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Biogeochemical Dynamics of Iron and Sulfur in Sediments from Hydro-electric Dams Submitted to Wetting and Drying Cycles

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Biogeochemical Dynamics of Iron and Sulfur in Sediments from Hydro-electric Dams Submitted to Wetting and Drying Cycles

by

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Abstract

Water level changes due to the decommissioning of hydro-electric dams can result in sediment exposure to air. Oxidation of sediments can decrease the pH as a result of iron sulfides changing into iron oxides. The present study was designed to simulate drying and wetting cycles of shallow lake sediments from two lakes (Stump and Black Donald Lakes in Ontario), in order to assess the mineralogical changes of Fe-rich minerals. Our results indicate that the total reactive iron fraction of the sediment increased after the wetting and drying cycles. This increase was caused by the weathering of pyrite and Fe-silicates and their subsequent transformation into more Fe-reactive mineral species. The pH of the surface sediments also decreased following the addition of simulated rainwater and the oxidation of iron sulfides in the sediments. This preliminary study shows that the decommissioning of hydro-electric dams will have an effect on the biogeochemical cycles of iron and sulfur.

Résumé

Les changements de niveau d'eau des lacs causés par le démantèlement de barrages hydro-électriques peuvent mener à l'aération des sédiments. L'oxydation des sédiments peut engendrer une baisse de pH suite à la transformation des sulfures de fer en oxydes de fer. La présente étude a simulé des épisodes de pluie et de sécheresse sur des sédiments provenant de deux lacs (lacs Stump et Black Donald en Ontario), afin d'évaluer les changements minéralogiques des minéraux riches en fer. Nos résultats indiquent que la fraction totale de fer réactif dans les sédiments a augmenté après les épisodes de pluie et de sécheresse. Cette augmentation est reliée à l'altération de la pyrite et des silicates et à leur transformation en espèces de fer plus réactives. Le pH des sédiments de surface a aussi diminué suite à l'addition de pluie acide et à l'oxydation des sulfures de fer dans les sédiments. Cette étude préliminaire démontre que le démantèlement des barrages hydro-électriques risque d'affecter les cycles biogéochimiques du fer et du soufre.

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The material for this project, i.e. sediment cores, porewater, etc., was also used by Kristi Hindle, a Master's of Biology candidate at the University of Ottawa. The focus of our theses was different however some data (pH, sulfide, sulfate, moisture and organic content) was shared to explain overall trends.

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1.0 Introduction

Altering the natural hydrology of our landscapes has been a human practice for thousands of years. Dam building has been rampant during the last century to meet the growing demands for power (Poff and Hart, 2002). In North America, Ontario's mills, logging operations, farm or residential ponds and early hydro impoundments date back to the late 1700s (Tiner, 1998).

Dams reduce flood hazard and allow settling and farming (irrigation), provide drinking water and improve navigation (Poff and Hart, 2002). These structures have a finite life span. As they age, they fall into a state of disrepair and become more susceptible to dam failure. Lack of appropriate funding can accelerate the ageing process. New technologies and cleaner, more efficient energy sources, such as nuclear and solar, have made some of the smaller, older hydro-electric dams obsolete.

All rivers in Ontario have been damned with only a few exceptions. As of September 30th, 2003, Ontario Power Generation is responsible for 36 hydro-electric stations, 29 small dams and 240 dams on 26 river systems (www.opg.com), for a total of 305 dams in Ontario alone. This number does not take into account the Ministry of Natural Resources' and privately run hydro-electric facilities or water impoundments. Many of these dams are very old, up to 80 and 90 years old. They are deteriorating and no longer profitable. In the future, energy producers may be interested in removing these dams to reduce costs. This would subsequently lower water levels in the upstream reservoirs.

A thorough literature review did not reveal any information on Canadian attempts at dam removal operations. In the United States, approximately 500 dams have been partially or completely removed (Poff and Hart, 2002). Most of these are small structures (<5 m in height) because they are in greater number and easier to remove. The observed effects of dam removal projects are without borders and can be applied to any ecosystem, Canadian or otherwise.

1.1 Dams and dam removal effects

The building of these hydro-electric dams is controversial because of the disturbances they cause to the natural environment. These include, regional hydrology changes, sediment retention, blocked fish migration routes, greenhouse gas emissions, and several social consequences such as community resettlement (Doyle et al., 2000; Rosenberg et al., 1997). Dams can impact rivers, damage nearby estuaries, beaches and ocean and adversely affect biodiversity on a regional scale (Babbitt, 2002).

Deteriorating dams now pose the problem of what to do with the deteriorating dams and the effects of their removal. Every river system has unique characteristics. Therefore a river's response to a dam removal may not necessarily be the same as another. Dam removals, which were done in the United States, were instigated by local necessity for reasons of safety, costs, health, imminent extinction, budgets and litigation (Babbitt, 2002). The decision to remove a dam is a difficult one and should take into account the economic, social, ecological, physical and geochemical ramifications associated with each individual impoundment. The effects of dam removal on sediment geochemical characteristics, more specifically the redox sensitive elements like iron and sulfur, have not been investigated to date.

1.1.1 Social effects

Society consists of many different groups of people with different points of view. When dealing with dam removal, everyone from cottagers, marina owners, farmers, energy consumers and ecologists to government officials and environmentalists, want to be heard and consulted. Hydro-electric dams have created water reservoirs for water sport enthusiasts to enjoy. Many people have also built their cottages and homes around these 'lakes' and may attribute nostalgic, historical or aesthetic value to dam structures (Doyle et al., 2000). The removal of a dam structure will inevitably change the watercourse, which will inadvertently lower property values and have a direct impact on local economy. It is for this reason that local communities should participate in dam removal decisions.

1.1.2 Ecological effects

Plant and animal communities can be affected by changes in water temperature, sediment loading and oxygen levels during the removal of dams (Tiner, 1998). Movements and migrations of fish, invertebrates and other wildlife can also be impacted (Tiner, 1998). In the United States, several dam removal projects have already been carried out. In a review by Doyle et al. (2000) some of the ecological impacts that were observed during dam removals are mentioned. They report that within 3 months of one removal, striped bass were able to return to previously inaccessible sections of the river. Plant communities changed due to newly available nutrient-rich sediments. In another study, the invertebrate communities were diversified within one year of the dam breach. With a change in flora and environmental conditions comes a natural change in aquatic and terrestrial fauna. Hart et al. (2002) reported a summary of positive effects from several studies (Table 1.1). In a few of these studies, negative ecological effects of dam removal were also observed (Table 1.1). Some of the effects of dam removal may be reversed over time. However, to date, no dam removal studies have continued long enough to determine the response rates of all ecosystem components (Hart et al., 2002).

Table 1.1 Observed positive and negative ecological effects of dam removal from past studies.

Positive	Negative
Increase in fish diversity	Decrease in mussel abundance downstream due to sedimentation
Restoration of fish passage	Decreased downstream fish habitat
Plant colonization	Reduction in wetland habitat
Changes in community composition (algae) (also negative)	Loss of reservoir species
Improvement of fish habitat	Shifts in species abundance and distribution
Re-established spawning	Exotic species invasion of upstream habitat

(Hart et al., 2002)

1.1.3 Physical and geochemical effects

Dam removals can affect river ecosystems in several ways. The downstream flux of water and sediment can be altered, which will impact biogeochemical cycles (Poff and Hart, 2002). Doyle and his colleagues are investigating the physical effects of dam removal (Doyle et al., 2000). Some of these effects include, downstream sediment transport, watercourse realignment, channel adjustment, sediment compaction and organic matter changes (Doyle et al., 2000; James et al., 2001; Stephens et al., 2001). Doyle et al. (2000) reported removal projects with sediment transport occurring within 6 months to one year after dam breach. These sediment movements carried 33 to 100% of the post-dam depositional sediments downstream. Excessive sediment loads increase turbidity and can be detrimental to fish spawning. Changes in the sediment load of rivers and lakes inevitably change the biogeochemical cycling of elements (Rosenberg et al., 1997). With sediment transport comes sediment-bound contaminant transport. The presence of contaminants would depend on activities and practices in the area surrounding the water reservoir.

In 4 out of 5 cases, watercourses did not return to pre-dam channel alignment and within the first and second year, channel adjustments were dominant (Doyle et al., 2000). James et al. (2001) measured a sediment density increase and moisture content decrease after lake drawdown, or in other words, sediment compaction. This can lead to increased runoff and a decrease in groundwater recharge. Lovely and Phillips (1986) showed irreversible ageing of sediment minerals after repeated wetting and drying cycles. The ageing process allows the minerals to become more crystalline and therefore they are less likely to undergo reduction. The structure of organic material has also been shown to change during drying and as a result, this would affect metal sorption (Stephens et al., 2001). To summarise, the physical effects of dam removal can include: (Hart et al., 2002)

1) alteration of flow regime,
2) erosion at dam,
3) increased sediment transport,
4) downstream channel aggradation,
5) channel formation,
6) channel substrate coarsening in former impoundment,

7) decreased water temperatures,
8) overall water quality improvement,
9) mobilization of P and/or N (while others did not),
10) mobilization of organic contaminant

1.2 Biogeochemical transformations in sediments

1.2.1 Sediment composition

The bulk composition of the Earth's crust mainly consists of silicates, oxides and sulfides. Sediment composition varies and depends on the geology, geography, climate and vegetation of the surrounding area. Of interest to this study, sulfide minerals are particularly important because they are sensitive to redox changes brought upon by drying and wetting events. In an anoxic environment, like reducing soils and sediments, the most commonly found sulfide minerals are pyrite (FeS_2), with smaller amounts of chalcopyrite ($CuFeS_2$), elemental sulfur and monosulfides, such as pyrrhotite ($Fe_{1-x}S$), sphalerite (ZnS) and galena (PbS) (Schippers and Jorgensen, 2002; Preda and Cox, 2001; Aström, 1998). In neutral to slightly alkaline and oxidizing environments, iron oxides, hydroxides and oxyhydroxides are the dominant Fe-rich minerals (Ehrlich, 2002).

1.2.2 Iron and sulfur cycling

Iron exists in the environment as ferrous iron (Fe (II)) and ferric iron (Fe (III)). Depending on the physico-chemical conditions, iron can be soluble or precipitated as Fe (II)-sulfides or carbonates under reducing conditions, or as Fe (III)-oxides under oxic conditions (Stumm and Morgan, 1996) (Figure 1.1).

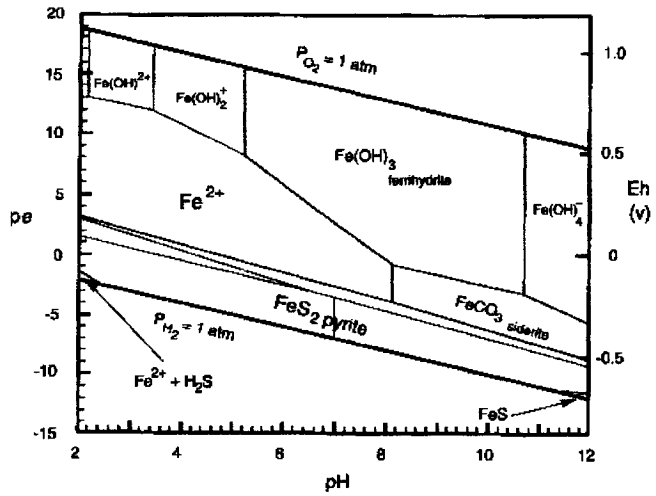


Figure 1.1 Iron stability in the system Fe-O-H_2O-S-CO_2 at 25°C as a function of pH and pE. (Drever, 1997)

Sulfur possesses several oxidation states, including S (+VI) (sulfate), S (0) (elemental sulfur) and S (-II) (sulfide). Under reducing conditions, Fe (II)-sulfide minerals are generally stable, but when exposed to oxygen, as would be the case after dam removal, they become oxidized and release ferric iron and sulfate, as shown by equations 1 and 2.

$$FeS_2 + {}^7/_2\, O_2 + H_2O \rightarrow Fe^{2+} + \underline{2\, SO_4^{2-}} + 2\, H+ \qquad (1)$$

$$2\, Fe^{2+} + \tfrac{1}{2}\, O_2 + 2\, H^+ \rightarrow \underline{2\, Fe^{3+}} + H_2O \qquad (2)$$

The conversion of ferrous to ferric iron is the rate-limiting step, and perpetuates the sulfide oxidation cycle. Ferric iron, a powerful oxidant, which is generated in equation 2, can then oxidize more pyrite as shown in equation 3.

$$FeS_2 + 14\, Fe^{3+} + 8\, H_2O \rightarrow 15\, Fe^{2+}\, 16\, H^+ + 2\, SO_4^{2-} \qquad (3)$$

The chemical oxidation of Fe (II) to Fe (III) is however pH dependent, i.e., the rate of oxidation becomes very slow under acidic conditions (Stumm and Morgan, 1996). Over a wide range in pH, including acidic and neutral, the oxidation of ferrous iron to ferric iron has been shown to be catalyzed by several types of microorganisms (Ehrlich, 2002; Schrenk et al., 1998). Bacteria act as catalysts in the Fe (II) to Fe (III) conversion reaction gaining

metabolic energy from the rate-limiting step (equation 2; Figure 1.2). They can increase the oxidation rate of iron and sulfur at low pH by a factor of several orders of magnitude, whereas at neutral pH conditions, both biotic and abiotic reactions can proceed (Emerson et al., 1999).

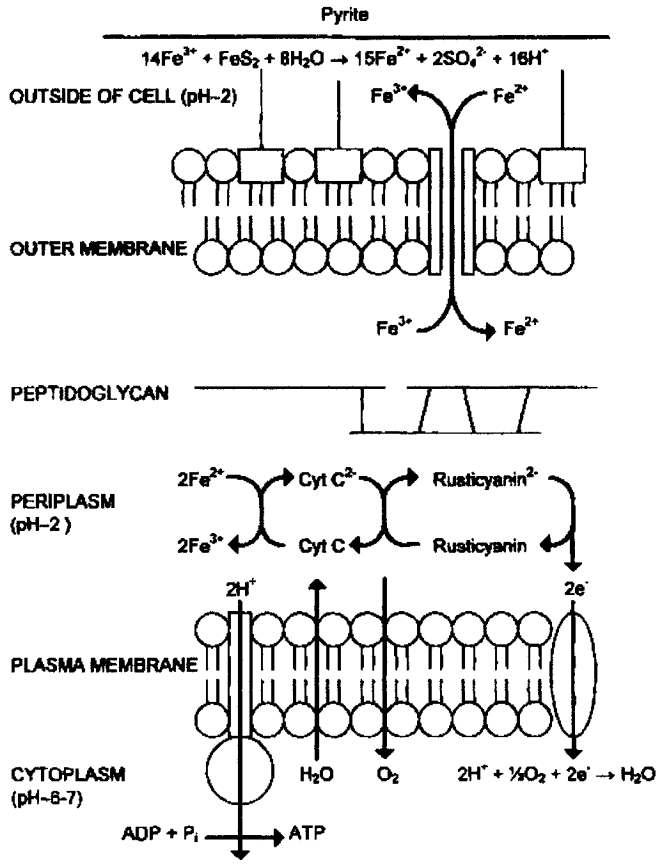


Figure 1.2 A bioenergetic model of iron oxidation by *Acidithiobacillus ferrooxidans* based on a bidirectional diffusion gradient for Fe (II/III). (Nordstrom and Southam, 1997)

The oxidation process can occur through the oxidative attack of the mineral substrate, i.e., as a result of direct attachment (Figure 1.3a), or indirectly, by the interaction involving the production of an oxidant (Fe (III)) that causes the solubilization to occur (Figure 1.3b) (Leduc, 1997). In addition, Mielke et al. (2003) recently demonstrated that *Acidithiobacillus ferrooxidans* can glue itself to the surface of pyrite in a biofilm containing secondary iron-oxide minerals. *A. ferrooxidans* and other bacteria can also produce exopolymers in order to enhance their attachment to the mineral surfaces (Little et al., 1997).

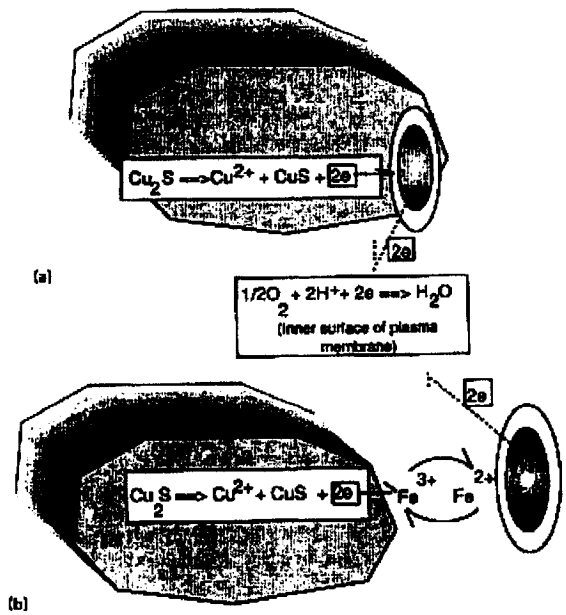


Figure 1.3 Schematic representation of direct and indirect oxidation of a particle of Cu_2S by *Acidithiobacillus ferrooxidans*. (Ehrlich, 2002)

In addition to iron oxidation, sulfide can be oxidized to sulfur and then to sulfate through a series of conversions of reduced intermediate sulfur species (Ehrlich, 2002). Certain microorganisms can catalyse this oxidative reaction. For example, a study of *Acidithiobacillus ferrooxidans*, by Mustin et al. (1992) showed sulfur being preferentially oxidized over iron before the pH dropped. However, at lower pH, the biological oxidation of iron was favored.

Sulfur oxidizing species are varied as well as the way they perform the oxidation. The specific details of the oxidation process are outside the scope of this research and will therefore not be discussed further. The important thing to remember is that the oxidation of reduced forms of sulfur, as well as iron, is possible in an aqueous environment, i.e. lake sediment, and that this oxidation is often enhanced by microorganisms present in the sediment.

1.2.2.1 Acidity generation during microbial and chemical oxidation reactions

Iron sulfide oxidation is of utmost importance because it has the potential of acidifying and releasing toxic heavy metals into the surrounding environment. As demonstrated by equations 1 to 3, a total of 18 protons are being generated during the sulfide

oxidation process where sulphuric acid is the ultimate product. Acid generation from sulfide oxidation and its consequences has been extensively studied in mining environments (Nordstrom and Southam, 1997 and references therein). The most striking example of sulfide oxidation and acidity generation can be found at Iron Mountain in California. The effluent from one of the mines corresponds to the most acidic waters ever reported anywhere in the world (down to pH –3.5) (Edwards et al., 2000).

Some studies have also looked at acidity generation following the drying and oxidation of sediments. Saeki et al. (1993) reported that as the pH decreased and the Eh increased in the sediments of Lake Teganuma, Japan, there was a corresponding sulfide decrease. Therefore, as sulfide minerals are dissolved, acidity is produced. Peverly and Kopka (1991) reported acidity and metal content increase in porewater and sediments after drawdown, whereas Aström (1998) reported the production of 'acid sulfate soils' after dredged sediment were exposed to air and water. Preda and Cox (2000) came to the following conclusions following their study of Australian floodplains:

1) Occasional showers during the dry season cause low pH and high amounts of dissolved metals in the water;
2) The first heavy rain of the wet season can produce very toxic conditions (low pH, high metal concentrations) that can result in a fish kill;
3) Towards the end of the wet season, prolonged flushing of pyrite oxidation products leads to short-term recovery of the aquatic system (neutral pH, low dissolved metals); and
4) A flood event can produce low pH, salinity and high metal concentrations, therefore lethal conditions to aquatic life.

The same authors also showed that the production of acid was not diminished during the wet season, it was only masked by dilution. Therefore, the environmental impact of sulfide oxidation depends on the amount and duration of rainfall. Fajtl (2002) carried out aeration experiments on shoreline sediment from a freshwater pond. These experiments produced results similar to Preda and Cox (2000). Table 1.3 summarises the experimental results done with the black layer of pond sediment. These results clearly show that with decreasing pH, there is a heavy metal content increase in the sediments (Fajtl, 2002)

Table 1.2 Black layer sediment aeration experiment results.

Parameter	Initial value (time = 0 hrs)	Final value (time = 362 hrs)
Redox potential (mV)	-124	+ 412
pH	6.72	2.7
SO_4^{2-} content (mg/kg of sed)	995	97 006
Zn content (mg/kg of sed)	13.7	527.9
Mn content (mg/kg of sed)	1	38.6
Fe content	variable	variable

(Fajtl, 2002)

1.2.2.2 Buffering capacity of sediments and soils

Alkalinity refers to a solution's capacity to resist changes in hydrogen ion concentration. Alkalinity is an index to the nature of the rocks within a drainage basin and to the degree to which they are weathered (Cole, 1983). Good sources of alkalinity are carbonate minerals and rocks. Calcite ($CaCO_3$), dolomite ($CaMg(CO_3)_2$), limestone and marbles are the most common carbonates found in nature. The presence of other ions, such as hydroxides, silicates, phosphates and borates, may also contribute to natural water alkalinity (Cole, 1983; Wetzel, 1975). Humic compounds, or organic acids, are usually poor buffers except between pH 4 and 5 (Cole, 1983; Schlesinger, 1997).

Carbon dioxide (CO_2) can also dissolve in natural waters and dissolve carbonate minerals. This reaction produces bicarbonate ions (equation 4) which can neutralise the acidity generated by sulfide oxidation (equation 5 and 6).

$$CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + 2\,HCO_3^- \quad (4)$$

$$HCO_3^- + H^+ \rightarrow H_2CO_3 \quad (5)$$

$$CO_3^{2-} + H^+ \rightarrow HCO_3^- \quad (6)$$

The alkalinity of most river waters is in the 10^{-3} meq/L range with the average being around 2.3 to 2.6 x 10^{-3} meq/L (Stumm and Morgan, 1996). According to Cole (1983), day-to-day fluctuations in alkalinity are negligible.

If the buffering capacity of the sediment is sufficient, porewater pH should remain unchanged during pyrite oxidation. Any iron released into solution by sulfide oxidation could therefore precipitate as iron oxides under favourable conditions. Blowes et al. (1998) showed that in sulfide-rich tailings dominated by siderite ($FeCO_3$), dolomite-ankerite ($CaMg(CO_3)_2$-$Ca(Fe,Mg)(CO_3)_2$) and calcite, sulfide oxidation was not associated with the generation of acidity and that neutral pH conditions prevailed in the residues. Previous unpublished work by Nugent (2000) also showed that sulfide-rich mine tailings rich in carbonate minerals displayed no acid generation and that heavy metals (Pb) existed in soluble carbonate complexes under neutral pH conditions.

1.2.2.3 By-products of metal sulfide oxidation

Sulfide oxidation products are well known. Southam and Beveridge (1992) reported the development of secondary minerals (iron chlorides and iron phosphates) in oxidized sulfide-rich tailings. Jarosite ($AFe_3(SO_4)_2(OH)_6$) can be formed during sulfide mineral oxidation as well as other Fe-bearing minerals capable of forming under these conditions, like goethite ($FeO(OH)$), ferrihydrite ($Fe(OH)_3$) and schwertmannite (Nordstrom and Southam, 1997). Bhatti et al. (1993) reported that pyrrhotite oxidation was also associated with the formation of potassic jarosite ($KFe_3(SO_4)_2(OH)_6$), goethite and schwertmannite.

1.2.2.4 Release of sorbed metals during oxidation of metal sulfides

Heavy metals, such as Ni, Cd, Cu, Zn, As, Pb, Hg, etc., are often adsorbed onto the surfaces of minerals (Fe-oxides and Fe-sulfides) (Tessier et al., 1996). The oxidation of iron sulfides can then release the metals into solution, whereas the formation of iron oxides under oxic conditions can immobilize the metals as a result of sorption reactions (Huerta-Diaz et al., 1998). Saeki et al. (1993), Aström (1998) and Stephens et al. (2001) studied heavy metal redistribution between the different sediment phases after sulfide oxidation. They observed a decrease of metals associated with sulfides and an increase of metals associated with iron and manganese oxides and oxyhydroxides. Dollar et al. (2001), conducted sequential chemical extraction experiments on inundated and drained wetland sediments. They used their drier wetland site to model trace metal redistribution in a hypothetical restoration of natural water flow to the wetland. Their results showed that re-flooding with near-neutral water for longer

periods of time would minimize the potential for leaching of metals by acidic precipitation and lower the redox potential thereby favoring the formation of metal scavenging sulfides. However, such process would also release metals bound to iron and manganese oxide coatings (Dollar et al., 2001). The same authors also suggested that metals strongly associated with the oxidizable fraction (organically or sulfide bound), like Cr and Cu, would be unlikely to be mobilized during re-flooding. None of these studies discussed the amount of time required for mineral transformations, i.e. sulfides to oxides and vice versa.

1.2.3 Bacteria involved in iron and sulfur cycling

Bacteria often derive their energy from the oxidation of organic and inorganic compounds. Some geomicrobially important groups of bacteria include iron-reducing and -oxidizing bacteria and sulfur-reducing and oxidizing bacteria, to name only a few. These bacteria participate in the redox cycling of iron and sulfur in the environment. Of particular interest to this study are the neutrophilic iron-oxidizing and sulfur-oxidizing bacteria (see section 1.2.3.2).

1.2.3.1 Acidophilic iron- and sulfur-oxidizing bacteria

Johnson (1998) defined extreme acidophiles as microoganisms that have an optimal growth pH of 3.0 or below. Chemical and biological dissolution of sulfide minerals is a highly exothermic reaction and can drive the pH down to values below 2.0 (Baker and Banfield, 2003). Therefore, only a select few organisms can thrive in these harsh environments where conditions of low pH, high temperature and high metal content prevail. These sites are commonly referred to as acid mine drainage (AMD) sites. Iron-oxidizing acidophiles include *Thiobacilli* spp., *Leptospirillum* spp., *Sulfobacilli* spp., *Acidimicrobium* spp. and *Ferromicrobium* spp. from the prokaryote taxa and *Ferroplasma* spp. from the Archaea taxa (Edwards et al., 2000; Johnson, 1998; Baker and Banfield, 2003). Most iron-oxidizing acidophiles are obligate aerobes (Johnson, 1998; Baker and Banfield, 2003). These bacteria use autotrophic carbon assimilation pathways (Johnson, 1998; Baker and Banfield, 2003). However, exceptions do exist. Sulfur-oxidizing acidophiles include *Thiobacilli* spp., *Sulfolobus* spp. and *Sulfobacilli* spp.. These sulfur-oxidizers are also aerobes and capable of autotropic and mixotrophic growth (Friedrich et al., 2001; Baker and Banfield, 2003).

1.2.3.2 Neutrophilic iron- and sulfur-oxidizing bacteria

There are a wide variety of bacteria that can oxidize iron and sulfur under pH neutral conditions. Tables 1.3 and 1.4 present a list of some important neutrophilic iron- and sulfur-oxidizing bacteria, respectively. The lists are not exclusive, new species are being identified every year, especially the sulfur-oxidizing species.

Most iron-oxidizing bacteria are Gram-negative, rod-shaped microorganisms with the exception of the *Siderococcus* family which are cocoid cells. Resting stages, or endospore formation, are not known for any of the bacteria listed in Table 1.3. As stated earlier, in the presence of oxygen and at high pH, ferrous iron autoxidizes quickly to ferric iron. Therefore, in order to be successful, bacteria that oxidize iron do so at low oxygen tension (Ehrlich, 2002). The iron-oxidizing bacteria 'family' includes sheathed and encapsulated bacteria. Member genera include *Shearotilus* spp., *Leptothrix* spp., and *Siderocapsaceae* spp. and a few, as yet, unnamed strains.

Most sulfur-oxidizing bacteria listed in Table 1.4 are Gram-negative, rod-shaped microorganisms. Resting stages, or endospore formation, are not known for any of these bacteria. According to Stubner et al. (1998), more research is needed to better understand the ecological niches of S-oxidizing bacteria in natural settings.

Table 1.3 Neutrophilic iron-oxidizing bacteria species and their growth requirements.

Genus name	O_2 demand	C/energy source	T° range (°C)	pH	Miscellaneous
Gallionella	Strictly aerobic to microaerophilic	Autotrophic or mixotrophic	20	6–7.6	- ferric hydroxide encrusted stalk Fe^{2+} only electron donor - "occur most abundantly in oligotrophic ferrous iron-bearing waters (freshwater of marine habitats)" [a] - "This is one of the most important iron bacteria…" [b] - Eh range +200 to +300mV
Species: *G. ferruginea*					
Leptothrix spp.		Chemoorganotrophs (sugars, organic acids and glycerol are C and energy sources)	10-35 (opt. 25)	6.5-7.5	- "no proof that these organisms oxidize Fe^{2+} to Fe^{3+}" [c] - usually thrive in "oligotrophic, slowly running, iron- an manganese-rich water" [d] - can oxidize Mn^{2+}
Species: *L. ochracea, L. pseudo-ochracea, L. discophora, L. cholodnii, L. lopholea*					
Sphaerotilus spp.	very low	Chemoorganotrophs (alcohols, organic acids and sugars are C and energy sources)	10-37 (optimum 20-30)	6.5-7.5	- thrive in organic nutrient-rich water [d] - cannot oxidize Mn^{2+}
Species: *S. natans*					
Siderococcus spp. (Family: *Siderocapsaceae*)	Aerobic to microaerophilic	believed to be organotrophic	10	6.2-7	- coccoid cells - iron depositions "may be considerable" and contain only ferric hydroxide [c]
Species: *S. limoniticus*					

(Source: [a] Bergey's Manual, 1984; [b] Erhlich, 2002; c Bergey's Manual, 1994; [d] Siering and Ghiorse, 1996

Table 1.4 Neutrophilic sulfur-oxidizing bacteria species and their growth requirements.

Genus name	O_2 demand	C/energy source	T° range (°C)	pH	Miscellaneous
Thermothrix **Species:** *T. thiopara*	Facultatively anaerobic	Facultatively chemolithotrophic	40-80	6-8	- "inorganic S compounds and organic compounds can be used as electron donors" [a] - O_2 or NO_3^- can be used as the terminal electron acceptor
Acidithiobacillus spp. (many species = wide range of growth possibilities) (neutrophilic) **Species:** *A. novellus*, *A. versutus*, *A. aquaesulis*, *A. thysasiris*	Obligate aerobes	Capable of autotrophic growth; can fix CO_2; some are obligately chemolithotrophic; others are chemoorganotrophic	20-43	2-8	- "energy is gained from oxidation of one or more reduced sulfur compound" [a] - "distribution is seemingly ubiquitous in marine, freshwater, and soil environments" [a]
Xanthobacter spp. **Species:** *X. autotrophicus*, *X. agilis*, *X. flavus*	Aerobic	Chemolithoautotrophs and chemoorganotrophs	25-30	5.8-9	- Gram-positive to variable - "The organisms occur free-living in wet soil containing decaying organic material, and also in water" [a]
Aquabacter spp. **Species:** *A. spiritensis*					
Azorhizobium *spp.* **Species:** *A. caulinodans*	Obligately aerobic	Only sugar oxidized is glucose			

(Source: [a] Bergey's Manual, 1994; Stubner et al., 1998; Graff and Stubner, 2003)

1.2.3.3 Bacterial community changes

Bacterial communities will vary as the surrounding environmental conditions change. In the case of anoxic sediments, oxygenation will likely inhibit the activity of anaerobic bacteria, such as sulfate reducing bacteria (Grossman and Desrocher, 2001), and lead to the establishment of a more aerobic microbial community. If the microbial population is capable of forming spores, (which is the case of some sulfur-reducing bacteria), then the anaerobic population will have the opportunity to flourish again once the environmental conditions return within their growth parameters.

As neutrophilic bacteria oxidize sulfides, acidity is generated as a by-product of the oxidation. If a sufficient buffering agent is not present, the neutrophilic bacteria will be responsible for their own annihilation and more acidophilic bacteria will prevail. Baldwin and Mitchell (2000) discussed the effects of drying and re-flooding of soils and sediments on nutrient availability and suggested that bacterial community changes were probable with drying and wetting cycles. Figure 1.4 shows the pH and Eh limits of sulfur- and iron-oxidizing bacteria.

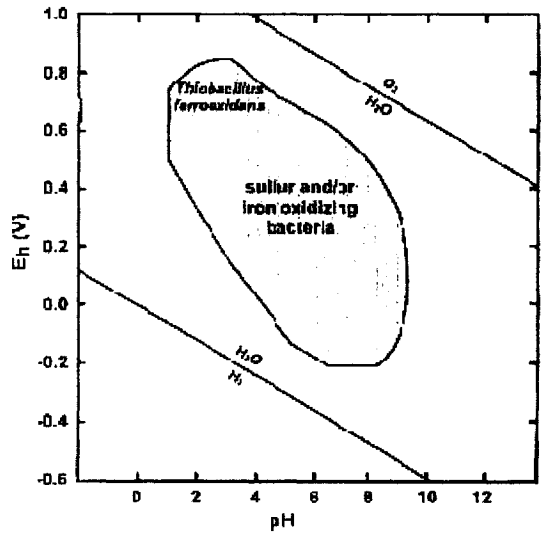


Figure 1.4 Diagram showing the environmental Eh and pH limits of S- and Fe-oxidizing bacteria. (McIntosh, 1997)

1.3 Objectives and hypotheses

The main objectives of the present study on the biogeochemical cycling of Fe and S in lake sediments submitted to wetting and drying events are:

1 To assess the importance of drying and wetting events on the mineralogy of Fe-rich minerals.
2 To assess the acidity generation caused by the oxidation of Fe-sulfides.
3 To determine the importance of Fe- and S-oxidizing bacteria in Fe and S cycling during wetting and drying events.

