Biogeochemical Zonation in an Athabasca Oil Sands Composite Tailings Deposit Undergoing Reclamation Wetland Construction

Biogeochemical Zonation in an Athabasca Oil Sands Composite Tailings Deposit Undergoing Reclamation Wetland Construction

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Abstract

As oil production increases in Alberta's Athabasca Oil Sands Region (AOSR), optimization of tailings management processes will be integral to the successful reclamation of tailings-based environments. Syncrude Canada Ltd. has established an innovative dry-storage method for their wastes known as composite tailings (CT) that supports mine closure objectives by providing a base for terrestrial reclamation landscapes. Syncrude's Sandhill Reclamation Fen is the first instrumented research wetland of its kind to be developed in the AOSR and it overlays a sand-capped composite tailings deposit in a retired open-pit mine site. This stratified sulfur-rich environment is highly anthropogenically altered and consists of three distinct zones: a constructed wetland, a 10m layer of sand, and 40m of CT. As oil sands tailings systems are becoming globally significant sulfur reservoirs due to their size, sulfur content, and diverse microbial communities, understanding the mechanisms behind H_2S generation in novel tailings structures will help inform our understanding of sulfur-rich environments. This study is the first to characterize the sulfur biogeochemistry in each zone of the Sandhill Reclamation Fen deposit in an effort to establish the potential for microbial sulfur cycling and explore the mechanisms controlling H_2S generation. Porewater $\Sigma H_2S_{(aq)}$ was detected at all depths, increasing with depth from the surface of the wetland (<1.1 μM) and peaking in the sand cap (549 μM). Across all sampling trips, $\Sigma H_2S_{(aq)}$ concentrations were consistently highest in the sand cap, with sampling-associated H_2S gas concentrations in the wells reaching 104-180 ppm. Abundance of dissolved sulfate (0.14-6.97 mM) did not

correlate to the distribution of ΣH_2S, and dissolved organic carbon (21.47-127.72 mg/L) only positively correlated with the observed maxima of ΣH_2S in the sand-cap. Identical sodium and chloride distributions in the sand and CT supported the model of upward migration of CT-derived porewater and fines into the sand cap. Functional metabolic enrichments established the ability of endemic microbial communities from all depths of the deposit to oxidize and reduce sulfur. Experimental microcosms demonstrated 1) the dependence of ΣH_2S generation on the presence of fine particles; 2) stimulation of endemic microbial sulfur reduction through amendment with labile carbon and 3) increased generation of ΣH_2S in the presence of thiosulfate over sulfate. Field and experimental results indicated that the bioaccessibility of recalcitrant organic carbon in the deposit likely controls rates of ΣH_2S generation at depth. While the mechanisms relating CT-derived fines to ΣH_2S in the sand cap are still unconstrained, the sand layer is clearly a bioreactive mixing-zone supporting optimal conditions for ΣH_2S accumulation. These findings inform our understanding of biogeochemical sulfur cycling in novel oil sands reclamation deposits and will advise on-going optimization of tailings-based landscape management practices.

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Introduction

By mass, sulfur is the 6th most abundant element on Earth and is found predominantly in its most oxidized state as sulfate, or its most reduced state in either sulfide or pyrite (Amend et al., 2004). It is highly reactive and found in a number of oxidation states including +6 (sulfate), +2 (thiosulfate), 0 (elemental sulfur) and -2 (sulfide) (Sturman et al, 2008). This redox sensitivity allows sulfur to participate in a number of biogeochemically significant reactions, including cycling through mineral, gas and aqueous phases (Amend et al., 2004). Due to its high reactivity, redox sensitivity and role as a metabolic substrate, sulfur is widely and rapidly cycled in a variety of environments. These reactions are highly influenced by system pH, salinity, temperature, oxygen availability, organic carbon concentrations and redox status.

The study of the marine sulfur cycle has received considerable attention in past decades due to its important ties to global carbon sequestration and the oxygenation of the ancient atmosphere. The microbial reduction of sulfate is the key pathway by which organic matter in the oceans is respired and recycled to CO_2, and the fixation of pyrite is a critical component in oxygenating the Earth's surface. Sulfur oxidation intermediates are largely associated with the formation of pyrite (Rickard & Luther, 2007), and they influence the transport and solubility of a variety of trace metals through sulfur complexes (Thamdrup et al, 1993). These studies have improved the ability to detect sulfur species in both very small concentrations and in their unstable intermediate forms, as analytical challenges related to sulfur quantification have hindered our understanding in a number of environments (Orr & Damste, 1990). Marine sulfur studies have explored

the role that changing organic carbon and oxygen regimes have on sulfur redox cycling by providing insight as to how these processes operate in saline environments.

Recent literature has focused on the role of sulfur in wetland systems, as sulfate loading by atmospheric deposition has increased the sulfur pool in remote wetlands (Sturman et al, 2008). A clear picture of sulfur dynamics in freshwater and saline wetlands has been hindered by analytical complications, so sulfur redox cycling pathways in wetlands are largely unconstrained (Giblin & Weider, 1992). Microbial sulfate reduction has been demonstrated as one of the most important methods of organic carbon mineralization in wetlands systems, and thus has important ties to the global carbon cycle (Berner, 1984). Additionally, sulfate reduction by bacteria is a key driver in metal sulfide precipitation, acting as a control on water quality in both natural and constructed wetlands (Feng & Hsieh, 1998, Webb et al., 1998). Therefore, an understanding of oxidative and reductive processes in wetlands is necessary to evaluate sulfur's contribution to global carbon cycling, as well as the rates of sulfur sequestration or gas release in these systems.

Sulfur cycling has widely been investigated in acidic mining environments where bacteria thrive in low pH environments (Johnson et al., 1993; Johnson & Hallberg, 2003; Baker & Banfield, 2003; Bernier & Warren, 2005; Druschel et al., 2004; Fortin et al., 1995). However, these processes have rarely been addressed in circumneutral saline mining deposits such as those in the Athabasca Oil Sands Region (AOSR). As dry-landscape reclamation proceeds, it is critical to have an understanding of sulfur dynamics in these environments. Comprehension of cycling processes both in high sulfate marine systems and organic-rich wetlands will provide a framework from which to investigate

sulfur cycling in novel oil sands reclamation deposits. As the distribution of sulfur metabolisms in the environment is largely constrained by system pH, oxygen levels, redox status, and substrate availability, consideration of sulfur environments with similar geochemical parameters to oil sands tailings deposits may help predict sulfur cycling processes in AOSR landscapes.

1.1 Aqueous Sulfur Chemistry

Sulfur exists in oxygenated surface waters and soils predominantly as the sulfate anion (SO_4^2). This is the most oxidized state of sulfur, and it is highly soluble in freshwater and saline systems, making it a prime oxidant for anaerobic microbial respiration (Rickard & Luther, 2007). The molecule is highly symmetrical, kinetically slowing the abiological reduction at typical surface temperatures (less than ~150 ° C) (Rickard & Luther, 2007). This kinetic inactivity implies that sulfate is often out of equilibrium with the more reactive reduced sulfur species discussed below.

1.1.1 Hydrogen Sulfide

Sulfide (S-II), the most reduced form of sulfur, is largely present in marine and freshwater systems in the forms of H_2S and HS^-. With a pK_1 (H_2S) of 6.98 ± 0.03 at 25° C, H_2S dominates in acidic systems, and HS^- is dominant under alkaline conditions (Rickard & Luther, 2007). While pK_2 (H_2S) is less well-constrained due to complications with polysulfide contamination, it is estimated to be >18, implying that S^{2-} is not significant in aqueous systems (Schoonen & Barnes, 1988). Issues in the literature concerning S(-II) speciation modeling are apparent, as a number of different pK_2 (H_2S)

values were presented in relatively recent work. These values ranged from 10 to >18, including a 2012 review that cited much older, uncorrected values that did not account for polysulfide artefacts (Jasinska et al., 2012). Despite this, Rickard and Luther (2007) explain that the activity of the S^{2-} ion in equilibrium with the total S(-II) activity is still thermodynamically significant. Their pH-Eh diagram depicting stable phases in the S-H_2O system at 25° C and 1 bar shows that significant HS^- is still present under oxidizing conditions at pH 8, and that SO_4^{2-} is still present under sulfide-dominated conditions. This shows that the oxic-anoxic boundary in sediments is not a true boundary between the sulfate and sulfide dominance fields (Rickard & Luther, 2007; Pourbaix, 1966). Sulfide may persist under oxygenated conditions when it is kinetically stabilized, either by bonding with metals or organic compounds (Luther et al.,1999).

When in the unstabilized forms of $H_2S_{(aq)}$ and $HS^-_{(aq)}$, sulfide is highly reactive, and rapidly abiotically oxidizes under oxygenated conditions. H_2S solubility is inversely coupled to both system salinity and temperature, as more H_2S will exist in solution as salinity and temperature decrease (Douabul & Riley, 1979). H_2S is much less soluble than HS^-, therefore sulfide in the unionized form is linked with the gas phase (Sturman et al, 2008) Changes in pH and pressure can influence partitioning between aqueous and gas phases, as Henry's Law dictates that the concentration of H_2S in solution will decrease with decreasing pressure (Krupp & Suleimenov, 1994). As the pH of a system becomes more acidic, the H_2S will become the more dominant phase, with the potential to off-gas and leave the system.

1.1.2 Sulfur Oxidation Intermediates

Sulfur also exists in a number of intermediate redox forms, and though they make up only a small component of the total sulfur pool they play an important role in the biogeochemical cycling of sulfur. Included in Table 1.1 are some of the various sulfur species that occur in aqueous media, and the most common species are bolded:

Table 1.1: Sulfur species found in aqueous media (Keller-Lehmann et al. 2006)

Sulfur species (Y)	Name	Oxidation state	pK	Oxidation products
$S_xO_6^{2-}$ x >= 3	polythionates	0, V		
$S_2O_8^{2-}$	peroxodisulfate	VII	0, 0.9	SO_4^{2-}
$S_2O_7^{2-}$	disulfate	VI		SO_4^{2-}
SO_4^{2-}	**sulfate**	**VI**	**1.98, -3**	**very stable**
$S_2O_6^{2-}$	dithionate	V		
$S_2O_5^{2-}$	disulfite	IV		SO_3^{2-} SO_4^{2-}
SO_3^{2-}	sulfite	IV	1.89, 7.21	SO_4^{2-}
$S_2O_4^{2-}$	dithionite	III	0.35, 2.45	$S_2O_3^{2-}$,SO_3^{2-} ,SO_4^{2-}
$S_4O_6^{2-}$	tetrathionate	II1/2		SO_4^{2-}
$M_m^{x+}(S_2O_3)_y^{(mx-2y)}$	metal thiosulfate complexes	II		
$S_2O_3^{2-}$	**thiosulfate**	**II**	**0.6, 1.72**	**SO_4^{2-}**
S^o & S_8	**elemental sulfur**	**0**		
$CH_3S_xCH_3$	dimethylpoly-sulfide (DMPS)	0,I		
RSH	sulfhydryl thiols	0		
SCN -	thiocyanate	0	-1.8	
S_x^{2-} x >= 2	polysulfides	0, I-		S^o, $S_2O_3^{2-}$
HS^-	**sulfide, hydrogen sulfide**	**II-**	**6.99, 12.9**	**$S_2O_3^{2-}$, SO_3^{2-},SO_4^{2-}**

Sulfate, sulfides, thiosulfate and elemental sulfur are generally the major components in natural systems, but tetrathionate and polysulfides also play important roles in sulfur cycling despite their low concentrations and the difficulty associated with their quantification. Tetrathionate is a chemical oxidation product of H_2S, FeS and FeS_2,

forming during the aerobic microbial oxidation of sulfide, elemental sulfur, or thiosulfate (Kelly, 1989; Sorokin, 1996). It can also form during abiotic thiosulfate oxidation with manganese oxides (Schippers & Jørgensen, 2001). While it is relatively well documented in laboratory experiments, tetrathionate has rarely been quantified in marine and wetland environments.

Polysulfides, chains of sulfur atoms, are of critical importance concerning the formation of pyrite, which is an iron(II) disulfide and the most abundant terrestrial iron sulfide mineral (Rickard and Luther, 2007). One of the greatest problems facing the modeling of S-cycling is the quantification of polysulfides: both the short chain (S_n(-II) where $n < 4$) and longer chain ($n \geq 4$) polysulfides have yet to be isolated individually in aqueous solution (Rickard & Luther, 2007). Therefore the role of polysulfides in aquatic systems is very poorly understood and is largely based on modeling assumptions.

Elemental sulfur is part of the solid fraction as it is insoluble in water (can be referred to as a Lewis acid), therefore it is much less reactive than other intermediates and accumulates readily in the sediment (Keller-Lehmann et al. 2006). Elemental sulfur is only produced during oxidative-S pathways. It is the main product of sulfide oxidation by manganese and iron oxides (Yao & Millero, 1996). Produced during both oxic and anoxic FeS oxidation, it can also be formed by microorganisms during bacterial disproportionation of thiosulfate and sulfide (Schippers & Jørgensen, 2001; Kelly, 1989). On average, elemental sulfur concentrations are greater under conditions of increased sulfate reduction rates, and its accumulation in sediments varies seasonally according to the balance of S^0-consuming and S^0-producing reactions (Zopfi et al., 2004; Moeslund et al.,1994). The interplay between sulfide and polysulfides generates a highly reactive

$H_2S/S^0/H_2S_x$ framework that is a dominant control on Eh in reducing sedimentary environments (Orr & Damste,1990).

A number of different biogenic organic sulfur compounds also exist in the environment. These may be in equilibrium with either the aqueous phase but have the ability to partition into the gas phase (Sturman et al, 2008). Microbial sulfur cycling or interactions with plant roots and litterfall can produce organic sulfur compounds. In many wetland and forest soils it is considered that 93% of the sulfur pool consists of organic sulfur, 41% of which is carbon-bonded (Inglett, 2008).

1.2 Sulfur Reduction

Sulfur reducing bacteria gain energy by coupling the reduction of sulfur compounds to the oxidation of organic substrates. These bacteria can be classified as either incomplete oxidizers (use volatile fatty acids to produce acetate) or complete oxidizers (use fatty acids to produce carbon dioxide) (Sturman et al, 2008, Widdel, 1988). Assimilatory sulfur reduction is performed by bacteria, fungi, algae and many plant species, whereas dissimilatory reduction is dominated by heterotrophic bacteria (Inglett, 2008).

Dissimilatory sulfur reduction is considered to be one of the most ancient metabolisms from early Earth, and as such there is a wide variety of organisms that are capable of reducing sulfur (Sturman et al, 2008). It is responsible for over half of the total organic carbon mineralization in a variety of environments, particularly in marine systems (Jorgensen, 1982). The rate of microbial sulfur reduction depends on the concentration of accessible sulfate, the quality and quantity of organic carbon,

temperature, and pH (Berner, 1984). The anaerobic zone that is most favourable for sulfate reduction occurs when the system Eh is between -200 to -100 mV, which is highly reducing (Inglett, 2008). Sulfur reducing activity consumes protons, and has been demonstrated to increase the pH of wetland systems (Sturman et al, 2008).

1.3 Sulfur Oxidation

Sulfur oxidation intermediates are primarily produced through the oxidative pathways of the marine sulfur cycle. While bacterial sulfate reduction is one of the most important processes for the decomposition of organic matter in the marine system, only a fraction of the reduced sulfur (5-20%) remains buried in the sediment (Jørgensen, 1983). This means that the vast majority of reduced sulfur avoids burial though re-oxidization to sulfate, emphasizing the importance of understanding the various sulfur oxidation pathways. Oxic sulfide oxidation involves the reaction of diatomic oxygen and reduced sulfur species. In order for this to occur in wetlands, S(-II) must move up into the oxic zone or the oxygenated water column. This suggests that the metal oxide layer that would normally trap H_2S as it diffuses upwards is exhausted or oxygenated water was somehow pumped downwards into the sulfidic sediment (Jørgensen & Kasten, 2006). This reaction can be facilitated by aerobic lithotrophic bacteria, which utilize different adaptive measures to out-compete the rapid abiotic reaction. Some of these adaptations include high enzyme affinities for oxygen and sulfide, allowing these reactants to be claimed by the cell before they react in the environment (Jørgensen, 1987). The ability to move throughout the sediment is highly beneficial to these bacteria, as they can orient themselves at the oxic/anoxic boundary where diffusion provides both reactants in small

concentrations. These bacteria are primarily chemolithotrophic, but those exposed to sunlight may facilitate the oxidation by using phototrophic mechanisms (Sturman et al, 2008). Aerobic chemolithotrophic sulfur oxidizers are largely linked to acidic environments, but some are able to thrive in neutral pH systems. They often occupy microaerophillic zones, where they can grown under competing gradients of oxygen and H_2S (Jorgensen, 1977).

