9	96.6
1999	4,5	8-16	1.0	0.3	1.3	1.481	14.3	4.0	18.7
1999	4,9	0-8	1.6	0.9	2.5	1.103	17.5	9.5	27.8
1999	4,9	8-16	0.3	0.2	0.5	1.351	4.5	2.2	6.9
1999	4,11	0-8	1.3	-0.2	1.1	1.307	17.3	-3.0	14.0
1999	4,11	8-16	0.6	-0.7	-0.2	1.388	7.9	-9.3	-2.2
1999	4,15	0-8	8.3	1.6	10.0	0.642	53.0	10.4	64.4
1999	4,17	8-16	0.3	0.4	0.7	1.439	4.3	5.0	9.7
1999	4,17	0-8	0.1	0.4	0.5	1.252	1.1	4.6	6.1
1999	6,3	0-8	0.2	0.2	0.4	1.489	3.0	3.4	6.6
1999	6,3	8-16	0.1	0.0	0.1	1.373	0.8	0.0	0.8
1999	6,3	8-16	-0.4	0.0	-0.4	1.373	-5.5	0.6	-4.9
1999	6,5	8-16	0.4	-0.1	0.3	1.386	5.7	-1.1	4.6
1999	6,5	0-8	3.7	1.4	5.3	0.814	30.3	11.5	42.8
1999	6,15	0-8	5.8	1.5	7.4	0.781	45.6	11.5	58.1
1999	6,15	8-16	0.6	-0.3	0.3	1.432	9.0	-4.7	3.9
1999	6,17	0-8	0.2	0.4	0.7	1.042	2.3	4.6	7.3
1999	6,17	8-16	0.4	-0.1	0.3	1.381	4.9	-0.8	4.0
1999	9,5	0-8	1.6	0.7	2.3	1.505	23.5	10.1	34.5
1999	9,5	8-16	1.1	-0.6	0.4	1.216	13.1	-7.1	5.4
1999	9,6	0-8	3.1	1.6	4.9	0.711	22.1	11.6	34.6
1999	9,6	8-16	-0.1	0.1	0.0	1.449	-1.4	1.0	-0.4
1999	9,17	0-8	1.9	1.0	3.0	1.016	19.1	10.2	30.2
1999	9,17	8-16	0.3	0.2	0.5	1.426	3.9	2.8	7.0
1999	11,5	0-8	8.4	2.4	11.1	0.904	76.1	22.0	100.0
1999	11,5	8-16	1.3	-0.2	1.2	1.461	19.5	-2.2	17.1
1999	11,11	0-8	3.5	1.0	4.6	0.963	33.4	10.0	44.2
1999	11,11	8-16	0.5	0.0	0.5	1.428	7.5	-0.3	7.1
1999	11,13	0-8	1.5	0.4	2.0	1.169	17.6	4.9	22.9
1999	11,13	0-8	1.4	0.3	1.7	1.169	15.8	3.3	19.4
1999	11,13	8-16	0.0	0.1	0.1	1.453	-0.5	2.0	1.7
1999	11,15	8-16	0.3	-0.2	0.1	1.450	4.4	-3.3	0.8
1999	11,15	8-16	0.2	-0.4	-0.2	1.450	3.4	-5.6	-2.8
1999	11,15	0-8	0.1	-0.2	0.0	1.036	1.5	-1.6	-0.2
1999	11,17	0-8	2.8	0.6	3.5	0.907	25.4	5.9	31.8
1999	11,17	8-16	0.3	0.2	0.5	1.324	3.7	2.6	6.5
1999	12,5	0-8	7.9	2.4	10.5	1.597	126.0	38.4	167.8
1999	12,5	8-16	0.9	-0.1	0.8	0.578	5.2	-0.6	4.5
1999	13,9	0-8	8.1	1.8	10.1	0.732	59.6	13.2	73.9
1999	13,9	8-16	0.3	0.0	0.3	1.608	4.4	0.3	4.7

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Sampling Date	x,y	Depth cm	Calcite %	Dolomite %	CCE %	Bulk density g dry wt/mL	Calcite mg/mL	Dolomite mg/mL	CCE mg/mL
1999	13,13	0-8	5.9	1.8	7.9	1.007	59.4	18.3	79.3
1999	13,13	8-16	0.3	0.0	0.3	1.328	3.4	0.0	3.5
1999	13,17	0-8	0.6	0.2	0.8	1.007	6.4	1.9	8.5
1999	13,17	8-16	-0.6	-0.3	-0.9	1.328	-7.4	-4.3	-12.0