Based on the literature review, the hypotheses of the study are:

1 Iron sulfides present in the sediments will undergo oxidation during drying events and revert back to iron sulfides during the wetting events. During the long exposure to dry conditions, iron oxides should accumulate in the sediments.
2 Acidity generation should occur during the drying events as a result Fe-sulfide oxidation. However, the *in situ* buffering capacity of the lakes should counteract the process and the pH should not significantly decrease with time.
3 Iron- and sulfur-oxidizing bacteria should be present at all sites. Their abundance should increase under oxidizing conditions. However, the long term exposure to dry conditions will result in a decrease in bacteria populations since water is essential to their survival.

2.0 Methodology

2.1 Study sites

Two different hydro-electric dam reservoirs were chosen based on their bedrock differences (i.e. buffering capacity), proximity to Ottawa, size of generating station and boat launch accessibility. The first reservoir lake sampled was Stump Lake (also known as Smiths & Thompsons Bay) and is situated 35 km southeast of Perth, ON (Figure 2.1). This shallow lake was created after the installation of a small generating station on the Mississippi River. High Falls generating station was placed in service in 1920 and has a generating capacity of 2 100 kW. The dam itself is ~155.4 m long (includes dam and wing wall) and ~7.9 m high. High Falls station is being maintained but is operated from North Bay, ON. It is apparent that, at the time, tree removal before flooding was not common practice. Trees and tree stumps litter the lakebed making Stump Lake sediments organic rich. The sediment has a clayey texture. According to an Ontario Geological Survey (OGS) report, the bedrock is from the Lavant Gabbro Complex which is mainly composed of granite, gabbros and diorites (Pauk, 1989).

The second study site is situated on the Madawaska River southeast of Arnprior, ON (Figure 2.1). Mountain Chute Generating Station was built in 1967. This station has an installed generating capacity of 139 500 kW. It is currently producing energy. Black Donald Lake already existed at this time. Its water level was raised when the downstream dam was installed. The water level rose to such an extent that a closed graphite mine and its buildings were completely submerged. Black Donald Lake is much deeper than Stump Lake. The sediment grains are fine to coarse sand and far less organic than Stump Lake. According to and OGS report, the lake overlies a carbonate metasediment bedrock (Lumbers, 1982) and is therefore assumed to have a higher buffering capacity than Stump Lake.

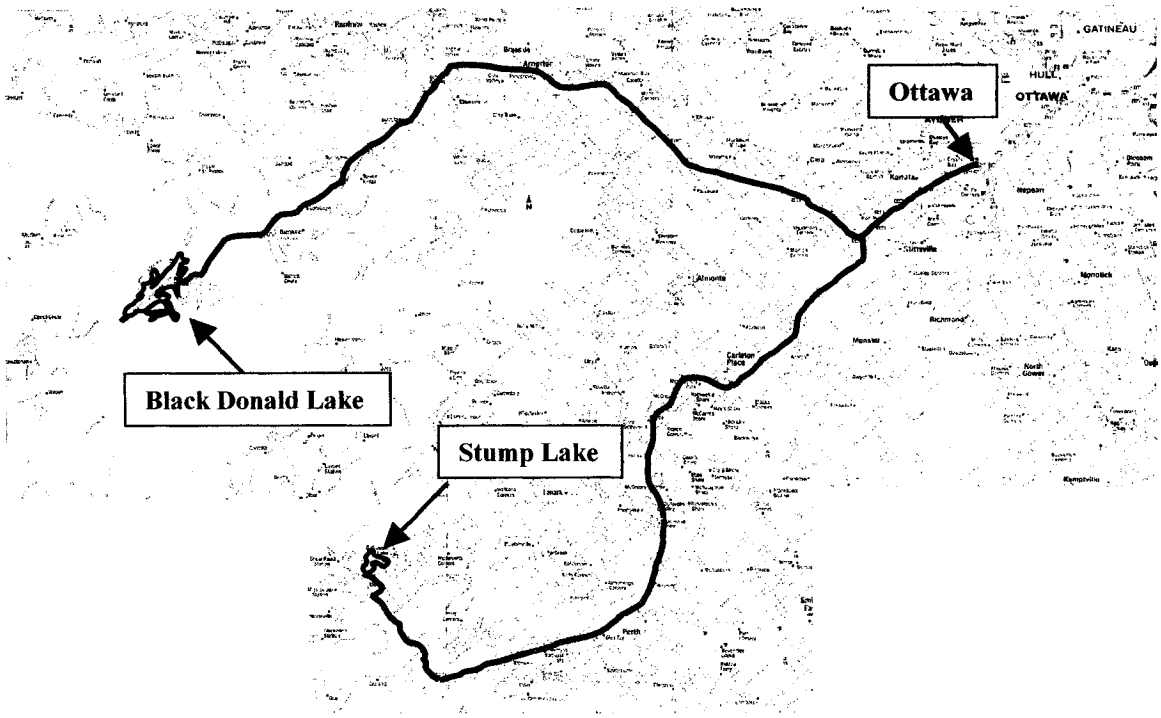
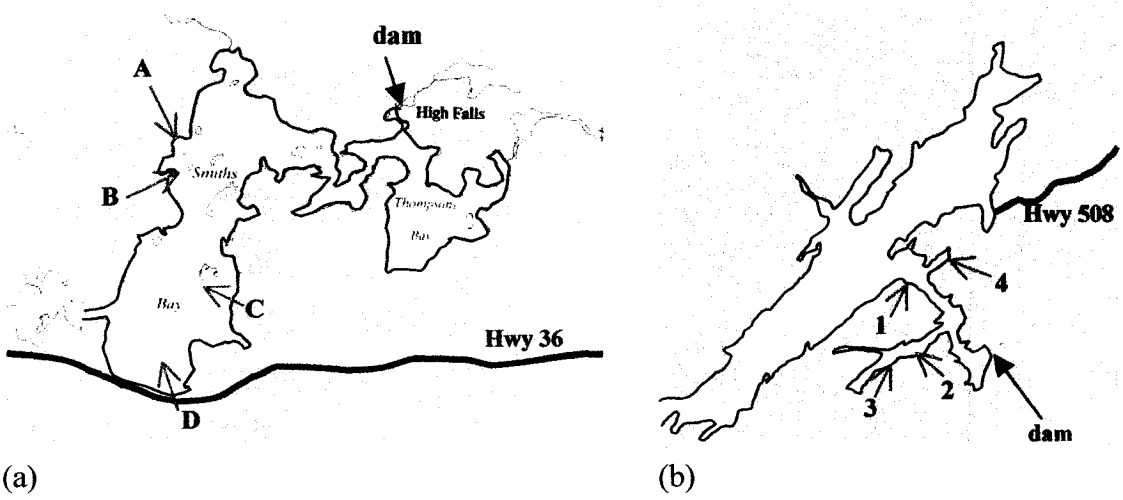


Figure 2.1 Location of Stump and Black Donald Lakes relative to Ottawa, ON. (MapArt, 2003)

2.2 Field sampling

Sediment cores and peepers were collected from August to November 2002. The general lake chemistry was determined in August 2003. Four different sampling sites were chosen for each lake (Figure 2.2). Table 1.2 shows the differences and similarities between sites. The selected sampling sites were near the shoreline and at shallow depths (2-3 m) in order to obtain sediments most susceptible to drying in the event of a dam removal. The sites chosen depended on boat accessibility and core retrieval feasibility. In total, two double peepers and four sediment cores were collected from each sampling site, for a total of 8 peepers and 16 sediment cores per lake.



(a) (b)

Figure 2.2 Location of (a) Stump Lake and (b) Black Donald Lake sampling sites.

Table 2.1 Characteristics of the sampling sites at Stump and Black Donald Lakes.

Stump Lake	Depth (m)	Position	Visual sediment characteristics
Site A	2	Set in a small bay away from the underlying river current	Protruding tree stumps litter this site; abundant tree litter.
Site B	3	Just to the side of the river current	Sandy to clayey sediment with none-to-little tree litter
Site C	2	In open area of lake; lightly affected by river current	Abundant tree litter and large tree stumps
Site D	2	In open area of lake nearest to boat launch; affected by river current	Abundant tree litter and weeds
Black Donald Lake*			
Site 1	2.5	In a small bay away from wind and river current	Lakebed littered with large rocks; few weeds
Site 2	2	Small indentation in small island just off the shore; near river current	Small to medium rocks litter lakebed; few weeds
Site 3	2	Very small bay; near river current	Abundant weeds; few rocks; large tree stumps on shoreline
Site 4	3	In a small sheltered bay away from wind and river current	Abundant weeds; few rocks; small gravel beach

*Water levels were ~50 cm higher than at the time the cores/peepers were collected.

Lexan polycarbonate plastic cores (60 cm high, 7 cm diameter) (distributed by GE® polymershapes), with 5 vertical sampling ports (1.3 cm wide; 2.5 cm apart vertically; and horizontally alternating 90° of each other (see Figure 2.3) situated at mid-core, were used to collect approximately 15-20 cm of shallow lake sediments and overlying water. The sediment cores were retrieved using a Kajak-Brinkhurst corer with a modified plunger. Before taking the sediment cores, silicon plugs were inserted into the mid-section sampling ports and secured in place with duct tape. Once out of the water, the top of the core was plugged with a plastic orange cap and the bottom with two butyl rubber stoppers, one covered with Teflon® (to prevent trace metal contamination). The ends of the core were then sealed with duct tape to prevent leaks and spills. All cores were kept cool and in a vertical position during transport to the laboratory. Upon arrival to the laboratory the cores were stored at 4°C.

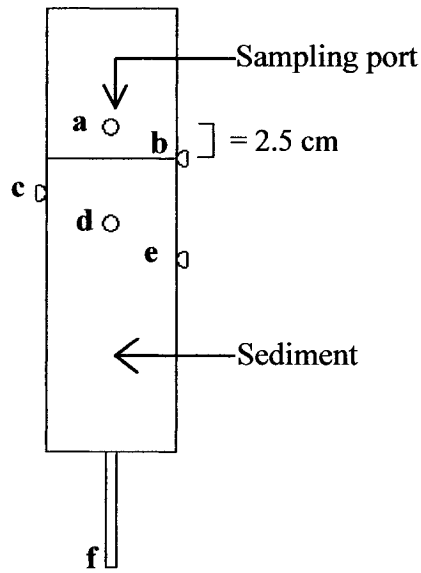


Figure 2.3 Schematic of vertical sampling ports of the sediment cores.

One sediment core from each site was sliced upon arrival to the laboratory and the fresh sediment was used for bacterial counts and sequential chemical extraction (sections 2.5 and 2.6.3). All cores were sliced in 2-cm increments down to a depth of 20 cm. To study the effects of extreme drought on the sediment, one of the three remaining cores was completely drained and remained dry for the remainder of the experiment (6 months). The remaining 2

sediment cores were submitted to repeated wetting and drying cycles (Figure 2.4). These cores as well as the cores simulating extreme drought were exposed to room temperature and ambient light.

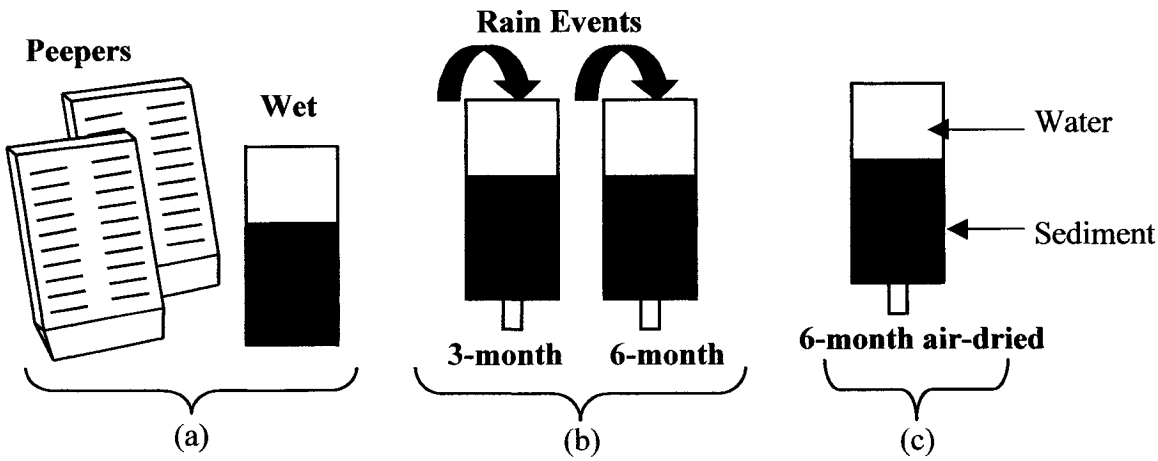


Figure 2.4 Schematic of experimental treatments used for (a) *in situ* conditions, (b) wetting and drying experiment and (c) 'extreme drought' conditions.

Each peeper, made of Lexan polycarbonate plastic, had two rows of 38 dialysis chambers each (total of 76 chambers/peeper). One dialysis chamber could hold approximately 4 mL of water. A pair of double peepers (Figure 2.5) was inserted into the sediment at each sampling site in order to collect *in situ* porewaters. Therefore, 16 mL of *in situ* porewater could be collected for one sampling depth, about every 1 cm. The number of analyses that needed to be done required all 16 mL of these water samples. There were no replicate porewater samples.

Once the membrane (Pall Gelman Tuffryn HT 200 membrane (0.2 μm membrane)) was mounted in the peepers, they were inserted into a large Nalgene tub filled with deionized water. For 2 weeks, this water was bubbled with nitrogen to purge the peepers and prepare them for insertion into the sediment. Before going out into the field, the peepers were taken out of the water and quickly sealed to prevent oxygen diffusion. Qualified divers unwrapped the peepers underneath the lake water surface and inserted them vertically within ~25 cm of the sediment in close proximity to the location where the sediment cores were collected in order to avoid lateral sediment heterogeneity. Approximately five dialysis chambers were left exposed to the water immediately above the sediment-water interface. Buoys were

attached to the peepers in order to facilitate retrieval. After approximately 3 weeks, the peepers were retrieved and the porewater samples were collected on site with sterile, N-purged, syringes. The samples were immediately analyzed for dissolved Fe (II) and sulfide and Eh and pH measurements (section 2.4). The remainder of the samples were acidified to 0.1%-1% with concentrated omnitrace HCl for sulfate and metal analyses (section 2.4).

Figure 2.5 Double peepers after insertion into sediments.

2.3 Wetting and drying cycles

The wetting cycles or 'rain events' consisted of a fixed volume (250 mL) of simulated rainwater (section 2.3.1) being added to the cores and letting the water settle for 4 hours (Figure 2.6). After this time, water samples were taken from the mid-section sampling ports along the core with sterile syringes and needles. After sampling was complete, the cores were drained to about 10 cm below the sediment surface and left exposed to room temperature and ambient light for 2 weeks. After 3 months, one core was sliced whereas the second was sliced after 6 months. The sediments were used for bacterial counts and sequential chemical extractions.

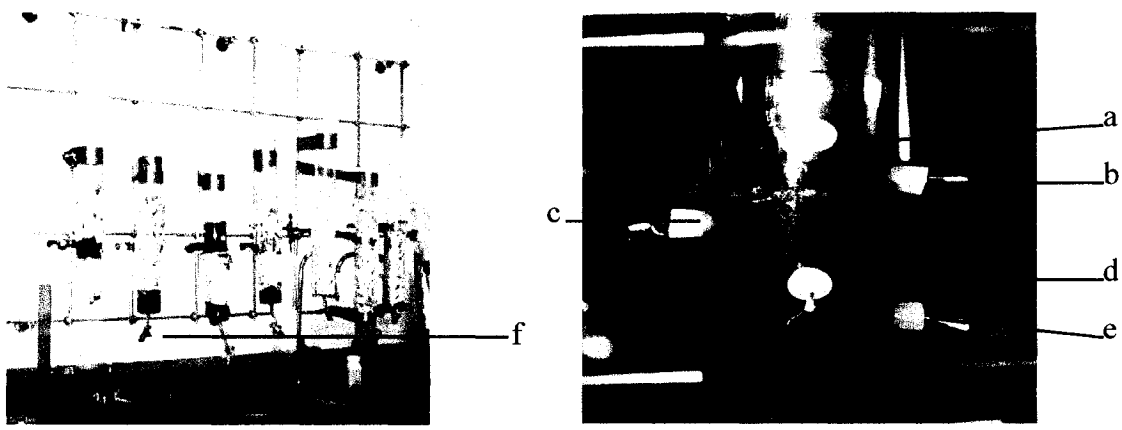
2.3.1 Simulated rainwater

The simulated rainwater composition (see appendix A) was based on the chemical data available for the average daily precipitation from the meteorological station closest to the study lakes, Chalk River, ON CAPMoN station. The average was taken over a five-year period (1995-2000) (http://www.msc.ec.gc.ca/capmon/index_e.cfm). In a clean 30 L HDPE barrel, chemicals were added to 25 L of deionized water. Over the course of the wetting and

drying experiment, three separate batches of rainwater were made, October 16 and November 28, 2002, and March 11, 2003.

2.3.2 Wetting and drying (w/d) method

All sediment cores were clamped side-by-side on a lab-frame (Figure 2.6 (a)). The sediment-water interface was manually positioned between the 2nd and 3rd mid-core hole (Figure 2.6 (b)). Top and bottom plugs were removed and vinyl tubing was inserted into the Teflon® covered plug at the bottom of the core to sample the leachate. The tubing was clamped during the 'drying' period to prevent loss of remaining water. No filtering agent was added to the tubing because this restricted the flow considerably and it was not necessary as the sediments acted as a filter. The cores were drained to 10 cm below the sediment surface. The leachate was acidified and stored for analysis. The top opening of the cores were covered with Parafilm®. The Parafilm® was punctured several times to expose the sediments to the atmosphere and to allow drying. This also allowed for a more even distribution of the rainwater on the sediment surface. The cores were then partially covered with foil, up to ~5 cm above the sediment surface to prevent light penetration to the deeper sediment which would, in a natural setting, not be exposed to surrounding light.



(a) (b)

Figure 2.6 (a) Setup of the cores in the laboratory and (b) position of the sampling ports.

Modified stainless steel needles (18 gauge 1½) were inserted into the silicon plugs in each mid-section sampling port for water sample collection. To prevent water from dripping from the needles, Teflon® tape was wrapped on the end of the needles (Figure 2.6 (b)).

After a 2-week drying period, the first 'rain' event occurred (October18th, 2002 for Stump Lake and October 24th, 2002 for Black Donald Lake). Approximately 250 mL of simulated rainwater was added to the cores of one lake every 2 weeks. This represents an average rainfall of 61.6 mm. The average monthly rainfall for Ottawa, Ontario is 61.1 mm (based on Environment Canada's database of climate normals and averages from 1971-2000 (www.climate.weatheroffice.ec.gc.ca/climate_normals/results_e.html)). After letting the rainwater settle for 4 hours, pH measurements and water samples (6-10 mL) were taken from each sampling port in descending order, ending with the tubing at the bottom of the core. The samples were either immediately analyzed for Fe (II) and S^{2-} concentrations or acidified to 0.1-1% with concentrated omnitrace HCl (up to November7th, 2002) or with 10% omnitrace HNO_3 (after November7th, 2002). After sampling, the water level was usually 10 cm or more below the sediment surface. The cores were covered in aluminum foil and a period of 2 weeks elapsed before the next rain event. The experiment ended with the last rain event (April 4th, 2003 for Stump Lake and April 9th, 2003 for Black Donald Lake) and subsequent sediment core slicing.

2.4 Porewater chemistry

Lake water, porewaters and water from the column experiment were analyzed for various parameters, including ferrous iron (Fe (II)), sulfide and sulfate concentrations as well as major metal ion concentrations and pH. Eh measurements were done on the lake water and porewater. Only lake water alkalinity and conductivity analyses were done.

2.4.1 Ferrous iron analysis

The ferrozine method (Stookey, 1970) was used to analyze the particulate and dissolved ferrous iron (Fe (II)) concentration. Three milliliters of unfiltered water sample were added to a vial already containing 0.3 mL of the ferrozine solution. The samples were kept cool until the optical density could be measured at 562 ηm using a Beckman DU-65

spectrophotometer (single cuvette; until November 8th, 2002) or with a Molecular Devices SpectraMax Plus spectrophotometer (96 micro-well plate; after November 8th, 2002). A factor of 2.2 was used to convert absorbency readings into concentration values. This factor was based on several calibration curves and a dilution factor. The detection limit for this protocol is 1 μm (S.D. 2%).

2.4.2 Sulfide analysis

Particulate and dissolved sulfide concentrations were determined with the Cline method (Cline, 1969). Cline reagents were added to 2-mL amber vials in an anaerobic chamber prior to sampling. A 1.5 mL unfiltered water sample was added to the Cline reagent mixture. The sample's optical density was then measured at 670 ηm using a Beckman DU-65 spectrophotometer (single cuvette; until November 8th, 2002) or with a Molecular Devices SpectraMax Plus spectrophotometer (96 micro-well plate; after November 8th, 2002). An average of several calibration curves was used. The detection limit for this protocol is 4 ηm.

2.4.3 Sulfate analysis

The turbidity method (Rodier, 1975) was used to analyze the particulate and dissolved sulfate content. Due to high concentrations, water samples from the cores were diluted 4 times (200μL of sample). However, due to the small volume of porewaters collected from the peepers, samples had to be combined (e.g. 500 μL each of the 1-cm and 2-cm depths were combined, 500 μL each of 3-cm and 4-cm depths were combined, etc.). The micro-well plates used for optical density measurements were briefly agitated before reading at 650 ηm using the Molecular Devices SpectraMax Plus spectrophotometer. Sulfate standards were freshly made. Calibration curves were done for each analysis session.

2.4.4 Metal analysis

A Varian Vista-Pro CCD Simultaneous ICP-OES was used for the analysis of metals. Several metals were below the ICP-OES detection limit, with the exception of Ca, Fe_{Total}, Mg, Mn and Na. Most samples were not filtered. Analysis was done using the "ICP

Expert" software. Standards were freshly made and tailored to the water samples. The simulated rainwater was used as matrix for this analysis. The detection limit for the major metal ions varied. Total iron and manganese was detectable above 1.8 μM for both lakes. The detection limit for sodium was above 22 μM and 87 μM for Black Donald Lake and Stump Lake, respectively. For both lakes, calcium and magnesium detection limits were 0.13 μM and 0.63 μM, respectively.

2.4.5 pH and redox measurements

Eh and pH measurements were performed with a VWR SympHony SP Series Waterproof Portable pH Meter Model SP21 (VWR Scientific Products) and a VWR SympHony 3-1 Gel pH electrode and a Corning Redox Platinum Comb electrode. The meter was calibrated before each use with pH 4 and 7 buffers and with the Zobell solution for the Eh measurements (Nordstrom, 1977). Measured Eh values were corrected with the reference hydrogen electrode (+199 mV) and a calibration factor measured before each use.

2.4.6 Conductivity

The conductivity of the lake water samples was measured upon return from the field with a Corning M-90 meter and a corresponding electrode on un-acidified samples. The meter was calibrated with a fresh conductivity standard (1413 μS/cm).

2.4.7 Total alkalinity

Total alkalinity was measured, *in situ*, at each site using a HACH digital titrator. The total alkalinity method outlined in the HACH Water Analysis Handbook (1989) was used for the titration along with the 1.6 N $\pm$ 0.005 H_2SO_4 acid titration cartridge.

2.5 Bacterial enumeration

Fresh lake sediments, sediments from the cores submitted to wetting and drying cycles and the air-dried sediment were analyzed for the presence of neutrophilic iron- and sulfur-oxidizing bacteria using the most-probable number (MPN) method. Sediment samples were taken at 2-cm depth intervals (except at 0 months which was taken at 1-cm intervals up to 6 cm then 2-cm intervals) down to a depth of 10 cm. The 2-cm intervals were chosen because of the slight sediment compaction that occurred during the wetting and drying cycles. There were no control cores used during bacterial enumeration.

2.5.1 Growth media for neutrophilic iron-oxidizing bacteria

The neutral growth medium used to estimate the approximate number of iron-oxidizing bacteria was the PTYP medium by Siering and Ghiorse (1996). It is however specific to the *Leptothrix* species. The PTYP medium consisted of 0.25 g/L of peptone, 0.25 g/L of Tripticase Soy Broth, 0.50 g/L of yeast extract, 0.6 g/L of $MgSO_4 \cdot 7H_2O$, 0.07 g/L of $CaCl_2 \cdot 2H_2O$ and 2.38 g/L of HEPES buffer. The pH was adjusted to 7.2 with 2 M NaOH. 1 mL of a sterile 100 μM iron sulfate stock solution and 1 mL of a sterile 1% sodium pyruvate stock solution were added to 8 mL of the PYTP medium in a sterile environment (final concentration of 10 μM iron sulfate and 0.1 % sodium pyruvate). Approximately 1 g of fresh sediments was then added to each tube and left to grow at room temperature for one month. The most probable number method (MPN) (section 2.5.3) was used to estimate the number of bacteria. Positive tubes had an apparent yellow precipitate.

According to Siering and Ghiorse (1996), the PTYP medium can be used to grow bacteria from the *Shaerotilus-Leptothrix* group, such as different strains of *L. discophora* and *L. cholodn*ii and *S. natans*.

2.5.2 Growth media for neutrophilic sulfur-oxidizing bacteria

The neutral growth medium used for the enumeration of thiosulfate-oxidizing bacteria was a modified version of the Harrison medium (Tv6) (Harrison, 1983) cited in Stubner et al. (1998). The Harrison method is said to be specific to *Acidiphilium cryptum* but some

Thiobacilli spp. may also grow (Harrison, 1983). The growth medium consisted of [A] 5.1 g/L of $Na_2HPO_4 \cdot 2H_2O$, 0.3 g/L of NH_4Cl, [B] 0.1 g/L of $MgSO_4 \cdot 7H_2O$, [C] 0.0244 g/L of biotin, 0.3 g/L of yeast extract and 5.0 g/L of $Na_2S_2O_3 \cdot 5H_2O$. The pH of solution [A] was adjusted to 8.5 with 2 M NaOH. Solutions [A], [B] and [C] were prepared separately and autoclaved or filter-sterilized. Then 1 mL each of [B] and [C] were added to [A] in a sterile environment. Fifty microliters of sterile Wolfe's vitamin solution (Wolin et al., 1963) was added to each culture tube. Growth of thiosulfate-oxidizing bacteria was assessed by turbidity measurement (Stubner et al., 1998). Absorbency readings at 578 ηm were done using a Molecular Devices SpectraMax Plus spectrophotometer after 2 months of growth. Positive tubes had an absorbance above 0.004 ηm after correcting absorbency readings with the absorbance value of a blank culture tube.

According to Stubner et al. (1998), the Harrison medium (Tv6) can be used to grow mixotrophic bacteria utilizing thiosulfate together with an organic substrate. Based on physiological properties and phylogenetic affiliations based on the 16S rDNA sequences, strains such as *Xanthobacter tagetidis*, *Aquabacter spiritensis*, *Azorhizobium caulinodans* and *Bosea thiooxidans* can be isolated (Stubner et al., 1998). All these bacteria are Gram-negative, facultative autotrophs that grow autotrophically with thiosulfate or tetrathionate.

2.5.3 Most probable number method (MPN)

The most probable number method gives an under-estimation of the actual number of bacteria present in a soil or sediment. Sediment depths were combined to reduce the number of MPN samples. For example, approximately 0.5 g from the 1-cm depth was combined with approximately 0.5 g from the 2-cm depth. This depth is represented on figures 3.13 to 3.16 as depth 1.5 cm. Approximately 1 g of the combined wet sediment sample was used to inoculate 10 mL of growth medium. This initial dilution was diluted five times. Each of these five dilutions had five replicates. Therefore each sample required a set of 25 MPN tubes. After the appropriate growth time, the MPN tubes were counted to see how many tubes from each sample set of 25 tubes were positive for bacterial growth. The most probable number table (Cochran, 1950) was used to estimate the number of Colony Forming Units per gram of dry sediments.

2.6 Sediment geochemistry

Geochemical analysis carried out on the fresh sediment, the sediment from the column experiment and the air-dried sediment included moisture content and sequential chemical extraction. The organic content of the sediment was estimated using the loss on ignition (LOI) method.

2.6.1 Gravimetric moisture content

The water content of the sediments was determined by drying approximately 1g of wet sediment in a drying oven (Stabil-Therm® Laboratory oven) at 100°C for 24 hours. The moisture content reported in section 3.4 is the percentage of the weight of water by weight of oven dried soil ratio. Dry weight measurements were used to express the bacterial counts and for the sequential chemical extraction data.

2.6.2 Organic carbon content

The Jackson (1958) loss on ignition (LOI) method was used to estimate the percentage of organic carbon in the sediment samples from the sliced cores. Approximately 1 g of sediment was weighed out in aluminum weighing dishes. These were then put in a drying oven (Stabil-Therm® Laboratory oven) at 100°C for 24 hrs. Samples were then transferred to a Lindberg oven for another 8-12 hrs at 400°C after which time the final weight was recorded.

2.6.3 Reactive iron extraction

The Kostka and Luther sequential extraction method (1994) separates the oxidized from the reduced iron minerals in sediment resulting in Fe (II) and Fe (III) concentration values. Frozen sediments were freeze-dried before undergoing the extraction. Duplicate samples from the surface (1-2 cm), middle (5-6 cm) and bottom (9-10 cm) of the sediment cores were used for these extractions. Samples were in clumps after freeze-drying, therefore a mortar and pestle was used on the sediment to break the clumps. From 0.2 to 0.5 g of freeze-dried sediment was added to Falcon tubes containing 10 mL of 5 M HCl. These were

placed on an orbital shaker for 1 hour at room temperature. The tubes were then vortexed and 0.1 mL of the sample was added to two different solutions: 1) 5 mL of a ferrozine and HEPES solution, and to 2) 5 mL of a HEPES and $NH_2OH{\cdot}HCl$ solution; the pH of the solutions was adjusted to 7 with 2 M NaOH. After 20 minutes on a rotisserie shaker, 0.1 mL of 10^{-2} M ferrozine solution was added to each Falcon tube (solutions 1 and 2). Approximately 50 minutes later, the extracts were filtered with a 0.2 μm filter and their optical density was measured at 562 ηm using a Molecular Devices SpectraMax Plus spectrophotometer. Fe (II) and Fe (III) concentration values were calculated with the same conversion factor (2.2) used for the ferrous iron analysis (section 2.4.1).

Most of the Fe (II) results were unreliable because the sediments had been freeze-dried before undergoing the extraction procedure. During the freeze-drying process, the sediment is exposed to oxygen, which leads to the oxidation of reduced iron species. Therefore, only the total reactive iron (poorly crystalline iron oxides and sulfides (not pyrite) extracted with HCl) will be used in section 3.6 of the results and for subsequent discussion purposes. Section 3.6 graphs show the average of the duplicate samples and their standard deviations (error bars).

2.7 Statistical analyses

To better understand the results, several different analyses of variance (ANOVA) were done on the data. An ANOVA test compares the variability between group means to the variability within each group. If a factor is found to have a significant effect on a dependent variable, it is because the variability between the group means is larger than the variability within the groups. The ANOVA test will not indicate which mean is different from which other mean. First, the observations, or data, needed to be separated into groups, or 'factors', that could affect the dependent variables, i.e., the physico-chemical parameters (pH, Fe (II), S^{2-}, SO_4^{2-} and bacteria population estimates). Statistical analyses were done using depth in the core (a, b, c, d, e and f), time ((week 1, 3, 5..., 23) or (0-, 3-, 6-month and 6-month air-dried sediment cores)), core (1 and 2 (for each site)), site (A, B, C and D (for both lakes)) and finally lake (SL and BDL) as factors. Kolmogorov-Smirnov tests, which

determine if the data is normally distributed, were done on the different data sets. No data transformations were needed. All statistical tests were done using a 95% confidence level.

In order to verify if the 3- and 6-month cores from the same site behaved similarly during the wetting and drying experiment, their means over time were compared at each individual depth using a one-way classification ANOVA. Tables B.1 and B.2 summarize these results (appendix B).

To determine if depth and time, the principal factors, had significant effects on the various dependent variables (pH, Fe (II), S^{2-}, SO_4^{2-} and bacteria population estimates) each site's data set (depth results over time) was analyzed with a two-way classification ANOVA (Tables B.3 and B.4; appendix B).

Assuming the sites were good replicates, the entire data set for each lake was analyzed using a two-way classification ANOVA with repetition. These results are summarized in sections 3.3.3.1 and 3.5.4.1 and in Table B.5 (appendix B).

To analyze the interaction between factors more thoroughly, a three-way classification ANOVA was also used on the data as a whole (including data from both lakes). These interactions, when found to be significant, would indicate if, for example, the effect of the depth (whatever it was) depended on which time was being considered and the effect of the time (whatever it was) depended on which depth was under consideration (Table B.6; appendix B).

To find the significant sources of variability in the experimental procedure, nested ANOVAs were used. A nested ANOVA is used when there is interchangeability within the levels of the experimental procedure hierarchy (lakes, sites, cores, samples). For example, the pH results from Site 1, core 2, depth 'a' cannot be taken instead of depth 'f'. There is no interchangeability at this, the depth level. However, the pH data from Site 1, core 2 can be exchanged with the pH data from Site 1, core 1. The nested ANOVA model for the wetting and drying experiment had three levels: 1) cores were nested within sites that were nested within the lakes, 2) sites were nested within lakes, and 3) lakes (Figure 2.7). Nested ANOVAs were generated for each dependent variable, namely pH, Fe (II), sulfide and sulfate concentrations. Note that for the bacteria population estimate nested ANOVAs, the hierarchal design only had two levels instead of three: 1) sites were nested within lakes, and 2) lakes (Table B.7; appendix B).