The overall reaction for aerobic H_2S oxidation can be written as follows (eqn 1):

$$(1)\ HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$$

The progression of this reaction is not as straightforward in the environment as it is idealized here. This reaction proceeds through a number of intermediate steps, but the exact mechanism for this reaction is still unconstrained (Zhang & Millero, 1993). The first step in this reaction is often the formation of sulfite (2). Sulfite then rapidly reacts with oxygen and further oxidizes (3), or it can form thiosulfate through reaction with HS^- (4) (Zopfi et al.,2004).

$$(2)\ HS^- + 1.5\ O_2 \rightarrow HSO_3^-$$

$$(3)\ SO_3^{2-} + 0.5\ O_2 \rightarrow SO_4^{2-}$$

$$(4)\ HS^- + SO_3^{2-} + 0.5\ O_2 \rightarrow S_2O_3^{2-} + OH^-$$

Jørgensen (1990) suggests that tetrathionate can also be produced via the incomplete oxidation of thiosulfate to sulfate, and this is thermodynamically favourable with a variety of other electron acceptors such as iron and manganese oxides. Equation 1 can also form elemental sulfur when proceeding in the presence of trace metals (5), which can then further react with sulfite (6) and sulfide (7) (Zhang & Millero, 1993). The

polysulfides produced will then rapidly form either thiosulfate or elemental sulfur, as they are highly unstable in an oxygenated environment:

$$(5)\ \ 2HS^- + O_2 \rightarrow 2S^0 + 2OH^-$$

$$(6)\ S^0 + SO_3^{2-} \rightarrow S_2O_3^{2-}$$

$$(7)\ (n-1)S^0 + HS^- \rightarrow HS_n^-$$

Anoxic sulfide oxidation also involves intermediate reaction steps, and other oxidants such dissolved iron and manganese are often involved in the suboxic zone. The ratios between MnO_2 and H_2S can have a large effect on which sulfur intermediate is the dominant product, as well as the extent to which solid phases such as pyrite can be oxidized (Schippers & Jørgensen, 2001). Iron does not easily facilitate the full oxidation of sulfide to sulfate, so elemental sulfur, thiosulfate and sulfite may also be produced (Peiffer et al., 1992).

1.4 Sulfur Disproportionation

Sulfur disproportionation is a form of inorganic fermentation facilitated by anaerobic bacteria, where the reactant is both oxidized and reduced. In reaction (8), sulfite is both oxidized to sulfate and reduced to sulfide:

$$(8)\ 4\ SO_3^{2-} + H^+ \rightarrow 3SO_4^{2-} + HS^-$$

Bak and Pfennig (1987) introduced the concept of microbial disproportionation during a study of thiosulfate metabolism in sulfate-reducing bacteria. The energy exchange associated with the internal electron transfer during thiosulfate disproportionation may support both heterotrophic and autotrophic anaerobes. Some bacteria are specialized to perform disproportionation reactions, but typical sulfate

reducers such as *Desulfovibrio desulfodismutans* are also capable of performing the reaction (Bak & Pfennig, 1987). Disproportionation reactions do not result in a net oxidation of sulfur, but the reactions act as a 'shunt' in the sulfur cycle that permits H_2S to be reoxidized to sulfur intermediates by metal oxides (Jørgensen & Nelson, 2004). Disproportionation reactions involving H_2S and manganese oxides may facilitate complete oxidation to sulfate: repeated cycling of elemental sulfur disproportionation and the removal of H_2S through a sulfide sink allows ¼ of the sulfur to completely oxidize on each cycle (Thamdrup et al, 1993). The formation of intermediate oxidation products is a key driver of the sulfur cycle, as they allow further transformation of products and mediate electron flow.

1.5 Iron and Sulfur Interactions

Iron sulfide minerals also play a critical role in sulfur cycling as pyrite is the reaction end-member by which sulfur is removed from the system through burial. Aqueous FeS clusters (FeS_{aq}) are important for their ability to transport Fe(II) in S(-II)-rich environments, either up into the oxidized zone, or down to sites of pyrite formation (Raiswell et al, 1993). The concentration of FeS_{aq} in sediments has a linear correlation with pyrite formation in estuarine sediments, and in combination with various polysulfides is likely a key driver in pyrite formation throughout marine deposits (Rickard et al.,1999). To date, the actual solubility of FeS at solution pH above 6 is not known, as failure to achieve reproducible results within the marine range of pH 6-8 is a current obstacle (Rickard & Morse, 2005). This means that the process controlling FeS solubility has not been constrained, and our current understanding of how sulfur

oxidation intermediates are involved with FeS solubility and precipitation is lacking. However, the formation of metastable FeS is kinetically very rapid, which indicates a threshold for how much Fe(II) and S(-II) can coexist in sedimentary porewaters (Rickard & Morse, 2005). Pyrite is more oxidized than iron (II) monosulfide, and it is more stable under higher oxidation potentials than FeS. Pyrite can be formed directly when Fe(II) reacts with S(-II), or as a result of S(0) and S(-II) reacting to produce polysulfides (Rickard & Morse, 2005). Burial of pyrite is an important process that decouples sedimentary iron sulfides from the more reactive sulfur pool, which has implications for both the rates of organic carbon decomposition and sulfide reoxidation, as well as the ability of $H_2S_{(g)}$ to evacuate the system.

1.6 Sulfur Cycling in the Environment

1.6.1 Marine Sulfur Cycling – A Stratified Redox Environment

In marine sediments, sulfur can predominantly be found as sulfate, sulfide, or pyrite (Zopfi et al, 2004). While the total rate of ocean sedimentary sulfate reduction is 3 x 10^{14} g of sulfate per year, only 5-20% of the resulting sulfides are sequestered in sediment and buried as pyrite (Jorgensen, 1982; Ferdelman et al, 1999). The remaining reduced sulfur participates in disproportionation reactions to produce a number of intermediate oxidation compounds that exist at small concentrations (D'Hondt, et al., 2002). Despite their extremely low concentrations relative to sulfate, these intermediates are critical components of the global biogeochemical sulfur cycle. The sulfite, thiosulfate, and tetrathionate concentrations are sub-micromolar, and elemental sulfur is the most abundant intermediate due to its high stability and low solubility (Zopfi et al., 2004).

Sulfur primarily enters the modern ocean through fluvial inputs, with minor contributions from atmospheric transport, volcanic emissions, and biologically generated H_2S gas (Fütterer, 2006). The availability of both organic carbon and oxygen is a key driver in the sulfur cycle, so coastal and deep-sea sediments exhibit different sedimentary profiles when undergoing early diagenesis.

Oxygen is the most thermodynamically favourable oxidant, and is thereby rapidly consumed in marine sediments. In waters where extensive degradation of organic matter occurs in the water column, such as very active phytoplankton communities where nutrient supply is high, oxygen is quickly depleted in the overlying sediment. The suboxic zone, defined as a region in which both oxygen and sulfide are present at very low concentrations, exhibits no strong gradient where the two species overlap. The thickness of the suboxic zone can range between a scale of millimeters to a few meters, particularly in anoxic basins such as the Black Sea (Murray et al, 1989). Beyond the oxic zone, nitrate, manganese oxides, iron oxides and sulfate are utilized as electron acceptors. Reducing conditions are maintained below the oxic zone as a result of bacterial heterotrophy. While nitrate, manganese oxides and iron oxides are more energetically favourable for use as oxidants, they are often in limited supply, rendering sulfate to be of much greater geochemical importance in the oceans. Sulfate is present in seawater at mean concentrations of 29 mmol/L, making it the second most abundant anion after chloride (Vairavamurthy et al. 1995). Due to this high availability of sulfate as an oxidant, it is the dominant electron acceptor in the marine oxidation of organic material (Henrichs & Reeburgh, 1987). While the redox ladder is an idealized vision of the chemical stratification in ocean sediments it can be altered by physical forcing.

Bioturbation enhances mixing between the oxic and anoxic zones by creating channels that facilitate exchange of reduced and oxidized species throughout the sediment (Aller, 1994). This creates a dynamic environment in which complex redox reactions can occur.

1.6.2 Sulfur in Natural Wetlands

Global wetland area is roughly estimated as 5.8×10^8 ha, and carbon storage in peatlands is estimated as twice the carbon storage of the world's forests (Giblin & Weider, 1992, Parish et al, 2008). Sulfur reduction is a ubiquitous process in wetlands as the waterlogged soil environment is often highly reducing. Despite their prominent role in wetland function and carbon cycling, microscale sulfur cycling processes in freshwater wetlands are still poorly constrained (Whitmire & Hamilton, 2005.) This is attributed to the diversity in nutrient source and plant composition of wetland environments, different solution chemistry between saline and freshwater systems, as well as the challenges of quantifying sulfur metabolic pathways beyond the redox end-members of sulfate and sulfide. Constraining the sulfur cycling processes in freshwater wetlands is of critical importance, as it can often be the most dominant pathway of organic carbon mineralization.

The dissolved sulfate concentration in wetlands exists in a wide range, with 0.48 mg/L sulfate in remote freshwater bogs (Gorham & Detenbeck, 1986) to much higher concentrations of 1.44 g/L in the soils of some salt marshes (Luther & Church, 1988). The average sulfate concentration of freshwater systems, estimated by Feng and Hsieh (1998) to be between 50-450 μM, is often deemed to be the rate-limiting factor in sulfate reduction, despite rapid redox cycling. The total sulfur content in soils also ranges

widely, from 0.2 to 16% of the dry weight, with much lower sulfur contents in freshwater wetlands than in the saline counterparts (Lowe & Bustin, 1986; Giblin & Weider, 1992). This sulfur is largely present as sulfate, organic sulfur, or reduced inorganic minerals such as pyrite, elemental sulfur, and a variety of iron monosulfides. It has been determined that gaseous sulfur and dissolved reduced sulfur contribute relatively little to most total sulfur wetland budgets (Wieder, 1985).

In the lighted surface waters of flooded wetlands, photosynthetic sulfur oxidizers may exist in microaerophillic or anaerobic niches on the surface sediment. While they often thrive on a gradient of H_2S as it diffuses upwards from anoxic layers below, many sulfur bacteria can generate and store elemental sulfur internally or extracellularly, where it is later used under conditions when sulfide is scarce (Milucka et al., 2013). Many chemolithotrophic sulfur oxidizers are found in association with the roots of wetland plants, where a microaerophillic microzone is maintained by oxygenated root exudates (Joshi & Hollis, 1977).

The majority of sulfur reduction in wetlands occurs in the anoxic sediments, driven by the degradation of organic material (Fortin et al., 2000). As H_2S is generated at depth, it precipitates with dissolved metals such as iron to form a number of metal sulfides. In wetlands with either low concentrations of dissolved metals or very high sulfate reduction rates, H_2S generation may exceed sequestration rates and diffuse higher into the sediment or water column (Sturman et al, 2008). This sulfide can either be reoxidized to sulfate (via bacterial oxidation or reaction with oxygen, ferric iron, manganese dioxide or nitrate) or to a variety of SOI by sulfur oxidizing bacteria. If the H_2S escapes oxidation, it has the potential to off-gas to the atmosphere.

Sulfur gas fluxes from wetlands are still poorly constrained, as a number of sulfur compounds have the potential to flow from wetlands. These include hydrogen sulfide, methylmercaptan, dimethyl sulfide (DMS), dimethyl disulfide, carbonyl sulfide and carbon disulfide (Giblin & Weider, 1992). H_2S and DMS are the dominant gaseous compounds produced by wetlands, but estimates of their emissions range over two orders of magnitude, with no concrete explanation for the variability (Giblin & Weider, 1992).

1.6.3 Sulfur in Constructed Wetlands

Constructed wetlands have become an important tool for managing wastewater from domestic, mining, and industrial effluent since the 1980's. In many influent streams, sulfur is present in highly reactive forms and participates readily in both abiotic and biotic redox transformations, playing an important role in the water chemistry along the flow path. Sulfur cycling in constructed wetlands differs from traditional wetlands in that sulfate concentrations are much higher as they enter the system though anthropogenic loading (Fortin, et al., 2000). These systems are often very shallow, and may differ in structure between surface flow and subsurface flow depending on the desired wastewater treatment.

The ability of a constructed wetland to sequester metals as sulfides is integral to its success at treating mining influent, and sulfur reducing bacteria are critical to this process. Metal removal from influent has been largely attributed to the adsorption and precipitation of metal sulfides via microbial sulfate reduction, thus immobilizing potentially toxic metals in metal-rich mining wastewater (Feng & Hsieh, 1998). The formation of iron sulfides in constructed wetlands is of particular importance because the

precipitates often inhibit flow through the sediment (Fortin et al., 2000). Pyrite and elemental sulfur precipitation are often linked to clogging in horizontal flow systems, so maintaining permeability in constructed wetlands while keeping metals from re-entering solution is an ongoing engineering challenge (Sturman et al, 2008). Clogging can also result from biofilm development and the transport of fine sediments through the soil matrix resulting in entrapment (Langergraber et al, 2003). The success of sulfur reduction in constructed wetlands is also closely coupled to the temperature, season, plant species. In an experiment by Stein et al (2007), planted constructed wetlands demonstrated higher redox potentials and lower sulfate reduction rates despite identical organic carbon concentrations, thus suggesting that oxygen exuded from roots is inhibitory for bacterial sulfate reduction. When the organic carbon concentration was increased, sulfur reduction increased across all treatments in all seasons. Therefore, further investigation into the pathways and requirements of bacterial sulfur cycling will aid industry in the successful employment of constructed wetlands.

1.7 Sulfur in Alberta's Athabasca Oil Sands

The Athabasca Oil Sands region of northern Alberta, Canada, holds one of the world's largest oil reserves. The deposit spans over 75,000 km^2 and is estimated to contain between 1.7-2.5 trillion barrels of bitumen (Ramos-Padron et al, 2010). With 20% of the oil reserves accessible within 75m of the surface, the resource is surface-mined (Ramos-Padron et al, 2010).

Sulfur emissions to the atmosphere have been a concern in the Athabasca oil sands region for over 30 years of mining development, as sulfur is released to the

atmosphere during open-pit mining activities and bitumen upgrading (Shewchuk, 1982; Sandhu & Blower, 1986). These emissions are largely in the form of SO_2 but become oxidized in the atmosphere to SO_4 (NPRI, 2011; Newman et al, 1991). It is difficult to constrain proportion of S-emissions contributed by mining vehicle exhaust, but SO_2 emitted from AOSR stacks are were estimated at 100,908t in 2010 (NPRI, 2011). Bulk and throughfall SO_4 deposition rates with ~29km of the industrial region are higher than surrounding forested regions, indicating that mining activities are contributing to local sulfur budgets (Proemse et al., 2012).

Recently, more focus has shifted to sulfur emissions from tailings containments, as sulfur-reducing bacteria have demonstrated the ability to produce H_2S from SO_4^{2-} in the waste slurry (Ramos-Padron et al, 2011). While this H_2S is rapidly oxidized upon contact with atmospheric O_2, the growing scale of tailings landscapes indicates that S-fluxes from these systems may contribute in unknown ways to the atmospheric sulfur budget. Recent $\delta^{34}S$ comparisons of sulfur emissions from tailings ponds have demonstrated that tailings pond-derived SO_4 is depleted in ^{34}S (Proemse et al., 2012). This isotopic signature was detected in atmospherically deposited sulfur near the oil sands properties, suggesting that sulfur emissions from tailings storage contribute to local sulfur deposition (Proemse et al., 2012). Understanding sulfur fluxes from tailings materials will be critical in determining how mining activities in the AOSR contribute to acid-forming sulfur emissions.

1.7.1 Tailings Management

As the largest producer of synthetic oil in the Athabasca oil sands, Syncrude Canada Ltd. produces approximately 90 million barrels of oil per day (Syncrude Canada Ltd, 2010). The oil sand extraction process employs a hot water separation protocol to separate the bitumen from the sands, which requires $3m^3$ of water and generates $4m^3$ of slurry waste for every cubic meter of processed sand (Salloum et al., 2002). The waste slurry produced in the extraction process contains residual bitumen (0.5-5% by mass), sand, fine particles of silt and clay (<0.44 um), naptha diluent (<1% by mass) and a large volume of process water (Chalaturnyk et al., 2002). This slurry is pumped to containment ponds, and once the sand has settled out during gravity densification, a thick slurry forms known as fluid fine tailings (Penner & Foght, 2010).

In order to comply with Alberta's zero-discharge policy, all process-affected water and tailings material must be stored on site (Holowenko, 2000). Syncrude currently manages of an excess of 300 million m^3 fine tailings in containment ponds (Holowenko, 2000). Due to the slow consolidation rates of fluid fine tailings in these storage ponds, densification of the tailings could span 125-150 years (Eckert et al, 1996). Projections indicate that only utilizing fluid-containment tailings strategies in the Athabasca region would require one billion cubic meters of storage by 2030, underscoring the importance of developing creative and effective waste management options. Recent engineering efforts have focused on removing water from the tailings waste to reduce the storage footprint required by wet reclamation strategies (which involve the containment of fluid tailings materials in storage ponds), thus allowing the recycled water to be reused in the extraction process. The development of dry reclamation landscapes has helped support

mine closure objectives of increased reclaimed land, as these deposits are trafficable and can support terrestrial vegetation (MacKinnon et al, 2001)

1.7.2 Microbial Sulfur Cycling in Oil Sands Tailings Ponds

While in situ microbial activity in oil sands tailings environments is still poorly understood, these systems appear to be microbially rich in a variety of metabolisms, including sulfur oxidation and reduction, iron reduction, fermentation, and methanogenesis (Fedorak et al., 2003; Ramos-Padron et al., 2011; Harner, et al., 2011). Mildred Lake Settling Basin, Syncrude's oldest and largest tailings pond, contains approximately > 200 million m^3 of fluid fine tailings, with most probable number estimations of 10^3 anaerobic heterotrophs and 10^4 sulfate-reducing prokaryotes per mL of tailings (Foght et al., 1985). In zones of intense sulfur cycling, SRB cell counts can reach 10^5-10^7 cells/mL of tailings, with sulfur reduction rates of ~90 nmol mL^{-1} day^{-1} (Stasik et al., 2014). Microbially-mediated methanogenesis has been the primary focus of microbial investigations in an effort to understand methane fluxes from tailings ponds (Penner & Foght, 2010; Siddique et al., 2012). In these systems, methanogens and sulfur reducers were shown to compete for low molecular weight hydrocarbons as the preferred carbon source (likely derived from naptha extraction diluent), with sulfur reduction inhibiting methane generation in gypsum-amended tailings (Ramos-Padron, 2011).

1.7. 3 Composite Tailings

Since 2000, Syncrude has explored the use of composite tailings (CT) at a commercial scale to reduce the space required to contain the growing fluid fine tailings inventory (Matthews et al., 2002). CT deposits are placed in previously mined-out areas of the mine property, providing a base for dry landscape reclamation activities. Syncrude projected in 2000 that two thirds of CT deposits will be capped with tailings sand for use in dry reclamation efforts, and one third will be left uncapped for amendment with peat and wetland vegetation (Matthews et al., 2002).

When creating CT, post-processed oil sand and a chemical coagulant are added to the slurry of fine tailings, clay minerals, residual bitumen and extraction solvent to facilitate water release from the solids and to prevent the segregation of fine and coarse-grained tailings materials (Matthews et al., 2002). While many different coagulants were investigated for usage in commercial scale CT creation, including lime, acid-lime, sodium aluminate, sulfuric acid, alum, lime-CO_2, and organic polymers, gypsum ($Ca_2SO_4 \bullet H_2O$) was deemed most appropriate for use due to the ease of handling, geotechnical performance, and quality of release water (Matthews et al., 2002). Gypsum is also readily available as a waste product from the fertilizer industry and flue-gas desulphurization, making it an easily accessible and affordable option for large-scale commercial use (MacKinnon et al, 2001). To obtain optimal functionality of the CT mixture, approximately one kg of gypsum is added for every m^3 of tailings slurry (Matthews et al., 2002). As gypsum is added to the CT mixture, the dissolved calcium participates in three different processes within the porewater: one third of the calcium

exchanges with sodium on the clay particle surfaces, one third precipitates as calcite and lowers system alkalinity, and the remaining third is found in solution as the Ca^{2+} ion (MacKinnon et al, 2001).

The quality of CT release water is strongly influenced by the choice in coagulant, and different additives can influence the salinity, pH, toxicity and permeability of the porewater. These coagulants act to increase the ionic strength of the fluid suspension and decrease the surface potential of suspended clays, which reduces the repulsive force between fine particles and allows them to aggregate. As these clay aggregates grow in size, they drop out of suspension and become entrained in the sand matrix of the CT mixture (MacKinnon et al, 2001). Water is then rapidly released from the deposited material and is available for reuse in the bitumen extraction process (Matthews et al, 2002). Dewatering of the CT happens rapidly over days to weeks, and the deposit can quickly reach 70% solids (Salloum et al., 2002). Slower long-term water release and densification will result in a 40% volume decrease of the deposited materials relative to the initial CT mixture (MacKinnon et al, 2001).

As CT is a mixture of FFT, fresh extraction tailings, and the chosen coagulant (gypsum), the quality and character of CT-release water is influenced by a number of waste management streams. The added FFT typically contributes half of the CT pore water, as well as most of the fine particulates (MacKinnon et al, 2001). This porewater chemistry will also be altered post-deposition by atmospheric exchange, clay surface interactions, dilution from groundwater and precipitation, and biological processes. In a study comparing the CT-release water quality of CT formed with different coagulants, most of the salinity increase in gypsum-treated CT was caused by the loading of

approximately 1000mg/L of sulfate to the CT release waters per CT treatment cycle. While MacKinnon et al concluded that sulfate was 'relatively conservative' in their CT system, preliminary unpublished work by Stephenson (2012) and Kendra (2013) has established the presence of sulfate-reducing bacteria in Syncrude's CT deposits. Microbial sulfur cycling has also been documented in tailings containment ponds, suggesting that sulfate is likely actively metabolized in CT systems (Penner & Foght, 2010; Ramos-Padron et al., 2011, Fedorak et al., 2003; Salloum et al., 2002; Stasik et al., 2014).

2.0 Research Scope

Syncrude Canada Ltd. has established an innovative dry-storage method for their wastes that facilitates dewatering while simultaneously reclaiming disturbed regions of the mine property. These composite tailings deposits represent a significant portion of Syncrude's fine tailings inventory, and support mine closure objectives by providing a base for terrestrial reclamation landscapes. With oil production in northern Alberta predicted to increase to 400 million barrels per year, tailings-based environments are increasing and will form a significant portion of the AOSR landscape (Holowenko et al., 2000). As mining efforts expand, oil sands tailings systems are becoming globally significant sulfur reservoirs due to their size, sulfur content, and diverse microbial communities. Active sulfur reduction has been observed in tailings ponds at rates ~90 mL^{-1} day^{-1}, and sulfate has become the dominant electron acceptor in these systems (Stasik et al., 2014). With recent evidence demonstrating that sulfur emissions from tailings storage contribute to local sulfur deposition, the increased prevalence of tailings

landscapes may influence the atmospheric sulfur budget in unknown ways. The incorporation of fine tailings into CT deposits underscores the likelihood of active biogeochemical cycling of sulfur occurring within CT landscapes. As tailings management practices are still being optimized, an understanding of the processes that influence sulfur geochemistry in these deposits is integral to the success of reclamation efforts. To date, sulfur biogeochemistry in oil sands tailings systems remains poorly understood. This research will help inform our understanding of the distribution of sulfur within tailings-based landscapes and the greater context of sulfur rich environments.

Sandhill Reclamation Fen represents the first stage of reclamation activity on Syncrude's East-In Pit, which contains a significant portion of Syncrude's composite tailings inventory. This instrumented research wetland is the first of its kind to be developed in the AOSR and overlays a composite tailings deposit in a retired open-pit mine site. This deposit is separated into three zones characterized by distinct porewater chemistry and solid material: 1) the Wetland, which is comprised of freshwater-flooded peat, woody debris and clay till; 2) the Sand Cap, a structural support layer which consists of 10 m of processed oil sand; and 3) the Composite Tailings, spanning ~40m of interbedded CT and tailings sand layers. This reclamation deposit represents a novel, stratified sulfur environment which is highly anthropogenically altered. There are no natural analogues for this site, but the CT shares similar characteristics with marine sedimentary systems (saline, high organic carbon, circumneutral/alkaline, anoxic) and tailings ponds (high sulfur content, naphthenic acids, highly reducing). As in tailings ponds, the distribution, abundance, and diversity of microorganisms depends on the availability of preferred carbon sources (such as labile hydrocarbons), accessibility of

terminal electron acceptors (TEAs, ie. sulfate, ferric iron), and the depletion of soluble nutrients and trace elements (Penner & Foght, 2010). Therefore, porewater and solid exchange between the wetland and the underlying tailings materials has the potential to stimulate microbial metabolisms at depth through the downward migration of labile organic carbon and TEAs.

During the initial phases of wetland construction, $H_2S_{(g)}$ was detected in a dewatering well, suggesting that $H_2S_{(aq)}$ was present in the deposit at higher levels than predicted by abiotic geochemical modeling of the porewater. Early field campaigns detected porewater $H_2S_{(aq)}$ throughout the deposit in addition to microbial communities capable of metabolizing sulfur (Stephenson, 2012). While the CT is thought to be the source of the reduced sulfur, porewater and solid-associated sulfur distribution has not been assessed in each layer of the deposit. Therefore, this thesis characterizes the sulfur biogeochemistry in each layer of the Sandhill Reclamation Fen deposit in an effort to establish the potential for microbial sulfur cycling and explore the mechanisms controlling H_2S generation.

2.1 Objectives and Hypotheses

This research combines a field and experimental approach to characterizing the sulfur biogeochemistry of the Sandhill Reclamation Fen deposit. The primary field objectives of this thesis are to:

1) Characterize the porewater and fines-associated sulfur and organic carbon geochemistry in the wetland, sand cap, and CT;

2) Evaluate the sulfur cycling metabolic capabilities of the endemic microbial community in each layer of the deposit
3) Identify microbial-geochemical mechanisms associated with H_2S generation and sequestration in each of the three layers.

To address the field research objectives, the following hypotheses were tested:

1) ΣH_2S will be present in the porewaters of each layer of the deposit, and concentrations will reflect geochemical parameters (eg. ORP, pH, dissolved oxygen).
2) Fines-associated pools of sulfur and organic carbon will differ between the wetland and tailings materials, reflecting the solid source materials
3) Microbial communities capable of both sulfur oxidation and reduction will be present in all layers of the deposit
4) Characterization of the wetland will reflect biogeochemistry of both constructed and natural wetlands; sand cap and CT layers will share similarities with sedimentary marine and tailings pond environments

The primary experimental objectives of this thesis are to:

1) Examine the ability of endemic microbial communities to metabolize ΣH_2S and SO_4^{2-} in the presence and absence of fine particulates
2) Identify the effect of organic carbon and sulfur (SO_4^{2-}, S^0, $S_2O_3^-$) stimulation on rates of ΣH_2S generation

To address the experimental objectives, the following hypotheses were tested:

1) Fines will exhibit a mechanistic control on ΣH_2S generation through stimulation of sulfur reduction;
2) ΣH_2S generation and SO_4^{2-} consumption rates will be decoupled, indicating the presence of a significant sulfur oxidation intermediate pool;
3) Carbon amendments of microcosms will stimulate ΣH_2S generation.

3.0 Methodology

3.1 Field Site Location and Description

Field sample collection occurred over three sampling campaigns: July 12-15 2013, September 10-12, 2013, and June 25-26, 2014 (Table 3.1). All field samples were collected from Sandhill Fen Reclamation Site, an instrumented research wetland overlaying the North East Pond of the East-In-Pit Composite Tailings Deposit at the Base Mine at Mildred Lake, Syncrude Canada Ltd., north of Fort McMurray, Alberta (Figure 3.1). Future mine closure plans for the North East Pond include an integrated series of watersheds that support constructed wetlands and boreal forest (Internal Document, Syncrude Canada Ltd., 2008). Sandhill Fen Wetland currently covers 15.5 hectares of the proposed watershed, with the adjacent Kingfisher Deposit still awaiting reclamation.

This wetland is the first of its kind to be developed in the AOSR, which overlays a retired open-pit mine site filled with ~40m of interbedded CT and tailings sand layers. Underlying the CT deposit is a layer of lean oil sands fill and limestone bedrock, over which CT mixtures of various consistencies have been discharged. Due to the highly variable nature of tailings management, the chemical composition and solids content of

the deposit is spatially heterogeneous. The CT is slowly dewatering over time, and the high water content causes the tailings to liquefy when disturbed, therefore making the surface of the deposit non-trafficable. To provide structural support for the developing wetland reclamation landscape, 10m of processed tailings sand was deposited over the unconsolidated CT. On top of this sand, a 0.5m thick layer of clay-till was installed as a base for wetland construction. Stockpiled and salvaged live peat materials stripped from active mine sites were placed on top in 2011, held in place by wood berms that horizontally cross-cut the deposit to prevent downstream migration of the peat. Flooded in the summer of 2012, a freshwater reservoir with a leaky dam sustains a trickle-fed surface pond (<0.5m deep), and a network of underground dewatering drains maintains the artificial water table. The drains prevent the upward migration of tailings water into the wetland, as the high salt content would negatively impact wetland vegetation. The deposit is therefore separated into three distinct zones that differ in solid composition and porewater chemistry: wetland, sand cap, and CT (Figure 3.2).

Porewater was sampled from a number of piezometers installed throughout the deposit. Table 3.1 lists the name, well depth, and dates sampled for each sample site. Figure 3.3 shows the location of these sampling sites within the Sandhill Fen deposit. Coloured circles indicate the sampling site number and associated wells (ie. ‘Site 5’ contains sampling sites Pond 5, Well 5C, and Well 5D).

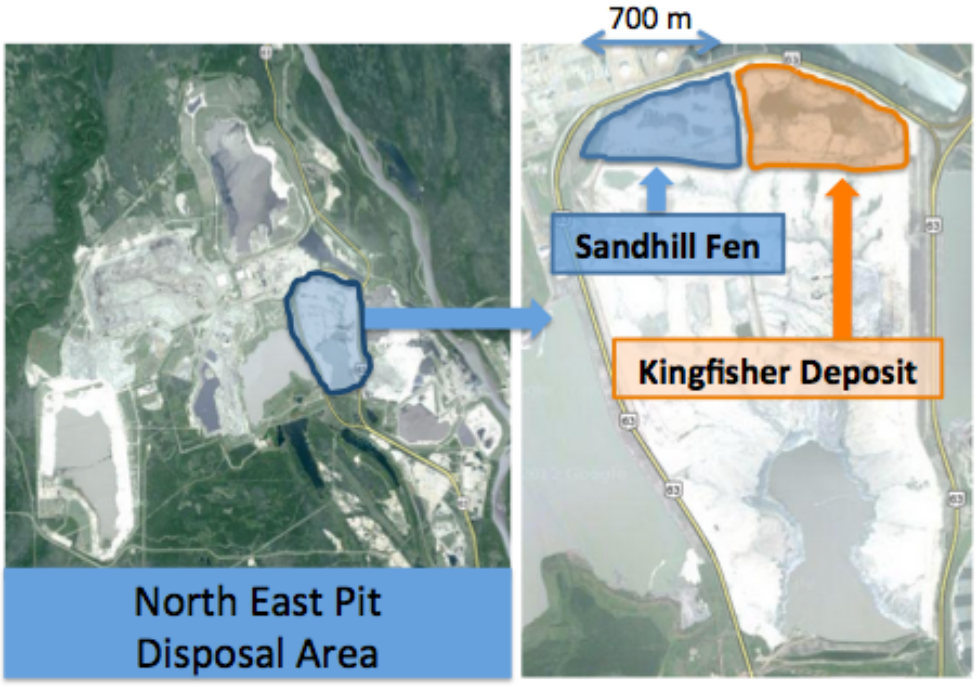


Figure 3.1. Sampling site location within East-In Pit, Base Mine at Mildred Lake, Syncrude Canada Ltd, located approximately 50 km north of Fort McMurray, Alberta. Images obtained from Google Earth on February 5, 2012.