Two-way classification ANOVAs were generated using time and depth as factors to see if they had a significant effect on sediment moisture and organic content values. Tables B.8 and B.9 (appendix B) summarize these results. Sections 3.4.1 and 3.4.2 however only report the results from the factor 'time'. This was the more important factor when considering the moisture and organic content.

The spreadsheet software EXCEL (version 98) was used to produce the one- and two-way ANOVAs and SYSTAT 10 was used to produce the three-way and nested ANOVAs. Because SYSTAT was used to produce the nested ANOVAs, the F-ratios generated by the program as well as the Mean Squared deviations (MS) of the error had to be recalculated.

SYSTAT uses MS_{Error} as the denominator in all F-ratio calculations. For more accurate F-ratios, the denominator of the 'parent' level F-ratios needed to be the MS of the 'daughter' level (Figure 2.7).

	SYSTAT 10	Recalculated
Lake ← parent Site(Lake) ← parent Core(Site(Lake)) ← daughter **Error** ← daughter	$F\text{-ratio}_{Lake} = \frac{MS_{Lake}}{MS_{Error}}$	$F\text{-ratio}_{Lake} = \frac{MS_{Lake}}{MS_{Site(Lake)}}$

Figure 2.7 F-ratio re-calculation.

Ordinarily a statistical model contains a random error (Figure 2.7 in bold) which is calculated from the residual random observations. The residual observations associated with this study were not taken at random. The variables themselves were linked. They were taken at a specific time and place. Therefore, the interactions 'depth*time' from the two-way classification ANOVAs with repetition were used to reintroduce a random component to the nested ANOVA results table. Since both 3- and 6-month core data was included in generating the nested ANOVAs, the MS and degrees of freedom (df) from their two-way classification ANOVA interactions were averaged for both lakes. The averages were then used in the equation 7 to calculate a pondered mean that would replace the MS_{Error} of the nested ANOVAs.

$$\text{Error} = \frac{[(MS_{SL} \times df_{SL}) + (MS_{BDL} \times df_{BDL})]}{(df_{SL} + df_{BDL})} \qquad (7)$$

The MS values (of each individual hierarchical level (i.e., site nested within lake) or factor (i.e., site or lake)) presented in the nested ANOVA tables can be re-calculated to give the actual value of the variance (σ^2) or variability of the nested factor. Equation 8 was used to calculate the variance of the factor itself (i.e., site), without it being nested (site within lake).

$$\sigma^2_{Site} = (\sigma^2_{Site(lake)} - MS_{Core}) / n(core) \qquad (8)$$

Where $\sigma^2_{Site(lake)}$ is the variance calculated by SYSTAT for the hierarchical level 'site nested within lake', MS_{Core} is the newly calculated Mean Squared deviation of the daughter level, i.e., core, and n(core) is the number of observations under the hierarchical level 'site'.

3.0 Results

3.1 General lake chemistry

The water chemistry of both lakes was measured on August 16 & 17th, 2003. Both lakes displayed an average surface temperature of 25°C. The pH values of surface and deeper water samples indicated that both lakes are alkaline, i.e., pH of 8.5 for Stump Lake and 8.1 for Black Donald Lake. Redox measurements of surface and deeper lake water samples showed that the water was oxic (i.e., 394 mV and 381 mV for Stump Lake and Black Donald Lake, respectively). However, redox values decreased with depth for both lakes. On average, Stump Lake has higher alkalinity and conductivity than Black Donald Lake (Tables 3.1 and 3.2). Alkalinity in both lakes increases slightly with depth. Iron and manganese concentrations in surface lake water samples were below the detection limit of the ICP-OES used in this study. Stump Lake surface waters had higher concentrations of calcium and magnesium than Black Donald Lake surface waters (Tables 3.1 and 3.2). Sodium concentrations show the inverse situation with 85 μM for Stump Lake and 124 μM for Black Donald Lake (Tables 3.1 and 3.2).

Table 3.1 *In situ* physico-chemical conditions of Black Donald Lake

Site (sampling depth)[1]		**Site 1** (183 cm)	**Site 2** (200 cm)	**Site 3** (190 cm)	**Site 4** (285 cm)	**AVG**	**Std error**
GPS Position		18T 0348364	18T 0348349	18T 0347518	18T 0349367		n/a
	UTM	5007642	5006159	5005710	5007503		n/a
Altitude (m.a.s.l)		257	251	263	256		n/a
Sediment-water interface depth (cm)		197	208	202	295	226	23.3
Temperature (°C)		25.2	25.2	25	25.4	25.2	0.08
pH	surface	8.11	8.13	8.23	8.24	8.18	0.03
	depth	8.08	8.08	8.21	8.06	8.11	0.03
Eh (mV)	surface	388	362	412	363	381	11.9
	depth	329	340	356	330	339	6.22
Total Alkalinity (mg $CaCO_3$/L)							
	surface	29.5	33	30	28.5	30.3	0.97
	depth	32	30	31	42	33.8	2.8
Conductivity (µS/cm)		102	102	103	102	102	0.21
Ca avg[2] (µM)		272	268	271	271	270	0.79
Fe_T avg[3] (µM)		<1.79	<1.79	<1.79	<1.79	<1.79	
Mg avg[2] (µM)		78.2	77.3	79.4	79.4	78.6	0.5
Mn (µM)		<1.82	0.13	<1.82	<1.82	<1.82	
Na avg (µM)		122	122	127	124	124	1.07

[1]Depth at which *in situ* pH, temperature and alkalinity samples were taken.

[2]Indicates the average of three ICP-OES wavelengths.

[3]Indicates the average of two ICP-OES wavelengths.

Table 3.2 *In situ* physico-chemical conditions of Stump Lake

Site (sampling depth)[1]		**Site A** (170 cm)	**Site B** (290 cm)	**Site C** (170 cm)	**Site D** (130 cm)	**AVG**	**Std error**
GPS Position		18 T 0370248	18T 0370772	18T 0370855	18T 0370199		n/a
	UTM	4977333	4978467	4977830	4977384		n/a
Altitude (m.a.s.l)		195	195	195	195		n/a
Sediment-water interface depth (cm)		183	310	178	145	204	36
Temperature (°C)		26.7	26.5	26.9	26.4	26.6	0.11
pH	surface	8.48	8.36	8.55	8.73	8.53	0.07
	depth	8.52	8.42	8.51	8.68	8.53	0.05
Eh (mV)	surface	413	390	391	384	394	6.26
	depth	377	377	377	372	376	1.27
Total Alkalinity (mg $CaCO_3$/L)							
	surface	51	51	52.5	53	51.9	0.52
	depth	66.3	57.5	52	47	55.7	4.1
Conductivity (µS/cm)		145	141	141	138	141	1.4
Ca avg[2] (µM)		424	415	410	408	415	3.5
Fe_T avg (µM)		<1.79	<1.79	<1.79	<1.79	<1.79	
Mg avg[2] (µM)		132	116	116	114	119	4.2
Mn (µM)		<1.82	<1.82	<1.82	<1.82	<1.82	
Na avg (µM)		83.5	85.3	87.9	84.4	85.3	0.94

[1]Depth at which *in situ* pH, temperature and alkalinity samples were taken.

[2]Indicates the average of three ICP-OES wavelengths.

[3]Indicates the average of two ICP-OES wavelengths.

3.2 Porewater chemistry

3.2.1 Black Donald Lake (BDL)

The porewater pH was more acidic than the surface water but remained in the neutral range (Figure 3.1). The pH of all BDL sites ranged between pH 5.5 and 7.4 and showed a decrease in depth. Redox values below the sediment-water interface ranged between 150 and 400 mV and remained stable with depth for sites 1 and 4, whereas they declined with depth for sites 2 and 3, indicating more reducing conditions in the sediments (Figure 3.1). However, it is important to mention here that the Eh measurements were done on site under open atmosphere and that oxygen likely diffused into the peeper chambers during the reading, therefore biasing the values. Ferrous iron concentrations ranged between 0 and 160 μM whereas sulfide concentrations varied between 0-100 μM (Figure 3.1). All sites showed an increase in Fe (II) concentrations just below the sediment-water interface, between 1 and 4 cm (Figure 3.1). Fe (II) levels greatly varied with depth for all sites. Sulfide was present in the porewaters at the sediment-water interface at sites 3 and 4 and fluctuated with depth (Figure 3.1). At site 1, sulfide levels were very low, whereas a zone of sulfide production was observed at site 2 between 1 and 6 cm (Figure 3.1). Porewater sulfate concentrations ranged between 30-100 μM with site 4 possessing the highest concentrations (Figure 3.1). At all sites, sulfate levels declined with depth, especially for sites 1 and 2 (Figure 3.1).

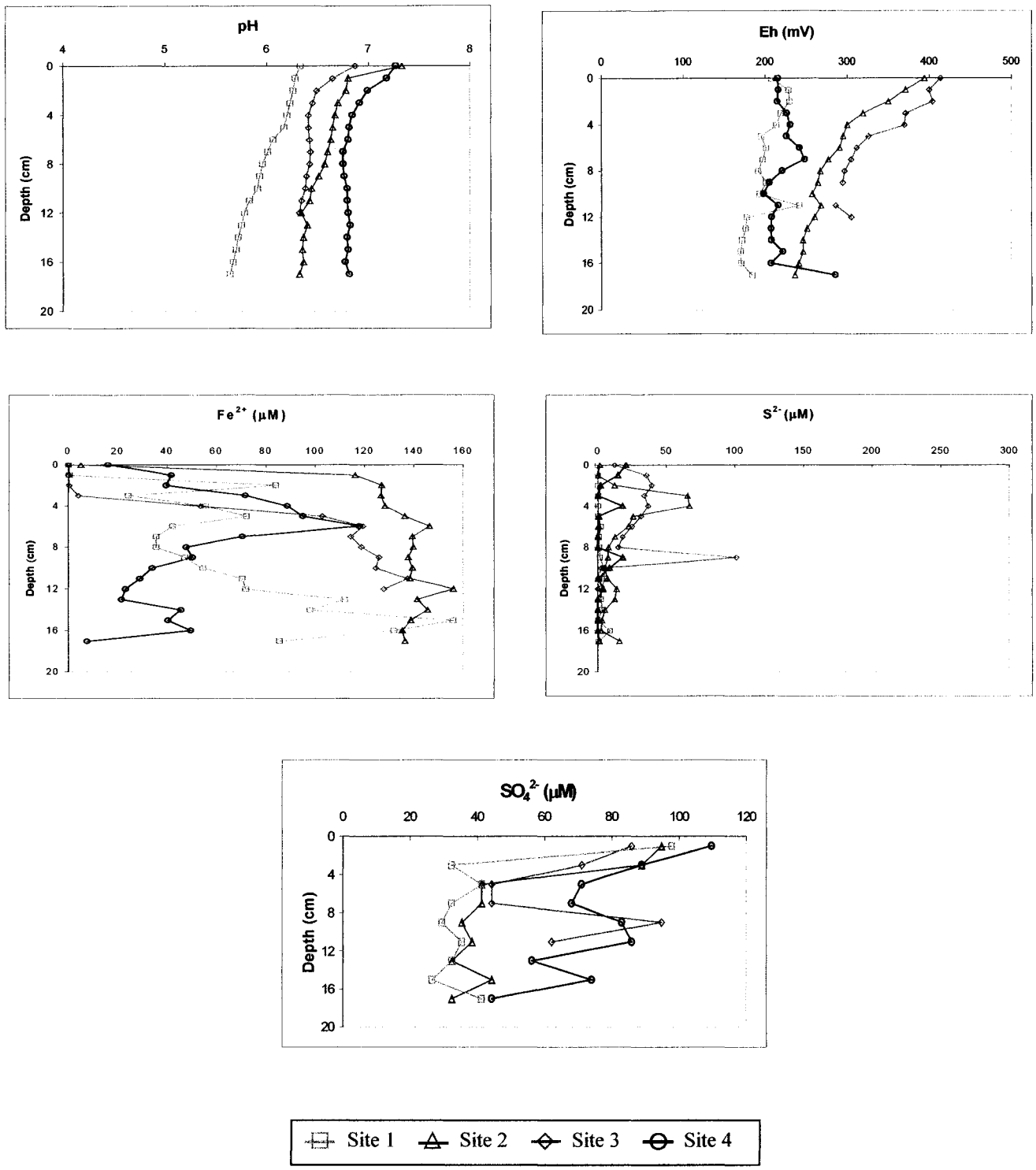


Figure 3.1 Porewater chemistry of Black Donald Lake (BDL) sediments as a function of depth. Data points indicate results from individual peeper dialysis chambers. Note: Incorrect Eh reading resulted in missing 10-cm depth value.

3.2.2 Stump Lake (SL)

Analysis of Stump Lake porewater showed the porewater to be more acidic than the surface water but still neutral (Figure 3.2). The pH of the four SL sites varied between pH 6.5 and 7.5 and showed a decrease with depth. Redox measurements ranged between 200 and 350 mV, indicating oxic conditions in the sediment profile (Figure 3.2). Due to the fact that the measurements were made in open air, these redox readings should be treated with a certain degree of skepticism. Fe (II) concentrations in the sediments varied between 0 and 50 μM (Figure 3.2). Soluble ferrous iron was present in the porewaters just below the sediment-water interface and decreased with depth at sites A, B and D, whereas it remained stable at site C (Figure 3.2). Sulfide concentrations for the Stump Lake sites were between 0-250 μM. Concentrations peaked near the sediment-water interface and then declined with depth for sites A and D, whereas they remained stable at site B. As for site C, sulfide levels increased with depth (Figure 3.2). Sulfate concentration ranged between 20 and 85 μM, with site B having the highest concentrations (Figure 3.2).

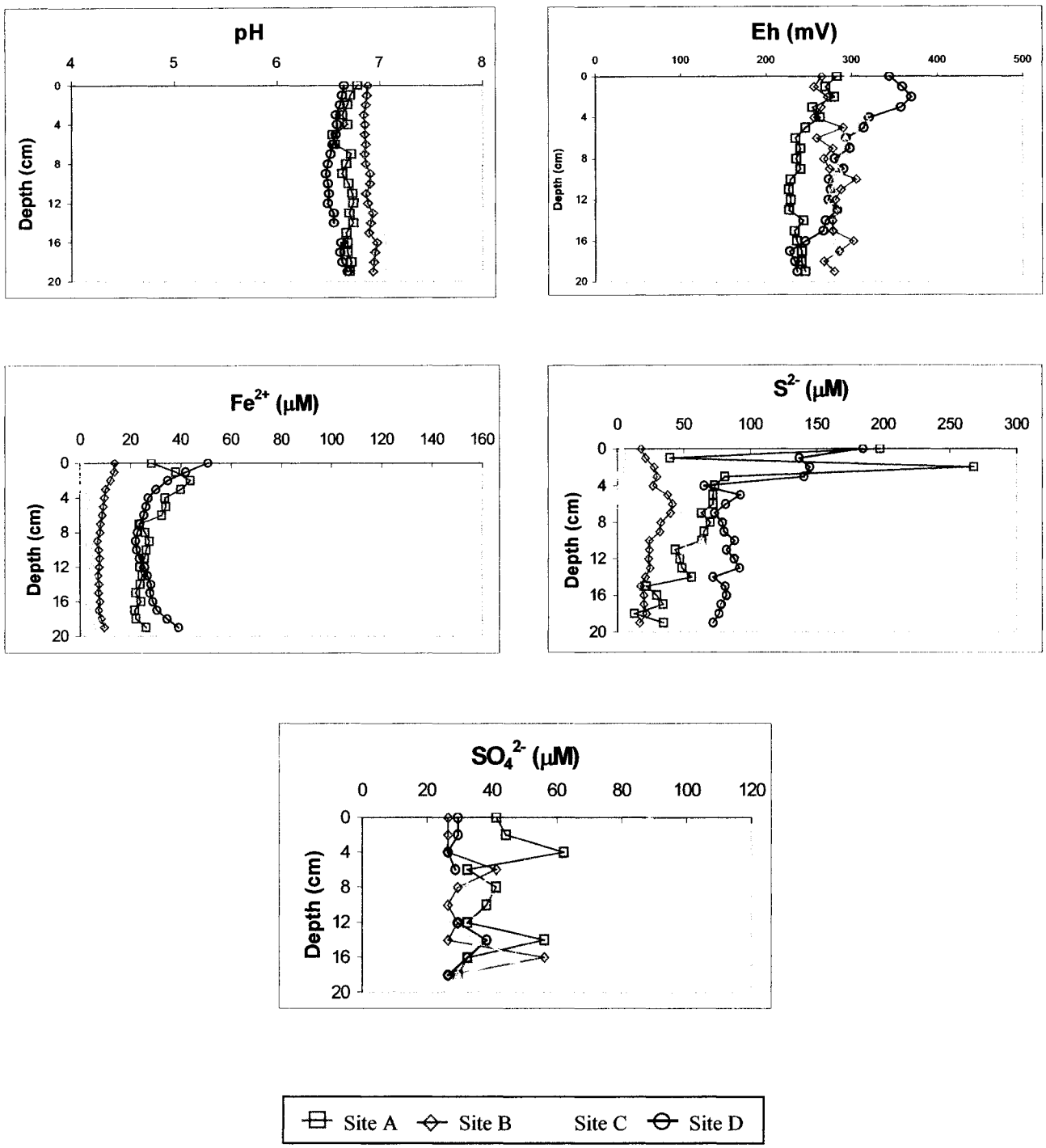


Figure 3.2 Porewater chemistry of Stump Lake (SL) sediments as a function of depth. Data points indicate results from individual peeper dialysis chambers.

3.3 Wetting and drying experiments

The following sections show the chemical changes (pH, Fe (II), sulfide and sulfate) that occurred during wetting and drying events. The figures in these sections are three-dimensional and show the chemical species concentrations as a function of time and by depth in the core (i.e., sampling ports 'a' to 'f'; Figure 2.3). The graphs in the following section represent the results from the 6-month cores. It would be redundant to show both the 3- and 6-month cores. The latter cores were used over a longer period of time and are more suitable for detecting trends in time. In general, statistical analyses comparing the results of both 3- and 6-month cores at each depth showed no significant differences between the means of both cores. Only 23% of the one-way ANOVA done to compare the 3- and 6-month cores were significant and these were mostly found in the lower sampling ports 'd, e and f' (see appendix B for more details on the statistical analyses presented in this section). Weeks 1,3,5,..., and 23 represent sampling times after wetting events. Weeks 2,4,6,..., and 22 were drying weeks.

3.3.1 Black Donald Lake

In general, the pH values for all four sites ranged between 4.5 and 7.0 (Figure 3.3). Site 4 has the highest pH throughout the experiment whereas site 1 has the lowest. For all sites, the pH was always the highest in the leachate (sampling port 'f') and the lowest in the first sampling port ('a') (Figure 3.3). The pH in the sediments (ports 'a' to 'e') tended to decline over time, whereas it stayed relatively stable in the leachate (port 'f'). For all sites, both depth and time had a significant effect on the pH (depth $p= 2x10^{-13}$ to $6.3x10^{-5}$; time $p= 2x10^{-9}$ to 0.0024).

The ferrous iron concentrations ranged from 0 to 160 μM (Figure 3.4). Sites 1 and 3 have the lowest ferrous iron concentrations (i.e., 0-10 μM). Fe (II) concentrations were the greatest for all 4 sites, especially for sites 2 and 4 (Figure 3.4). For those sites, the ferrous iron concentrations remained stable over time. Some Fe (II) was present in the deeper sediments (ports 'c' to 'e') at sites 2 and 4, especially during the first 10 weeks of the experiment. Sites 1, 2 and 4 showed significant effect of depth on the Fe (II) concentration ($p= 7.3x10^{-8}$, $2.2x10^{-17}$ and $3x10^{-16}$, respectively) whereas this same significance could not be

detected for site 3 (p= 0.122). We could not detect a significant effect of time on the Fe (II) concentration for sites 1, 2 and 3 (p= 0.31, 0.20 and 0.09, respectively). Time had a significant effect on the Fe (II) concentration for site 4 (p= 0.007).

Sulfide concentrations varied between 0 and 70 μM (Figure 3.5). Very low sulfide levels were observed in the sediments of sites 1 and (with the exception of the sample in port 'c' after 1 week at site 3), even in the leachate (port 'f') and they did not appear to vary with time (Figure 3.5). In the cores of sites 2 and 4, there was an apparent release of sulfide in the porewaters (ports 'c' to 'e') (Figure 3.5). For site 2, sulfide was also detected in the leachate at the beginning of the experiment, whereas it was released later on in the core from site 4 (Figure 3.5). Depth had a significant effect on the sulfide concentration of sites 1, 2 and 4 (p= 0.0005, 0.0003 and 0.042, respectively). No significant effect of depth on sulfide concentration could be detected at site 3 (p= 0.158). For sites 1, 3 and 4, a significant effect of time on the sulfide concentration could not be detected (p= 0.28, 0.25 and 0.31, respectively). Time had a significant effect on site 2's sulfide concentration (p= 0.012).

Sulfate concentrations ranged from 0-1800 μM (Figure 3.6). Sulfate concentrations generally increased from the top (port 'a') to the bottom of the cores (port 'e'), with the exception of site 3 (Figure 3.6). For all sites, sulfate levels were higher in the core than in the leachate (port 'f'), with the exception of site 3 (Figure 3.6). For sites 1 and 3, sulfate concentrations slightly increased over time but declined near the end of the experiment (week 21). For site 2, sulfate levels declined over time in the upper portion of the core (port 'b'), whereas they increased in the rest of the core (Figure 3.6). For sites 1, 2 and 3, a significant effect of depth on sulfate concentration was found (p= $1.2\text{x}10^{-10}$, $2.7\text{x}10^{-6}$ and $7.6\text{x}10^{-12}$, respectively). We could not detect a significant effect of depth on the sulfate concentration at site 4 (p= 0.52). Time had a significant effect on sulfate concentration of sites 2 and 3 (p= 0.033 and 0.0006). No significant effect of time on sulfate concentration could be detected for sites 1 and 4 (p= 0.1 and 0.56).

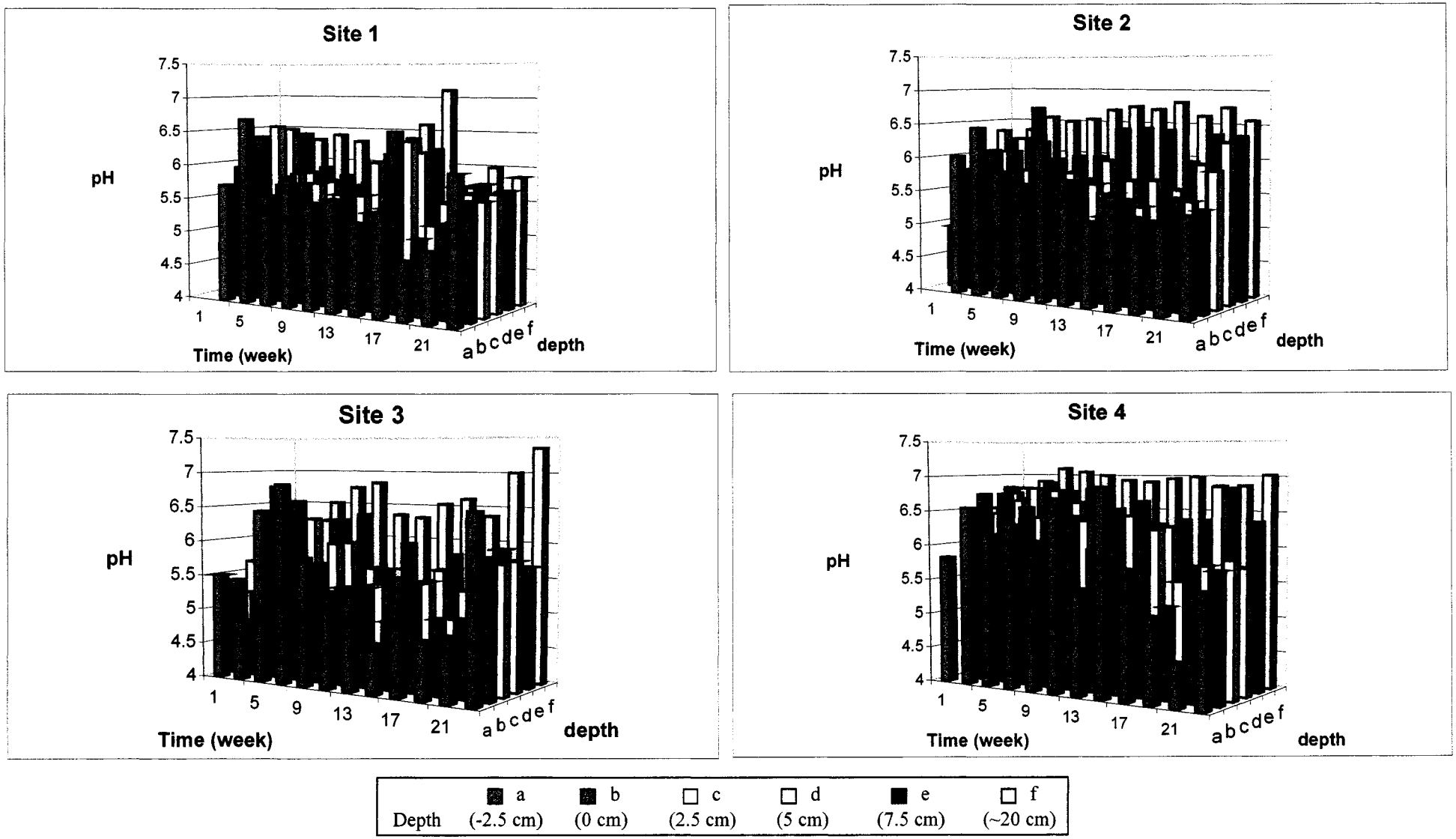


Figure 3.3 pH in the four BDL sediment cores during the wetting and drying cycles as a function of time.

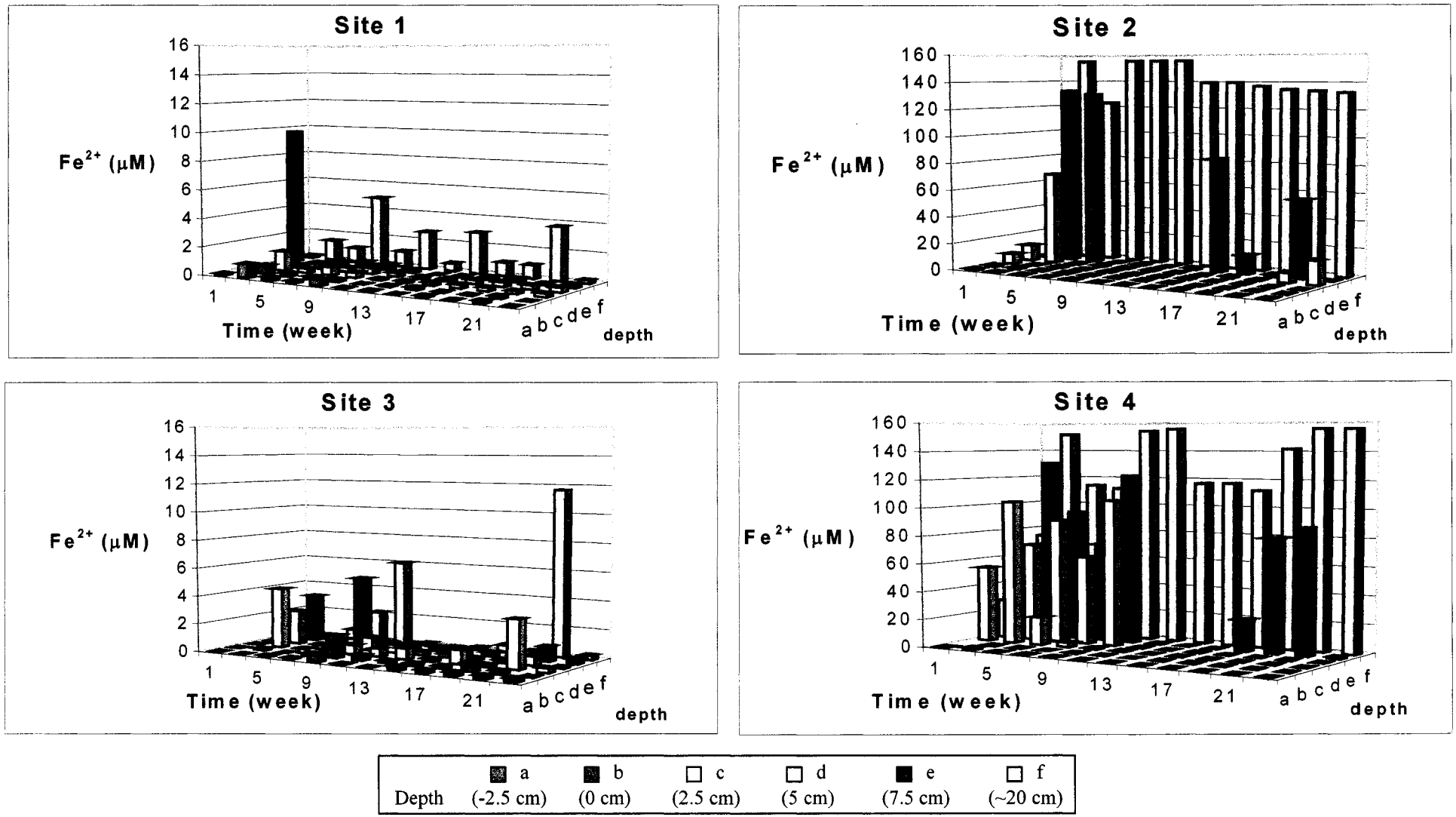


Figure 3.4 Ferrous iron concentrations in the four BDL sediment cores during the wetting and drying cycles as a function of time. (NOTE: Sites 2 and 4 are on a scale 10x larger than sites 1 and 3.)

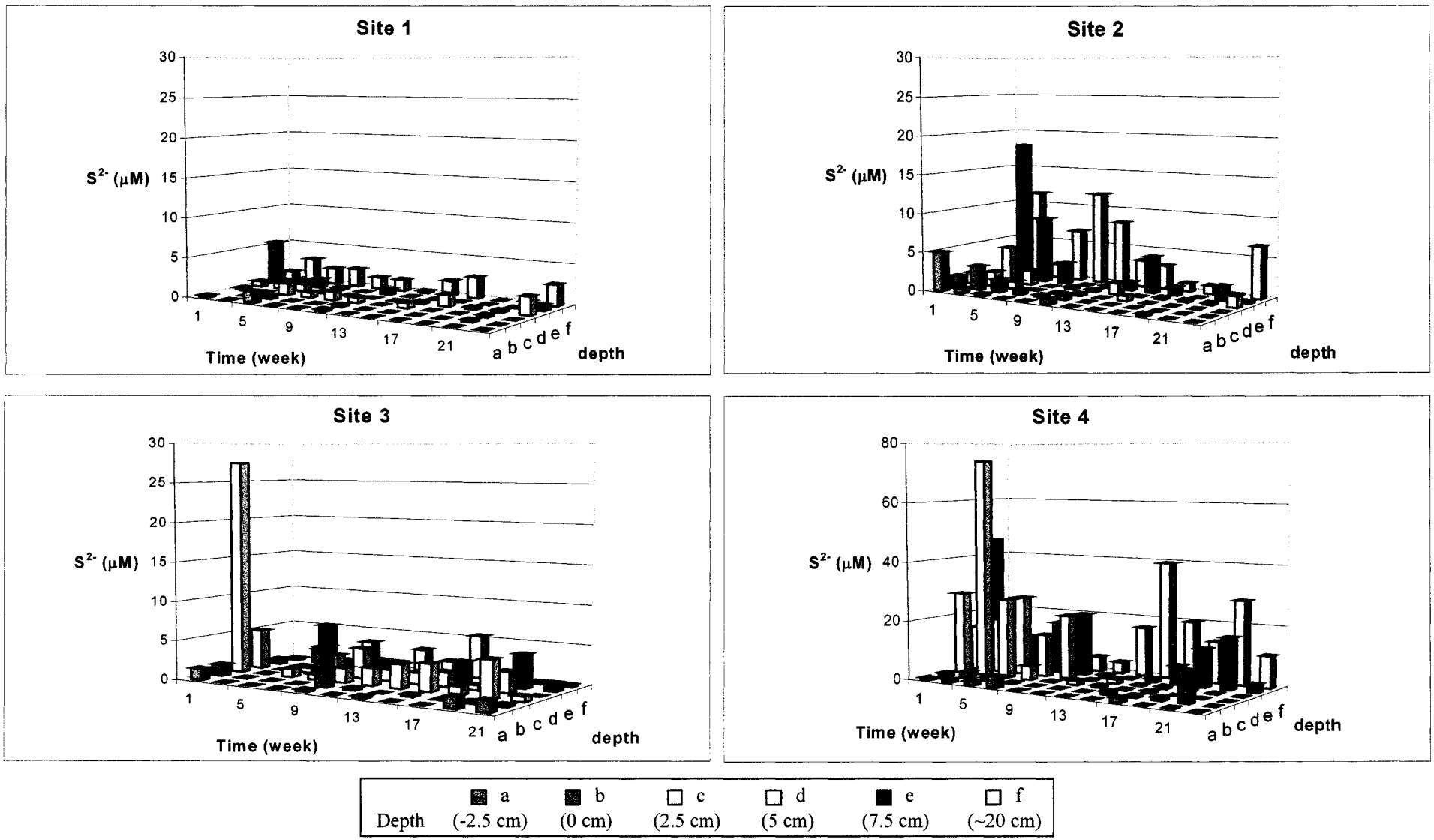


Figure 3.5 Sulfide concentrations in the four BDL sediment cores during the wetting and drying cycles as a function of time. (NOTE: The scale is larger for site 4).