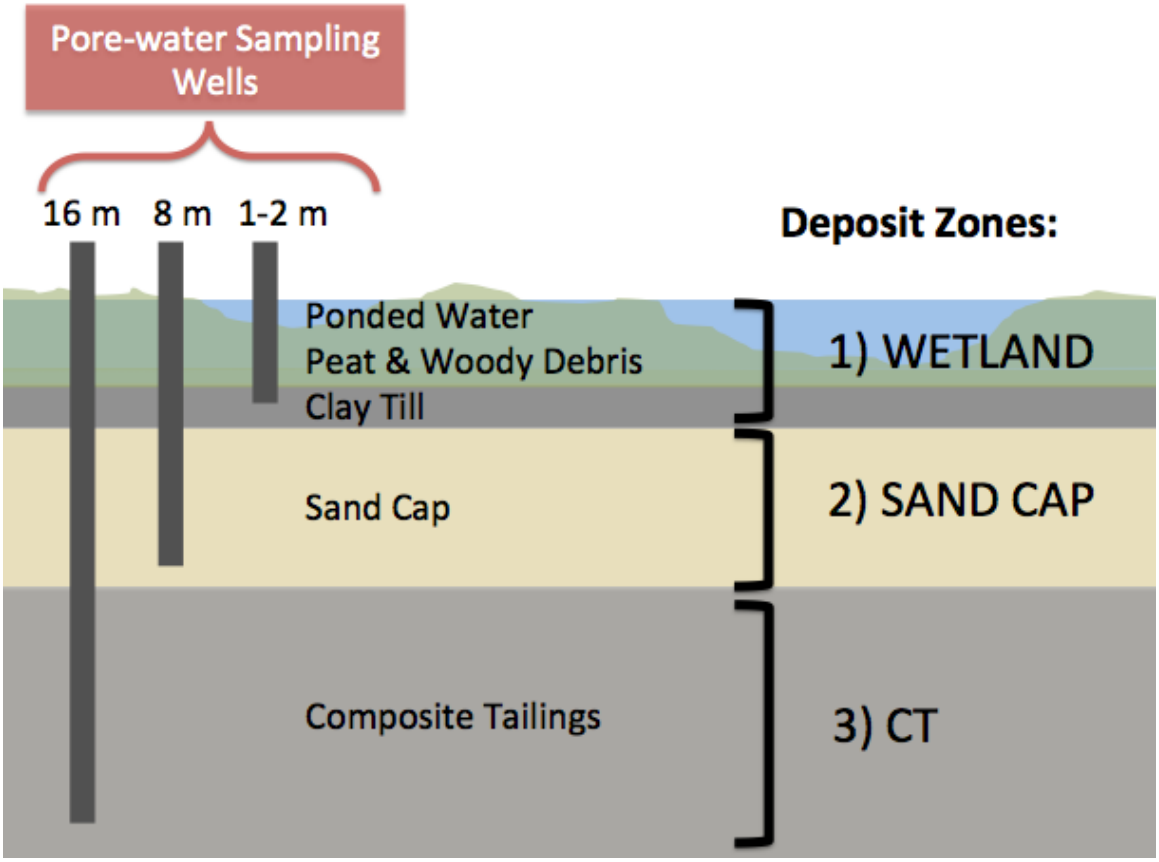


Figure 3.2: The deposit is divided into three distinct layers, which differ in solid composition and porewater chemistry.

Table 3.1 Sampling well descriptions and field sampling campaigns

Well Name	Well Depth	Sampling Layer	July 2013	Sept. 2013	June 2014
Surface Ponded Water (Ponds 1,2,4,5)	0m	Flooded Fen Water	✔	✔	
Fen-Sand Interface Wells (Wells W1,W2,W4)	1-2m	Lower layer of fen	✔	✔	✔ (W2)
4C	8m	Sand Cap	✔		✔
5C	8m	Sand Cap	✔	✔	✔
6A	8m	Sand Cap	✔	✔	
5D	16m	Composite Tailings	✔	✔	✔

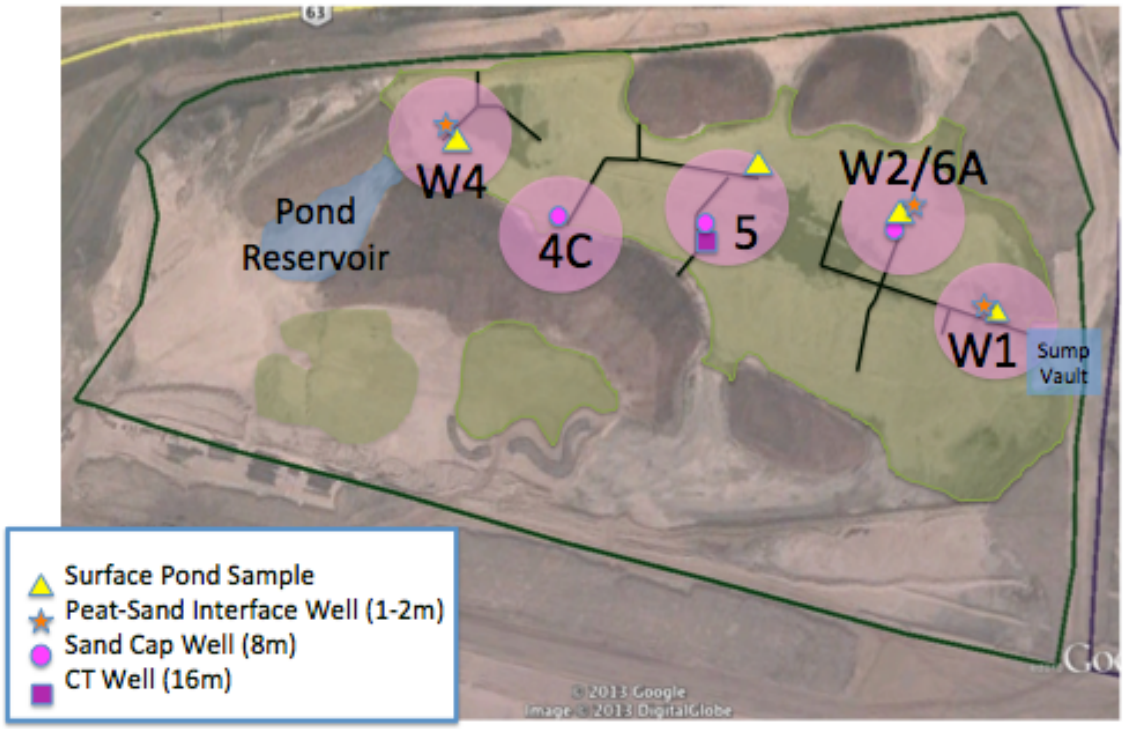


Figure 3.3: Sampling locations within Sandhill Fen. Wetland peat is indicated in green, and the brown regions represent constructed uplands made from sand to aid in water drainage towards the wetland. The pond reservoir provides a slow stream of freshwater to the deposit. The CT dewatering drains lead to the sump vault, where water is pulled from the deposit as it is released by the consolidation of the tailings. Image modified from T. Colenbrander and Google Maps.

3.1.1 Porewater Well Sampling and Analysis

Porewater was pumped from sampling piezometers using high-density polyethylene tubing and foot valves (Waterra). Prior to sampling, three well volumes were purged from each well to discard standing water and particulates that had accumulated in the well casing. Water was continuously pumped into a bucket or bottle with a submerged multi-probe to characterize pH, dissolved oxygen, temperature,

conductivity, salinity, total suspended solids, and oxidative-reductive potential of the sample (YSI Professional Plus 6-Series Sonde, YSI Incorporated).

Total dissolved $\Sigma H_2S_{(aq)}$ was determined colorimetrically via methylene blue analysis (Hach Method 8131) using a Hach Spectrophotometer. To prevent the rapid oxidation of O_2-sensitive analytes, 15mL polypropylene centrifuge tubes were pre-spiked with the appropriate stabilizing reagent prior to sample collection. Sample water was pipetted directly from the stream of pumped water and added immediately to the pre-spiked tubes. After a reaction time of 5 minutes, samples were then read for sulfide and sulfate on the spectrophotometer. Samples for total dissolved SO_4^{2-} were filtered (<0.7 um) to remove particulates, and then analyzed colorimetrically on the Hach Spectrophotometer (SulfaVer4 Method 8051, Hach Company).

Sample water for total dissolved sulfur analysis was filtered with a 0.45 um filter into a 50mL acid-clean polypropylene centrifuge tube that was pre-spiked with 0.2% v/v Optima-Grade concentrated Nitric Acid. All acid-clean tubes were soaked overnight in a 4% v/v HCl bath, rinsed 7 times with MilliQ Ultrapure water, and allowed to dry before use in the field. Tubes were stored at 4°C until shipment to the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia for analysis by ICP-AES.

Ferrous and Ferric iron were preserved by the addition of 2% v/v Optima-Grade Hydrochloric Acid, and analyzed colorimetrically by a modified ferrozine method. Standard curves for determination of Fe(II)/Fe(III) concentrations were prepared by serial

dilution of an acidified $FeCl_3$ solution (1.786 $x10^{-2}$ mol/L $FeCl_3$, in 2% v/v HCl). Fe(II) and Fe(III) were quantified on a single preserved water sample before and after a reduction step as described by Viollier et al. (2000).

Water for total organic carbon (TOC) and inorganic carbon (TIC) was pumped into carbon-clean amber glass 120mL bottles. Bottles were cleaned with detergent, rinsed with ethanol, and placed in a 10% HCl bath overnight. After 7 rinses with MilliQ water, bottles were muffled in a furnace at 450°C for 8h to remove any residual carbon. Lids were rinsed with a 1:1:1 mixture of dichloromethane, hexane, and methanol and allowed to evaporate dry. In the field, each bottle was rinsed twice with sample before filling and then frozen at -20°C until filtration for dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) characterization in the laboratory. Analyses on the filtrate were conducted using a Shimadzu TOC-L Total Organic Carbon Analyzer with an autosampler ASI-L using the 680°C combustion catalytic oxidation method as per manufacturer recommended protocols (Mandel Scientific). Particulate total carbon and inorganic carbon were measured on the 0.7um filters by combustion in the solid sampler SSM-5000A attachment for the Shimadzu TOC-Analyzer (Mandel Scientific). For total carbon, filters were placed in carbon-free ceramic sample boats and heated at 900°C, where the oxidized carbon released as CO_2 was measured by infrared gas analyzer. For TIC, filters were placed in carbon-free ceramic sample boats and equilibrated inside the analyzer for 2 minutes to remove atmospheric CO_2 influence. After the addition of 0.5 mL of 50% H_3PO_4 acid, the sample was immediately pushed into the sampling chamber for heating at 200°C, where the evolved CO_2 was measured by infrared gas analyzer (Shimadzu Corporation, 2013).

Samples for routine chemical analyses (trace dissolved metals, routine anions, alkalinity, inorganic nonmetallic parameters) were collected for characterization by Syncrude's commercial laboratory according to APHA Standard Methods for the Examination of Water and Wastewater (Exova Edmonton, Alberta, Canada).

Microbial enrichment samples were pumped into sterile 1L Nalgene bottles or 50mL Corning tubes (sterilized with 70% v/v ethanol, rinsed with sample twice before filling). Bottles were sealed with no headspace and stored at 4°C until microcosm set-up in the laboratory.

To characterize the solid components present in the well water, 50mL Corning tubes were filled during the first purge of the well and mid-sampling. This allowed for comparison between standing sediment in the well casing and fine sediment carried by the pumped porewater. Acid-clean 50mL Corning tubes were filled and immediately frozen at -20°C for characterization of acid-volatile sulfides, acid-extractable sulfate, and total suspended solids.

3.1.2 Surface Water Sampling

Prior to sampling, surface ponded water was analyzed for pH, dissolved oxygen, temperature, conductivity, salinity, total suspended solids, and oxidative-reductive potential using a YSI Professional Plus 650 Series probe (YSI Incorporated). Depth profiles were collected at the air-water interface and sediment-water interface depending on the depth of the surface water. For water depths of less than 0.5m, one reading in the middle of the water column was taken.

Ponded surface water samples for iron, DOC/DIC, and routine chemical characterization were collected by submerging bottles underwater or pipetting directly into tubes spiked with preservative. Sulfide and sulfate were determined colorimetrically by Hach spectrophotometry.

In addition to grab samples, a passive sampling approach was developed for measuring ΣH_2S gradients between the air/water interface and sediment/water interface. To measure dissolved ΣH_2S at the surface of the water, Float-A-Lyzer dialysis devices (Spectrum Labs), were installed and sampled after 3-4 days (Figure 3.4). To measure sulfide and iron at the sediment/water interface, Slide-A-Lyzer casettes (Figure 3.4) were buried vertically in the sediment with the porewater membranes approximately 1cm below the surface of the sediment. The floating samplers were installed directly above the sediment samplers and were contained within a 25 x 45 cm grid by the associated 'box gas trap' (described in detail below). These devices consist of cellulose ester membranes filled with Milli-Q water, allowing for chemical equilibration with surrounding sample water. The membranes have a 5mL (Float-A-Lyzer) and 12mL (Slide-A-Lyzer) volume and allow compounds with less than 20,000 dalton molecular weights (equivalent to 20,000 g/mol) to diffuse across the membrane. After 3-4 days of submersion in the wetland water, the Milli-Q solution within the device was sampled for ΣH_2S colorimetrically. A previous laboratory experiment demonstrated that 3 days of submersion in a known solution of sulfate-enriched water provided sufficient time for equilibration with sample concentrations (Kendra & Colenbrander, unpublished data).

Figure 3.4: Float-a-lyzer dialysis membrane (Left) and Slide-a-lyzer dialysis cassette (Right) used for sampling the air-water interface and sediment-water interface.

3.1.3 H_2S Gas Detection During Well Sampling

The presence of gas-phase H_2S was determined colorimetrically using lead acetate strips (Sigma Aldrich). At each site prior to sampling, 10mL of pumped well water was quickly sealed in a 50mL Corning tube with a lead acetate strip affixed to the lid. After 5-10 minutes, the presence of a black precipitate on the strip indicated a concentration of H2S gas over 1ppm. If the strip test identified that H_2S gas was evolving from the well water, the gaseous concentration in ppm was measured using the CES Landtech GEM 2000 Plus landfill gas analyzer (Hoskin Scientific). The gas analyzer could detect H_2S concentrations up to 500 ppm with a detection limit <1ppm and accuracy ±10% full scale. The gas analyzer tubing was inserted 1m into the well pipe, a depth that ensured that the sampling inlet was well above the height of the water but deep enough to determine a reading before the H_2S mixed with atmospheric O_2. Readings were taken prior to well pumping to assess any passive venting of $H_2S_{(gas)}$. Values were monitored during well pumping and the peak concentrations were recorded.

3.1.4 H_2S Gas Detection in Surface Waters

To test for the evolution of H_2S gas from ponded surface water, a passive gas trap sampler was installed at each pond site (Figure 3.5). A clear plastic container (25 x 25 cm) was attached to spikes that would support its weight above the water over the 3-day sampling period. The bottom edge of the container just touched the surface of the water, such that the pressure inside the container was still in equilibrium with the atmosphere. A 50mL polypropylene centrifuge tube was vertically affixed to the inside of the container, such that the opening faced up. Once the container was installed at the pond site, 15mL of alkaline zinc acetate solution was pipetted into the tube. Modified from acid volatile sulfide protocols from Burton et al. (2007) and Hsieh et al. (2002), evolved H_2S gas from the ponded water would precipitate as zinc sulfide on contact with the solution. After 3 days in the pond, the tubes were removed from the container and sealed until analysis in the lab. Zinc sulfide was then quantified colorimetrically by methylene blue analysis. While this method only captures a portion of the evolved sulfide from the pond over a defined area, it provided a simple mechanism for detecting small fluxes of H_2S gas while preventing contamination of air-borne sulfur particles.

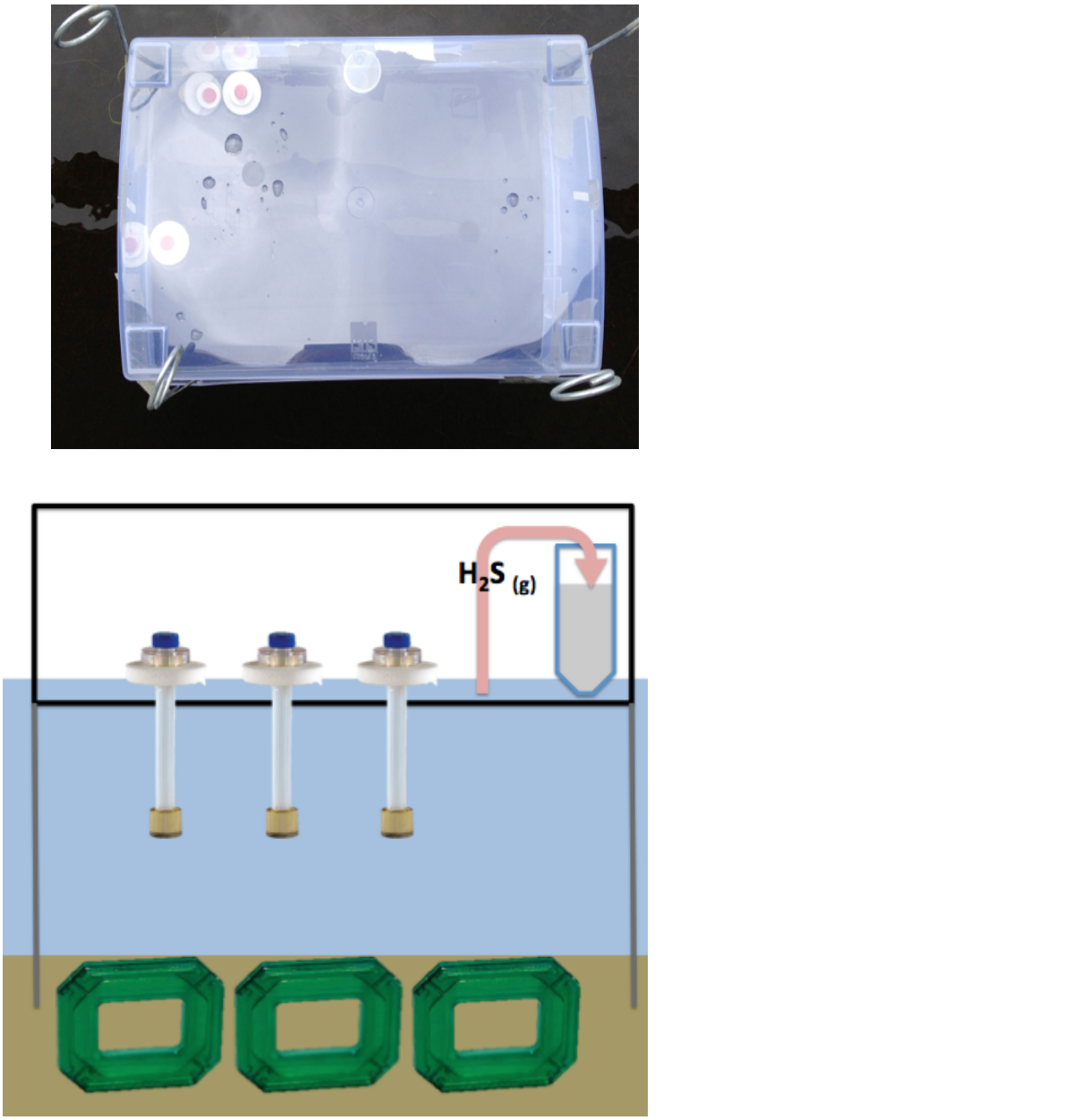


Figure 3.5: Passive 'Box Sampler' schematic used for quantifying dissolved and gaseous H_2S in the wetland. Top: Top-down photo of gas trap employed in the field over ponded water. Bottom: side-view schematic of box sampler set-up. Floating dialysis peepers were contained within the box boundary while sediment-water interface peepers were installed in the sediment below the box.

3.1.5 Solid Sampling

To quantify the mass of fines in the well water, samples were filtered on 0.2um cellulose acetate filters. Filters were weighed before filtration and after drying at room temperature to determine the mass of dry sediment per unit volume of well water. The fines-associated pool of acid volatile sulfide was determined via a modified extraction process from Burton et al. (2007) and Hsieh et al. (2002). Fines were filtered onto a 0.2um cellulose acetate filter and the volume of filtered water was recorded. Filters were added to acid-clean 50 mL polypropylene centrifuge tubes in the anaerobic chamber. A round-bottom polypropylene culture tube filled with 7mL of 3% alkaline zinc acetate solution was inserted into each centrifuge tube. Lastly, 10mL of 6M HCl/0.1M ascorbic acid solution was added to the centrifuge tube such that the filters were submerged completely, and the tubes were tightly sealed. Fines-bound AVS was extracted for 18h in the anaerobic chamber, during which the liberated sulfide precipitated in the zinc acetate solution as zinc sulfide. After 18h, the alkaline zinc acetate solution was removed from the centrifuge tube and zinc sulfide was quantified colorimetrically by methylene blue analysis. AVS values were normalized per gram of sediment, and extractions were run in triplicate. Acid extractable sulfate was quantified on the remaining HCl solution via colorimetric analysis by SulfaVer4 Method 8051 (Hach Company) as outlined in Burton et al. (2007).

3.1.6 Environmental Microbial Enrichments

Functional enrichments for sulfur metabolizing bacteria were conducted to establish the potential for active sulfur cycling within each layer of the deposit. Batch enrichments utilized liquid enrichment media targeted to enhance the growth of neutrophilic sulfur oxidizers and reducers in the endemic community. By manipulating growth media to provide the necessary environment and substrates for microbial metabolic requirements, we can interpret positive growth as the presence of specific functional metabolisms within the endemic community. Functional enrichments provide insight into the metabolic capacity of a community from a given system, but positive growth does not necessarily imply that these processes are occurring at depth in the environment. Therefore, the successful establishment of sulfur reducing and oxidizing enrichments only indicates the potential for these microbial metabolisms to be active within the reclamation deposit.

Well water from the wetland (W2), sand cap (4C, 5C) and CT (5D) from June 2014 was stored in the dark at 4°C until experimental set-up within one week of sample collection. Samples were slowly brought to 25°C in the anaerobic chamber, after which 5mL were aseptically transferred to a sterile 50mL polypropylene centrifuge tube (Corning) containing 45mL of media. Minimal headspace remained in the tubes. All surfaces within the chamber were sterilized with 70% v/v ethanol prior to opening each sample. Enrichments were prepared in duplicate from both unfiltered and 0.45 um filtered samples to test for positive growth in the presence and absence of fine particles. All samples were grown without agitation in the dark. Media components were prepared in bottles autoclaved at 121°C for 30 minutes, degassed under N_2 flow for 45 minutes, and

combined in the anaerobic chamber after filter sterilization via 0.22um filter Nalgene vacuum filter column (Thermo Scientific).

Sulfur Reducing Bacteria

Heterotrophic sulfur reducers were grown in brackish media according to a procedure outlined by Burlage et al. (1998). For enrichments targeting sulfate-reducing bacteria, media was amended by the addition of $CaSO_4$ and $MgSO_4 \bullet 7H_2O$. For enrichments assessing the potential for reduction of sulfur oxidation intermediates, 1mM sodium thiosulfate ($Na_2S_2O_3$) or ~10mM precipitated elemental sulfur was added in addition to amending the enrichments with a 50:50 solution of sodium acetate trihydrate and sodium lactate (final concentration per tube of 100mg/L of organic carbon). Positive growth in all enrichments was confirmed by development of a black FeS precipitate.

Neutrophilic Sulfur Oxidizing Bacteria

Chemolithotrophic, neutrophilic sulfur oxidizing bacteria were grown in media containing thiosulfate as the sole sulfur substrate (Burlage et al., 1998). System pH was adjusted using sterile NaOH until the phenol red indicator turned pink (pH>8). Positive growth was indicated by colour change from pink to yellow, as acid generated via sulfur oxidation dropped system pH below 8.

3.2 Microcosm Experimental Design

3.2.1 Microcosm Experiment 1: Microbial Community and Fine Particle Controls on Sulfur Cycling

Positive growth in targeted enrichments establishes the potential for microbial sulfur cycling at depth, but it is difficult to extrapolate the influence that these communities may have on sulfur dynamics in situ. Therefore, an experiment was designed to measure sulfur reduction rates in well water from each layer of the deposit by tracking the evolution of SO_4^{2-} and $\Sigma H_2S_{(aq)}$ over 7 weeks. The objective of the experiment was to compare the ability of bulk microbial communities from the wetland, sand, and CT to generate $\Sigma H_2S_{(aq)}$, and to determine what effect, if any, the presence of fine particles ("fines") had on sulfur reduction rates.

Water samples for the microcosms were collected from the wetland (Well W2, June 2013), sand cap (Well 5C, July 2013) and CT (Well 5D, May 2012). Samples were stored sealed in the dark at 4°C until experimental set-up, during which they were slowly brought to 25°C in the anaerobic chamber. All surfaces within the chamber were sterilized with 70% v/v ethanol prior to opening each sample. After physicochemical characterization (pH, temperature, Eh, total dissolved solids, conductivity and salinity, Orion 5-Star pH/DO/Conductivity portable multiparameter meter, Thermo Scientific) samples were either filtered or left unfiltered as described in the filtering process below. The samples were divided into six main treatments based on source water and the presence or absence of fines (Table 3.2). One microcosm per treatment was sacrificed at each of the five time points were selected (t=0, 10, 21, 33, 53), generating a total of 90

sacrificial microcosm tubes. Figure 3.6 illustrates the microcosm construction process. Each microcosm treatment consisted of one 50 mL polycarbonate centrifuge tube containing the following:

1) Sterile filtered well water

2) Sterile filtered well water amended with microbial-enriched filter

3) Unfiltered well water

4) Unfiltered well water amended with microbial-enriched filter

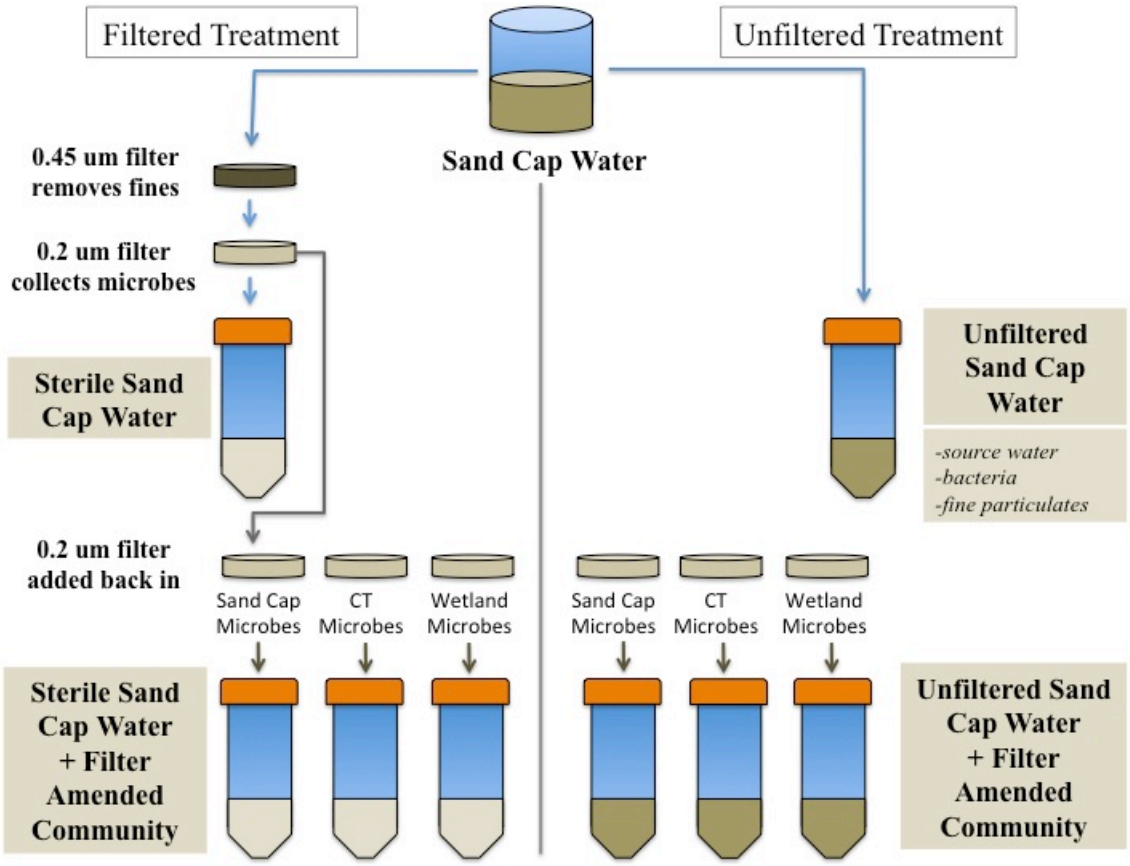


Figure 3.6: Experimental set-up for Microcosm Experiment 1. The same procedure was carried out for microcosms with source water from the wetland and CT.

Samples designated to contain no fines were first pre-filtered with a 0.45um cellulose acetate membrane (GE Water & Process Technologies) to remove almost all fine sediment particles. Membranes were pre-rinsed with ultrapure MQ water to prevent sample contamination. After prefiltration to remove solids, the sample was filtered through a 0.2um polycarbonate membrane (Millipore) to collect the microbial community not associated with solid fines. Therefore, the 0.45um filter was considered to contain all of the fines, while the 0.2um filter contained only the microbial community. While it is acknowledged that some fines <0.45um in diameter will have passed through to the second filter, this prefiltration step was used as a mechanism to limit the influence of fines on $\Sigma H_2S_{(aq)}$ generation while trying to leave the microbial community relatively intact. Visual inspection of the 0.2um filters showed little to no particulate material, and the added mass to the filter was below the detection limit of the balance <0.01g. Samples were filtered in 30mL aliquots to ensure that approximately the same volume of water passed through each filter. Filters containing microbes from each well were added to source water from different levels of the deposit (ie. Wetland microbial community added to unfiltered sand cap water) to assess how the new community composition affected $\Sigma H_2S_{(aq)}$ generation.

Table 3.2 : Matrix of treatment combinations in Microcosm Experiment 1

Treatment Name	Source Water	Fines	Added: 0.2 µm Filter	Microbial Community Composition - Amended by Filter Addition
Unfiltered CT Water	CT	CT	none	CT
Unfiltered CT Water + Sand Cap Filter	CT	CT	Sand Cap	CT + Sand Cap
Unfiltered CT Water + Wetland Filter	CT	CT	Wetland	CT + Wetland
Sterile Filtered CT Water	CT	none	none	Sterile
Filtered CT Water + CT filter	CT	none	CT	CT
Filtered CT Water + Wetland Filter	CT	none	Wetland	Wetland
Filtered CT Water + Sand Cap Filter	CT	none	Sand Cap	Sand Cap
Unfiltered Sand Cap Water	Sand Cap	Sand Cap	none	Sand Cap
Unfiltered Sand Cap Water + CT Filter	Sand Cap	Sand Cap	CT	Sand Cap + CT
Unfiltered Sand Cap Water + Wetland Filter	Sand Cap	Sand Cap	Wetland	Sand Cap + Wetland
Sterile Filtered Sand Cap Water	Sand Cap	none	none	Sterile
Filtered Sand Cap Water + CT Filter	Sand Cap	none	CT	CT
Filtered Sand Cap Water + Wetland Filter	Sand Cap	none	Wetland	Wetland
Filtered Sand Cap Water + Sand Cap Filter	Sand Cap	none	Sand Cap	Sand Cap
Unfiltered Wetland Water + CT Filter	Wetland	Wetland	CT	Wetland + CT
Unfiltered Wetland Water	Wetland	Wetland	none	Wetland
Unfiltered Wetland Water + Sand Cap Filter	Wetland	Wetland	Sand Cap	Wetland + Sand Cap
Sterile Filtered Wetland Water	Wetland	none	none	Sterile
Filtered Wetland Water + CT Filter	Wetland	none	CT	CT
Filtered Wetland Water + Wetland Filter	Wetland	none	Wetland	Wetland
Filtered Wetland Water + Sand Cap Filter	Wetland	none	Sand Cap	Sand Cap

At each sampling point, the water column was removed from the microcosm for chemical analysis. Samples were aliquoted for colorimetric quantification of $\Sigma H_2S_{(aq)}$ (Methylene Blue Method 8131, Hach Company) and SO_4^{2-} (SulfaVer4 Method 8051, Hach Company).

3.2.2 Fines Microcosm Experiment: Amendments

After 33 days, the remaining microcosms were each aseptically divided into three sterile 50 mL polypropylene centrifuge tubes. Amendments were made to the tubes to test if $\Sigma H_2S_{(aq)}$ generation could be stimulated through the addition of labile organic carbon or sulfur oxidation intermediates. One tube in each treatment was left unamended, and this data is included in the time series displayed in Figure 5.1. One tube was amended with 1 mM sodium thiosulfate to determine if $\Sigma H_2S_{(aq)}$ generation in the microcosms was limited by the absence of sulfur intermediates. The final treatment was designed to test carbon limitation, and each tube was amended with a 50:50 solution of sodium acetate trihydrate and sodium lactate (final concentration per tube of 100mg/L of organic carbon). At t=53, each treatment was sampled for colorimetric quantification of $\Sigma H_2S_{(aq)}$.

3.2.3 Microcosm Experiment 2: Carbon and Sulfur Stimulation of Sand Cap $\Sigma H_2S_{(aq)}$ Generation

The first microcosm experiment tracked the evolution of $\Sigma H_2S_{(aq)}$ and SO_4^{2-} over several weeks, approximating 'in situ' sulfur reduction processes by providing the microbial community with both porewater and solid phase substrates sourced from the deposit. As discussed in results section 5.1 and supporting field observations of peak $\Sigma H_2S_{(aq)}$ in the sand cap, the unfiltered sand cap water generated the highest observed $\Sigma H_2S_{(aq)}$. While it was observed that $\Sigma H_2S_{(aq)}$ peaked in the sand cap microcosms, there was no apparent correlation to sulfate reduction rates, suggesting that porewater sulfate is not the dominant control on the rate of $\Sigma H_2S_{(aq)}$ generation. Across all CT and sand cap

treatments it was apparent that the presence of fines played a critical role in $\Sigma H_2S_{(aq)}$ generation, but the mechanism behind this influence was not clear. The fines may provide an easily metabolized form of labile organic carbon or preferred sulfur species. Conversely, the sulfur-reducing microbial community may be largely solid-associated, bound to the fines on a surficial biofilm. Understanding the ability of fines-associated microbial communities to generate $\Sigma H_2S_{(aq)}$ under different stimuli may help explain the mechanism behind $\Sigma H_2S_{(aq)}$ accumulation in the deposit. In an attempt to determine the optimal metabolic substrates for sand cap $\Sigma H_2S_{(aq)}$ generation, the second microcosm experiment was designed to tightly constrain the sources of sulfur and carbon available to the microbial community. The objective of this experiment was to compare the rates of $\Sigma H_2S_{(aq)}$ generation by fines-associated SRB in the presence of different carbon and sulfur sources.