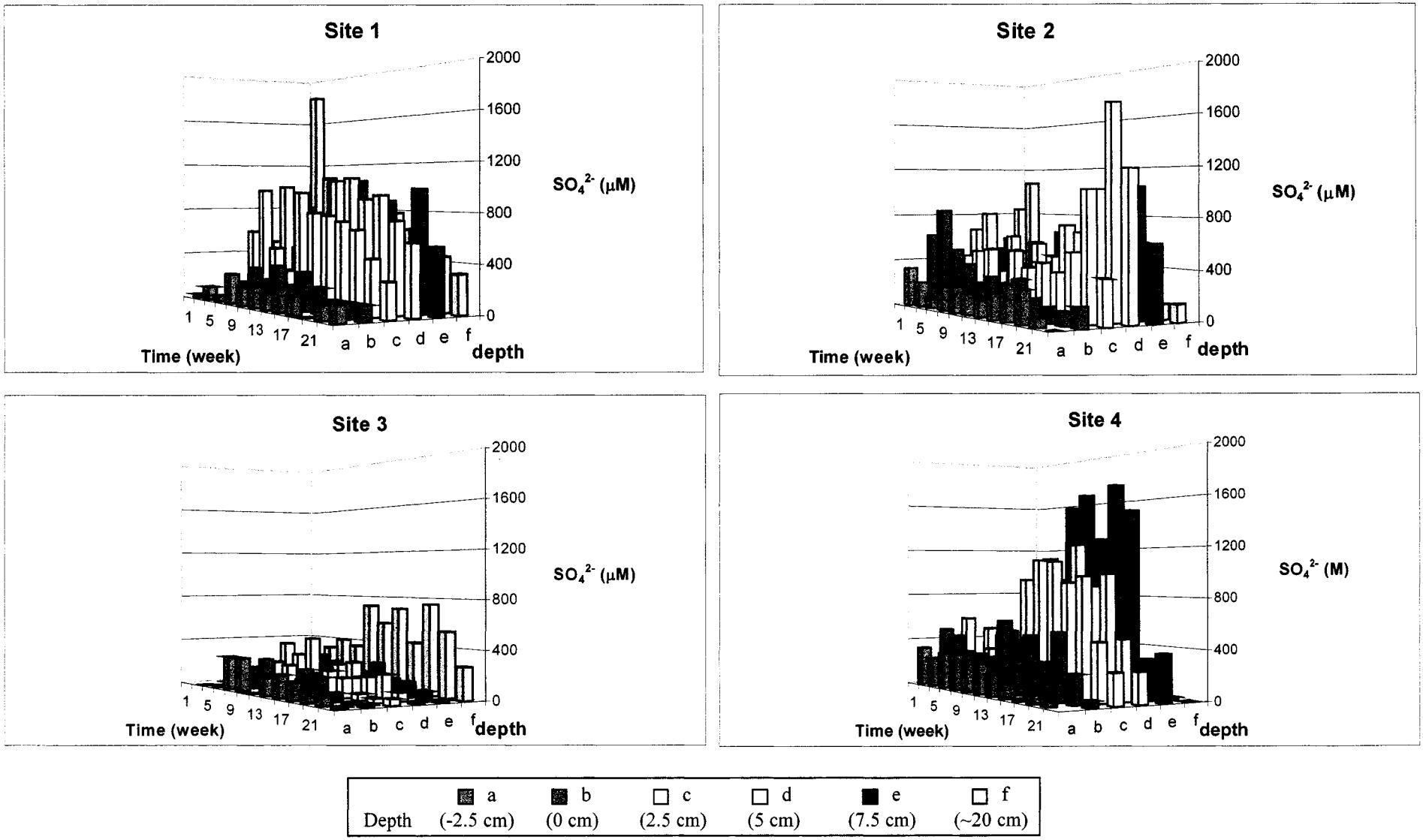


Figure 3.6 Sulfate concentrations for the four BDL sediment cores during the wetting and drying cycles as a function of time.

3.3.2 Stump Lake

The pH values of the porewaters in Stump Lake (SL) are similar to the values measured for BDL, namely 4.5 to 6.7 (with the exception of a peak at week 15/sampling port 'f'/site C). The highest pH values during the experiment were observed in site C whereas the lowest were in site B (Figure 3.7). As is the case with the Black Donald Lake cores, the pH was always the highest in the leachate (port 'f'), with the exception of site D (Figure 3.7). For sites A, B and C, the pH remained fairly stable over time, whereas it decreased with time in the sediments for site D (Figure 3.7). Depth had a significant effect on the pH for all sites ($p= 7.4x10^{-20}$ to $2.4x10^{-4}$). Time also had a significant effect on pH but only for sites A, B and D ($p= 3.3x10^{-9}$, $2.9x10^{-9}$ and $1.2x10^{-14}$, respectively). A significant effect of time on pH for site C could not be detected ($p= 0.06$).

Ferrous iron concentrations for Stump Lake ranged between 0 and 38 μM. Fe (II) concentrations were very low in the sediments from sites B, C and D, whereas for site A, there was an apparent release of Fe (II) in the deeper sediments (ports 'c' to 'e') at the beginning of the experiment (Figure 3.8). Fe (II) concentrations were generally high in the leachate, with the exception of site C (Figure 3.8). Fe (II) levels varied with time in the leachate, but appeared to be higher in the first weeks of the experiment, especially for sites A and D. A significant effect of depth on Fe (II) concentration could not be detected for site D ($p= 0.1$). However, sites A, B and C showed a significant effect of depth on the Fe (II) concentration ($p= 9.6x10^{-8}$, $1.3x10^{-7}$ and $3.1x10^{-10}$, respectively). Time had a significant effect on the Fe (II) concentration for sites C and D ($p= 1.2x10^{-5}$ and 0.012) whereas this significance could not be detected for sites A and B ($p= 0.082$ and 0.46).

The sulfide concentrations ranged between 0-350 μM (Figure 3.9). Sulfide was detected in the sediment porewaters, but only sporadically (Figure 3.9). Most sites showed depth 'a, b and c' varying between 0 and 5 μM while depth 'd and e' were between 0 and 50μM. On the other hand, sulfide was constantly present in the leachate (port 'f') of all cores. Leachate from sites A and C had the highest sulfide concentration and showed a slow decline with time (Figure 3.9). Site D however showed a faster decline with time starting at week 7. Site B's sampling port 'f' sulfide concentration increased with time, peaked at week 11 and then decreased. For sites A, B and C, depth had a significant effect on the sulfide

concentration ($p= 10^{-6}$, $3.9x10^{-4}$ and $4.3x10^{-14}$, respectively) whereas a significant effect of time could not be detected (p= 0.47, 0.52 and 0.44, respectively). Site D showed a significant effect of time on sulfide concentration (p= 0.027) but a significant effect of depth on sulfide concentration could not be detected (p= 0.08).

Sulfate values were between 0-5300 μM (Figure 3.10). Sulfate concentrations increased with depth (i.e., from port 'a' to port 'd') for all sites, but declined in port 'e' (Figure 3.10). The sulfate levels in the sediments also appeared to slightly decrease over time (Figure 3.10). Sulfate levels were always higher in the leachate than in the sediments at all sites, with the exception of site A, where they remained below the concentrations measured in the sediments (Figure 3.10). In the leachate, sulfate concentrations varied with time and only appeared to decline at site D. Depth was found to have a significant effect on the sulfate concentration of all sites ($p= 3.3x10^{-20}$ to $1.1x10^{-5}$). We could not detect a significant effect of time on sulfate concentrations for all SL sites (p= 0.11 to 0.50).

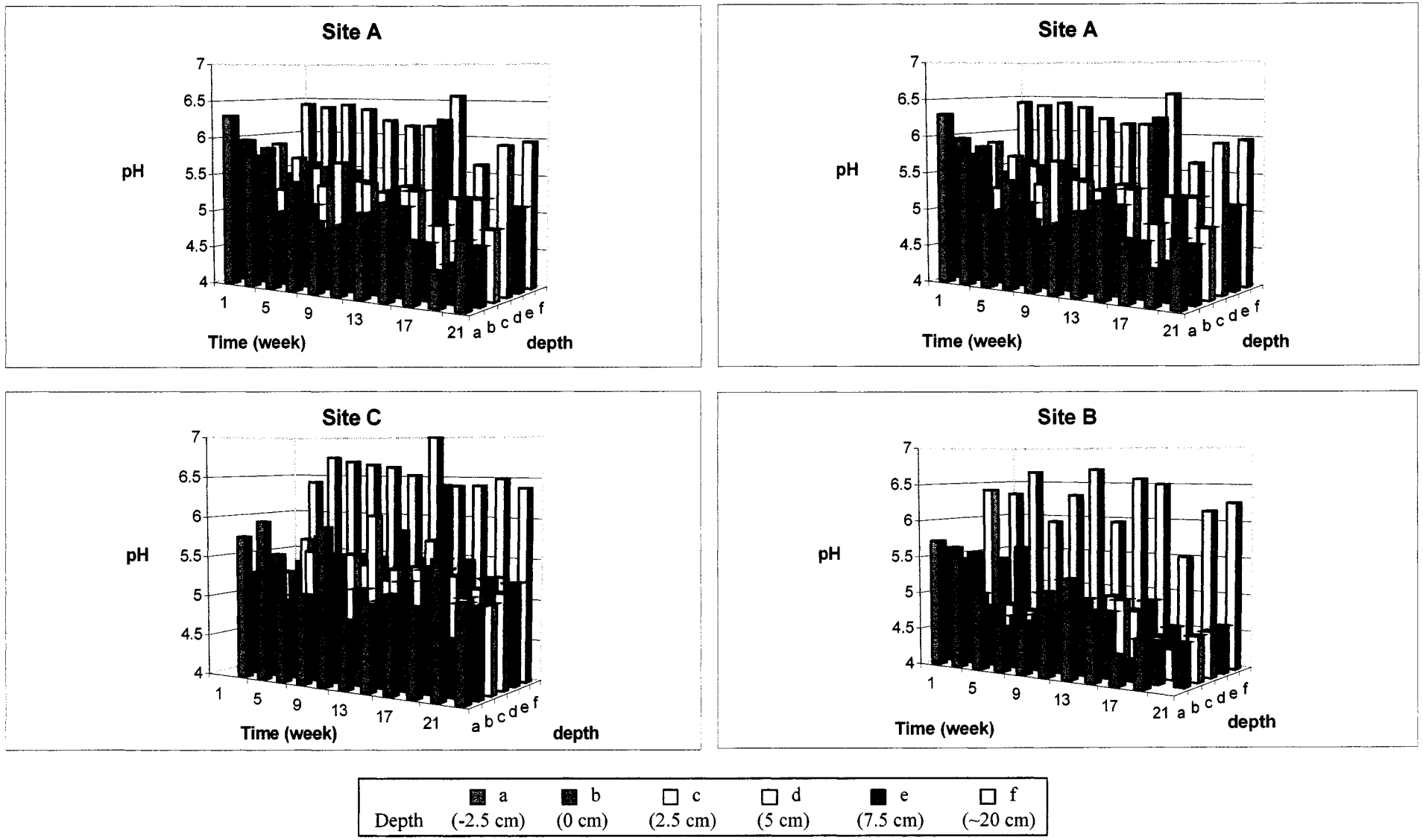


Figure 3.7 pH of the four SL sediment cores during the wetting and drying cycles as a function of time.

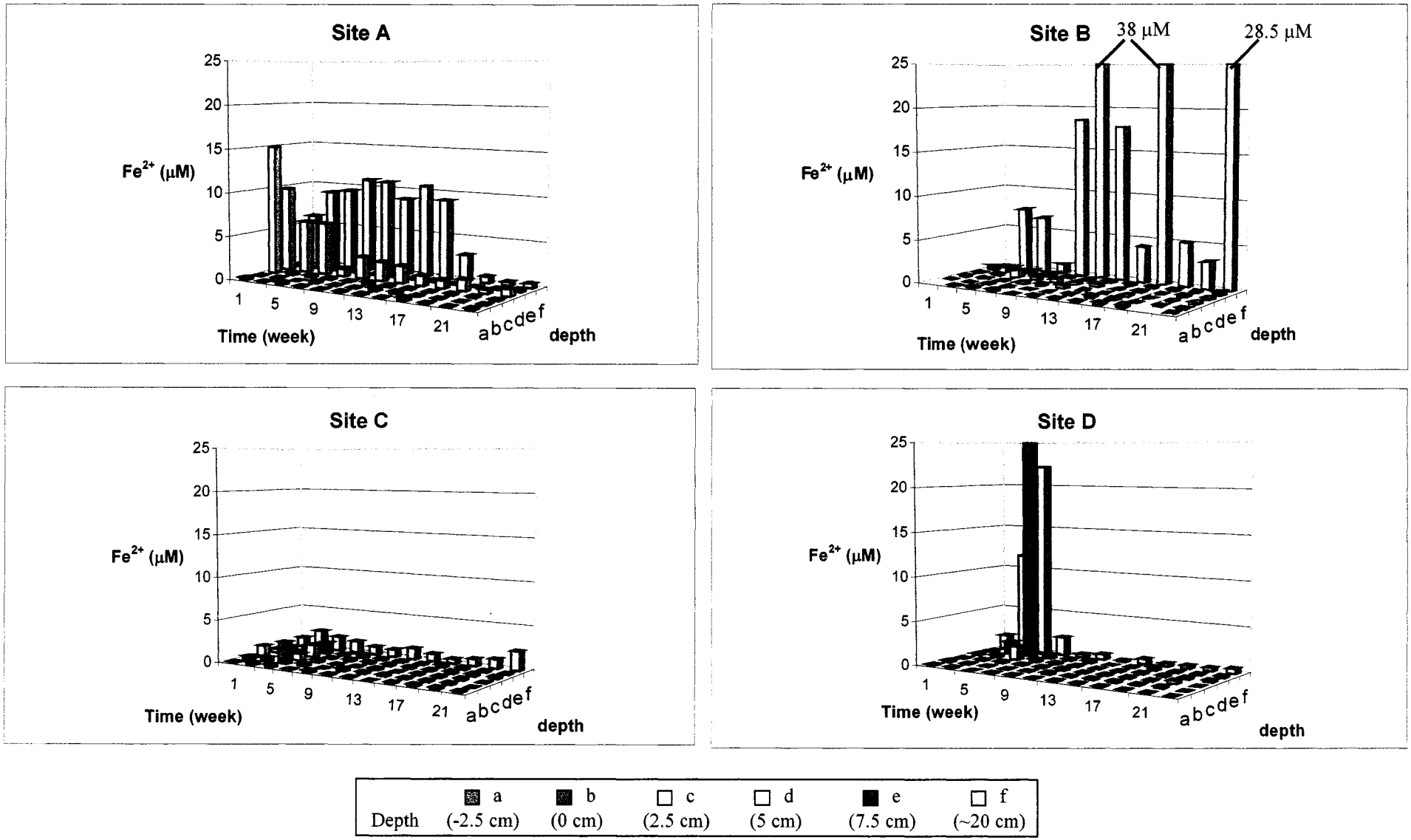


Figure 3.8 Ferrous iron concentrations in the four SL sediment cores during the wetting and drying cycles as a function of time.

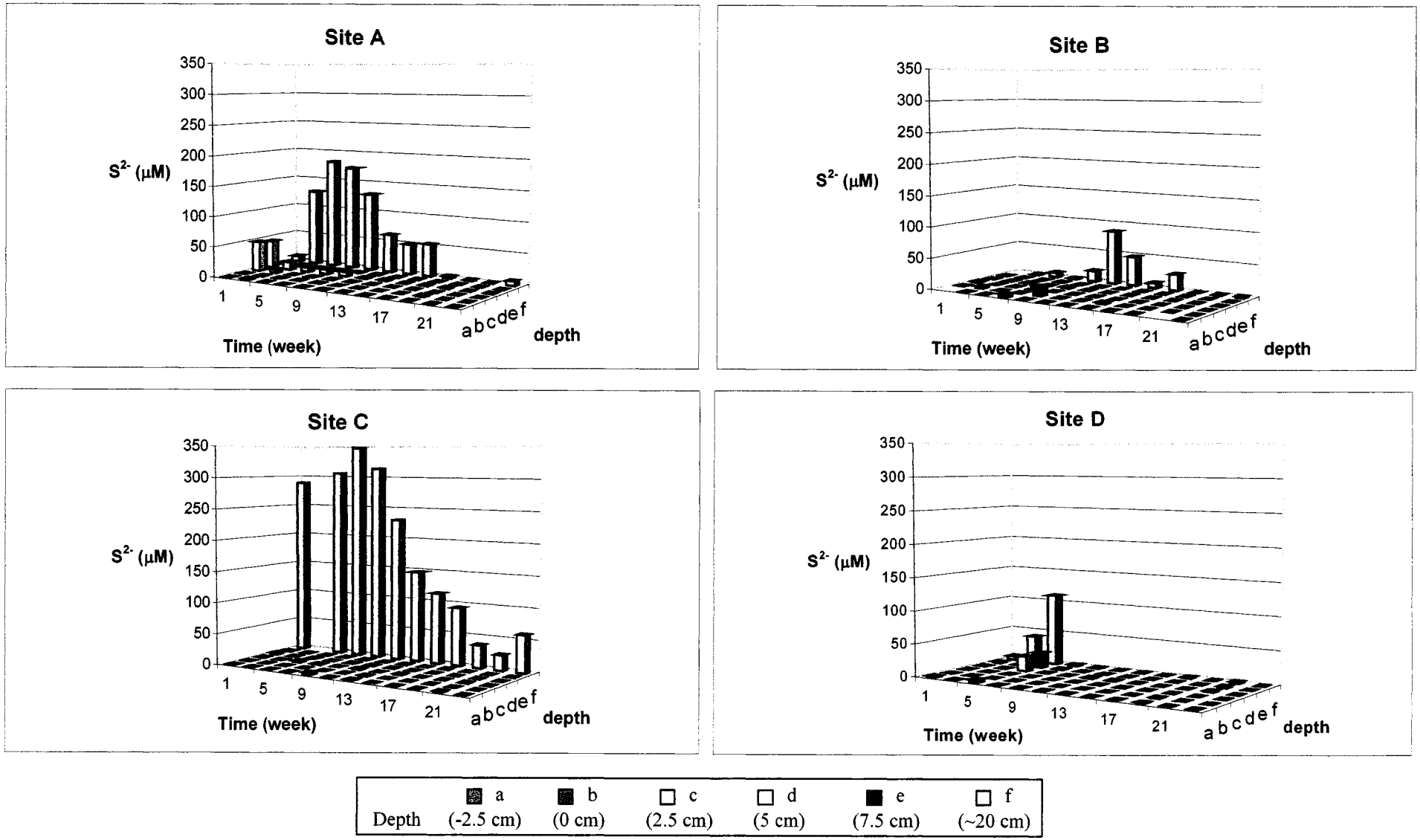


Figure 3.9 Sulfide concentrations in the four SL sediment cores during the wetting and drying cycles as a function of time.

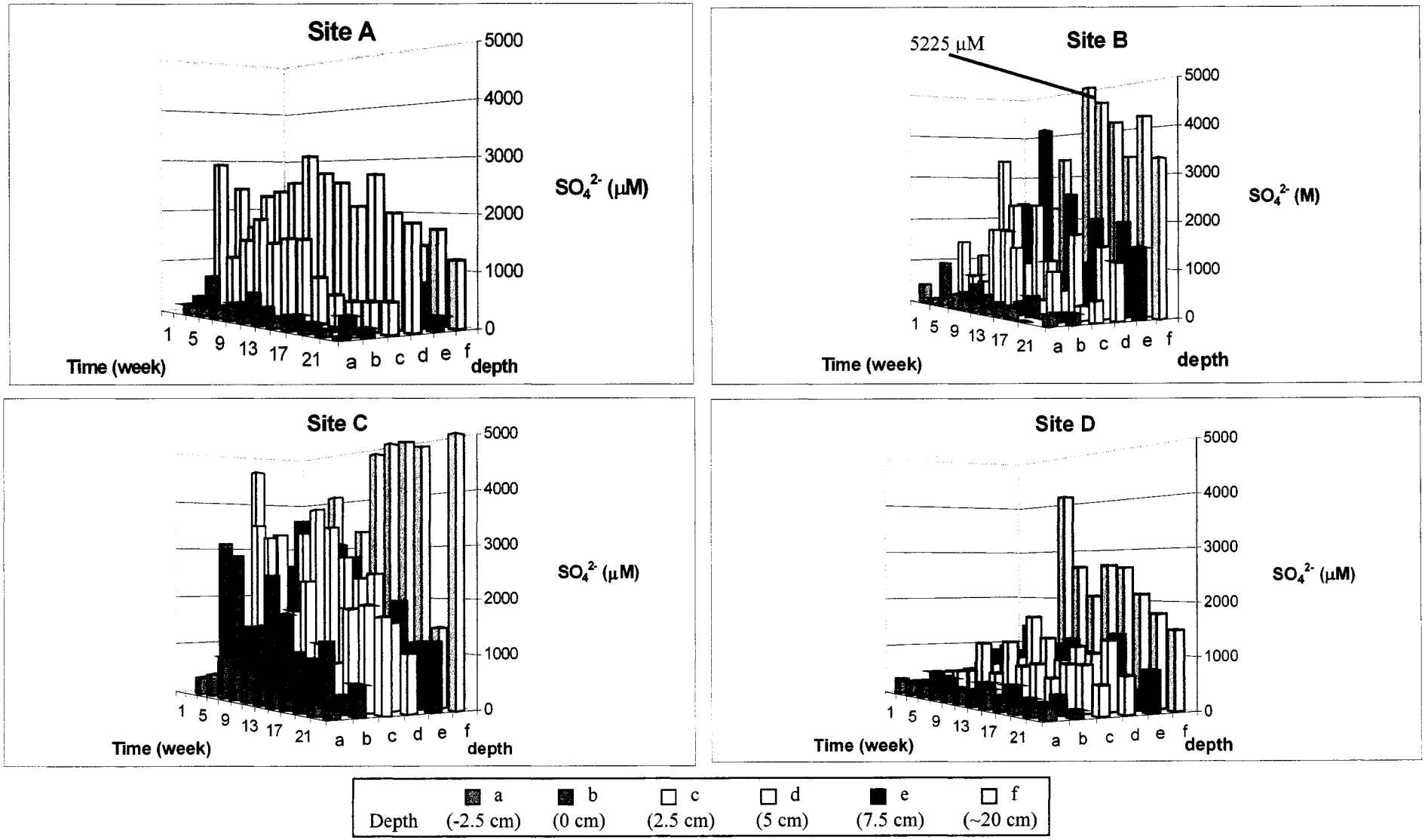


Figure 3.10 Sulfate concentrations in the four SL sediment cores during the wetting and drying cycles as a function of time.

3.3.3 Statistical analyses

3.3.3.1 Effect of depth, time and lake

Six-month cores from both lakes showed that depth and time had a significant effect on pH (SL $p= 2.9x10^{-21}$ and $5.4x10^{-10}$; BDL $p= 1.3x10^{-20}$ and $3.9x10^{-11}$, respectively). Depth had a significant effect on the Fe (II) and sulfide concentrations of both lakes (SL $p= 3.2x10^{-10}$ and $2.8x10^{-16}$; BDL $p= 4.6x10^{-17}$ and $8.9x10^{-5}$, respectively). However we could not detect a significant effect of time on the Fe (II) and sulfide concentrations of the study lakes (SL p= 0.47 and 0.13; BDL p= 0.65 and 0.68, respectively). Black Donald Lake's 6-month cores showed that we could not detect a significant effect of depth on the sulfate concentrations (p= 0.07). Stump Lake's 6-month cores showed that depth had a significant effect on sulfate concentrations ($p= 3.2x10^{-26}$). No significant effect of time could be detected for either Stump Lake or Black Donald Lake (p= 0.48 and 0.53, respectively).

A 3-way analysis of variance showed that the factor lake had a significant effect on pH ($p= 1.7x10^{-11}$), Fe (II) ($p= 2.6x10^{-11}$), sulfide ($p= 2.8x10^{-5}$) and sulfate ($p= 1.9x10^{-11}$) concentrations. These same analyses showed that the interactions of lake with the other significant factors, depth and time, also had a significant effect on pH, Fe (II), sulfide and sulfate concentrations. The interaction 'lake*time' (p= 0.004) had a significant effect on pH only, i.e. the effect of lake depended on week and the effect of week depended on lake. The 'lake*depth' interaction had a significant effect on pH (p= 0.004), Fe (II) ($p= 1.6x10^{-11}$), sulfide ($p= 1.5x10^{-11}$) and sulfate ($p= 1.4x10^{-11}$) concentrations. Therefore, the effect of lake depended on depth and vice versa.

3.3.3.2 Variability between factors

In the case of the dependent variable pH, lakes were a significant source of variability for depths 'a, b and c' (p= 0.006, 0.016 and 0.026, respectively). Cores were also a significant source of variability for pH for those same depths ($p= 7x10^{-8}$, $1.6x10^{-5}$ and $8.5x10^{-5}$, respectively). There is significant variability of the means of the lakes, sites and cores at depth 'd and e' (p= 0.030, 0.012 and 0.002; p= 0.035, 0.003 and 0.007, respectively). Only the cores introduced a significant source of variability for pH at depth 'f' ($p= 1.6x10^{-5}$).

The only source of variability introduced to the Fe (II) concentration was from the cores at depth 'd, e and f' ($p= 1.1x10^{-3}$, $2.6x10^{-11}$ and $1.6x10^{-57}$, respectively).

A significant source of variability for sulfide concentration was found at depth 'f' and was caused by lakes and cores ($p= 0.024$ and $5.3x10^{-21}$, respectively).

Depth 'c, d, e and f' showed a significant source of variability was caused by the lakes ($p= 0.016$, 0.001, 0.015 and 0.02, respectively) and by the cores ($p= 4.4x10^{-6}$, $9.8x10^{-13}$, $1.6x10^{-18}$ and $7.8x10^{-22}$, respectively).

3.3.4 Major cations in water samples

Due to the high detection limit of the ICP-OES, only the major metal ions, i.e., Ca, Fe_T, Mg, Mn and Na, were measured. The following section summarizes the metal analysis data. The graphs of major metal ion concentrations as a function of time over depth are presented in appendix E.

In general, the major cation concentrations of both lakes behaved similarly. The highest concentrations were seen in the leachate samples (sampling port 'f'). Iron and manganese concentrations were very low and close to the detection limit. Calcium and magnesium concentrations were a hundred times higher than those of iron and manganese. Concentrations decreased with time, in some cases, like iron and manganese, to below detection limit. BDL Na concentrations followed the same trends as the other cations whereas SL Na concentrations were completely below the detection limit after 7 weeks.

3.4 Gravimetric moisture and organic carbon contents of the sediments

The analyses were performed on fresh sediment cores, sediment cores submitted to several wetting and drying events for 3 and 6 months and on air-dried sediment that simulated extreme drought for 6 months. For more details on the statistics presented in this section, see appendix B.

3.4.1 Black Donald Lake

The water content of the fresh sediments varied between 20 and 70 % for all 4 sites and generally decreased from the surface to the bottom of the cores (Figure 3.11). As expected, dried cores (for 6 months) showed the lowest moisture content (Figure 3.11). As for the cores that were submitted to 3 and 6 months of wetting and drying events, the moisture content was generally higher at the top of the cores than at the bottom, with the exception of site 4 (Figure 3.11). In addition, the water content of these cores was generally higher than in the fresh cores, with the exception of site 3. Sites 1, 3 and 4 showed a significant effect of time on the moisture content of the sediment ($p= 4.0x10^{-4}$, $4.7x10^{-4}$ and $8.1x10^{-7}$, respectively) whereas for site 2, no significant effect could be detected ($p= 0.111$).

The organic carbon content of the fresh sediments from all sites varied between 0 and 20 % and decreased with depth (Figure 3.11). The wetting and drying events did not affect the carbon content of the sediments in the cores from sites 2 and 3, whereas the organic carbon content increased after 6 months in the cores from sites 1 and 4. The sediment cores left to dry for 6 months had a similar carbon content to the 3 month-old sediments (Figure 3.11). All sites showed a significant effect of time on the organic content of the sediment ($p= 2.9x10^{-8}$ to 0.029).

3.4.2 Stump Lake

The moisture content of the sediments of Stump Lake varied between 70-90 %, with the exception of site B (Figure 3.12). The moisture content of the sediments did not vary much with depth, with the exception of site B (Figure 3.12). The dried cores showed the lowest moisture content, whereas the fresh cores and the cores submitted to wetting and

drying events did not show any apparent differences (Figure 3.12). Time had a significant effect on the moisture content of the sediments from all SL sites ($p= 1.9x10^{-8}$ to $1.3x10^{-4}$).

The organic carbon content of the sediments in Stump Lake was higher than in the sediments of Black Donald Lake, with values ranging from 50 to 80 % in the sediments from sites A, C and D, and from 10 to 40 % in site B (Figure 3.12). The organic carbon content of the fresh sediments was not determined for sites A, B, C and D. The wetting and drying events (3 and 6 months) and the complete drying of the sediments (6 months dry) did not greatly affect the organic carbon content of the sediments (Figure 3.12). No significant effect of time on the organic carbon content from sites A, B and C could not be detected (0.219, 0.147 and 0.456, respectively). Time did however have a significant effect on the organic carbon content of SL's site C sediment ($p= 0.022$).

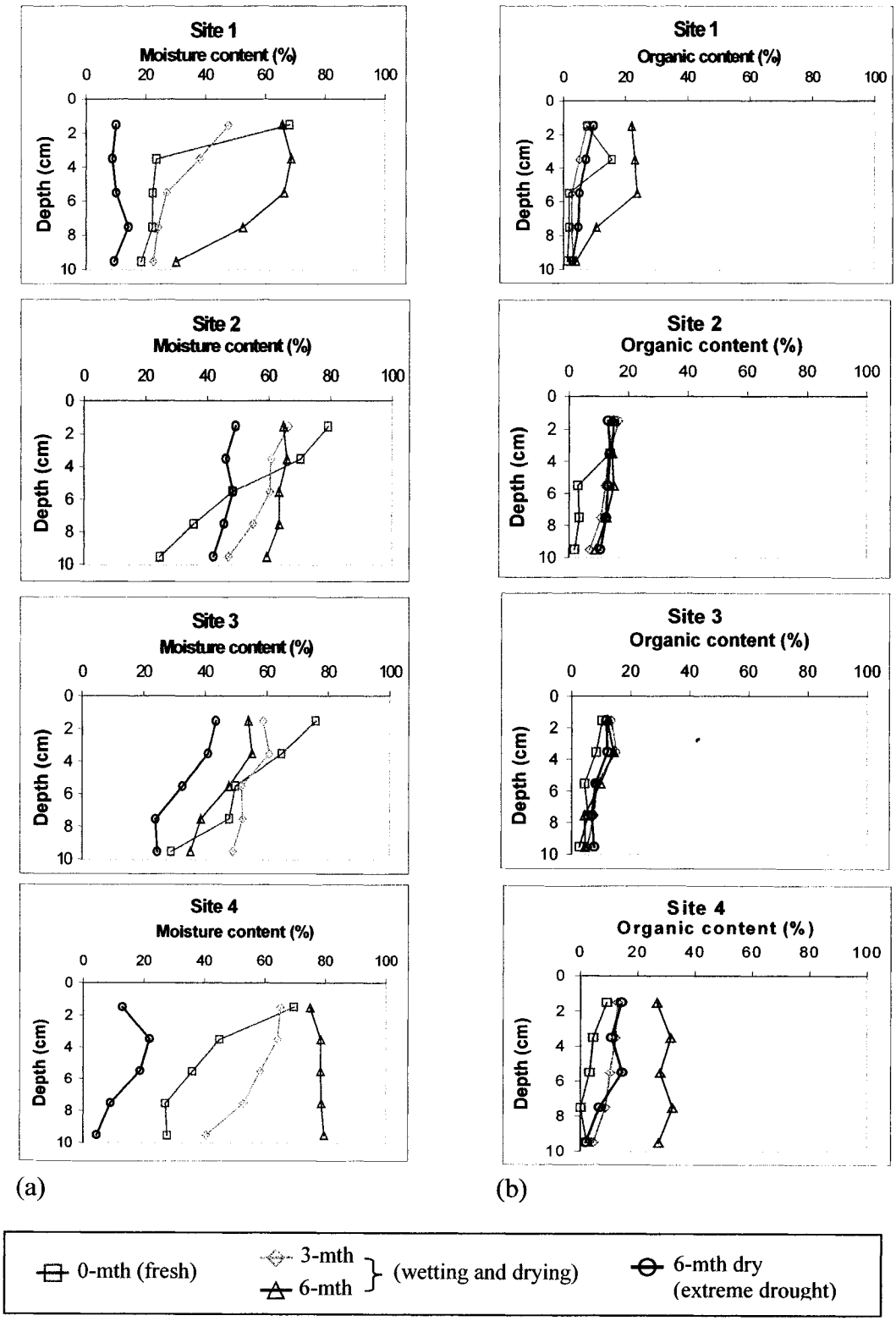


Figure 3.11 Moisture (a) and organic carbon (b) contents of the Black Donald Lake sediment cores as a function of time.