Water from the sand cap (Well 5C, June 2014) was collected in sterile 50mL polypropylene centrifuge tubes and stored sealed in the dark at 4°C until experimental set-up, approximately 5 days post-sampling. Immediately before experimental set-up, the tubes were slowly brought to 25°C in the anaerobic chamber and the initial $\Sigma H_2S_{(aq)}$ concentrations were determined colorimetrically by methylene blue analysis. All surfaces within the chamber were sterilized with 70% v/v ethanol prior to opening each sample. To capture all of the fines and the associated microbial community, sand cap water was vacuum filtered through a 0.2um polycarbonate membrane (Millipore) that was pre-rinsed with ultrapure water to remove impurities. Samples were shaken before being filtered in 15mL aliquots to ensure that each filter contained approximately the same mass of fines. Filters were then added to sterile 50 mL polypropylene centrifuge tubes.

To ensure that all sources of sulfur and carbon were tightly controlled, a synthetic salt-water media was modified from Burlage et al. (1998). To each centrifuge tube, 30 mL of media was added and then vigorously shaken to resuspend all sand cap fines attached to the filter. Two treatments were made up with sterile 0.2 um filtered sand cap water and sterile 0.2 um filtered wetland water in the place of salty media, to evaluate how fines-associated SRBs performed when provided with dissolved substrates from the tailings environment. Treatments were then amended with magnesium sulfate, sodium thiosulfate, precipitated elemental sulfur, or solution of sodium lactate and sodium acetate trihydrate as described in the treatment matrix (Table – Treatment matrix). In addition to sterile filtered wetland and sand cap water, tubes containing combinations of salty media and each sulfur and carbon source were also measures as abiotic controls. To determine how closely solid-associated sulfur generation could approximate bulk $\Sigma H_2S_{(aq)}$ generation, unfiltered sand cap samples were run alongside the amended treatments.

The microcosms were sealed in the dark in the anaerobic chamber for 10 days, after which $\Sigma H_2S_{(aq)}$ was quantified colorimetrically methylene blue analysis (Method 8131, Hach Company). Initial $\Sigma H_2S_{(aq)}$ concentrations were subtracted from the detected value. $\Sigma H_2S_{(aq)}$ values detected for abiotic controls were subtracted from amended treatments. As there was high variability between triplicate microcosms, the enrichment from each treatment with the peak $\Sigma H_2S_{(aq)}$ was selected for discussion purposes.

Table 3.3: Matrix of treatment combinations in Microcosm Experiment 2

Treatment Name	**Growth Media**	**Treatment Amendment**	**Source Water for Filter (Fines + Microbial Community)**
Sand Cap Filter in Media	Synthetic Salt Water	none	Sand Cap - 5C June 2014
Sand Cap Filter + DOC	Synthetic Salt Water	50 mg/L Acetate 50 mg/L Lactate	Sand Cap - 5C June 2014
Sand Cap Filter + SO_4^{2-}	Synthetic Salt Water	1mM $MgSO_4$	Sand Cap - 5C June 2014
Sand Cap Filter + S^0	Synthetic Salt Water	~10 mM S^0	Sand Cap - 5C June 2014
Sand Cap Filter + $S_2O_3^{2-}$	Synthetic Salt Water	1 mM $Na_2S_2O_3$	Sand Cap - 5C June 2014
Sand Cap Filter + $S_2O_3^{2-}$ & DOC	Synthetic Salt Water	2 mM $Na_2S_2O_3$ 50 mg/L Acetate 50 mg/L Lactate	Sand Cap - 5C June 2014
Sand Cap Filter in Filtered Sand Cap Water	Sterile Sand Cap Water	none	Sand Cap - 5C June 2014
Sand Cap Filter in Filtered Wetland Water	Sterile Wetland Water	none	Sand Cap - 5C June 2014
Sterile Filtered Sand Cap Water	Sterile Sand Cap Water	none	-
Sterile Filtered Wetland Water	Sterile Wetland Water	none	-
Unfiltered Sand Cap Water	Unfiltered Sand Cap Water	none	-

4.0 Field Results and Discussion

4.1 Geochemical Properties: Physicochemical Characterization and Conservative Elements

Throughout the entire deposit, well water was circumneutral pH and brackish (Table 4.1). The pH of the ponded water above the deposit ranged from 7.42-8.68, and pH was consistently higher in the July 2013 sampling period compared to September values. Across the three zones of the CT deposit, pH showed an increasing trend with depth, with values ranging from 6.44-7.55 in the wetland-sand interface, increasing to a 7.95-8.36 in the CT. These ranges are comparable to previously reported pH values for Syncrude tailings ponds (Mildred Lake Settling Basin: Holowenko et al., 2000; Salloum et al., 2002; Penner & Foght, 2010) and CT pH (Fedorak et al, 2003; Stephenson, 2012, unpublished data; Kendra et al., 2013, in prep), in both magnitude and increasing trend with depth. High concentrations of bicarbonate were detected throughout the deposit, supporting strong buffering of system pH. Bicarbonate concentrations were lowest in the surface ponded water (174-462 mg/L), and highest in the sand cap (1700 mg/L).

Table 4.1: Geochemical characterization for well water pH, specific conductance, temperature, oxidation reduction potential (ORP), and dissolved oxygen saturation of during each sampling period. BDL indicates that dissolved oxygen levels were below detection limits.

	Sample Site	Date	Depth (m)	pH	Specific Conductance (uS/cm)	Temperature (°C)	Oxidation Reduction Potential (mV)	Dissolved Oxygen Saturation (%)
Surface Ponded Water	W1 Pond	July 2013	0-0.5 m	8.32	420	13.46	137.4	107.1
		Sept. 2013	0-0.5 m	7.42	653	18.95	73.6	66.6
	W2 Pond	July 2013	0-0.5 m	8.58	392	12.33	117.4	104.2
		Sept. 2013	0-0.5 m	7.51	675	11.74	154.2	60.6
	5 Pond	July 2013	0-0.5 m	8.66	520	11.88	113.3	107.2
		Sept. 2013	0-0.5 m	7.67	396	16.56	125.3	89.5
	W4 Pond	July 2013	0-0.5 m	8.68	596	10.26	99.6	90.1
		Sept. 2013	0-0.5 m	7.68	605	12.62	125.6	62.4
Wetland - Sand Interface Wells	W1 Well	July 2013	1-2 m	7.33	2028	17.68	101.5	102.4
		Sept. 2013	1-2 m	6.44	1067	16.23	52.0	57.8
	W2 Well	July 2013	1-2 m	7.55	451	20.53	-40.2	14.2
		Sept. 2013	1-2 m	7.22	675	16.79	-36.8	22.8
		June 2014	1-2 m	6.98	1031	19.90	-52.4	88.0
	W4 Well	July 2013	1-2 m	6.49	612	18.95	-5.0	N/A
		Sept. 2013	1-2 m	6.54	690	16.40	-8.0	35.5
Sand Cap Wells	4C Well	July 2013	8 m	7.63	4297	13.35	-354.4	BDL
		June 2014	8 m	7.42	4221	10.90	-299.8	BDL
	6A Well	July 2013	8 m	8.07	559	13.41	-117.0	BDL
		Sept. 2013	8 m	7.52	1253	13.78	-211.0	BDL
	5C Well	July 2013	8 m	7.78	4345	14.68	-251.0	BDL
		Sept. 2013	8 m	7.51	3462	14.56	-350.6	BDL
		June 2014	8 m	7.41	4413	13.57	-294.9	BDL
CT Wells	5D Well	July 2013	16 m	8.36	4183	14.69	-228.3	BDL
		Sept. 2013	16 m	7.97	3419	14.56	-350.6	BDL
		June 2014	16 m	7.95	4149	13.60	-136.9	BDL

Temperature varied with depth, as the sand cap and CT water appeared to be thermally insulated from the ambient surface temperatures. Surface ponded water reflected the temperature of ambient air during sampling time, with maximum temperatures (20.53°C) recorded during mid-afternoon, and minimum values (10.26°C) recorded early in the morning. The peak temperatures were found in the wetland/sand interface layer, where the dark peat likely had low albedo and therefore retained heat during summer sampling. Sand Cap and CT temperatures did not fluctuate seasonally,

suggesting thermal decoupling from surface heating in the summer and cooling in winter. Thermal insulation with depth has also been observed in tailings ponds, where temperatures at depth do not fluctuate seasonally, and average between 11°C and 15°C all year (Penner & Foght, 2010; Holowenko et al., 2000). The oxidation-reduction potential (ORP) of the porewater became more reducing with depth, decreasing to -354.4 mV in Well 4C. Oxygen concentrations in the sand cap and CT were below levels of detection, and based on both the highly negative ORP and presence of reduced sulfur, these layers were assumed to be anoxic.

Conductivity and salinity both increased 10x from the surface pond to the sand cap. The salinity in both sand and CT wells was ~1/15th of seawater (CT is 2.32 ppt, seawater is 35 ppt), classifying this system as brackish (Roychoudhury et al., 2013) The magnitudes of conductivity, dissolved chloride, dissolved sodium, and salinity indicated a decoupling between the surface wetland and the sand/CT wells. Dissolved chloride concentrations were three to five times higher in sand and CT than in the wetland (Figure 4.2). These concentrations are two times higher than observed concentrations in water overlying MLSB tailings, and three to ten times higher than levels observed at a depth of 20m in the tailings (Holowenko, et al., 2000). The similar concentrations in sand cap and CT of conservative elements indicates that water chemistry in the sand cap is dominated by CT porewater and less influenced by wetland water exchange. The corresponding concentrations in sand and CT salt contents suggest that upward water movement from the CT into the sand layer has occurred.

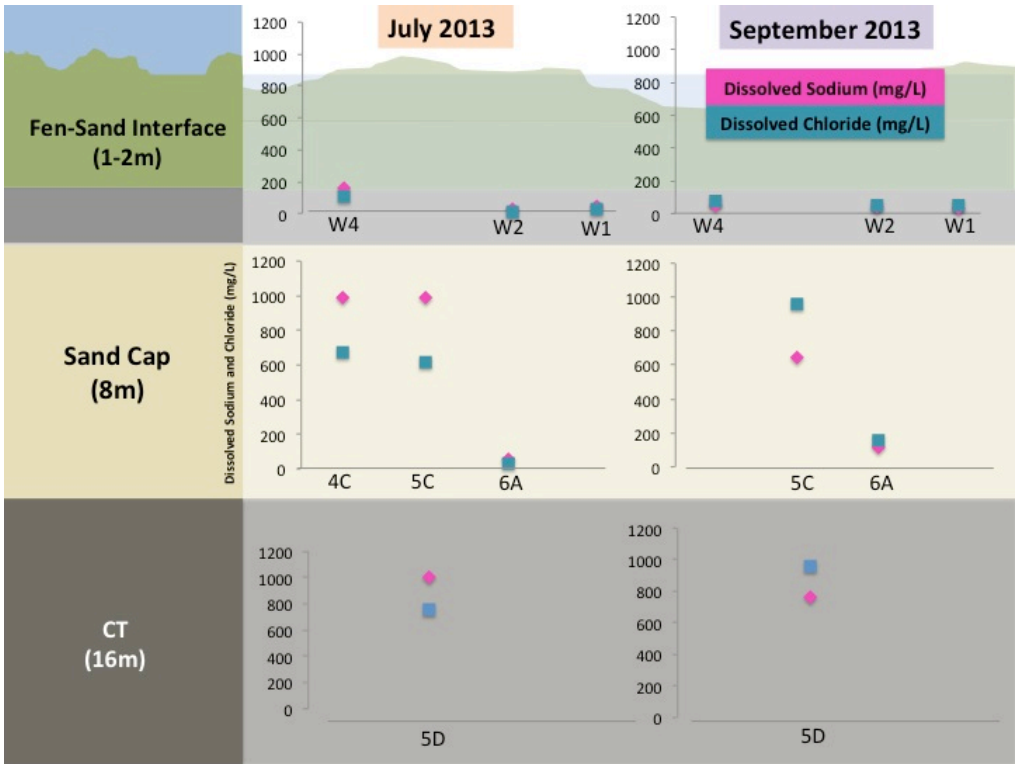


Figure 4.1: Conservative salt tracers in well water indicate a decoupling of water exchange between the wetland and the sand/CT layers. Lower values at Well 6A supported field observations of a leaky well pipe, which likely facilitated the downward movement of wetland water into the sand layer. Conservative tracer values from 6A reflected the magnitudes observed in the wetland, indicating that samples are likely diluted by oxygenated wetland water.

4.2 Porewater Sulfur

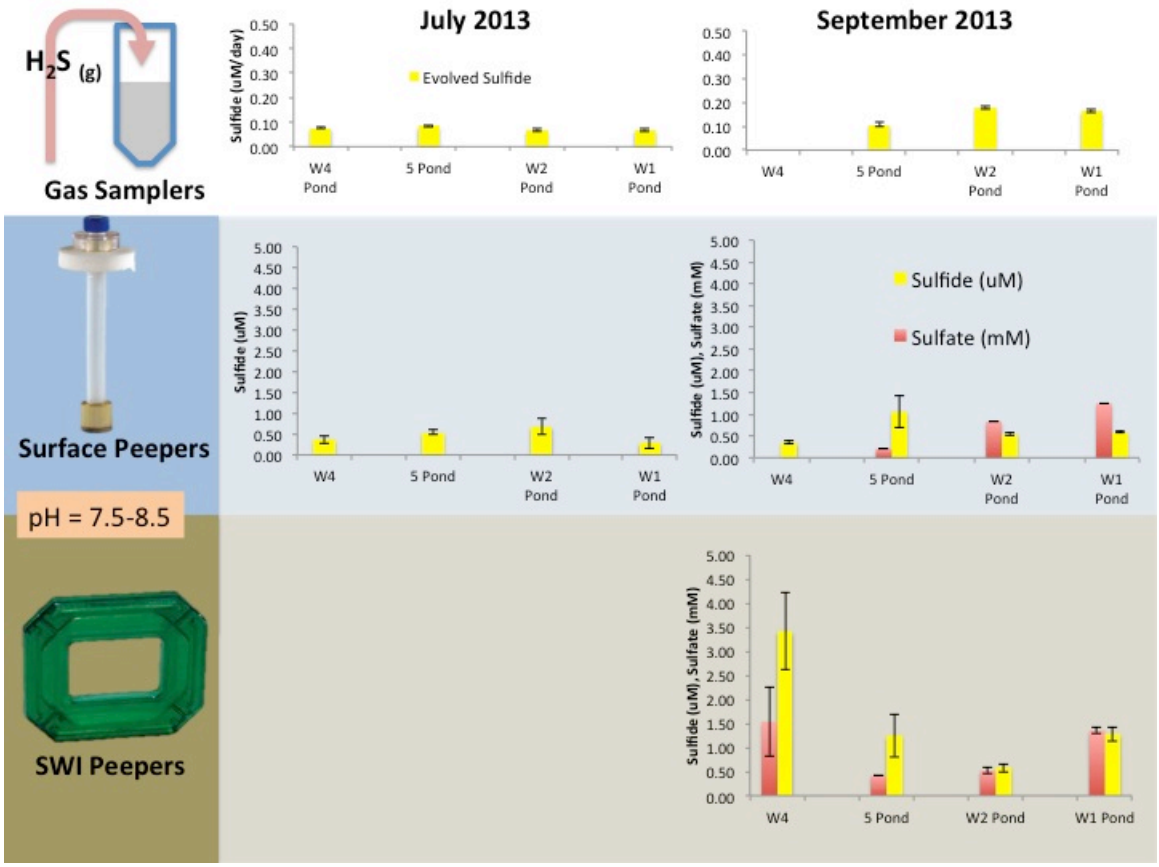


Figure 4.2: Total dissolved sulfide (uM, $\Sigma H_2S = H_2S + HS^- + S^{2-}$) and sulfate (mM) in surface pond water sampled July and September 2013. Values displayed represent the mean of multiple analyses (n=3), error bars represent one standard error of the mean. Note that the scale for the gas samples is expressed as a rate/day, at smaller scale than the peeper samples. Values marked with * indicate that no data is available for this sample. In July, only sulfide was measured, and sediment-water interface peepers were not used.