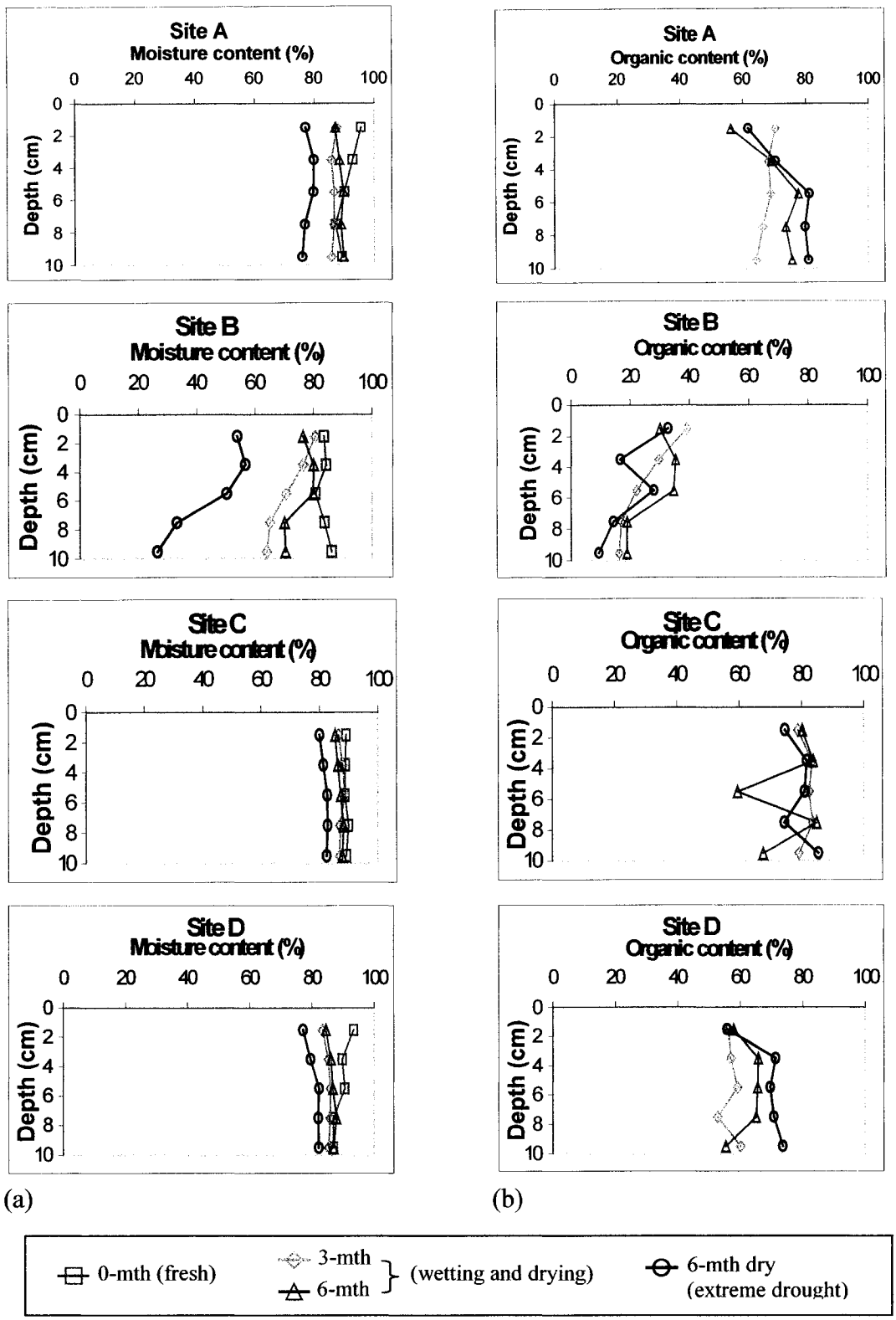


Figure 3.12 Moisture (a) and organic carbon (b) contents of the Stump Lake sediment cores as a function of time.

3.5 Iron- and sulfur-oxidizing bacteria

3.5.1 Black Donald Lake

The neutrophilic iron-oxidizing bacteria (NIOB) populations varied between 10^2 and 10^6 CFU/g dry wt. sed. (Figure 3.13). NIOB populations were the highest in the fresh sediments and generally declined with depth (Figure 3.13). NIOB populations in the dried sediment cores (6 months dry) remained stable with depth at all sites, whereas they fluctuated with depth during the wetting and drying cycles (Figure 3.13). For all BDL sites, no significant effect of depth on the NIOB population estimate could be detected ($p= 0.42$ to 0.61). Sites 2, 3 and 4 showed that a significant effect of time on the NIOB population estimate could not be detected ($p= 0.08$, 0.26 and 0.07, respectively). However, time had a significant effect on the NIOB population estimate for site 1 ($p= 0.03$).

The neutrophilic sulfur-oxidizing bacteria (NSOB) populations were in the range of 10^4 to 10^7 CFU/g dry wt. sed. (Figure 3.14). In general, NSOB populations varied with depth during the wetting and drying cycles at all BDL sites (Figure 3.13). In the completely dry samples (6 months dry), NSOB populations remained as high as in the fresh samples. We could not detect a significant effect of depth or time on the NSOB population estimate for all sites (depth $p= 0.08$ to 0.71; time $p= 0.25$ to 0.72, respectively).

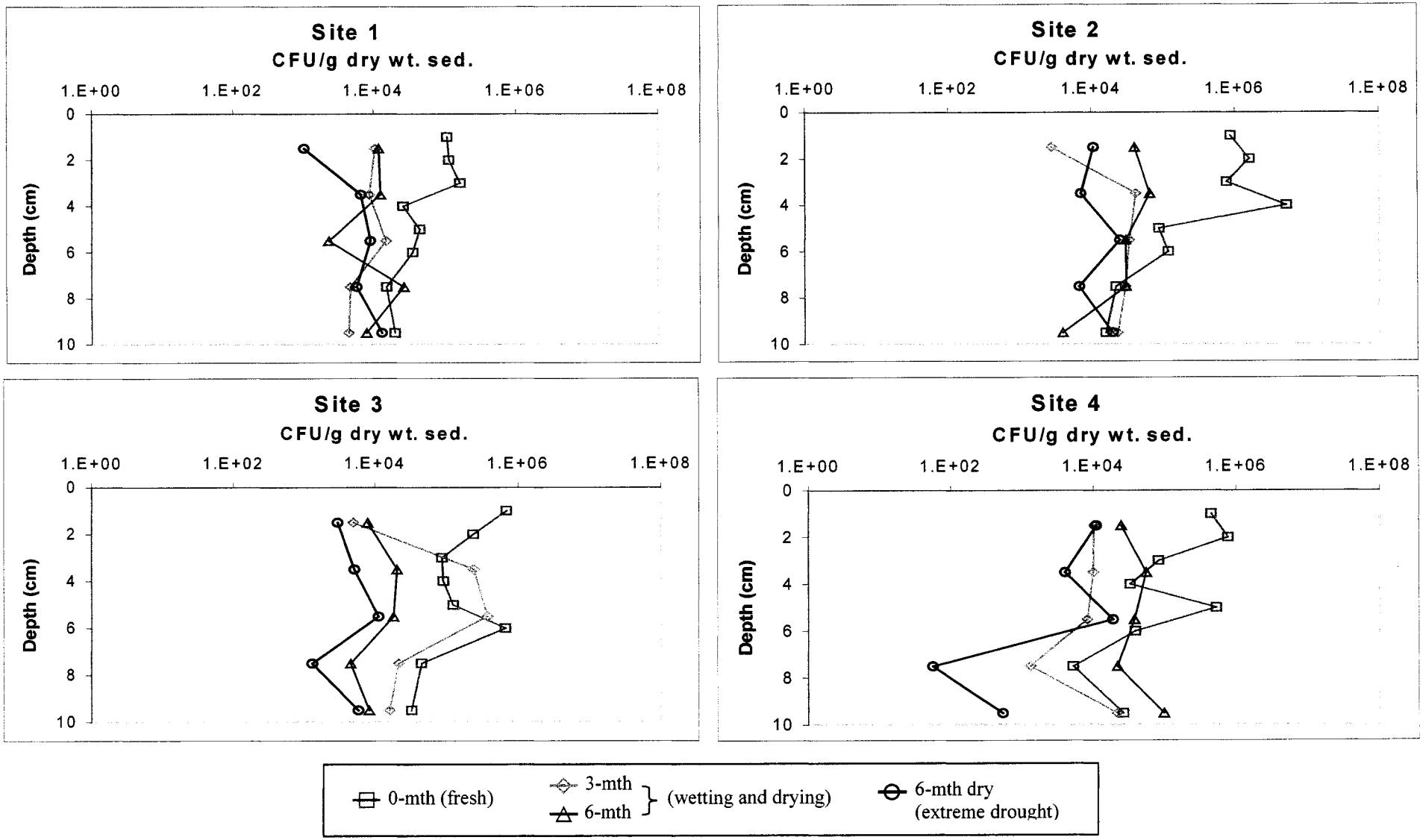


Figure 3.13 NIOB populations in the sediment cores of Black Donald Lake at four separate experimental times.

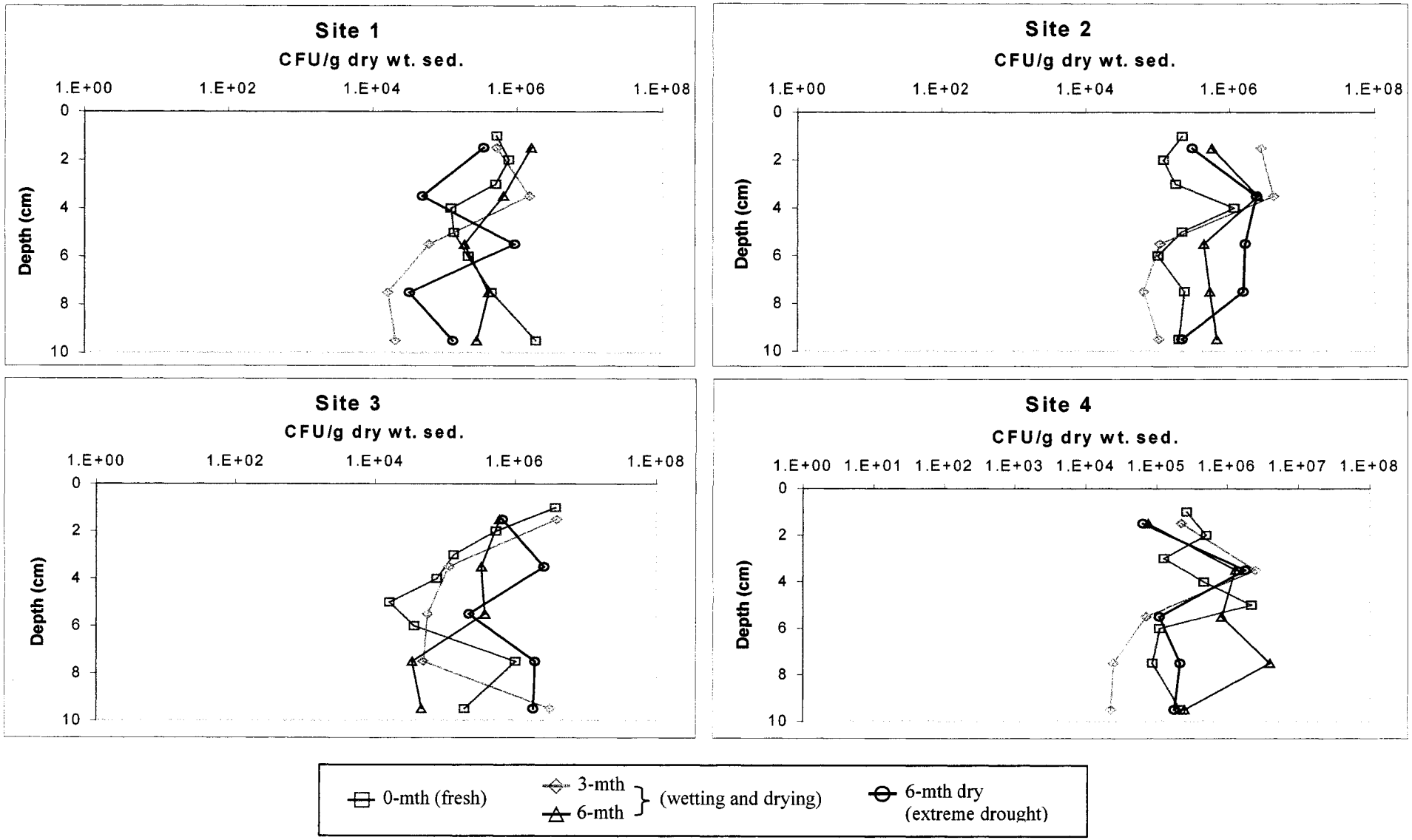


Figure 3.14 NSOB populations in the sediment cores of Black Donald Lake at four separate experimental times.

3.5.2 Stump Lake

The neutrophilic iron-oxidizing bacteria (NIOB) populations ranged from 10^3 to 10^7 CFU/g dry wt. sed. (Figure 3.15). NIOB populations were the highest in the fresh sediments of cores A and B and generally declined with depth (Figure 3.15). In the sediments of sites C and D, NIOB populations in the fresh sediments varied with depth, but remained as abundant as the populations in the sediments submitted to 3 and 6 months of wetting and drying events (Figure 3.15). The NIOB populations in the completely dry samples were generally low at all sites and slightly fluctuated with depth (Figure 3.15). No significant effect of depth on the NIOB population estimate of SL could be detected for all sites ($p= 0.25$ to 0.50). For site A and C, no significant effect of time on NIOB population estimate was detected ($p= 0.09$ and 0.08, respectively). A significant effect of time on the NIOB population estimate was detected for sites B and D ($p= 0.01$ and 0.02, respectively).

The neutrophilic sulfur-oxidizing bacteria (NSOB) populations were in the range of 10^4 and 10^7 CFU/g dry wt. sed. (Figure 3.16). For all sites, there were no obvious population differences between the fresh and dry samples and the ones submitted to wetting and drying cycles (Figure 3.16). We could not detect a significant effect of depth or time on the NSOB population estimate for all SL sites (depth $p= 0.72$ to 0.98; time $p= 0.08$ to 0.74, respectively).

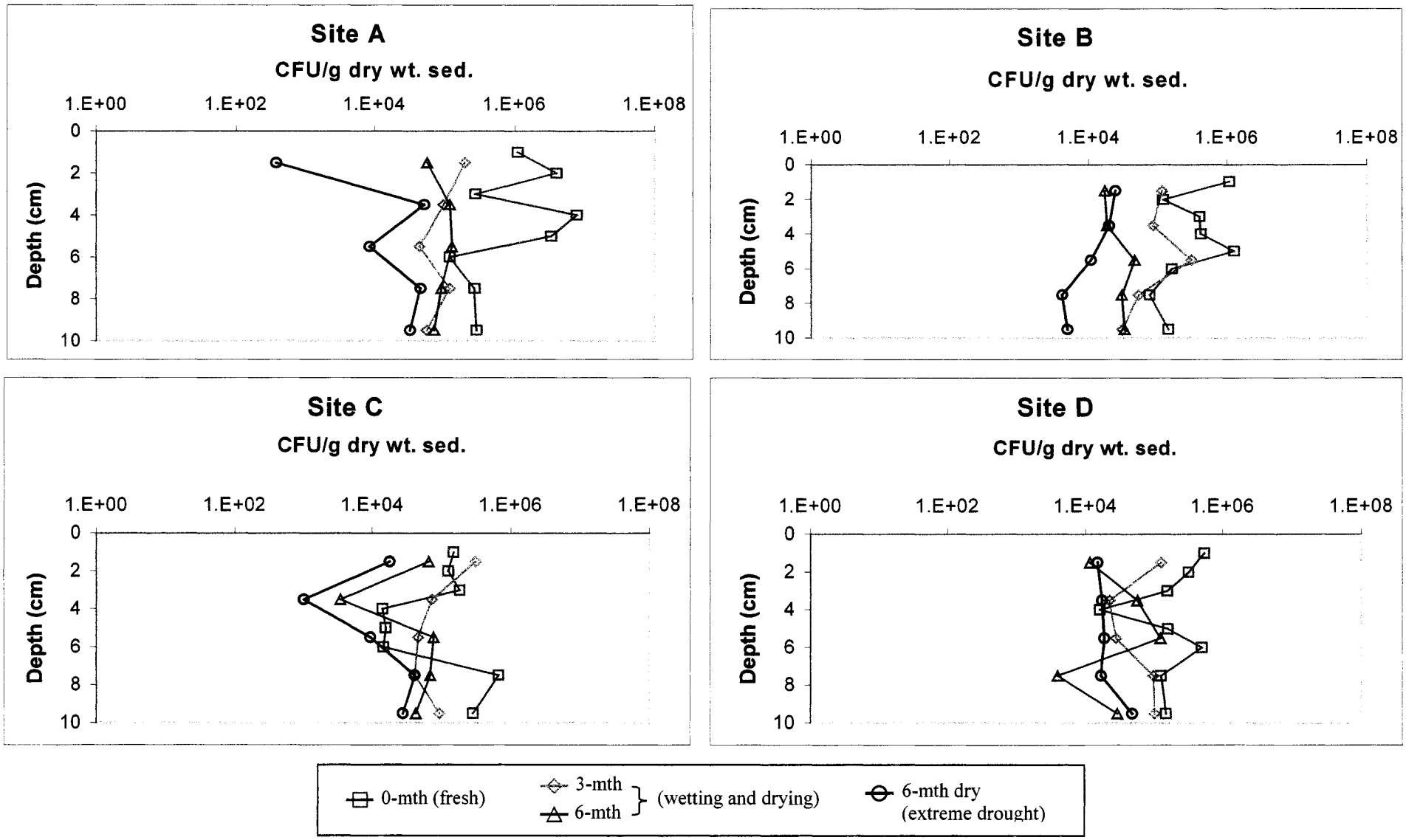


Figure 3.15 NIOB populations in the sediment cores of Stump Lake at four separate experimental times.

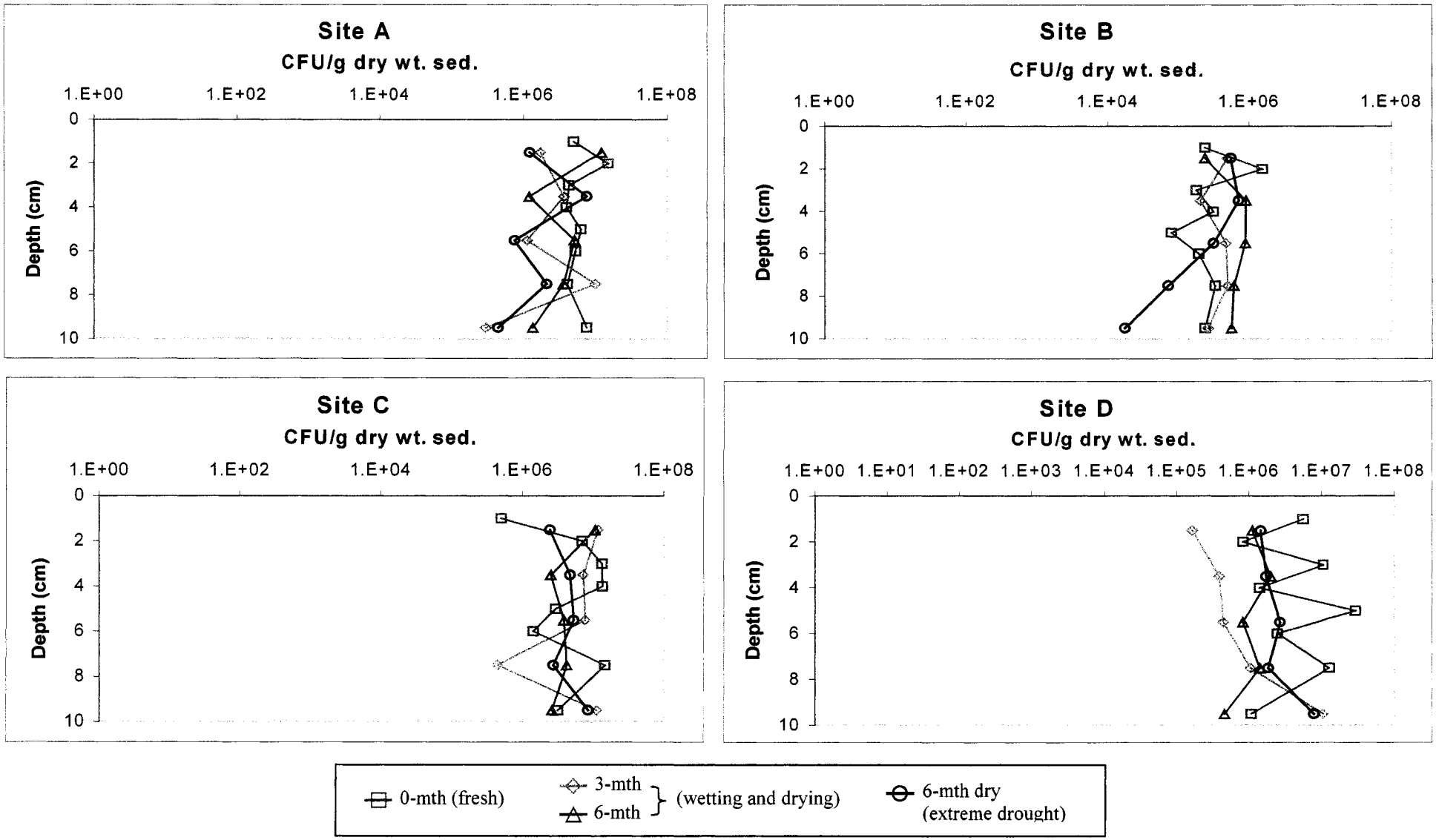


Figure 3.16 NSOB populations in the sediment cores of Stump Lake at four separate experimental times.

3.5.3 MPN estimate from experimental sediment as % of fresh sediment estimate

The neutrohpilic iron-oxidizing bacteria (NIOB) population ratios for both lakes declined with time (i.e., between 0 and 3 months) (Figure 3.17), indicating that all the NIOB population estimates decreased from their 0-month population sizes. BDL's site 3 behaved slightly differently but the population estimates remained below 100% and decreased with time. In the 6-month cores, the population estimates were either lower than the 3-month ones or remained unchanged (Figure 3.17). Both lakes show a 90-98 % drop in population size from the original 0-month estimate (Figure 3.18). Therefore the NIOB populations' decrease with drying is constant in both lakes and for all sites.

The 3-month % NSOB population ratios of site 1 (BDL) and sites A and D (SL) show an initial decrease from the original 0-month value (Figure 3.17). The % ratios of the 6-month cores do not follow any decreasing or increasing trend. Stump Lake's site C 3-month NSOB population estimates increased by 10% and then decreased. The remaining sites, namely BDL sites 2, 3 and 4 and SL site B were all above 100 % of their original NSOB estimate values (Figure 3.17). This would indicate that, for these sites, the NSOB population estimates increased with drying. With the exception of BDL sites 2, 3 and SL site B, all the sediment cores left to dry for 6 months were between 20 to 74 % below their original NSOB population estimates (Figure 3.18).

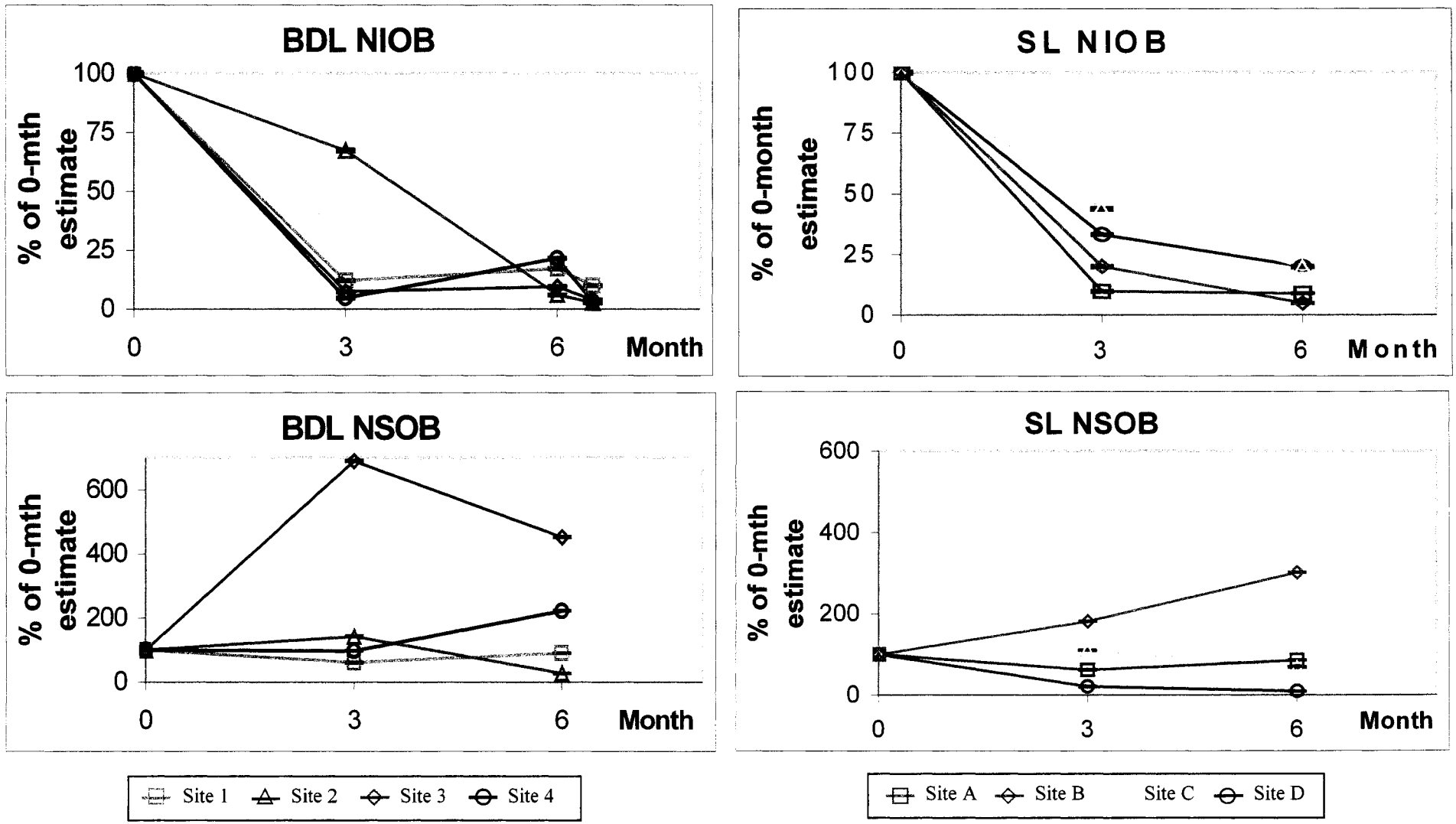
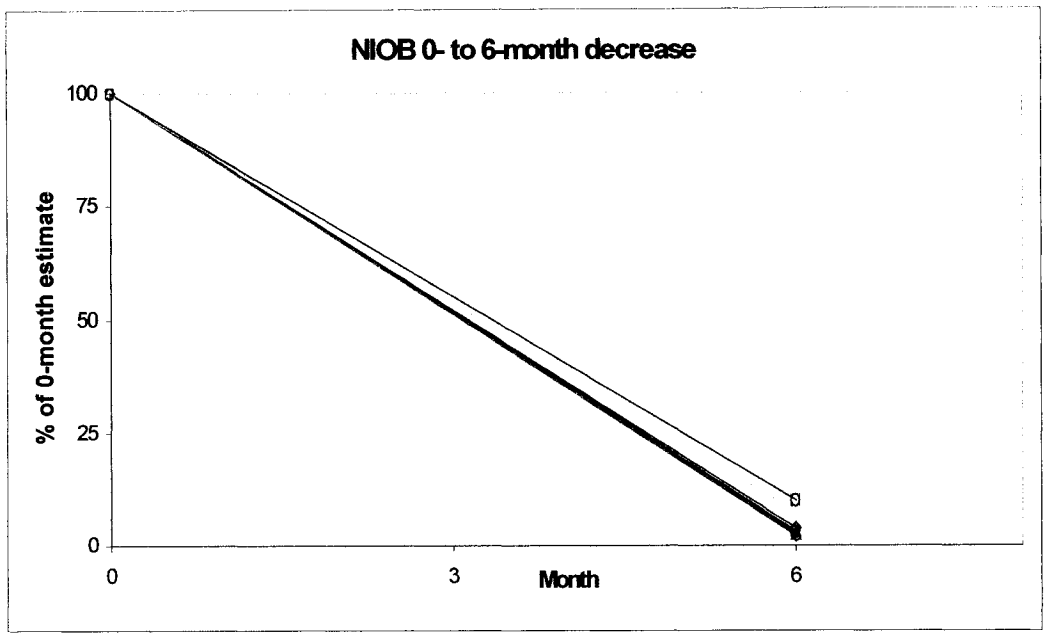


Figure 3.17 Average NSOB and NIOB 3- and 6-month MPN values as percentages of the average 0-month core MPN estimates. (n= 5) Error bars indicate the standard errors. (NOTE: The larger scale for the NSOB.)



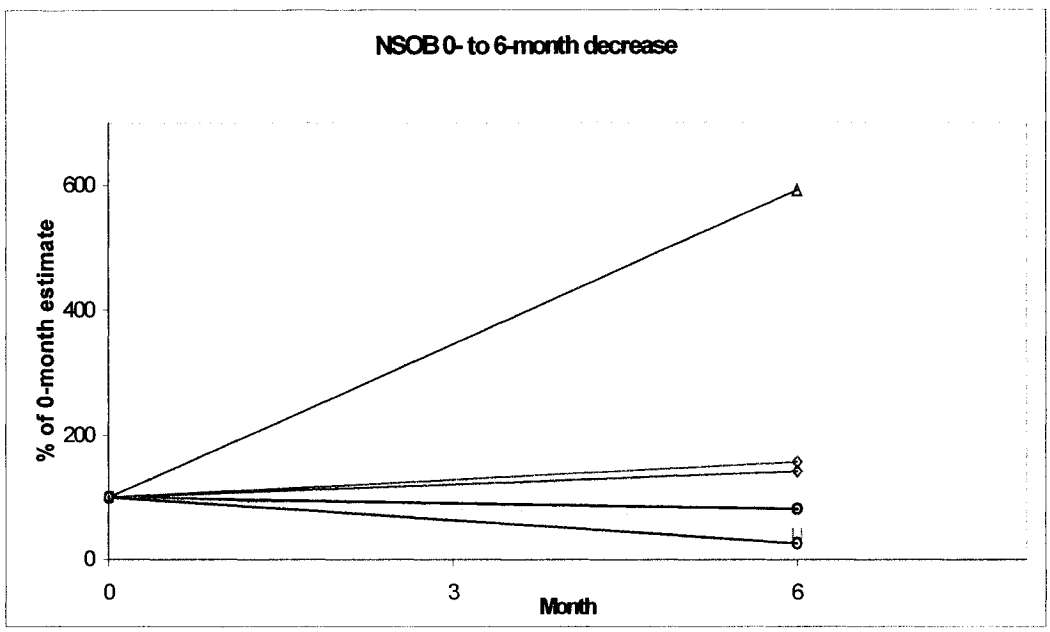


Figure 3.18 Average NSOB and NIOB 6-month Dry MPN values for both lakes as percentages of the average fresh sediment core MPN estimates. (n= 5) (NOTE: The NSOB graph is on a larger scale.)

3.5.4 Statistical analyses

3.5.4.1 Effect of depth, time and lake

No significant effect of depth or time on NIOB population estimates could be detected for BDL 6-month cores (p= 0.38 and 0.15, respectively). Stump Lake's 6-month cores had a significant effect of time on the NIOB population estimate ($p= 2.9x10^{-5}$) but no significant effect of depth could be detected (p= 0.37). We could not detect a significant effect of depth or time on the NSOB population estimate from both Stump Lake and Black Donald Lake (depth p= 0.83 and 0.057; time p= 0.75 and 0.86, respectively).

A 3-way analysis of variance showed that the factor lake had a significant effect on the NIOB population estimate means (p= 0.015) and on the NSOB population estimate means ($p= 5.3x10^{-7}$). The 'lake*core' interaction (the term 'core' (0-, 3-, 6-month) can be substituted with 'time') had a significant effect on the NIOB population estimate means ($p= 2.7x10^{-8}$). The effect of lake depended on the time and the effect of time depended on the lake.

3.5.4.2 Variability between factors

At a depth of 9.5 cm, significant variability between the means of the NIOB population estimate means existed (p= 0.004). The factor 'site' was a source of variability between the means from depth 1.5, 3.5, 5.5 to 7.5 cm ($p= 3.2x10^{-5}$, 0.0029, $8.5x10^{-5}$ and 0.023, respectively).

The only significant source of variability between the NSOB population estimate means introduced by the lake factor was found at depth 7.5 cm (p= 0.038). There was significant variability between the NSOB population estimate means introduced by factor 'site' at depth 1.5 and 5.5 cm (p= 0.04 and 0.012, respectively).