Sulfide concentrations in the deposit are lowest in the surface peepers and increase with depth until peaking in the sand cap (Figure 4.3). Across all sampling trips, sulfide values are consistently highest in the sand cap, with well 5C showing significantly higher concentrations than all other wells (peak: 549 uM). These values are comparable to maximum reported values for sulfidic, anoxic Black Sea sediment porewater (435 uM, Zopfi et al., 2004), anoxic coastal marine sediment porewater in Aarhus Bay, Denmark

(250 uM, Zopfi et al., 2004), hydrothermal fluids from shallow submarine vents, wells and seeps at Vulcano Island, Italy (22.0-375.6 uM, Amend et al., 2004), and Syncrude MFT microcosms amended with sulfate (586.9 uM,Fedorak et al., 2003). System pH dictates that HS^- is the dominant species in most wells. CT sulfide levels (21.0 -70.1 uM) are lower than those found in the sand, but higher than wetland levels.

$\sum H_2S$ was concentrations at the surface of the wetland were very low, with <1.1 uM dissolved sulfide at the air-water interface, and < 3.5 uM at the sediment-water interface. Despite being the lowest detected levels of sulfide in the deposit, these values are higher than the 0.1-2 nM detected in ocean surface waters (Cutter and Oats, 1987; Luther and Tsamakis, 1989). This suggests that H_2S at the wetland surface may be kinetically stabilized through the formation of metal sulfides (Luther et al., 1999) and/or sorbed to organic matter (Ciglenecki & Cosovic, 1996).

Very small (<0.2 uM/day) fluxes of sulfide gas were detected from the surface ponded water using the passive sampler described in section 3.1.4. While pumping the wells during sampling, sulfide gas was noticeable at all wells due to its characteristic "rotten egg" smell, but it was challenging to quantify at low concentrations due to rapid oxidation. Peak sulfide gas was detected using a landfill gas analyzer in the sand cap and CT wells, which reached maximum values of 180 ppm (5C), 104 ppm (4C) and 147 ppm (5D) during pumping.

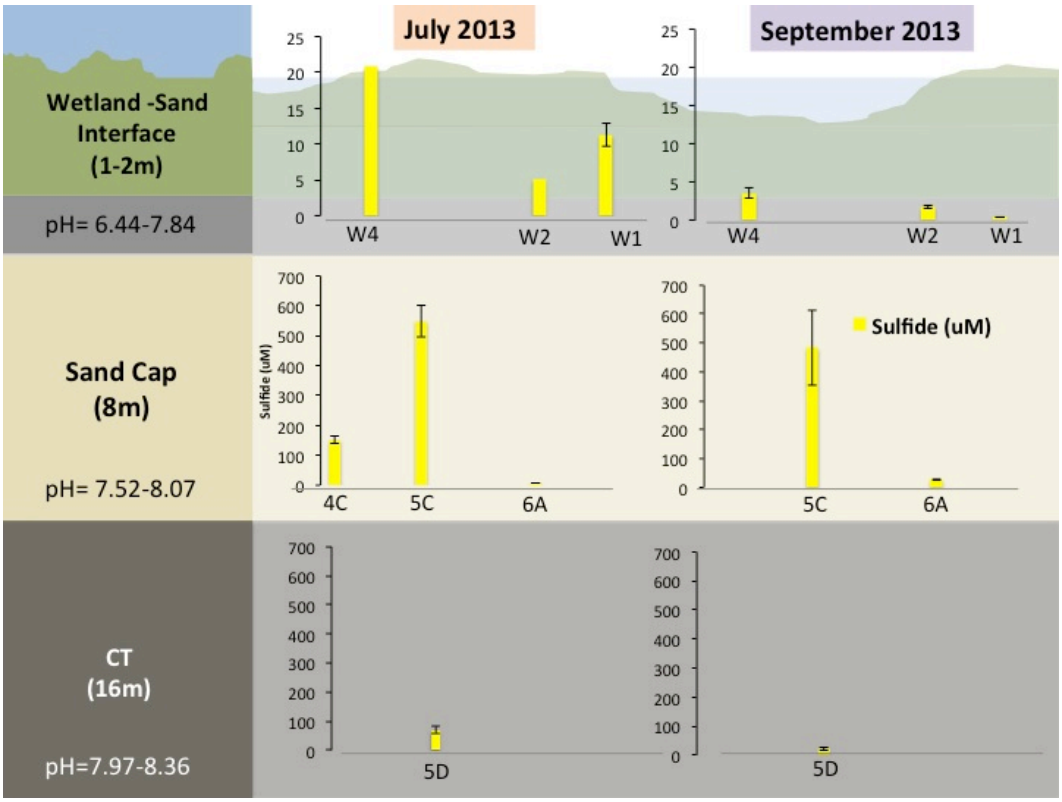


Figure 4.3: Total aqueous sulfide ($\Sigma H_2S = H_2S + HS^- + S^{2-}$) in well water sampled July and September 2013. Values displayed represent the mean of multiple analyses (n=3-8), error bars represent one standard error of the mean. Note that the scale for the wetland-sand interface is an order of magnitude smaller.

Dissolved Sulfate was variable with depth, but many orders of magnitude higher than sulfide (Figure 4.4). Both the highest (6.97 mM) and lowest (0.14 mM) detected levels of sulfate were found in the wetland-sand interface wells, speaking to the heterogonous nature of the interface layer. Sulfate levels detected in the wetland are much higher than found in most natural wetland systems (50-450 uM, Feng and Hsieh, 1998), including remote freshwater bogs (5 uM, Gorham and Detenbeck, 1986), and salt marshes (5 uM 14.9 uM, Luther and Church, 1988). The values more closely resemble a constructed wetland designed to treat storm run-off (<1.8 mM, Fortin, Goulet, and Roy, 2000).

Sulfate concentrations in the sand cap and CT wells were all < 2.6 mM. These concentrations are much lower than seawater (29 mM, Vairavamurthy et al. 1995), slightly lower than observed for fresh CT (9.0-13.4 mM, Fedorak et al, 2003), and more closely resemble values found in a Syncrude tailings pond (<0.5-4.7 mM, Stasik et al, 2014). Despite sharing the same underlying tailings bed, they are much higher concentrations than observed in porewater from adjacent Kingfisher CT deposit (< 0.79 uM, Kendra, unpublished 2013). There were no discernible seasonal or depth-dependent trends for sulfate concentrations.

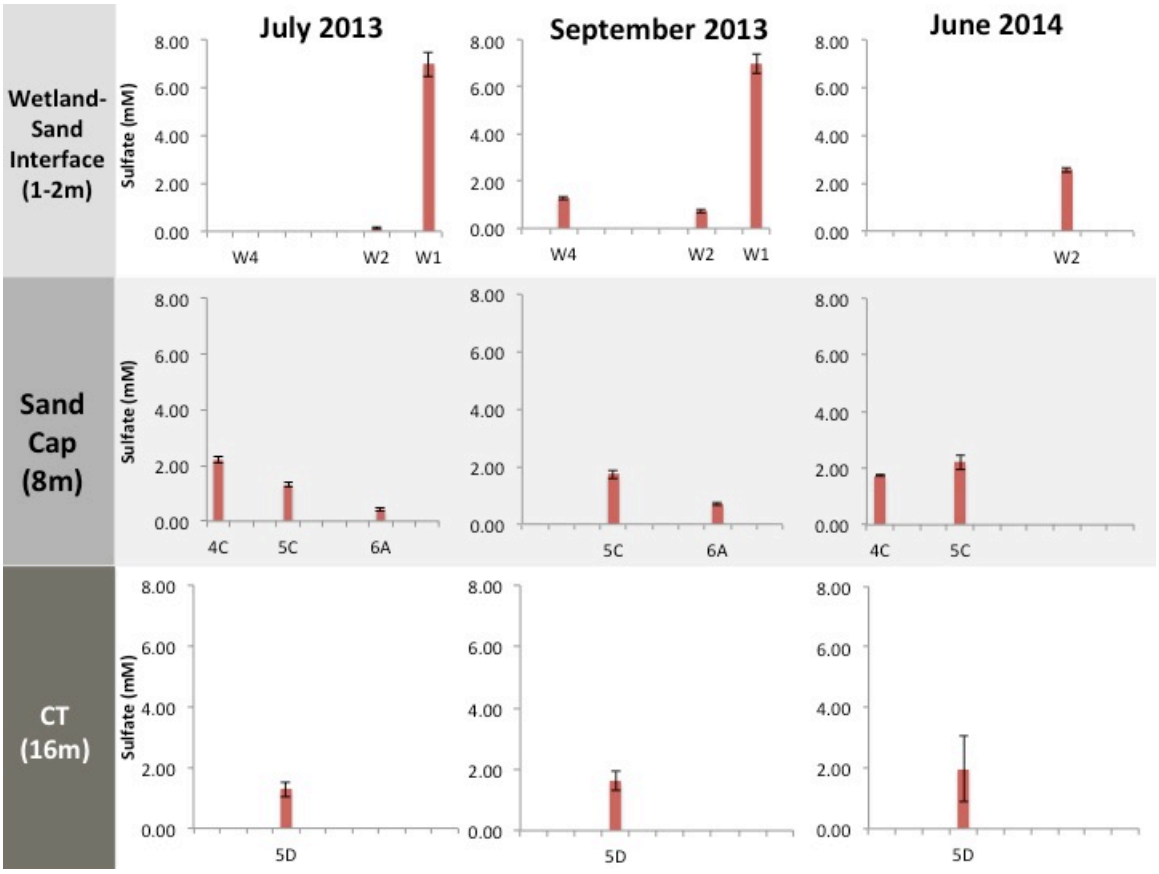


Figure 4.4: Total dissolved sulfate in porewater samples from July 2013, September 2013, and June 2014. Values displayed represent the mean of multiple analyses (n=3-8), error bars represent one standard error of the mean.

4.3 Iron

The presence of reduced dissolved iron at depth establishes the potential for H_2S sequestration through incorporation into amorphous iron sulfides. Ferrous iron (Fe^{2+}) was detected in all wells, with the highest concentrations observed in the sand cap (peak value: 6297.2 uM in Well 4C, July 2013, Figure- all Fe2/3 graphs). The presence of reduced iron is consistent with the existence of iron reducing bacteria at depth, suggesting that iron reduction is occurring in the sand cap (Stephenson, 2012). Ferric iron (Fe^{3+}) was detected in small concentrations at almost all wells, and was also found to be highest in sand cap at (peak value: 616.1 uM, Well 5C). Ferrous and ferric iron concentrations decreased considerably in all wells from July 2013 to June 2014, with ferric iron only detected in the wetland-sand interface well W2 in 2014.

Table 4.2: Porewater Fe^{2+}/Fe^{3+} concentrations

	July 2013				September 2013				July 2014			
Site Name	**Fe^{2+} (uM)**	**± 1 Std. Err.**	**Fe^{3+} (uM)**	**± 1 Std. Err.**	**Fe^{2+} (uM)**	**± 1 Std. Err.**	**Fe^{3+} (uM)**	**± 1 Std. Err.**	**Fe^{2+} (uM)**	**± 1 Std. Err.**	**Fe^{3+} (uM)**	**± 1 Std. Err.**
W1 Surface	2.5	0.1	5.5	0.1	-	-	-	-	-	-	-	-
W2 Surface	2.1	0.0	10.3	0.1	-	-	-	-	-	-	-	-
5 Surface	1.2	0.0	2.5	0.0	-	-	-	-	-	-	-	-
W4 Surface	0.2	0.1	0.0	0.0	-	-	-	-	-	-	-	-
W1 Pond	11.8	0.1	7.3	0.2	5.0	0.1	26.9	0.1	-	-	-	-
W2 Pond	12.2	0.2	0.0	0.0	8.8	0.5	3.9	0.3	-	-	-	-
5 Pond	1.4	0.1	2.2	0.1	0.5	0.1	4.2	<0.1	-	-	-	-
W4 Pond	0.7	0.0	32.1	0.4	0.8	0.0	7.0	0.1	-	-	-	-
W1 Well	81.4	0.6	160.0	1.3	200.0	1.6	266.1	5.2	-	-	-	-
W2 Well	1540.0	27.7	292.1	41.0	558.3	9.0	380.8	7.2	210.0	2.2	65.4	1.7
W4 Well	4627.7	72.6	ND	ND	1499.3	9.5	407.4	36.4	-	-	-	-
6A Well	125.8	1.5	75.2	1.2	7.2	1.1	48.6	1.0	-	-	-	-
4C Well	6297.2	69.8	ND	-	-	-	-	-	489.3	<0.1	ND	-
5C Well	6227.3	58.2	616.1	48.9	1087.8	7.9	<0.1	<0.1	166.5	<0.1	ND	-
5D Well	394.5	4.9	555.3	16.1	0.6	<0.1	41.38	<0.1	4.9	<0.1	ND	-

These values for both ferric and ferrous iron are considerably higher than previously documented for this deposit. Unpublished field data from 2012 shows maximum values for ferrous iron at 4.88 uM and ferric iron at 25.7 uM, both in the CT (Stephenson, unpublished 2012). Porewater concentrations in Kingfisher deposit were also significantly lower, with 38.5 uM ferrous iron detected near the surface of the deposit, and no detectable ferric iron (Kendra et al., 2013). These field values are also much higher than observed for tailings pond total dissolved iron (< 5.4 uM) but lower than total reactive iron extracted from the tailings (~ 44-53 mM, Stasik et al, 2014). X-ray diffraction analysis on CT minerals from the adjacent CT deposit showed that Fe-bearing minerals can account for ~20-50% of overall CT mineralogy, with 64% of total extractable Fe coming from easily reducible (amorphous oxyhydroxides) and reducible (crystalline (hydr)oxides) fractions (Kendra et al., 2013 – in prep). The preservation method chosen for the field campaign utilized acid to maintain iron speciation until later analysis, and this likely dissolved colloidal iron and amorphous iron sulfides in the porewater (like metastable mackinawite (FeS) and greigite (Fe_3S_4)), leading to an increased total iron concentration (Viollier et al, 2000). These field values represent more than enough iron to precipitate sulfide from solution, suggesting that the high concentrations are likely an artefact of the sampling process rather than reflecting dissolved iron values.

4.4 Dissolved Carbon

Dissolved organic carbon (DOC) values were variable throughout the deposit, with no apparent depth-dependent or seasonal trends (Figure 4.3). DOC concentrations ranged from 21.5-127.7 mg/L. This range is comparable to reported values for Mildred Lake Settling Basin (44-56 mg/L, Penner and Foght, 2010), fluid fine tailings (50-70 mg/L, Fedorak et al, 2003), and fresh CT (91-100mg/L, Fedorak et al, 2003). Dissolved inorganic carbon (DIC) had a wide range from 16.13 mg/L to 278 mg/L, with the highest values in the sand cap and CT wells. These values are similar in magnitude to an active tailings pond (~150-275 mg/L, Stasik et al, 2014).

Table 4.3: Porewater dissolved carbon

	Site Name	Total Dissolved Carbon (mg/L)	± 1 Std. Err	Inorganic Dissolved Carbon (mg/L)	± 1 Std. Err	Dissolved Organic Carbon (mg/L)	± 1 Std. Err
July 2013	W1 Pond	72.16	0.17	24.58	0.19	47.58	0.26
	W2 Pond	63.55	0.34	26.96	0.10	36.59	0.36
	5 Pond	84.47	0.70	20.93	0.14	63.54	0.71
	W4 Pond	60.13	0.23	25.12	0.16	35.01	0.28
	W1 Well	76.95	0.76	29.68	0.02	47.27	0.76
	W2 Well	74.30	0.77	29.61	0.33	44.69	0.84
	W4 Well	86.78	0.43	57.46	0.23	29.32	0.48
	6A Well	65.19	0.35	23.50	0.12	41.68	0.37
	5C Well	100.62	0.83	27.87	0.29	72.75	0.88
	4C Well	277.95	1.40	215.96	3.19	61.99	3.49
	5D Well	62.68	0.39	21.12	0.23	41.55	0.46
September 2013	W1 Pond	80.49	0.44	16.79	0.29	63.70	0.53
	W2 Pond	75.64	0.59	17.93	0.28	57.71	0.65
	5 Pond	43.15	0.37	21.68	0.23	21.47	0.44
	W4 Pond	68.01	0.47	16.13	0.28	51.88	0.55
	W1 Well	126.25	0.22	53.96	0.36	72.29	0.42
	W2 Well	87.52	0.82	26.31	0.18	61.20	0.84
	W4 Well	169.40	0.65	41.68	0.31	127.72	0.72
	6A Well	74.60	1.00	29.26	0.21	45.34	1.02
	5C Well	348.17	1.43	272.43	1.32	75.73	1.94
	5D Well	285.12	0.70	192.60	1.33	92.52	1.51
June 2014	W2 Well	90.75	0.30	55.25	0.32	35.50	0.44
	5C Well	345.30	2.86	278.67	2.90	66.63	4.08
	4C Well	315.60	2.43	229.07	1.62	86.53	2.92
	5D Well	261.27	2.35	185.95	1.84	75.32	2.98

A significant positive correlation between DOC and $\Sigma H_2S_{(aq)}$ values was only observed for the sand cap zone ($R^2 = 0.97$, Figure 4.3). This strong exponential relationship indicates that the concentration of DOC is an important control on the generation of sulfide in the sand cap. As the bacterial metabolizability of organic carbon differs greatly depending on the origin and structure of the carbon, the reactivity of

carbon in the deposit is an important rate-controlling parameter for sulfur reduction (Westrich & Berner, 1984). Sulfate-reducing bacteria rapidly utilize the more labile fractions of the available carbon pool, and the remaining fraction of less-reactive carbon accumulates in the deposit, as it is unable to support the same rate of sulfur reduction. The exponential relationship between DOC and $\Sigma H_2S_{(aq)}$ in the sand cap represents a first-order *G* model, where the rate of organic carbon decomposition through sulfur reduction depends on the amount and decomposability of the available carbon (Westrich & Berner, 1984). This organic matter limitation relationship between carbon and sulfide was not observed in the CT porewater or surface ponded water.