3.6 Reactive iron fraction in the sediments

3.6.1 Black Donald Lake

The concentration of reactive iron (extracted with 5 M HCl) in Black Donald Lake's sediments ranged from 10 to 250 μM/g sediment. (Figure 3.19). Given the large error associated with each measurement, trends are difficult to assess. However, reactive iron seems to decline with depth at site 1 in the fresh core and in the cores submitted to wetting and drying cycles (Figure 3.19). In the other sites, iron appears to remain fairly stable with depth. Given the fact that each experiment was done on individual cores, it is difficult to assess the effect of the wetting and drying cycles because the chemical composition of the sediments is heterogeneous, even though the cores were taken in close proximity to each other.

3.6.2 Stump Lake

The concentration of reactive iron varies between 20 and 200 μM/g sediment in Stump Lake sediments (Figure 3.20). Most surface concentrations were higher than those at depth for all sites, with the exception of the fresh sediments at site A. The apparent chemical heterogeneity of the sediments does not allow us to compare the fresh sediments to the sediments submitted to 3 and 6 months of wetting and drying cycle

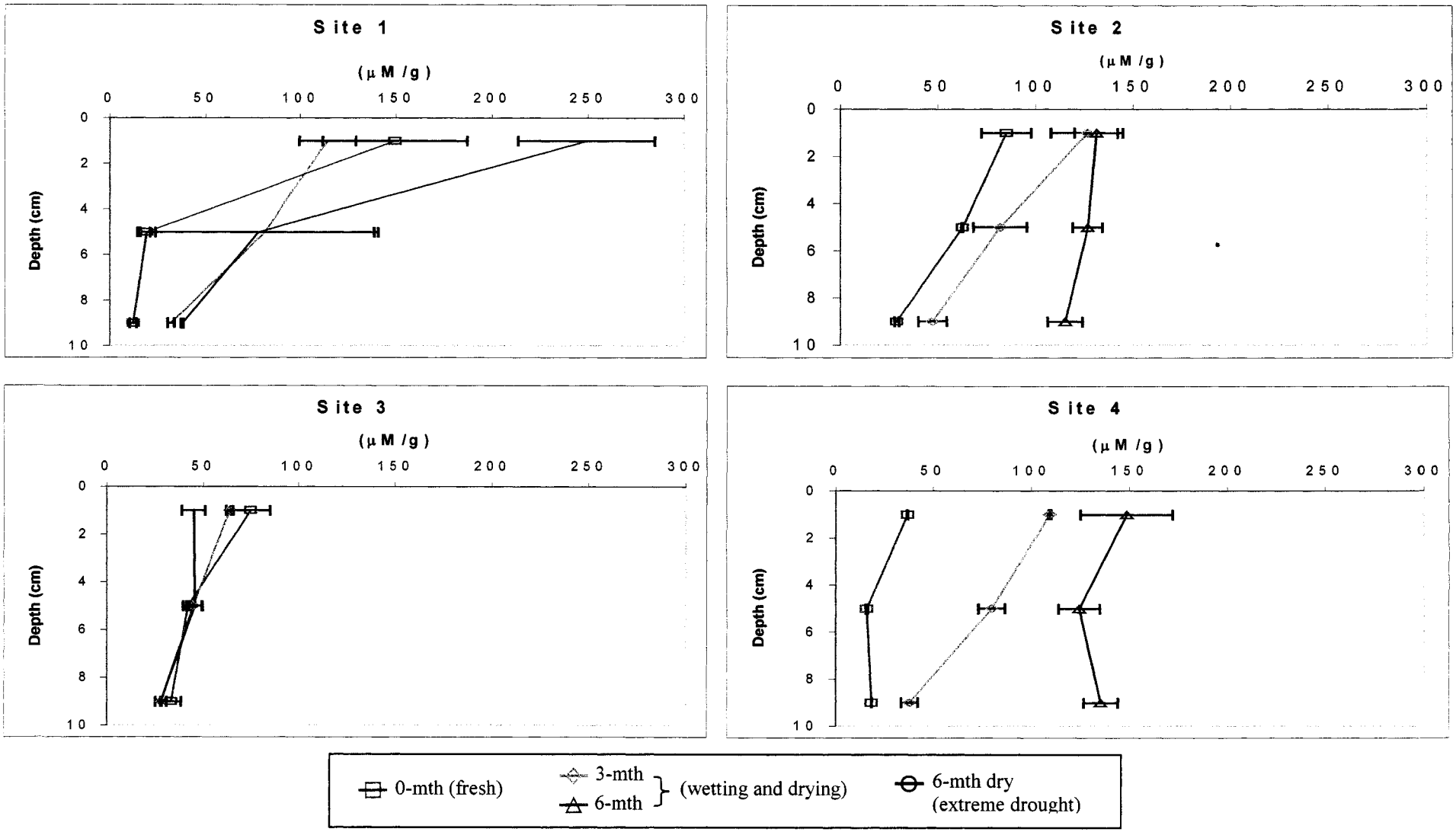


Figure 3.19 Average total reactive iron in the Black Donald Lake sediments at three separate experimental times. (n= 2) Error bars indicate standard deviations.

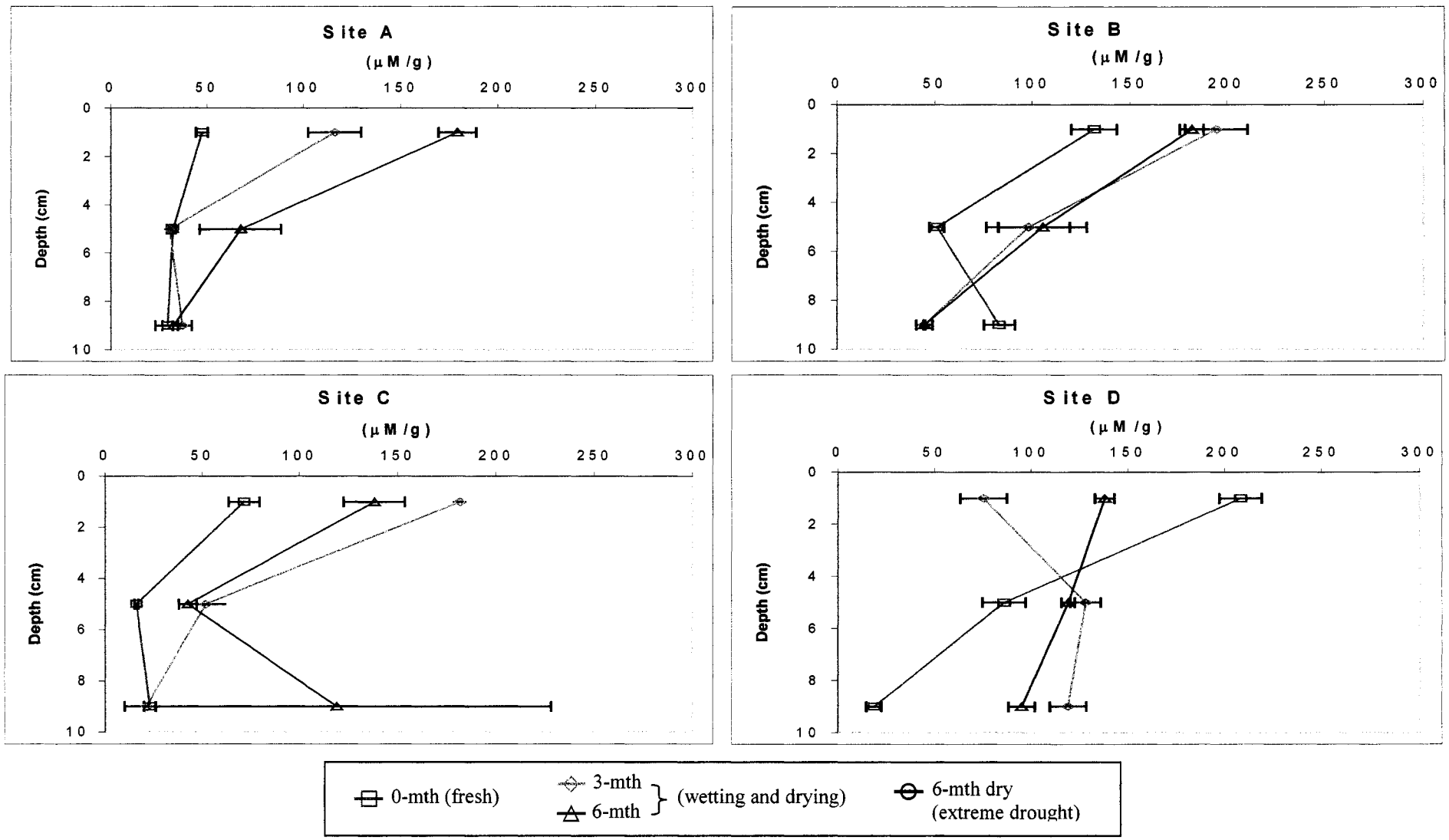


Figure 3.20 Average total reactive iron in the Stump Lake sediments at three separate experimental times. (n= 2) Error bars indicate standard deviations.

4.0 Discussion

4.1 Physico-chemical characteristics of the two study lakes

This study was designed to compare how wetting and drying episodes affected the sediments from two lakes with apparent different buffering capacities. As it turned out, the greatest similarity between both study lakes was their inherent buffering capacities. Black Donald Lake has mineral alkalinity, originating from its carbonate bedrock (Table 3.1) (Lumbers, 1982). One of the criterion for choosing Stump Lake as a study site was its assumed lack of buffering capacity due to its granitic bedrock (Pauk, 1989). A closer look at the sources of alkalinity of both study lakes was not done during the course of this study. Due to the presence of large amounts of decomposing organic matter originating from dead trees, Stump Lake turned out to have a higher alkalinity than Black Donald Lake. Naturally occurring organic substances, i.e. lignin or chlorophyll from trees and leaves, are transformed into humic acids (Stumm and Morgan, 1996). The chemical formula of these substances has not yet been determined due to their complexity. However, it is known that they possess functional groups (i.e., carboxyl and/or hydroxyl groups) which allow them to bind protons and metals (Cai et al., 1998; Tipping, 1998). Cai et al. (1998) reported that in some freshwater systems the dissolved organic matter (DOM) might be a more important acid-base species than carbon dioxide. It is likely that the large number of trees that have been decomposing in the Stump Lake sediments over the last 80 years have contributed to the pool of DOM in the lake and to its alkalinity. The higher conductivity of Stump Lake can also be attributed to the presence of decomposing organic matter. Both lakes showed oxic conditions and circumneutral pH.

Black Donald Lake (BDL) showed large concentrations of reduced iron (Fe (II)) in the porewaters, when compared to Stump Lake (SL) (Figures 3.1 and 3.2). This first indicates that reducing conditions prevailed below the sediment-water interface of both lakes and that Fe-oxides underwent reduction. Such Fe (II) profiles are often encountered in freshwater lake sediments (Fortin et al, 1993). The porewater results also showed that the pool of reactive iron, i.e., Fe fraction present in either iron oxides or iron monosulfides, appeared to be greater in the BDL sediments than in the Stump Lake sediments. This is inferred from the larger concentrations of Fe (II) in the porewaters of BDL. As a result, the

wetting and drying cycles of the BDL sediments led to the release of larger quantities of Fe (II) (Figure 3.4) than in the SL sediments (Figure 3.8). On the other hand, the porewaters of Stump Lake displayed larger sulfide concentrations than BDL, despite having similar sulfate concentrations (Figures 3.1 and 3.2). These results first show that microbial sulfate reduction occurred in both lakes because sulfide production can only be generated at low temperature (i.e., <150°C) as a result of microbial activity (Trudinger et al, 1985). The larger sulfide concentrations observed in the SL sediments could indicate higher microbial sulfate reduction rates in the sediments, but given the fact that the pool of reactive iron in the same lake was relatively low when compared to Black Donald Lake, it is more likely that sulfide accumulated in the porewaters, whereas it reacted with Fe (II) in the BDL sediments to form iron monosulfide precipitates. *In situ* sulfate reduction rates (Kostka et al., 2002) would have been required here to fully assess the activity of sulfate-reducing bacteria in both lakes. Sulfide concentrations during the wetting and drying cycles were larger in the leachate of the SL cores (Figure 3.9) when compared to the BDL cores (Figure 3.5), because there was an excess of sulfide over ferrous iron in the SL cores, due to the apparent low pool of reactive iron.

4.2 Effect of wetting and drying events on the mineralogy of Fe-rich minerals

One of the objectives of this study was to examine the effect that wetting and drying cycles would have on the mineralogy of Fe-rich minerals such as iron oxides and sulfides. It was hypothesized that the most affected would be the amorphous iron minerals since these are less stable (Fortin et al., 1993). With longer drying than wetting periods, an accumulation of iron oxides in the sediments was expected. Unfortunately, a suitable sequential chemical iron extraction procedure that could differentiate between amorphous iron sulfides and amorphous iron oxides was not found and could not be developed due to time constraints. Instead, it was thought that the Kostka and Luther (1994) protocol, designed to estimate the ratio of reactive Fe (II) to Fe (III) in sediments, could be used in this study. Variations of the Fe (II)/Fe (III) ratio in the sediments would indicate changes in the redox state of the iron minerals and give indirect information of the mineral transformations during the wetting and drying events. However, because the sediments had been freeze-dried

prior to the extraction, in preparation for a less suitable extraction method, it became impossible to assess the quantity of reactive Fe (II) in the sediments because Fe (II)-monosulfides are very unstable and oxidize quickly during freeze-drying (Kostka and Luther, 1994). The total fraction of reactive iron (Fe (II) + Fe (III)) was however determined for each core and it was found to be within the same range in both lakes (Figures 3.19 and 3.20). This is not in agreement with the porewater chemistry data of both lakes (section 4.1), which indicated that there was likely a larger pool of reactive iron in the BDL sediments than in the SL sediments. The presence of larger Fe (II) concentrations in the BDL porewaters must then be related to the type of iron-rich minerals in the sediments. Fortin et al. (1993) showed that diagenetic iron oxides in freshwater lake sediments occur as amorphous iron oxides, poorly ordered ferrihydrite and lepidocrocite. The susceptibility of these oxides to undergo reductive dissolution in the anoxic portion of the sediments depends on their mineralogy, surface reactivity and surface areas (Urrutia et al., 1998). The results found in this study therefore suggest that the type of Fe (III)-oxides in the sediments of both lakes was different, even though the overall amount of reactive iron was the same. The larger Fe (II) concentrations in the porewaters of BDL indicate that the iron oxides were likely more amorphous and therefore more soluble than the ones in SL. As a result, they underwent reductive dissolution more easily and led to more Fe (II) in the porewaters and in the leachate of the cores during the wetting and drying cycles. A more precise mineralogical analysis of the Fe (III)-oxides present in the sediments of both lakes would however be necessary to confirm this hypothesis.

The iron extraction data also showed that the sediment that underwent 3 and 6 months of wetting and drying had higher total Fe concentrations than the 0-month cores. It is unlikely that the wetting and drying cycles increased the iron content (reactive iron) of the sediments because Fe was not added as a constituent of the artificial rainwater. It is however possible that some Fe could have originated from stable Fe-rich minerals (such as pyrite (FeS_2) or Fe-silicates) undergoing weathering during the wetting and drying events and forming new diagenetic iron oxides or iron sulfides. Pyrite, which is not extracted with the Kostka and Luther protocol, was likely oxidized during the drying events and provided more reactive iron. The oxidation of pyrite over time is consistent with the general decline of pH, as described in section 4.3. In addition, the presence of grabbros in the bedrock near Stump

Lake indicates that Fe-rich silicates (olivine and pyroxene) present in such rocks (Klein and Hurlbut, 1999), were likely present in the sediments of the lake itself (Pauk, 1989). Olivine and pyroxene have a higher chemical and microbial weathering rate than quartz and transform into Fe-oxides. Such mineral transformation is possibly responsible for the increase of reaction iron over time. On the other hand, it is also possible that the increase of reactive iron over time was caused by sediment heterogeneity between the cores from each lake. At the present time, it is impossible to differentiate the two processes.

4.3 Acidity generation caused by oxidation of Fe-sulfides due to drying events

The second objective of the study was to assess the acidity generation caused by the oxidation of Fe-sulfides present in the sediments during the drying events. Iron sulfides (pyrite and iron monosulfides) form under anoxic conditions as a result of microbial sulfate reduction (Berner, 1984). Iron monosulfides easily transform into pyrite, a more stable mineral (Berner, 1984). Such minerals were likely present in the sediments of both lakes because the porewater results indicated that Fe (II) and soluble sulfide were present (Figures 3.1 and 3.2). The amount of iron monosulfides present in the sediment of both lakes could however not be determined or assessed with the Kostka and Luther (1994) extraction protocol because the sediments had been freeze-dried. As a result, it is impossible to assess the importance of the pool of Fe-sulfides on the pH trends observed during the wetting and drying cycles. In addition, the amount of Fe-pyrite in the sediments of both lakes was not determined. As a result, it is impossible to quantify the acidity generated by the oxidation of Fe-sulfides, because the fraction of Fe-monosulfides and Fe-pyrite is not known.

It is however clear that during the wetting and drying cycles, time and depth had a significant effect on the pH of the wetting and drying experiment of both lakes (Figures 3.3 and 3.7). This indicates that the means for time and depth were in fact different. The initial pH of the water samples taken at the beginning of the experiment was near-neutral, pH 5.5 to 6.5. The pH of the porewater taken along the core (ports 'a' to 'e') became more acidic at the end of the 6-month experimental period, i.e., pH 4 to 5.5. These results are in agreement with the studies by Saeki et al. (1993) and Peverly and Kopka (1991) who reported that acidity was generated in sediments after exposure to air. The likely source of acidity in the

BDL and SL sediment cores during the wetting and drying events was the oxidation of Fe-sulfides and the formation of Fe (III)-oxides, since the processes generate sulfate and consume hydroxyl ions, respectively (Aström, 1998; Schippers and Jorgensen, 2002). There are two lines of evidence suggesting that pyrite oxidation might be responsible for the acidity generation in the cores. First, the pool of reactive iron increased over time, likely as a result of Fe originating from pyrite oxidation and re-precipitating as Fe (III)-oxides. Second, the sulfate levels measured in the leachate were far greater (up to 5000 μM) than the concentration present in the artificial rainwater (~25 μM) indicating a release of sulfate from the sediment. However, one must keep in mind that the artificial rainwater was also acidic (pH 4.5; appendix A) and that it likely contributed to the acidification of the sediments, along with *in situ* biogeochemical reactions.

Both lakes possessed some form of alkalinity (organic matter and mineral). Stump Lake's buffering capacity may come from the humic substances that are able to bind protons (Cai et al., 1998; Stumm and Morgan, 1996), whereas for Black Donald Lake, the buffering capacity originates from the carbonate bedrock. Over the course of the experiment, the acidity produced by the Fe-sulfide oxidation and by the rainwater itself depleted the neutralizing capacity of the surface sediments. As a result, there was a gradual decrease in pH over the length of the core (ports 'a' to 'e') (Figures 3.3 and 3.7), whereas the leachate samples (sampling ports 'f') remained near-neutral and there were no significant decreases over the course of the experiment. This indicates that the buffering capacity of the deeper sediment of both lakes possessed a higher acid neutralizing capacity (ANC) than the upper sediments. The increased ANC of the deeper sediments in the core was partially caused by the natural buffering capacity of the sediments, but also by *in situ* biogeochemical reactions, such as microbial sulfate reduction and iron reduction (Stumm and Morgan, 1996). Both reactions generate bicarbonate as a by-product. Evidence of iron and sulfate reduction within the deeper portion of the cores is indicated by the present of Fe (II) and sulfide in the leachate of the cores of both lakes (Figures 3.4, 3.5, 3.8 and 3.9), which indicates that reducing conditions prevailed in the core. It is likely that sulfate-reducing bacteria (SRB) were growing in the deeper portion of the cores and participated in sulfate reduction and sulfide production. The presence of SRB was monitored during the course of this study, by a

colleague (K. Hindle) and it was observed that SRB were indeed abundant in the sediments of both lakes.

As hypothesized, Fe-sulfide oxidation occurred in the sediment of both lakes as evidenced by the high sulfate release in the porewaters. Acidity generated by this oxidation was however buffered by the natural buffering capacity of the sediments and, to a smaller degree, by the by-products of sulfate-reducing bacteria metabolism.

4.4 Importance of Fe- and S-oxidizing bacteria in Fe and S cycling during wetting and drying cycles

An important aspect that needed to be investigated was the presence of iron- and sulfur-oxidizing bacteria and how their populations were affected by the wetting and drying cycles (objective 3). The hypothesis was that iron- and sulfur-oxidizing bacteria should be present at all sites and should be more abundant under oxidizing conditions. The long term exposure to dry conditions should decrease the bacterial populations since water is essential to their survival. A number of studies have shown that soil biomass decreases after repeated drying and rewetting cycles (Bottner, 1985; Fierer and Schimel, 2002; Baldwin et al., 2000). The specific reasons for this decrease are not yet known, but Fierer and Schimel (2002) stated that soil water potential associated with soil rewetting events may cause microbes to undergo osmotic shock.

Given the circumneutral pH condition of both lakes, only neutrophilic iron- and sulfur-oxidizing bacteria populations were estimated during the course of the wetting and drying experiment. Over the 6-month period, neutrophilic iron-oxidizing bacteria (NIOB) in the cores of both lakes declined (Figures 3.13 and 3.15), whereas the neutrophilic sulfur-oxidizing bacteria (NSOB) remained fairly stable over time (Figures 3.14 and 3.16). Several factors affect the survival of these bacteria, i.e., moisture and aerobic conditions, organic carbon content, temperature and pH. The conditions preferred by several neutrophilic Fe- and S-oxidizing bacteria are listed in Tables 1.3 and 1.4. For optimal growth, neutrophilic iron-oxidizing bacteria require temperatures from 10 to 30°C and pH conditions from 6 to 7.5 (Table 1.3). On the other hand, neutrophilic sulfur-oxidizing bacteria can thrive at temperatures from 20 to 40°C and at pH from 2 to 9 (Table 1.4).

Of the factors mentioned above, it is unlikely that moisture and aerobic conditions affected the microbial populations because the wetting cycles provided sufficient moisture within the sediment cores (Figures 3.11 and 3.12), whereas drying cycles provided aerated conditions. Fe- and S-oxidizing bacteria also require organic carbon, which was present in the sediments of both lakes, as estimated by the LOI fraction of the sediment (Figures 3.11 and 3.12). The organic carbon content of Stump Lake sediments was greater than that of Black Donald Lake, however, both lakes had sufficient organic material to sustain microbial growth. The presence of organic acids (originating from the degradation of dead trees) in Stump Lake may have favored some Fe- and S-oxidizers, because such acids represent a good growth substrate, as shown in Table 1.3. Temperature, another important factor affecting bacterial growth (Grossman and Desrocher, 2001), remained constant during the course of the experiment (i.e., ~23°C) and had little effect on the growth of bacteria, since it is within the optimal growth temperature range of both groups of bacteria (Tables 1.3 and 1.4). The only factor that may have played an important role is pH, since neutrophilic iron-oxidizing bacteria do not tolerate acidic conditions, whereas sulfur-oxidizers can (Ehrlich, 1996).

The Siering and Ghiorse (1996) bacterial growth medium is specific to the *Leptothrix* species, a neutrophilic iron-oxidizing species. The optimal pH range for these particular bacteria is 6.5 to 7.5. The NIOB population estimates from both study lakes showed that the populations decreased as the wetting and drying experiment progressed. At the beginning of the wetting and drying experiment, the pH of the sediment porewater coincided with *Leptothrix*'s optimal pH range. However, when the pH of the surface sediments decreased to 4 – 5.5 over time (Figures 3.3 and 3.7), some of the NIOB started to die off, as indicated by the lower populations after 6 months (Figures 3.13 and 3.15). However the pH increased in the deeper sediments (Figures 3.3 and 3.7) and it is possible that some NIOB may have been able to better survive at depth. The results show that for BDL sites 3 and 4 and SL site C, the NIOB population estimates seem to increase at the 10-cm depth (Figures 3.13 and 3.15). The pH of the leachate was within the *Leptothrix* species' optimal growth range, however, it is unlikely that they were present because the deeper sediments were anoxic, as inferred by the presence of Fe (II) and sulfide.

In addition, the Tv6 growth medium (Harrison, 1983), capable of sustaining acidophilic sulfur-oxidizing bacteria, was modified by Stubner et al. (1998) to allow the growth of neutrophilic sulfur-oxidizing bacteria (NSOB) capable of oxidizing thiosulfate. The modified version of the Harrison medium was used in this study (section 2.5.2). Stubner et al. (1998) found that the isolates that thrived in the modified Harrison medium were closely associated with strains of *Xanthobacter* spp., *Azorhizobium* spp. and *Aquabacter* spp.. These bacteria are aerobic and grow within an optimal pH range of 5.8 to 9 (Table 1.4).

The bacterial population estimates indicated that neutrophilic sulfur-oxidizing bacteria (NSOB) population did not decrease over time (Figures 3.14 and 3.16), even though the pH of the surface sediments in the cores declined. The pH of the Black Donald Lake sediments over the course of the experiment remained within the *Xanthobacter* species' (one of the species growing in the Stubner et al. (1998) method) optimal growth pH range. The pH of the porewater did not sufficiently decrease to negatively affect NSOB populations in BDL. On the other hand, the pH of Stump Lake sediments decreased over time to below *Xanthobacter* species' optimal growth pH. However, no decrease in NSOB populations was observed over time. It is possible that neutrophilic sulfur-oxidizing bacteria are more tolerant to pH changes than iron-oxidizing bacteria, but since no specific pH tests were performed with the cultures present in the sediments of Stump Lake, the effect of pH on the survival of NSOB can only be speculated. It is also possible that some acidophilic sulfur-oxidizing bacteria were isolated with the Stubner et al. (1998) medium used in this study, but since no molecular analyses were performed, it remains a hypothesis.

During the wetting and drying cycles of the sediment cores, iron- and sulfur-oxidizing bacteria were detected at all study sites. These cycles promoted the oxidation of iron sulfides, which partially lowered the pH of the sediments. As a result, populations of iron-oxidizing bacteria declined over time, where NSOB remained fairly stable. Due to their different optimal growth pH ranges, the wetting and drying cycles had a more distinct effect on the NIOB populations than on the NSOB populations. Were the buffering capacities of the lakes' sediments not so high, the pH of the porewater would have likely decreased more quickly and more considerably and a more dramatic decrease in iron- and sulfur-oxidizing bacteria populations would have been observed. Future wetting and drying experiments on

lake sediments should include more specific bacterial population enumeration and characterization.

5.0 Conclusions

The present study was conducted to further our knowledge of the biogeochemical changes that can occur when shallow sediments are exposed to the atmosphere after hydro-electric dam removal. Iron sulfides present in the sediments of both lakes underwent oxidation as a result of the wetting and drying events. Oxidation of iron monosulfides present in freshwater sediments increased sulfate levels in the porewaters and partially decreased the pH. Our study also showed that pyrite (FeS_2), a stable iron disulfide, was likely oxidized, which increased the pool of reactive iron in the sediments. The resulting sulfate was leached out of the column and a portion of Fe (II) was oxidized back to Fe (III) and formed iron oxides. A better assessment of the transformations of iron sulfide to iron oxide should be performed in future studies in order to quantify the Fe (II)/Fe (III) ratios of the sediments and the acidity potential of such biogeochemical transformations. In addition, a suitable extraction protocol must be developed to differentiate between amorphous iron oxides and iron sulfides. Preliminary work performed at the beginning of this study proved unsuccessful and indicate that a proper extraction method is difficult to establish given the high reactivity of both amorphous iron oxides and sulfides in the presence of weak acids and reducing agents.

Further studies should also focus on quantifying the acidity generated by the oxidation of the sulfide minerals, because the decline of pH in the sediment cores might have also originated from the acidic rainwater added during the wetting events. Incorporating a control core (wet with deionized water) into the experimental set-up, as well as using homogeneous sediments, would be beneficial to assessing the relative importance of the two sources of acidity. The effect of variations in the frequency of the wetting events also warrants further study. To better understand the environmental ramifications for wetting and drying events on freshwater sediment, study lakes with more diverse buffering capacities would be useful to future studies. Chemical profiles of potential study lakes would also be beneficial.

Fe- and S-oxidizing bacterial populations were detected in the sediment cores of both study lakes. The decrease in moisture content of the sediment cores certainly had an effect on the microbial communities. However, the decrease in porewater pH was most likely the

major factor which caused the neutrophilic iron-oxidizing bacteria (NIOB) populations to decrease over time. The NIOB were more susceptible to porewater pH changes, i.e., pH values lower than their optimal growth pH range. The neutrophilic sulfur-oxidizing bacteria (NSOB) on the other hand, were more tolerant of the lower pH values. Therefore, wetting and drying events did affect the microbial community of freshwater sediments. The amount of stress that these microbial communities can endure before dying out is unknown. Whether or not these bacteria (NIOB and NSOB) form spores, as well as their spores' tolerance to wetting and drying events, is as yet unknown.

In conclusion, decommissioning of hydro-electric dams will have an affect on the exposed sediments and as a consequence the surrounding environment. The degree to which these sediments are affected will depend on the sediment and rainwater compositions and on the amount and frequency of rainfall (Preda and Cox, 2000). Before exposing anoxic sediments to the atmosphere, thorough biogeochemical, limnological and mineralogical profiles of the sediments in question are recommended.

6.0 References

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Appendix A

Simulated Rainwater Recipe

This rainwater recipe was created from daily precipitation chemistry values collected from the Chalk River CAPMoN station, ON for 1995-2000. The ion concentrations of the various elements found in the daily precipitation (Table A.1) were converted to their respective molar concentration. Suitable chemicals were selected to deliver these ions and no other to the simulated rainwater. The appropriate weight needed to produce the desired ion concentrations similar to the daily precipitation were calculated (Table A.2). 25 L of deionized water was added to a clean 30 L HDPE barrel. The chemicals were then carefully weighed out and added to the barrel. The pH of the simulated rainwater was usually ~ 4.5 once all the chemical had been added. No other adjustments were needed. (Note: The October 16, 2002 simulated rainwater batch needed pH adjustment. This was done with 0.1M HNO_3 and 0.1M NaOH.) When not in use, the simulated rainwater was kept at 4°C. The barrel was taken out of the cold room approximately 16 hours before a rain event and left to acclimate to room temperature.

Table A.1 Major ions of average daily precipitation (DP) and simulated rainwater (RW) and their respective concentrations.

Major ions	Concentration of major DP ions (mg/L)	Concentration of major RW ions (mg/L)
H^{+} [b]	0.05	0.029
SO_4^{2-} [a]	2.14	2.14
NO_3^{-}	2.90	4.54
Cl^{-}	0.14	0.14
NH_4^{+} [a]	0.54	0.96
Na^{+}	0.06	0.06
Ca^{2+}	0.24	0.24
Mg^{2+}	0.04	0.04
K^{+}	0.04	0.04
pH	4.45	~ 4.5

Table A.2 Chemicals used to prepare simulated rainwater and their respective weights added to 25 L of deionized water.

Chemical Name	Weight added
$MgSO_4 \cdot 7H_2O$	10.13 g
$Ca(NO_3)_2 \cdot 4H_2O$ [a]	35.333 g
KCl	1.9067 g
NH_4NO_3 [a]	106.5 g
$NaNO_3$	5.545 g
0.001 M H_2SO_4	516.4 mL
0.1 M HCl	0.7315 mL
0.1 M HNO_3	133.6 mL

[a] The concentration of these chemicals differs from what was necessary to match the DP because of a calculation error made during the preparation of the first batch of simulated rainwater. This mistake was purposefully perpetuated to avoid introducing a bias.
[b] The concentration of protons [H^+] could not be increased because all other element concentration requirements had been met or exceeded.

CAPMoN database

website: http://www.msc.ec.gc.ca/capmon/index_e.cfm

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Appendix B

Table B.1 Black Donald Lake one-way classification ANOVA tables.

Site 1	Degrees of freedom	F	P-value	F crit
pH				
a	1	0.547	0.481	5.318
b	1	0.881	0.375	5.318
c	1	0.245	0.634	5.318
d	1	1.276	0.291	5.318
e	1	0.829	0.389	5.318
f	1	5.802	0.043	5.318
Fe (II) concentration				
a	1	0.220	0.652	5.318
b	1	0.986	0.350	5.318
c	1	4.114	0.077	5.318
d	1	0.966	0.355	5.318
e	1	1.918	0.203	5.318
f	1	4.719	0.062	5.318
sulfide concentration				
a	1	0.220	0.652	5.318
b	1	0.986	0.350	5.318
c	1	2.456	0.156	5.318
d	1	1.333	0.282	5.318
e	1	0.642	0.446	5.318
f	1	0.731	0.417	5.318
sulfate concentration				
a	1	1.391	0.272	5.318
b	1	1.849	0.211	5.318
c	1	12.582	0.008	5.318
d	1	13.137	0.007	5.318
e	1	66.740	0.000	5.318
f	1	22.548	0.001	5.318

Site 2	Degrees of freedom	F	P-value	F crit
pH				
a	1	0.302	0.598	5.318
b	1	1.702	0.228	5.318
c	1	2.356	0.163	5.318
d	1	0.758	0.409	5.318
e	1	0.079	0.786	5.318
f	1	0.270	0.617	5.318
Fe (II) concentration				
a	1	0.281	0.608	4.965
b	1	0.175	0.685	4.965
c	1	0.916	0.361	4.965
d	1	1.421	0.261	4.965
e	1	0.148	0.709	4.965
f	1	23.759	0.001	4.965
sulfide concentration				
a	1	1.606	0.234	4.965
b	1	0.998	0.341	4.965
c	1	0.506	0.493	4.965
d	1	1.853	0.203	4.965
e	1	0.197	0.667	4.965
f	1	5.949	0.035	4.965
sulfate concentration				
a	1	3.310	0.099	4.965
b	1	3.080	0.110	4.965
c	1	0.589	0.461	4.965
d	1	5.136	0.047	4.965
e	1	0.016	0.902	4.965
f	1	0.198	0.666	4.965

Site 3	Degrees of freedom	F	P-value	F crit
pH				
a	1	1.685	0.231	5.318
b	1	0.939	0.361	5.318
c	1	0.998	0.347	5.318
d	1	1.372	0.275	2.318
e	1	2.555	0.149	5.318
f	1	0.002	0.969	5.318
Fe (II) concentration				
a	1	0.029	0.869	4.965
b	1	0.007	0.934	4.965
c	1	0.818	0.387	4.965
d	1	1.447	0.257	4.965
e	1	2.546	0.142	4.965
f	1	13.921	0.004	4.965
sulfide concentration				
a	1	0.434	0.529	5.318
b	1	0.273	0.616	5.318
c	1	1.188	0.308	5.318
d	1	4.931	0.057	5.318
e	1	1.292	0.289	5.318
f	1	4.863	0.059	5.318
sulfate concentration				
a	1	0.207	0.661	5.318
b	1	0.298	0.600	5.318
c	1	1.373	0.275	5.318
d	1	1.049	0.336	5.318
e	1	0.240	0.638	5.318
f	1	8.286	0.021	5.318

Site 4	Degrees of freedom	F	P-value	F crit
pH				
a	1	2.542	0.150	5.318
b	1	4.201	0.075	5.318
c	1	11.067	0.010	5.318
d	1	13.411	0.006	5.318
e	1	22.778	0.001	5.318
f	1	2.959	0.124	5.318
Fe (II) concentration				
a	1	2.369	0.155	4.965
b	1	0.019	0.893	4.965
c	1	3.182	0.105	4.965
d	1	13.790	0.004	4.965
e	1	13.759	0.004	4.965
f	1	87.926	0.000	4.965
sulfide concentration				
a	1	0.017	0.899	4.965
b	1	0.037	0.851	4.965
c	1	3.710	0.083	4.965
d	1	15.963	0.003	4.965
e	1	7.443	0.021	4.965
f	1	21.644	0.001	4.965
sulfate concentration				
a	1	1.051	0.329	4.965
b	1	1.379	0.267	4.965
c	1	5.502	0.041	4.965
d	1	4.736	0.055	4.965
e	1	5.911	0.035	4.965
f	1	3.793	0.080	4.965

Table B.2 Stump Lake one-way classification ANOVA tables.

Site A	Degrees of Freedom	F	P-value	F crit
pH				
a	1	0.727	0.4187	5.318
b	1	0.03	0.8665	5.318
c	1	2.103	0.185	5.318
d	1	3.612	0.0939	5.318
e	1	2.987	0.1222	5.318
f	1	10.25	0.0126	5.318
Fe (II) concentration				
a	1	2.046	0.1904	5.318
b	1	0.865	0.3796	5.318
c	1	4.365	0.0701	5.318
d	1	6.647	0.0327	5.318
e	1	5.744	0.0434	5.318
f	1	19.45	0.0023	5.318
sulfide concentration				
a	1	0.127	0.7305	5.318
b	1	0.305	0.5959	5.318
c	1	0.088	0.7738	5.318
d	1	10.67	0.0114	5.318
e	1	0.084	0.7796	5.318
f	1	14.41	0.0053	5.318
sulfate concentration				
a	1	0.089	0.7737	5.318
b	1	0.143	0.7154	5.318
c	1	0.817	0.3926	5.318
d	1	0.057	0.8173	5.318
e	1	18.38	2.70E-03	5.318
f	1	6.434	0.0349	5.318

Site B	Degrees of Freedom	F	P-value	F crit
pH				
a	1	1.675	0.2317	5.318
b	1	0.62	0.4539	5.318
c	1	0.042	0.842	5.318
d	1	1.881	0.2075	5.318
e	1	0.312	0.5917	5.318
f	1	0.002	0.9632	5.318
Fe (II) concentration				
a	1	0.158	0.7016	5.318
b	1	8.664	0.0147	4.965
c	1	2.268	0.163	4.965
d	1	0.517	0.4887	4.965
e	1	0.238	0.6359	4.965
f	1	0.562	0.4708	4.965
sulfide concentration				
a	1	0.685	0.432	5.318
b	1	0.945	0.3539	4.965
c	1	0.31	0.5899	4.965
d	1	0.796	0.3932	4.965
e	1	3.645	0.0853	4.965
f	1	4.025	0.0797	5.318
sulfate concentration				
a	1	0.069	0.7979	4.965
b	1	1.844	0.2043	4.965
c	1	5.039	0.0486	4.965
d	1	8.185	0.0169	4.965
e	1	3.899	9.58E-02	5.987
f	1	30.26	0.0006	5.318

Site C	Degrees of Freedom	F	P-value	F crit
pH				
a	1	0.873	0.3774	5.318
b	1	1E-04	0.9918	5.318
c	1	0.484	0.5248	7.789
d	1	0.978	0.3516	5.318
e	1	0.308	0.5943	5.318
f	1	5.214	0.0625	5.987
Fe (II) concentration				
a	1	1.11	0.3229	5.318
b	1	0.894	0.3721	5.318
c	1	0.467	0.5137	5.318
d	1	0.423	0.5336	5.318
e	1	0.029	0.869	5.318
f	1	8.014	0.0221	5.318
sulfide concentration				
a	1	1.466	0.2652	5.592
b	1	0	1	5.318
c	1	0.669	0.4371	5.318
d	1	1.997	0.1954	5.318
e	1	1.016	0.3431	5.318
f	1	110.5	5.84E-06	5.318
sulfate concentration				
a	1	21.46	0.0017	5.318
b	1	13.88	0.0058	5.318
c	1	0.085	0.7777	5.318
d	1	1.59	0.2428	5.318
e	1	1.273	2.92E-01	5.318
f	1	4.627	0.0637	5.318

Site D	Degrees of Freedom	F	P-value	F crit
pH				
a	1	1.322	0.2835	5.318
b	1	0.138	0.7198	5.318
c	1	4.906	0.0576	5.318
d	1	7.622	0.0246	5.318
e	1	0.051	0.8271	5.318
f	1	6.683	0.0324	5.318
Fe (II) concentration				
a	1	0.39	0.5465	4.965
b	1	2.236	0.1657	4.965
c	1	6.848	0.0257	4.965
d	1	0	1	4.965
e	1	1.07	0.3254	4.965
f	1	23.25	7.00E-04	4.965
sulfide concentration				
a	1	1.869	0.2015	4.965
b	1	2.304	0.16	4.965
c	1	3.288	0.0999	4.965
d	1	0.691	0.4254	4.965
e	1	4.171	0.0684	4.965
f	1	0.998	0.3414	4.965
sulfate concentration				
a	1	1.051	0.3295	4.965
b	1	0.7	0.4223	4.965
c	1	14.46	0.0052	5.318
d	1	18.33	0.0016	4.965
e	1	41.63	7.33E-05	4.965
f	1	0.561	0.471	4.965

Table B.3 Black Donald Lake two-way classification ANOVA without repetition tables (6-month cores only).

Site 1	Degrees of Freedom	F	P-value	F crit
pH				
depth	5	6.832	6.3e-5	2.400
time	10	9.675	9.9e-9	2.026
Fe (II) concentration				
depth	5	12.444	7.3e-8	2.400
time	10	1.206	0.310	2.026
sulfide concentration				
depth	5	5.286	0.0005	2.383
time	11	1.254	0.2754	1.968
sulfate concentration				
depth	5	19.33	1.2e-10	2.400
time	10	1.725	0.101	2.026
NIOB population estimate				
depth	4	0.889	0.500	3.259
time	3	4.258	0.028	3.490
NSOB population estimate				
depth	4	0.544	0.707	3.259
time	3	0.448	0.723	3.490

Site 2	Degrees of Freedom	F	P-value	F crit
pH				
depth	5	28.091	2.0e-13	2.400
time	10	3.294	0.0024	2.026
Fe (II) concentration				
depth	5	41.06	2.2e-17	2.383
time	11	1.392	0.203	1.968
sulfide concentration				
depth	5	5.680	0.0003	2.383
time	11	2.534	0.0116	1.968
sulfate concentration				
depth	5	9.004	2.7e-9	2.383
time	11	2.129	0.0331	1.968
NIOB population estimate				
depth	4	1.048	0.423	3.259
time	3	2.958	0.0752	3.490
NSOB population estimate				
depth	4	2.695	0.082	3.259
time	3	1.558	0.251	3.490

Site 3	Degrees of Freedom	F	P-value	F crit
pH				
depth	5	19.362	1.1e-10	2.400
time	10	10.717	2.0e-9	2.026
Fe (II) concentration				
depth	5	1.838	0.122	2.400
time	10	1.757	0.0938	2.026
sulfide concentration				
depth	5	1.673	0.158	2.400
time	10	1.308	0.252	2.026
sulfate concentration				
depth	5	22.804	7.6e-12	2.400
time	10	3.886	0.0006	2.026
NIOB population estimate				
depth	4	0.694	0.610	3.259
time	3	1.526	0.258	3.490
NSOB population estimate				
depth	4	1.504	0.262	3.259
time	3	0.968	0.440	3.490

Site 4	Degrees of Freedom	F	P-value	F crit
pH				
depth	5	12.622	6.0e-8	2.400
time	10	5.747	1.1e-5	2.026
Fe (II) concentration				
depth	5	39.578	3.0e-16	2.400
time	10	2.88	0.0065	2.026
sulfide concentration				
depth	5	2.498	0.0415	2.383
time	11	1.205	0.306	1.968
sulfate concentration				
depth	5	0.849	0.521	2.383
time	11	0.883	0.561	1.968
NIOB population estimate				
depth	4	0.935	0.476	3.259
time	3	3.104	0.0671	3.490
NSOB population estimate				
depth	4	0.995	0.447	3.259
time	3	0.567	0.647	3.490

Table B.4 Stump Lake two-way classification ANOVA without repetition tables (6-month cores only).

Site A	Degrees of Freedom	F	P-value	F crit
pH				
depth	5	34.390	4.6e-15	2.400
time	10	10.385	3.3e-9	2.026
Fe (II) concentration				
depth	5	11.742	9.6e-8	2.383
time	11	1.770	0.0821	1.968
sulfide concentration				
depth	5	9.780	1e-6	2.383
time	11	0.983	0.473	1.968
sulfate concentration				
depth	5	55.193	3.3e-20	2.383
time	11	1.083	0.391	1.968
NIOB population estimate				
depth	4	0.958	0.465	3.259
time	3	2.719	0.0911	3.490
NSOB population estimate				
depth	4	0.357	0.835	3.259
time	3	0.647	0.600	3.490

Site B	Degrees of Freedom	F	P-value	F crit
pH				
depth	5	59.425	7.4e-20	2.400
time	10	10.482	2.9e-9	2.026
Fe (II) concentration				
depth	5	11.490	1.3e-7	2.383
time	11	0.999	0.459	1.968
sulfide concentration				
depth	5	4.465	0.0019	2.400
time	10	0.946	0.500	2.026
sulfate concentration				
depth	5	23.69	4.0e-12	2.400
time	10	1.142	0.351	2.026
NIOB population estimate				
depth	4	1.547	0.251	3.259
time	3	5.522	0.0129	3.490
NSOB population estimate				
depth	4	0.526	0.719	3.259
time	3	2.959	0.0752	3.490

Site C	Degrees of Freedom	F	P-value	F crit
pH				
depth	5	39.502	3.2e-16	2.400
time	10	1.951	0.0597	2.026
Fe (II) concentration				
depth	5	17.26	3.1e-10	2.383
time	11	5.317	1.2e-5	1.968
sulfide concentration				
depth	5	28.352	4.3e-14	2.383
time	11	1.017	0.444	1.968
sulfate concentration				
depth	5	7.951	1.1e-5	2.383
time	11	0.966	0.487	1.968
NIOB population estimate				
depth	4	0.882	0.504	3.259
time	3	2.958	0.0752	3.490
NSOB population estimate				
depth	4	0.0923	0.983	3.259
time	3	0.426	0.738	3.490

Site D	Degrees of Freedom	F	P-value	F crit
pH				
depth	5	5.870	2.4e-4	2.400
time	10	21.142	1.2e-14	2.026
Fe (II) concentration				
depth	5	1.974	0.0969	2.383
time	11	2.511	0.012	1.968
sulfide concentration				
depth	5	2.078	0.082	2.383
time	11	2.208	0.027	1.968
sulfate concentration				
depth	5	17.833	1.8e-10	2.383
time	11	1.671	0.105	1.968
NIOB population estimate				
depth	4	0.9519	0.468	3.259
time	3	4.629	0.0226	3.490
NSOB population estimate				
depth	4	0.515	0.726	3.259
time	3	2.932	0.0768	3.490

Table B.5 Two-way classification ANOVA with repetition tables (6-month cores only).

BDL	Degrees of Freedom	F	P-value	F crit
pH				
depth	5	26.74	1.3e-20	2.26
time	10	8.247	3.9e-11	1.879
Interaction	50	0.835	0.772	1.415
Fe (II) concentration				
depth	5	21.35	4.6e-17	2.26
time	10	0.779	0.649	1.879
Interaction	52	0.256	1	1.415
sulfide concentration				
depth	5	5.549	8.9e-5	2.26
time	9	0.732	0.679	1.93
Interaction	45	0.552	0.99	1.44
sulfate concentration				
depth	5	2.105	0.0663	2.26
time	10	0.908	0.527	1.879
Interaction	50	0.848	0.751	1.415
NIOB population estimate				
depth	4	1.067	0.384	2.579
time	2	2.01	0.146	3.204
Interaction	8	0.779	0.623	2.152
NSOB population estimate				
depth	4	2.48	0.0573	2.579
time	2	0.153	0.858	3.204
Interaction	8	1.193	0.324	2.152

SL	Degrees of Freedom	F	P-value	F crit
pH				
depth	5	28.561	2.9e-21	2.264
time	9	8.051	5.4e-10	1.932
Interaction	45	0.316	1	1.44
Fe (II) concentration				
depth	5	12.232	3.2e-10	2.264
time	9	0.969	0.468	1.932
Interaction	45	0.530	0.993	1.44
sulfide concentration				
depth	5	20.665	2.8e-16	2.264
time	9	1.546	0.135	1.932
Interaction	45	1.327	0.100	1.44
sulfate concentration				
depth	5	37.46	3.2e-26	2.264
time	9	0.951	0.482	1.932
Interaction	45	0.674	0.940	1.44
NIOB population estimate				
depth	4	1.095	0.370	2.579
time	2	13.282	2.9e-5	3.204
Interaction	8	1.904	0.0829	2.152
NSOB population estimate				
depth	4	0.364	0.833	2.579
time	2	0.293	0.748	3.204
Interaction	8	0.812	0.596	2.152

NOTE: 'Interaction' involves the interaction between factors 'depth' and 'time'.

Table B.6 Three-way classification ANOVA tables (6-month cores only).

		Degrees of freedom	F-ratio	p-value
pH	LAKE	1	349.5	1.71E-11
	DEPTH	5	66.779	1.06E-11
	WEEK	10	22.801	1.01E-11
	WEEK*LAKE Interaction	10	2.605	0.004
	DEPTH*LAKE Interaction	5	3.525	0.004
	WEEK*DEPTH Interaction	50	1.036	0.41
	LAKE*DEPTH*WEEK Interaction	50	0.658	0.967
Fe (II) concentration				
	LAKE	1	44.389	2.64E-11
	DEPTH	5	31.195	1.30E-11
	WEEK	11	0.381	0.964
	WEEK*LAKE Interaction	11	0.402	0.956
	DEPTH*LAKE Interaction	5	17.355	1.57E-11
	WEEK*DEPTH Interaction	55	0.535	0.998
	LAKE*DEPTH*WEEK Interaction	55	0.517	0.999
sulfide concentration				
	LAKE	1	17.783	2.81E-05
	DEPTH	5	26.666	1.36E-11
	WEEK	11	1.056	0.395
	WEEK*LAKE Interaction	11	0.869	0.571
	DEPTH*LAKE Interaction	5	21.423	1.46E-11
	WEEK*DEPTH Interaction	55	0.67	0.968
	LAKE*DEPTH*WEEK Interaction	55	0.831	0.802
sulfate concentration				
	LAKE	1	215.21	1.86E-11
	DEPTH	5	36.071	1.25E-11
	WEEK	11	1.571	0.103
	WEEK*LAKE Interaction	11	1.464	0.14
	DEPTH*LAKE Interaction	5	24.491	1.40E-11
	WEEK*DEPTH Interaction	55	0.649	0.977
	LAKE*DEPTH*WEEK Interaction	55	0.756	0.902
NIOB population estimate				
	LAKE	1	6.042	0.015
	DEPTH	4	2.417	0.052
	MONTH	3	14.891	2.70E-08
	LAKE*DEPTH$	4	1.009	0.406
	DEPTH*MONTH	12	1.819	0.052
	MONTH*LAKE	3	2.88	0.039
	DEPTH*MONTH*LAKE	12	1.069	0.393
NSOB population estimate				
	LAKE	1	28.129	5.30E-07
	DEPTH	4	0.091	0.985
	MONTH	3	1.8	0.151
	LAKE*DEPTH	4	0.185	0.946
	DEPTH*MONTH	12	0.87	0.579
	MONTH*LAKE	3	2.342	0.077
	DEPTH*MONTH*LAKE	12	0.896	0.553

* Indicates an interaction between the factors.

Table B.7 Nested ANOVA tables.

pH

Depth	Source of variability	Degrees of freedom	σ^2	F-ratio	p-value
a	LAKE$	1	0.027	17.743	0.006
	SITE$(LAKE$)	6	0.006	0.796	0.599
	CORE$(SITE$(LAKE$)	8	0.013	7.392	7.032E-08
	Error	113	0.102		
b	LAKE$	1	0.039	11.001	0.016
	SITE$(LAKE$)	6	0.014	2.594	0.106
	CORE$(SITE$(LAKE$)	8	0.009	5.167	1.6371E-05
	Error	114	0.102		
c	LAKE$	1	0.030	8.563	0.026
	SITE$(LAKE$)	6	0.014	2.935	0.081
	CORE$(SITE$(LAKE$)	8	0.007	4.529	8.544E-05
	Error	112	0.102		
d	LAKE$	1	0.042	7.954	0.030
	SITE$(LAKE$)	6	0.021	5.922	0.012
	CORE$(SITE$(LAKE$)	8	0.005	3.392	0.002
	Error	114	0.102		
e	LAKE$	1	0.052	7.346	0.035
	SITE$(LAKE$)	6	0.028	9.705	0.003
	CORE$(SITE$(LAKE$)	8	0.004	2.794	0.007
	Error	114	0.102		
f	LAKE$	1	0.004	0.966	0.364
	SITE$(LAKE$)	6	0.017	3.202	0.066
	CORE$(SITE$(LAKE$)	8	0.009	5.186	1.58E-05
	Error	113	0.102		

Fe (II) concentration

Depth	Source of variability	Degrees of freedom	σ^2	F-ratio	p-value
a	LAKE$	1	4.3E-05	0.93878	0.37001
	SITE$(LAKE$)	6	0.02998	3.26667	0.06271
	CORE$(SITE$(LAKE$)	8	-2.7387	0.00011	1
	Error	120	135.856		
b	LAKE$	1	0.0008	1.25095	0.30614
	SITE$(LAKE$)	6	0.03226	2.52885	0.11231
	CORE$(SITE$(LAKE$)	8	-2.7369	0.00077	1
	Error	123	135.856		
c	LAKE$	1	0.58555	2.21218	0.18749
	SITE$(LAKE$)	6	1.06478	0.74925	0.62727
	CORE$(SITE$(LAKE$)	8	-0.0765	0.97208	0.46113
	Error	121	135.856		
d	LAKE$	1	1.71876	1.84169	0.22358
	SITE$(LAKE$)	6	3.68038	0.73097	0.63879
	CORE$(SITE$(LAKE$)	8	6.89149	3.51603	0.00108
	Error	123	135.856		
e	LAKE$	1	16.0122	2.56155	0.16061
	SITE$(LAKE$)	6	24.8225	1.60659	0.26113
	CORE$(SITE$(LAKE$)	8	26.5638	10.6983	2.6E-11
	Error	122	135.856		
f	LAKE$	1	75.7062	3.81915	0.09848
	SITE$(LAKE$)	6	75.4951	0.39694	0.86175
	CORE$(SITE$(LAKE$)	8	372.804	137.108	1.6E-57
	Error	123	135.856		

Table B.7 (cont'd) Nested ANOVA tables.

sulfide concentration

Depth	Source of variability	Degrees of freedom	σ^2	F-ratio	p-value
a	LAKE$	1	0.00139	0.59469	0.4698818
	SITE$(LAKE$)	6	0.11438	2.2271	0.1458274
	CORE$(SITE$(LAKE$)	8	-10.771	0.00093	1
	Error	121	562.652		
b	LAKE$	1	0.00711	1.09933	0.3348005
	SITE$(LAKE$)	6	0.13015	3.36396	0.0583824
	CORE$(SITE$(LAKE$)	8	-10.765	0.00149	1
	Error	124	562.652		
c	LAKE$	1	0.12666	0.80563	0.4039914
	SITE$(LAKE$)	6	0.72819	0.96645	0.5024192
	CORE$(SITE$(LAKE$)	8	-9.462	0.12237	0.9982565
	Error	123	562.652		
d	LAKE$	1	0.00396	0.0482	0.8335113
	SITE$(LAKE$)	6	0.52698	0.84102	0.5716912
	CORE$(SITE$(LAKE$)	8	-9.7503	0.09563	0.9992895
	Error	124	562.652		
e	LAKE$	1	0.08992	0.22034	0.6553547
	SITE$(LAKE$)	6	1.79983	2.30962	0.1355822
	CORE$(SITE$(LAKE$)	8	-9.3	0.1374	0.9973651
	Error	124	562.652		
f	LAKE$	1	246.802	8.99193	0.0240499
	SITE$(LAKE$)	6	107.658	0.89673	0.5399451
	CORE$(SITE$(LAKE$)	8	234.338	22.7355	5.323E-21
	Error	123	562.652		

sulfate concentration

Depth	Source of variability	Degrees of freedom	σ^2	F-ratio	p-value
a	LAKE$	1	-3.6275	0.00654	0.93819
	SITE$(LAKE$)	6	4557.11	1.7497	0.22707
	CORE$(SITE$(LAKE$)	8	-59.958	0.98862	0.44842
	Error	120	270989		
b	LAKE$	1	7445.49	2.4394	0.16935
	SITE$(LAKE$)	6	12234.6	3.21789	0.06502
	CORE$(SITE$(LAKE$)	8	2350.02	1.44607	0.1842
	Error	122	270989		
c	LAKE$	1	123260	11.1659	0.01558
	SITE$(LAKE$)	6	43956.3	2.97596	0.07819
	CORE$(SITE$(LAKE$)	8	24432.2	5.63757	4.4E-06
	Error	121	270989		
d	LAKE$	1	277836	30.693	0.00146
	SITE$(LAKE$)	6	35646	1.12685	0.42535
	CORE$(SITE$(LAKE$)	8	59016.6	12.2022	9.8E-13
	Error	123	270989		
e	LAKE$	1	213299	11.4758	0.01472
	SITE$(LAKE$)	6	73469.5	1.45862	0.30268
	CORE$(SITE$(LAKE$)	8	96759.4	19.3663	1.6E-18
	Error	121	270989		
f	LAKE$	1	514122	9.98953	0.01955
	SITE$(LAKE$)	6	204871	3.22013	0.06491
	CORE$(SITE$(LAKE$)	8	122717	24.2933	7.8E-22
	Error	120	270989		

Table B.7 (cont'd) Nested ANOVA tables.

NIOB population estimate

Depth	Source of variability	Degrees of freedom	σ^2	F-ratio	p-value
1.5 cm	LAKE	1	9.57E+08	2.346	0.176
	SITE(LAKE)	6	1.48E+09	9.066	3.18E-05
	Error	24	3.67E+09		
3.5 cm	LAKE	1	-8.34E+06	0.0001	0.992
	SITE(LAKE)	6	6.69E+08	4.648	0.0029
	Error	24	3.67E+09		
5.5 cm	LAKE	1	6.68E+09	1.825	0.225
	SITE(LAKE)	6	1.49E+10	82.025	8.51E-15
	Error	24	3.67E+09		
7.5 cm	LAKE	1	8.87E+08	6.341	0.045
	SITE(LAKE)	6	3.79E+08	3.067	0.023
	Error	24	3.67E+09		
9.5 cm	LAKE	1	4.94E+08	20.845	0.004
	SITE(LAKE)	6	-8.89E+07	0.515	0.791
	Error	24	3.67E+09		

NSOB population estimate

Depth	Source of variability	Degrees of freedom	σ^2	F-ratio	p-value
1.5 cm	LAKE	1	5.73E+11	2.896	0.140
	SITE(LAKE)	6	4.99E+11	2.662	0.040
	Error	24	6.01E+12		
3.5 cm	LAKE	1	6.29E+11	3.388	0.115
	SITE(LAKE)	6	4.49E+11	2.495	0.051
	Error	24	6.01E+12		
5.5 cm	LAKE	1	1.39E+12	5.292	0.061
	SITE(LAKE)	6	7.59E+11	3.527	0.012
	Error	24	6.01E+12		
7.5 cm	LAKE	1	9.71E+11	6.988	0.038
	SITE(LAKE)	6	2.57E+11	1.856	0.130
	Error	24	6.01E+12		
9.5 cm	LAKE	1	8.50E+11	4.487	0.078
	SITE(LAKE)	6	4.63E+11	2.540	0.048
	Error	24	6.01E+12		

Table B.8 Moisture content two-way classification ANOVA without replication tables.

SL		Degrees of Freedom	F	P-value	F crit
Site A	depth	4	0.885	0.502	3.259
	weeks	3	45.375	8.0E-07	3.490
Site B	depth	4	2.735	0.079	3.259
	weeks	3	30.028	7.3E-06	3.490
Site C	depth	4	2.954	0.065	3.259
	weeks	3	88.254	1.9E-08	3.490
Site D	depth	4	0.560	0.696	3.259
	weeks	3	16.899	1.3E-04	3.490

BDL		Degrees of Freedom	F	P-value	F crit
Site 1	depth	4	3.136	0.056	3.259
	weeks	3	13.315	4.0E-04	3.490
Site 2	depth	4	2.673	0.084	3.259
	weeks	3	2.476	0.111	3.490
Site 3	depth	4	10.562	0.001	3.259
	weeks	3	12.848	4.7E-04	3.490
Site 4	depth	4	2.734	0.079	3.259
	weeks	3	45.234	8.1E-07	3.490

Table B.9 Organic content two-way classification ANOVA without replication tables.

SL		Degrees of Freedom	F	P-value	F crit
Site A	depth	4	2.233	0.155	3.838
	weeks	2	1.849	0.219	4.459
Site B	depth	4	7.072	0.010	3.838
	weeks	2	2.455	0.147	4.459
Site C	depth	4	0.569	0.693	3.838
	weeks	2	0.869	0.456	4.459
Site D	depth	4	1.357	0.330	3.838
	weeks	2	6.414	0.022	4.459

BDL		Degrees of Freedom	F	P-value	F crit
Site 1	depth	4	3.867	0.030	3.259
	weeks	3	8.946	0.002	3.490
Site 2	depth	4	5.632	0.009	3.259
	weeks	3	4.271	0.029	3.490
Site 3	depth	4	21.967	1.9E-05	3.259
	weeks	3	6.151	0.009	3.490
Site 4	depth	4	7.648	0.003	3.259
	weeks	3	81.940	2.9E-08	3.490

Appendix C

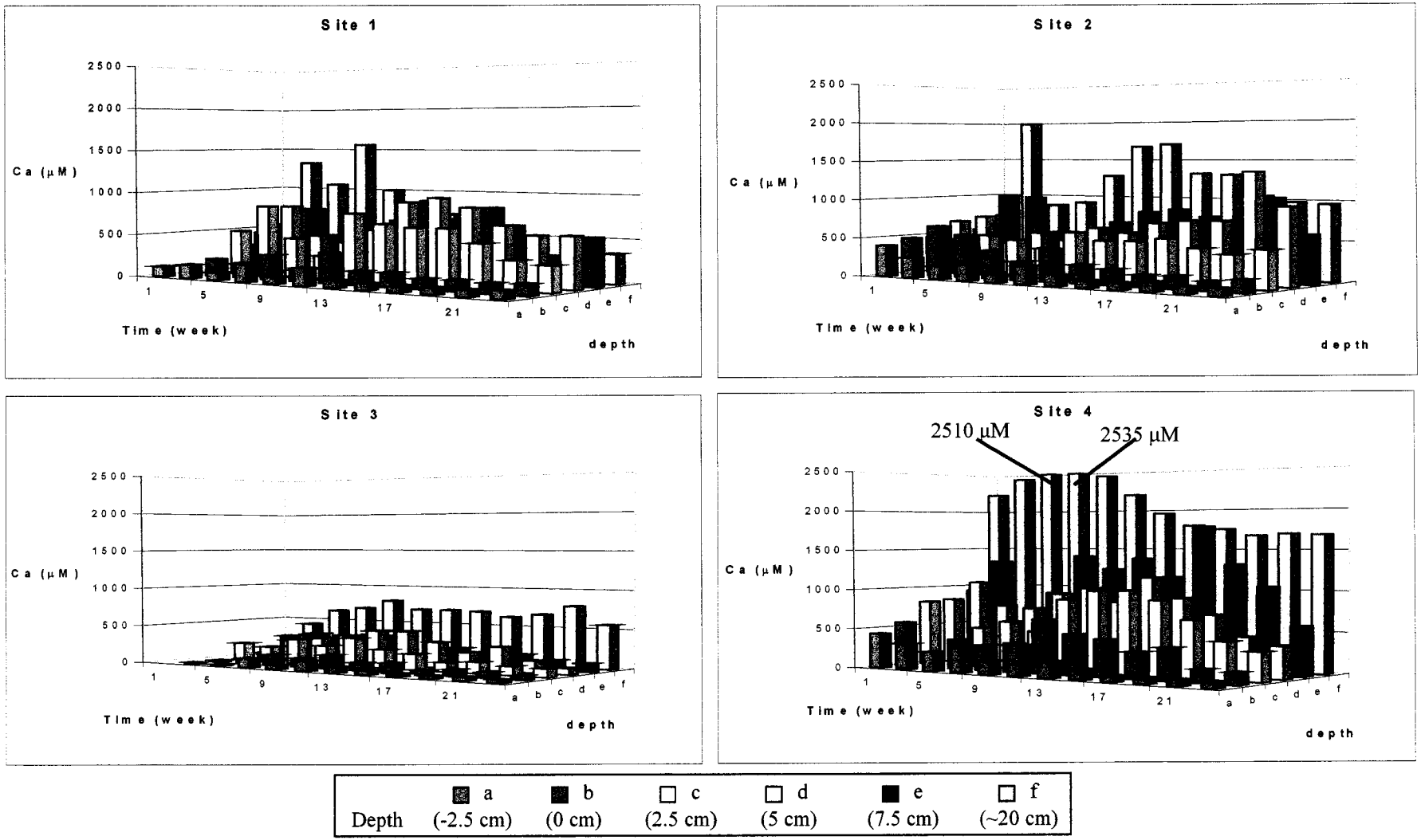


Figure C.1 Calcium concentration in the four BDL sediment cores during the wetting and drying cycles as a function of time.

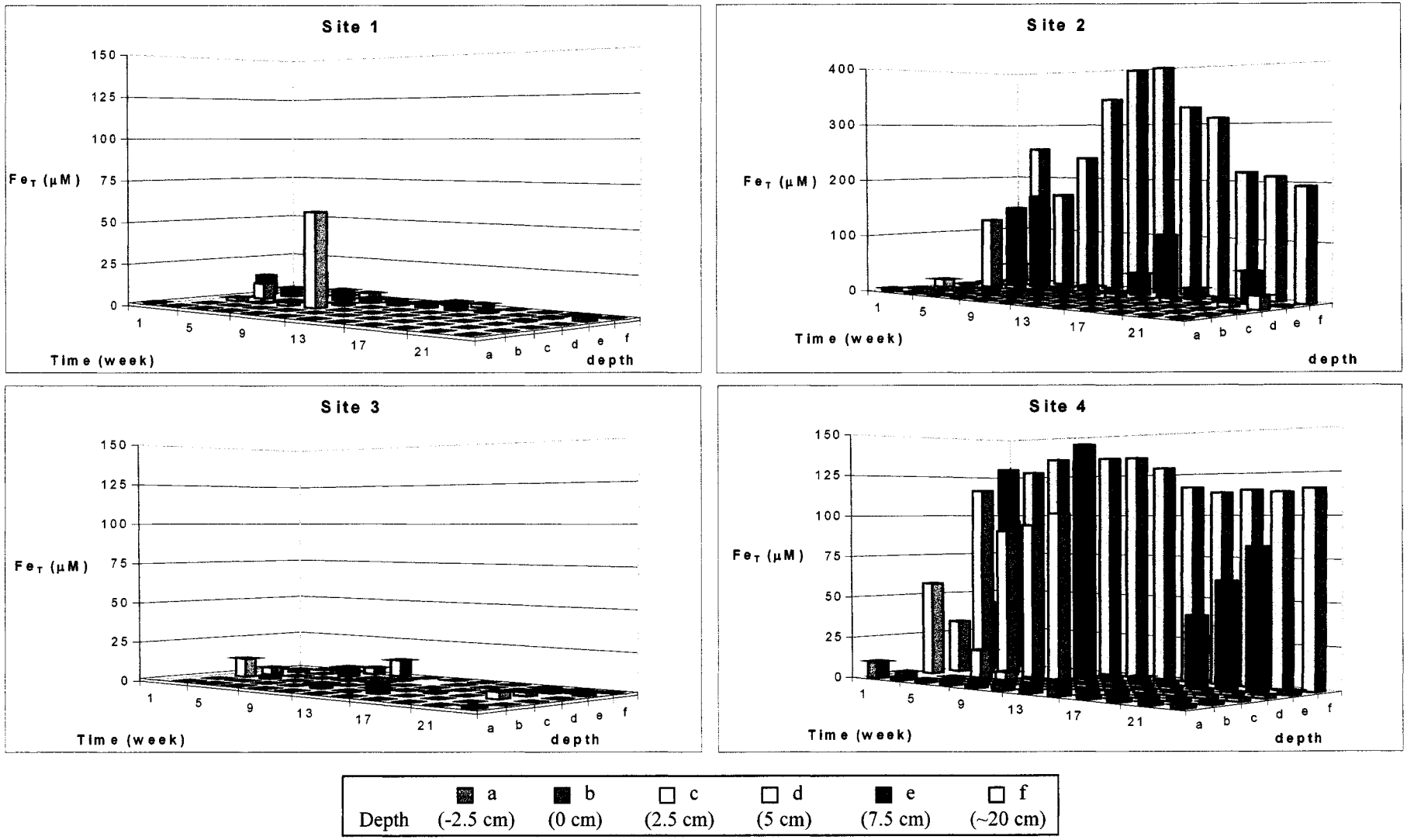
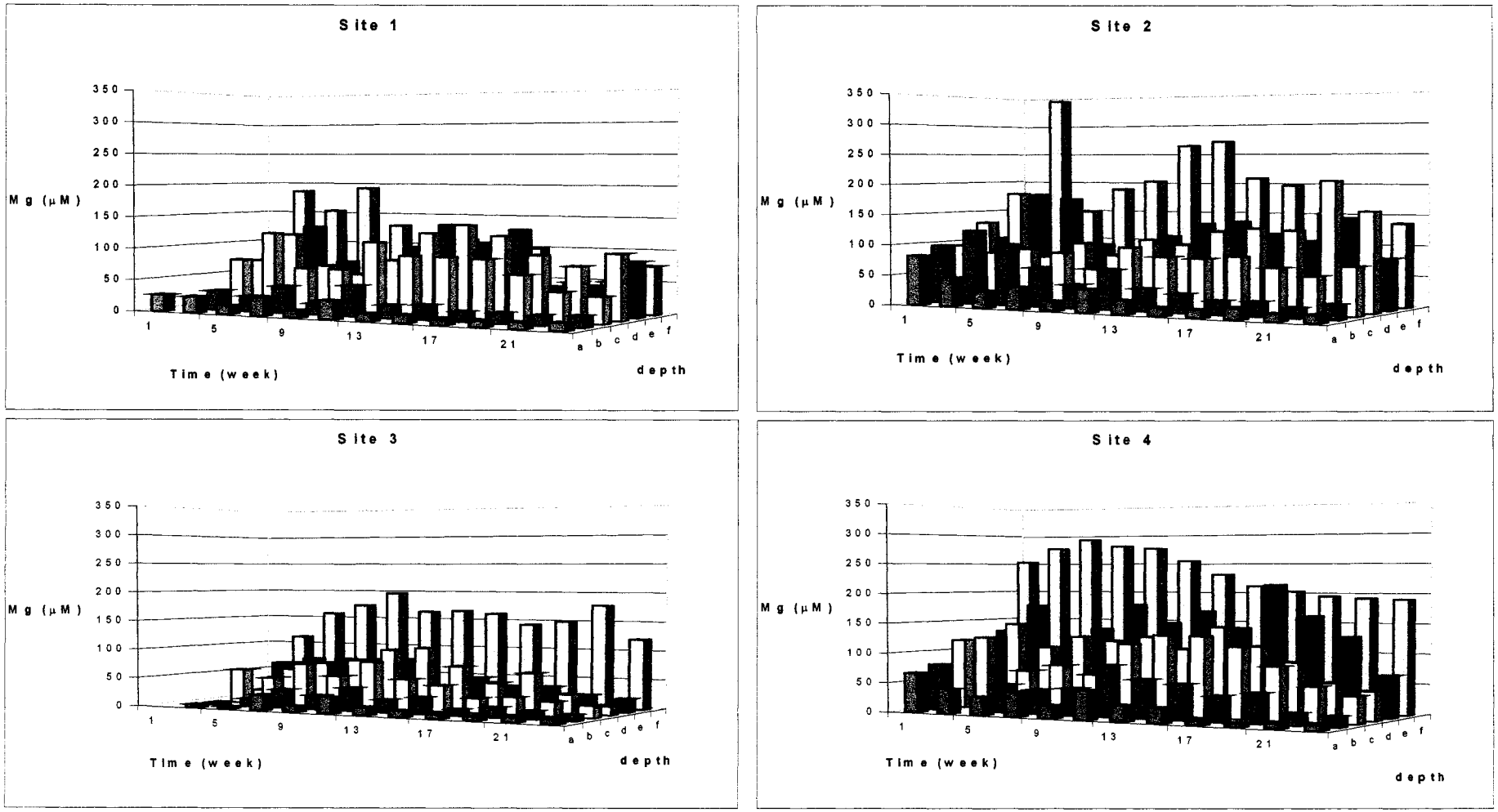


Figure C.2 Total iron concentration in the four BDL sediment cores during the wetting and drying cycles as a function of time. NOTE: The XY floor plane crosses at the detection limit (1.8 μM). Site 2 is on a different scale than the other sites.



Depth	a (-2.5 cm)	b (0 cm)	c (2.5 cm)	d (5 cm)	e (7.5 cm)	f (~20 cm)

Figure C.3 Magnesium concentration in the four BDL sediment cores during the wetting and drying cycles as a function of time.

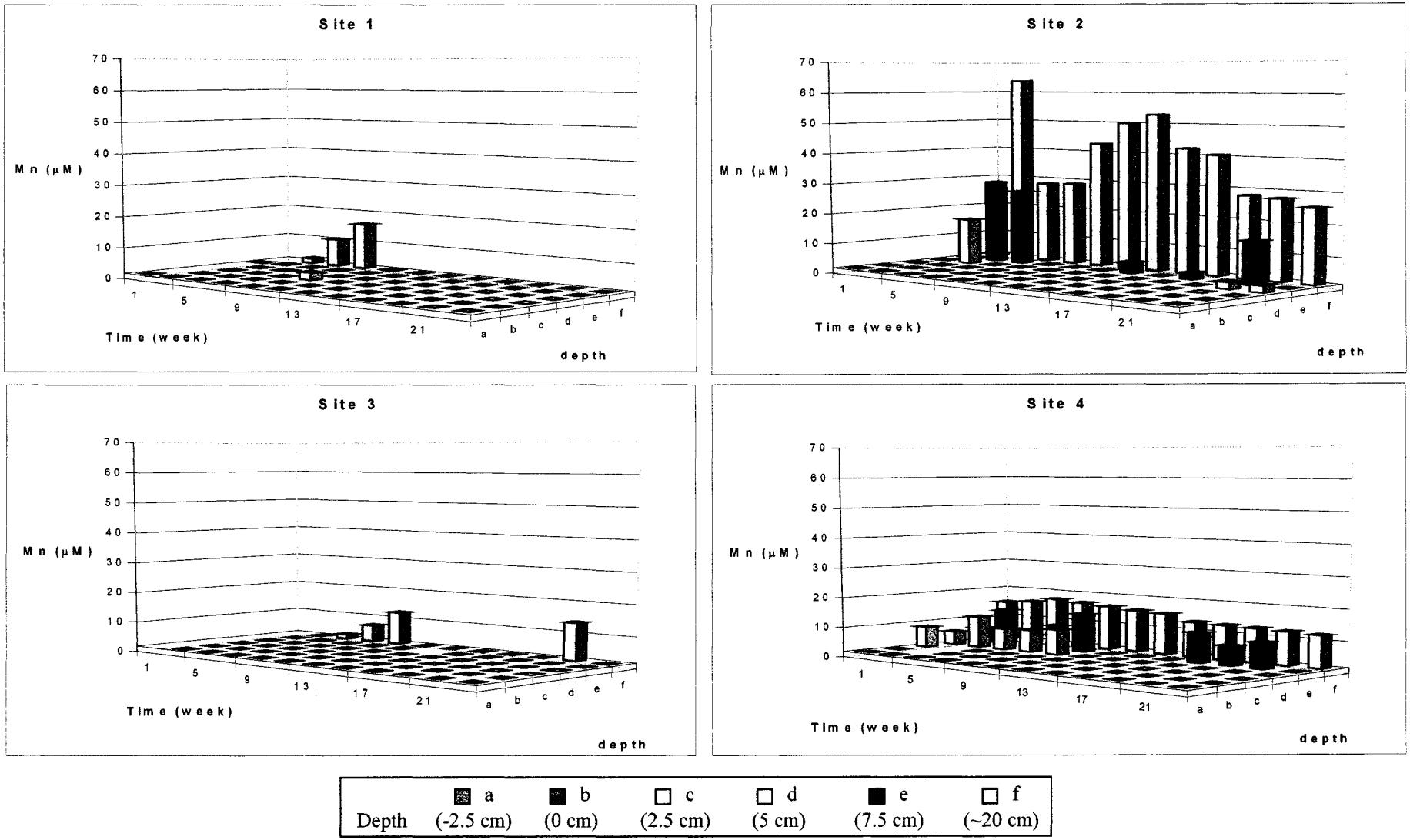


Figure C.4 Manganese concentration in the four BDL sediment cores during the wetting and drying cycles as a function of time. NOTE: The XY floor plane crosses at the detection limit (1.8 μM).

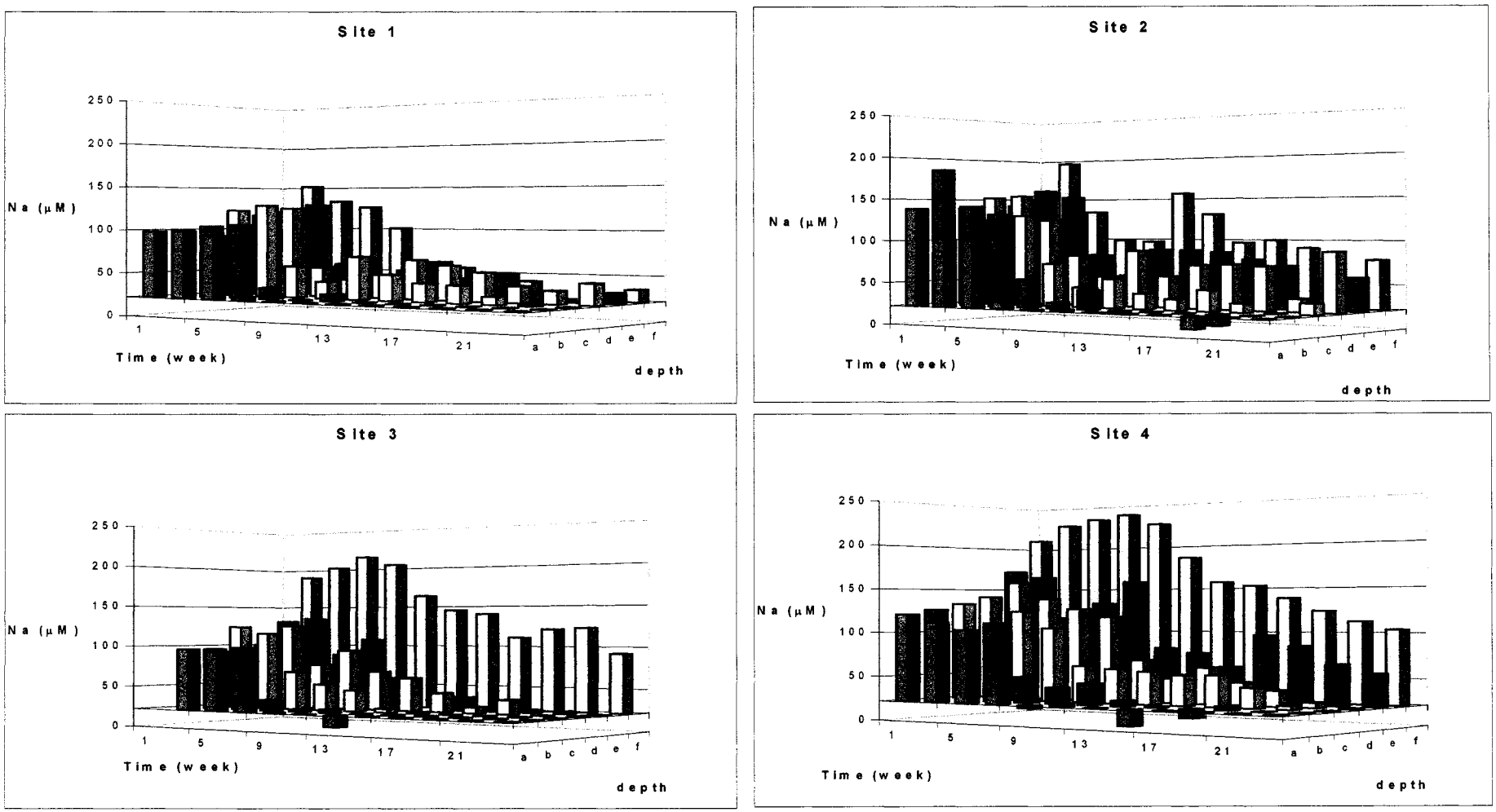


Figure C.5 Sodium concentration in the four BDL sediment cores during the wetting and drying cycles as a function of time. NOTE: The XY floor plane crosses at the detection limit (22 μM).

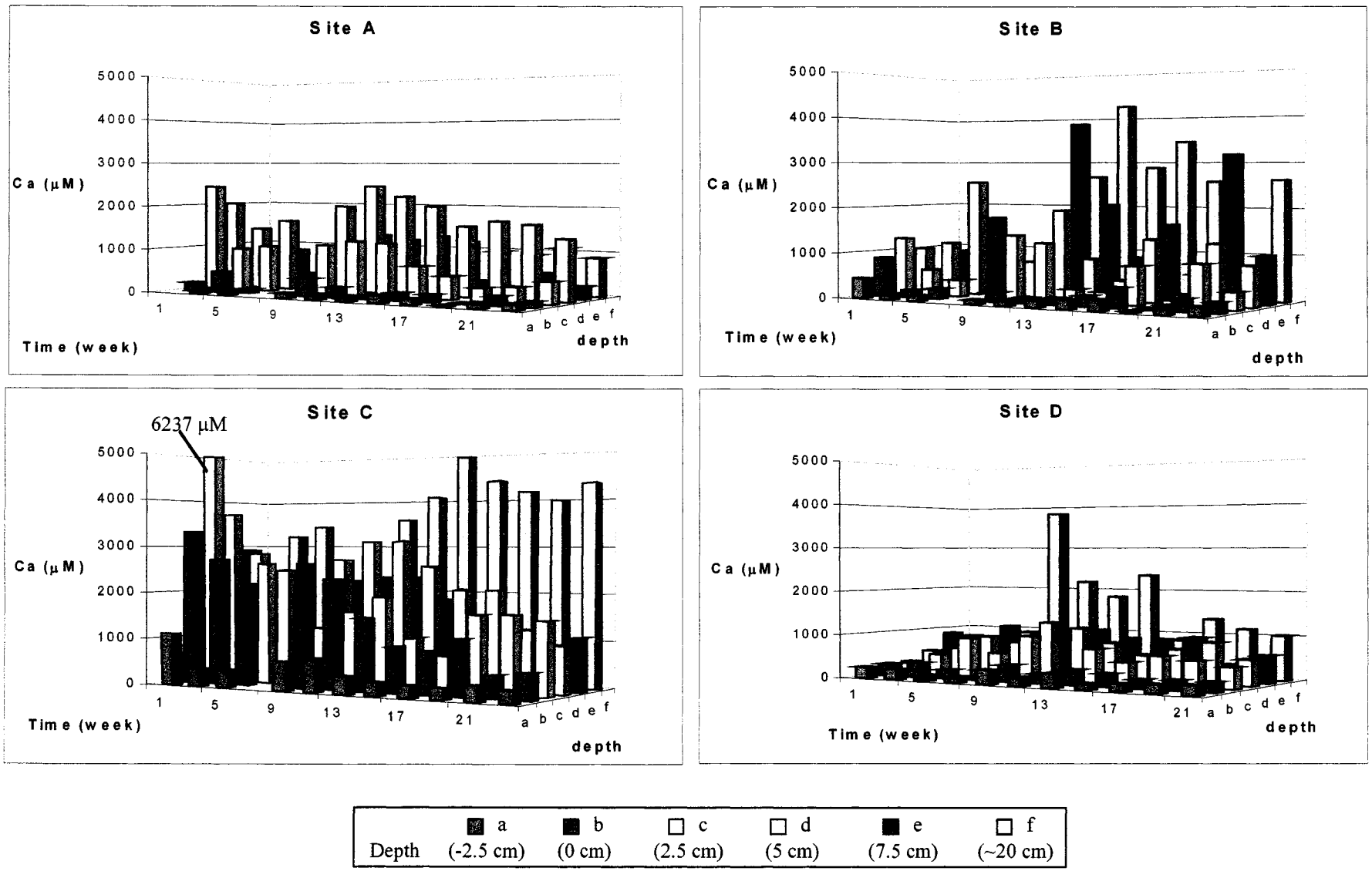


Figure C.6 Calcium concentration in the four SL sediment cores during the wetting and drying cycles as a function of time.

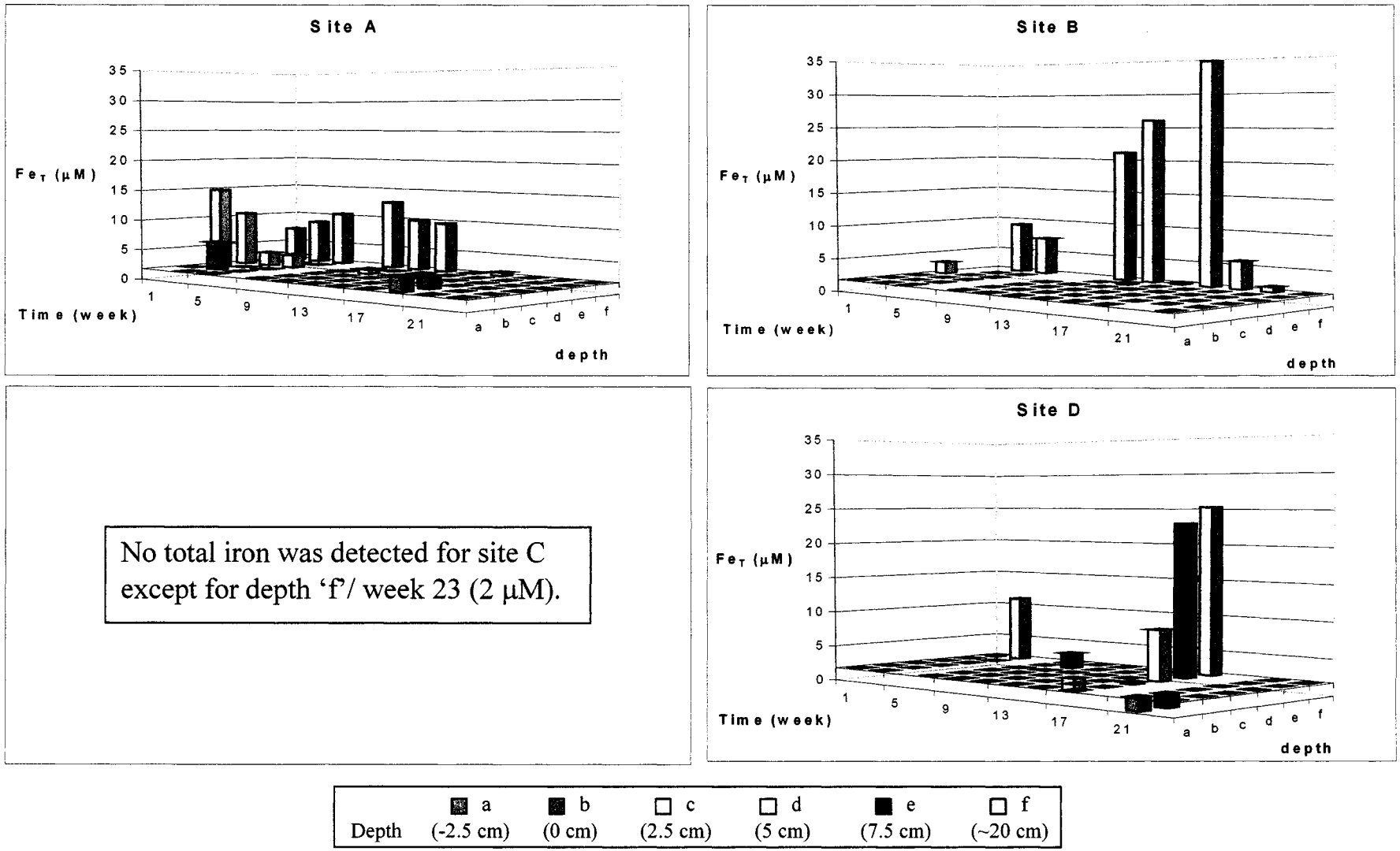


Figure C.7 Total iron concentration in the four SL sediment cores during the wetting and drying cycles as a function of time. NOTE: The XY floor plane crosses at the detection limit (1.8 μM).

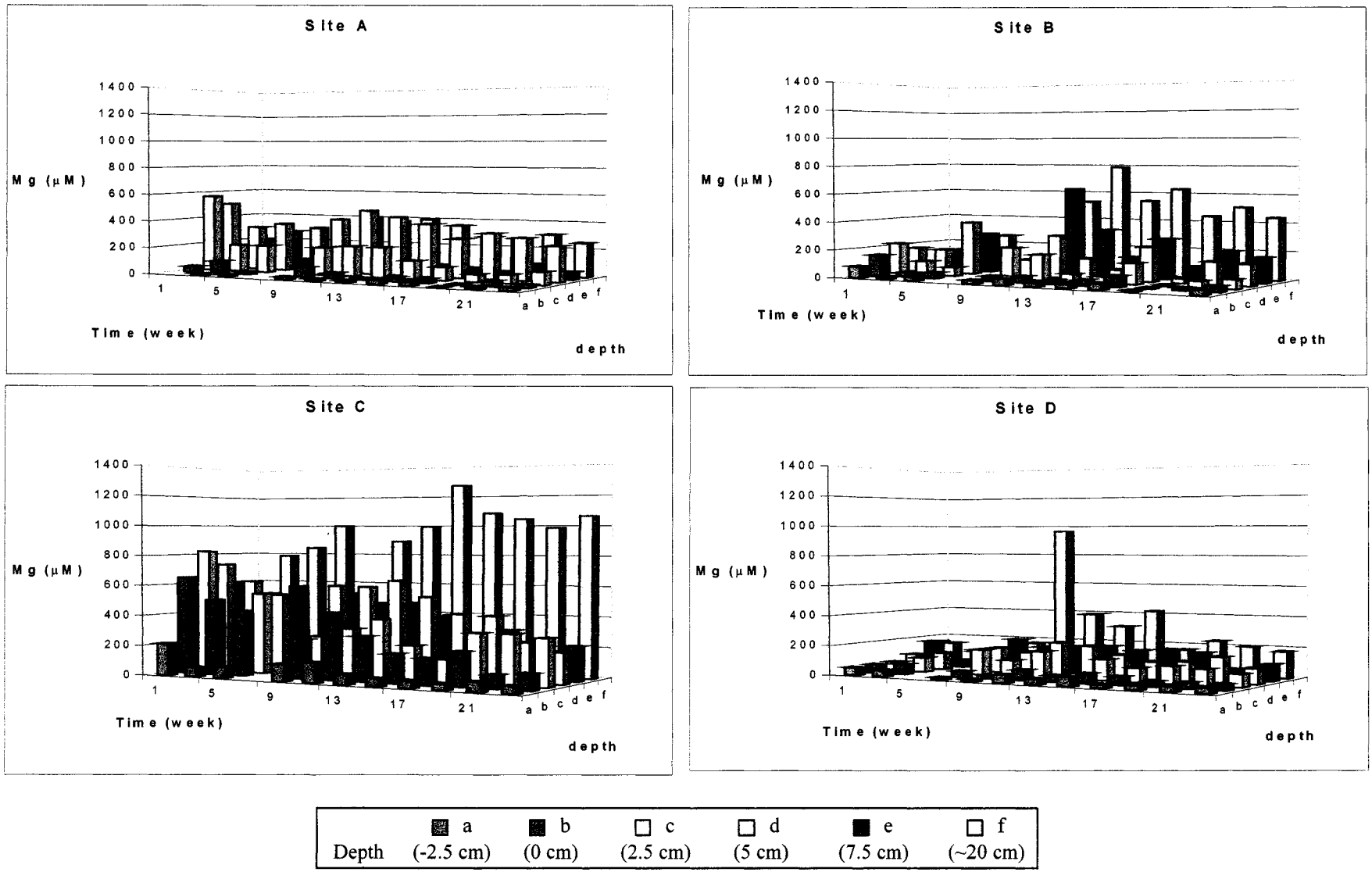


Figure C.8 Magnesium concentration in the four SL sediment cores during the wetting and drying cycles as a function of time.

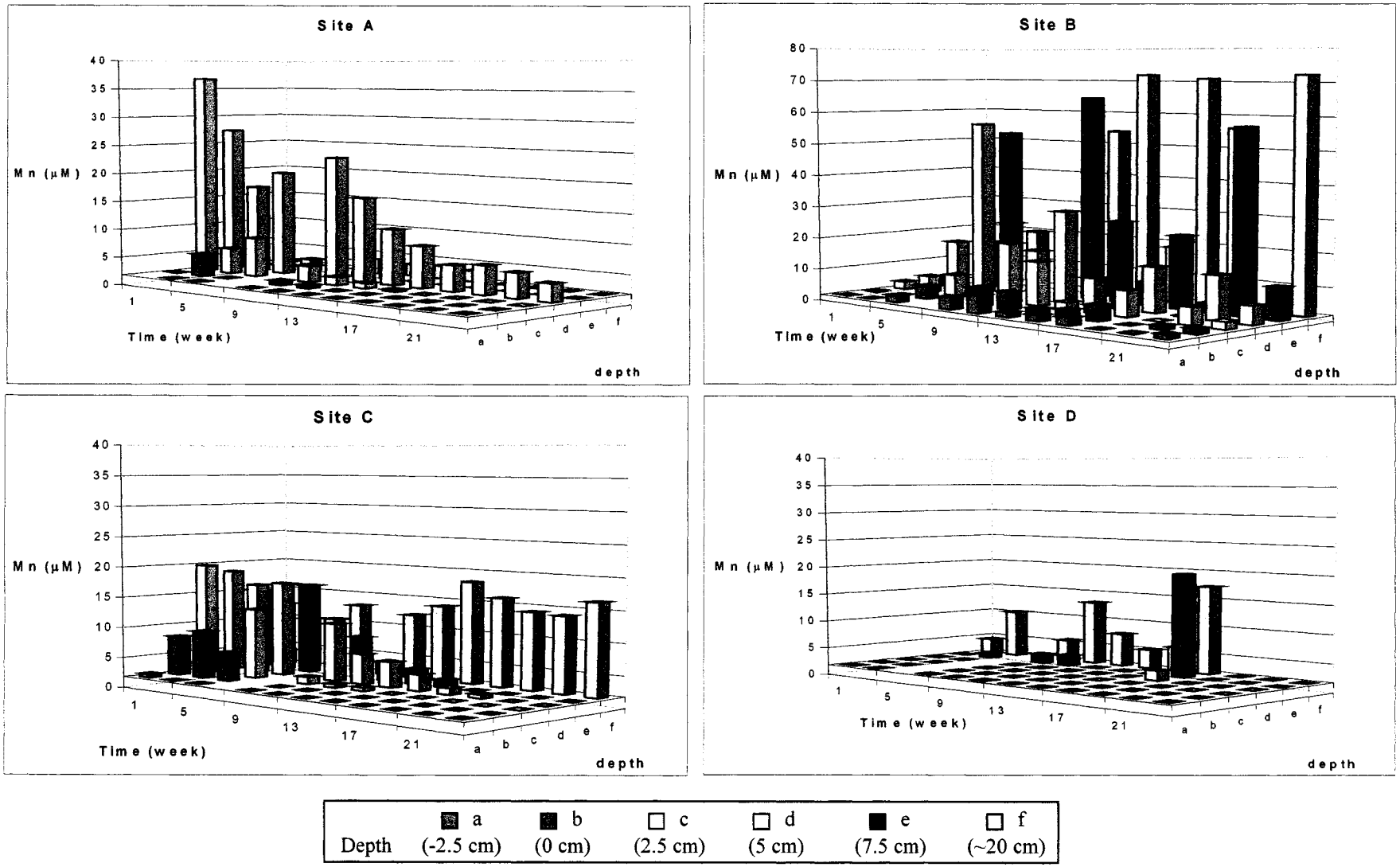


Figure C.9 Manganese concentration in the four SL sediment cores during the wetting and drying cycles as a function of time. NOTE: The XY floor plane crosses at the detection limit (1.8 μM). Site B is twice the scale of the other sites.

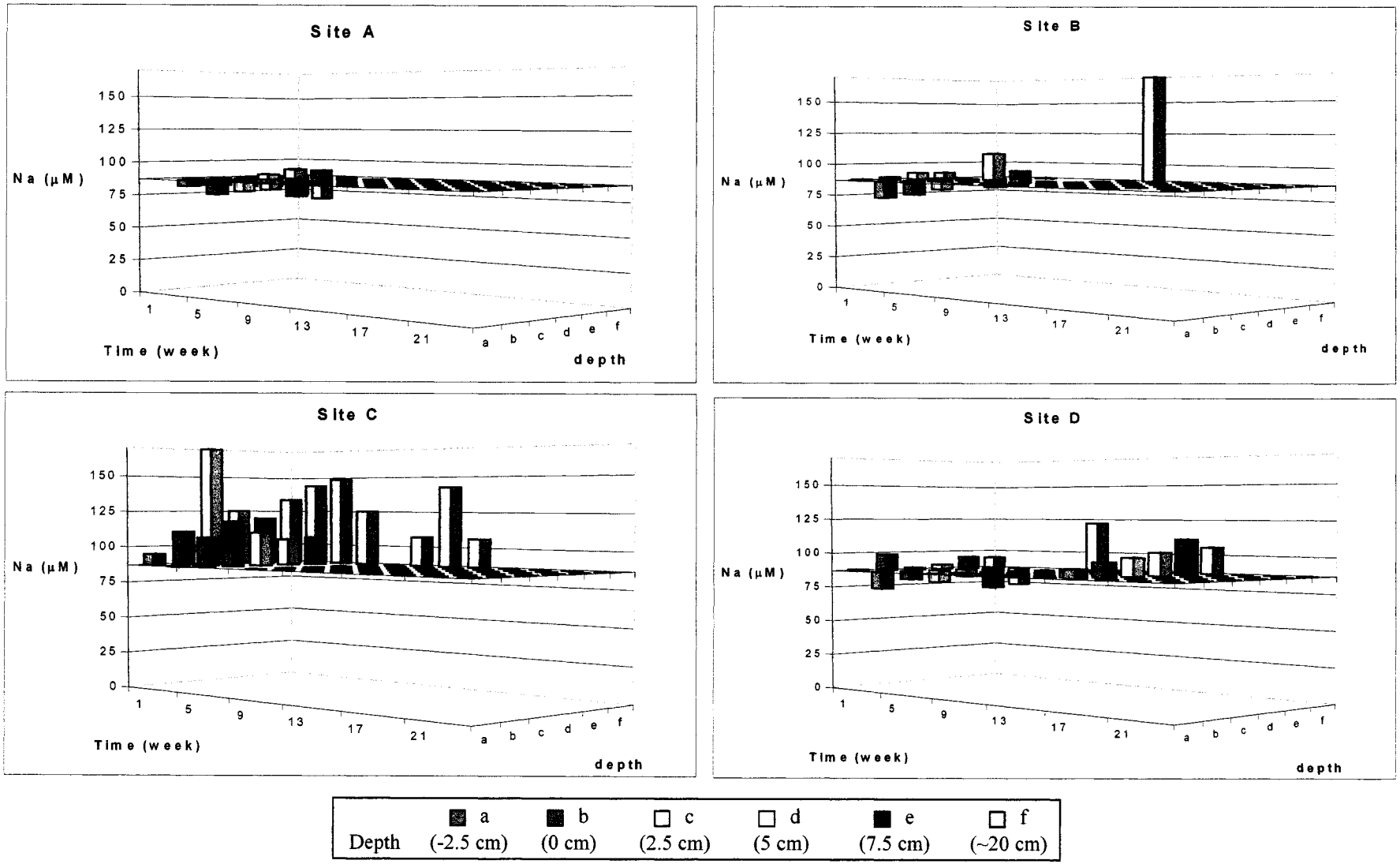


Figure C.10 Sodium concentration in the four SL sediment cores during the wetting and drying cycles as a function of time. NOTE: The XY floor plane crosses at the detection limit (87 μM).