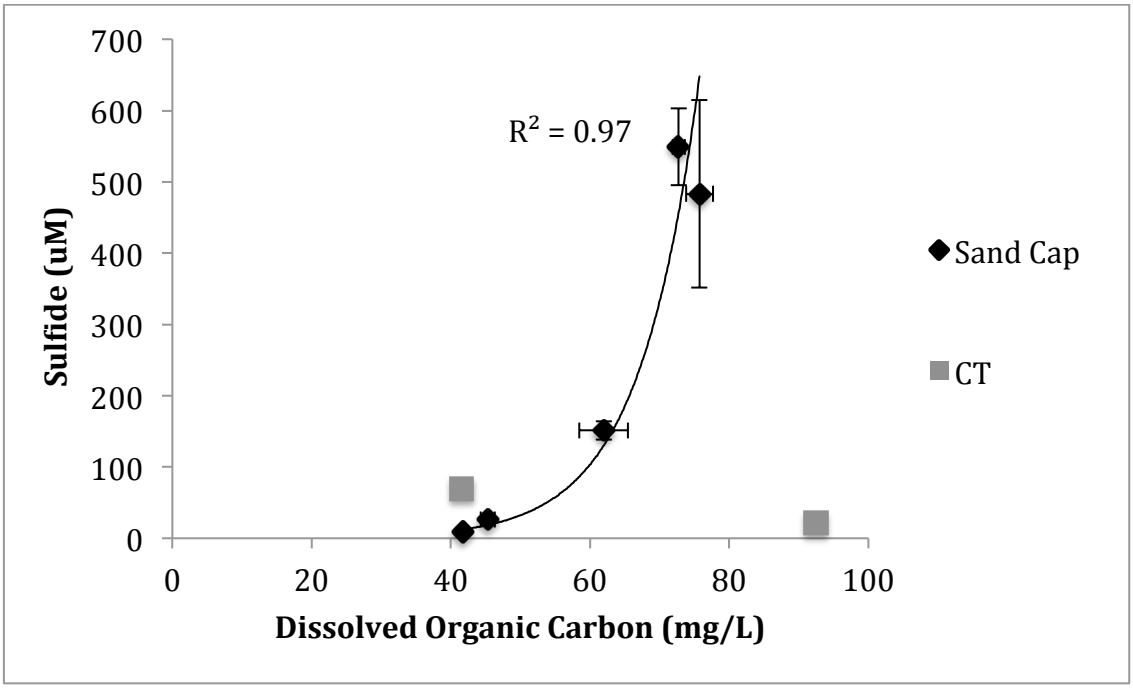


Figure 4.5: A correlation between $\Sigma H_2S_{(aq}$ and DOC values was observed only in the sand cap.

4.5 Characterization of Fines

Field observations from previous campaigns have described an increase in well water fine particulates ('fines') over time. The abundance of fines in the well water often necessitated prefiltration of samples to remove turbidity interferences during spectrophotometric analyses. Wells with high fines contents had slow recharge rates during pumping, suggesting that the migration of fines throughout the deposit could negatively affect hydraulic conductivity. Many of these wells ran dry during the sampling process, with wetland-interface wells drawing water down from the surface ponded water. Well 5C in the sand cap rapidly went dry during the pre-sampling purge process, which could indicate the presence of unsaturated lenses throughout the sand layer.

Characterization of fines from the June 2014 field campaign demonstrated depth-dependent differences in both the mass and composition of fines in the well water (Figure 4.5). Visual inspection showed that sand cap fines closely resembled CT fines, as they were both uniform in colour and size distribution (almost all sediment retained on a >0.45 um cellulose acetate membrane filter). Fine particles filtered from the wetland interface wells had a heterogeneous size distribution, containing pieces of woody debris and organic material from the peat layer. Fines content decreased with depth, with the highest reported solids content found in the wetland interface wells (1.83 g/L), and the lowest found in the CT layer (0.05 g/L).

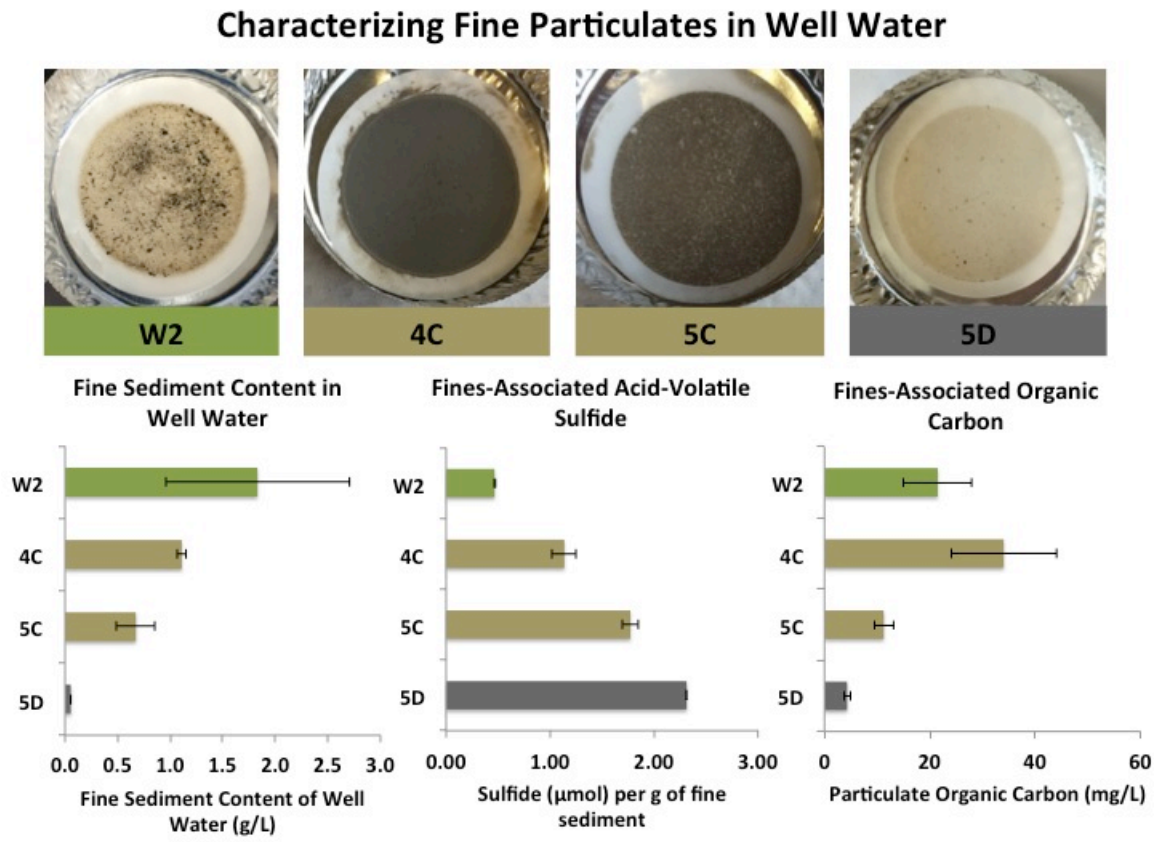


Figure 4.6: Characterizing fine particulates in well water. Error bars represent 1 standard error of the mean, from replicates of n=3-5.

Normalized for mass of sediment in each well, fines-associated acid-volatile sulfide (AVS) increased with depth. AVS per gram of CT fines was 5 times higher than AVS detected in the wetland-interface sample. Therefore, the pool of particle-associated acid-volatile sulfides at depth is highly dependent on the abundance of fines in each layer. AVS, which includes dissolved S(-II) (H_2S, HS^-, $FeHS^+$), FeS clusters, and nanoparticulate iron sulfides, is generally the dominant product of microbial sulfate reduction, suggesting that sulfate reduction is occurring at all depths in the deposit

(Stasik, 2014). Acid-extractable sulfate values were below detection limits, indicating that sulfate in the deposit is likely entirely dissolved.

The pool of fines-associated organic carbon was lower in the CT than in the overlying sand and wetland. As wells W2 (wetland-interface) and 4C (sand cap) contained the highest concentrations of fine particulates per litre of porewater, the fines-associated organic carbon pool is also highest in these wells. The decreasing particulate organic carbon pool with depth suggests that wetland particulate carbon is migrating downwards into the sand cap.

4.6 Potential Microbial Metabolisms Present At Depth

As abiotic sulfur reduction at temperatures below 150°C is kinetically very slow, the presence of reduced sulfur in the deposit must be attributed to the presence of sulfur reducing bacteria (SRB). To assess the metabolic capacity of the endemic microbial community, porewater enrichments were designed to promote the growth of sulfur oxidizing bacteria and sulfur reducing bacteria. At ~25°C, and under anoxic, neutrophilic conditions, positive growth was observed for both metabolisms in all depths. Positive growth of sulfur reducing bacteria was observed using sulfate, thiosulfate, and elemental sulfur as the sole sulfur sources, indicating that heterotrophic and disproportionating metabolisms exist in all three layers of the deposit. Consistent with high levels of sulfide in the sand cap, the enrichments from wells 5C and 4C demonstrated a very rapid positive growth response in the sulfur reducing media. Well 5D showed a very slow positive growth, which may be linked to low levels of particulate matter (and therefore low biomass) in the sample.

5.0 Experimental Results and Discussion

Initial characterization of microcosm water showed that sand cap and CT water are physicochemically identical (Table 5.1). Both solutions are circumneutral, reducing, and brackish. In comparison, the wetland microcosms were more acidic and less salty.

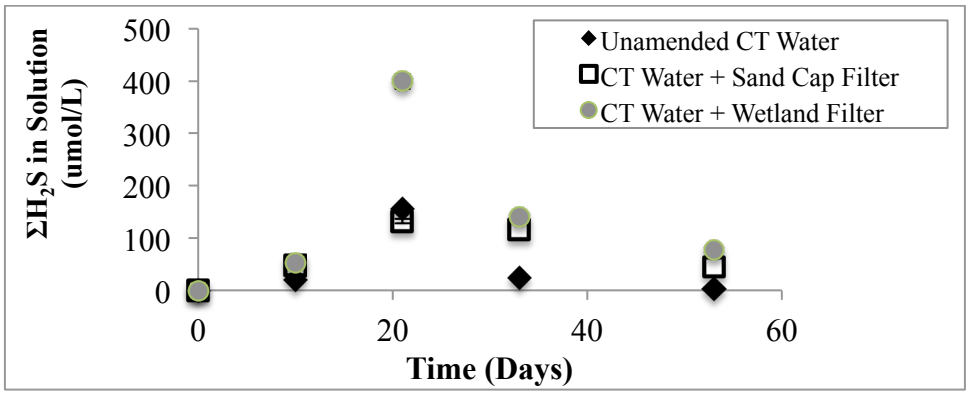
Table 5.1: Physicochemical characterization of microcosms at t=0

Treatment Name	pH	Temp. (°C)	Eh (mV)	Total Dissolved Solids (mg/L)	Conductivity (mS/cm)	Salinity (ppt)
Unfiltered CT Water	7.22	22.2	-13.9	1856	3.79	2
Unfiltered CT Water + Sand Cap Filter	7.28	24	-14.6	1845	3.81	2
Unfiltered CT Water + Wetland Filter	6.86	24.6	-9.2	1860	3.80	2
Sterile Filtered CT Water	7.18	23	-11.6	1827	3.73	2
Filtered CT Water + CT filter	7.17	23.9	-11.5	1827	3.73	2
Filtered CT Water + Wetland Filter	7.17	23.7	-11.3	1820	3.71	2
Filtered CT Water + Sand Cap Filter	7.14	23.3	-9.4	1836	3.75	2
Unfiltered Sand Cap Water	7.23	22.3	-15.1	1953	3.99	2.1
Unfiltered Sand Cap Water + CT Filter	7.19	24.2	-12.1	1942	3.96	2.1
Unfiltered Sand Cap Water + Wetland Filter	7.21	22.2	-19.8	1953	3.99	2
Sterile Filtered CT Water	7.15	22.7	-10.5	1940	3.95	2.1
Filtered Sand Cap Water + CT Filter	7.22	23.2	-14.5	1930	3.94	2.1
Filtered Sand Cap Water + Wetland Filter	7.19	23.7	12.9	1915	3.91	2.1
Filtered Sand Cap Water + Sand Cap Filter	7.22	23.7	-13.7	1928	3.94	2.1
Unfiltered Wetland Water + CT Filter	4.87	24.6	120	302	0.61	0.3
Unfiltered Wetland Water	5.03	23.1	111.4	314	0.64	0.3
Unfiltered Wetland Water + Sand Cap Filter	4.69	24.2	130.1	318	0.65	0.3
Sterile Filtered Wetland Water	4.66	23.5	132.3	288	0.59	0.3
Filtered Wetland Water + CT Filter	4.86	25.1	121.1	272	0.56	0.3
Filtered Wetland Water + Wetland Filter	4.6	24.9	130	277	0.57	0.3
Filtered Wetland Water + Sand Cap Filter	4.8	25.2	121.2	292	0.60	0.3

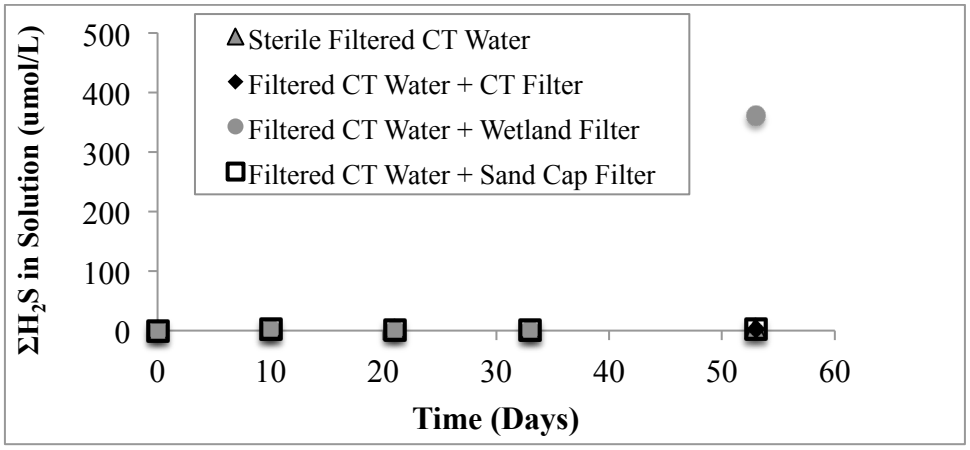
5.1 $\Sigma H_2S_{(aq)}$ Generation

In all treatments but one, $\Sigma H_2S_{(aq)}$ generation was significantly higher (P>0.05) in the unfiltered samples than in the filtered samples, suggesting that fines play a critical role in stimulating sulfur reduction (Figure 5.1, Table 5.2). $\Sigma H_2S_{(aq)}$ concentrations in the unfiltered treatments peaked at t=21 and decreased thereafter, indicating that optimal growth conditions were exceeded beyond this time point and sulfur reduction was likely substrate limited. Peak $\Sigma H_2S_{(aq)}$ concentrations at t=21 were 399.4 uM in the CT treatment (Unfiltered CT water + Wetland filter) and 389.0 uM in the sand treatment (Unfiltered sand cap water + CT filter). While the highest observed value of $\Sigma H_2S_{(aq)}$ was detected in a CT treatment, all other CT treatment concentrations fell below 200 uM. Treatments containing unfiltered sand cap water demonstrated higher $\Sigma H_2S_{(aq)}$ values as a group. Wetland treatments did not exceed 1 uM, supporting the hypothesis that CT-derived fines have a more significant mechanistic control on $\Sigma H_2S_{(aq)}$ generation than wetland-derived fines. Treatments from t=0 to t=33, solution $\Sigma H_2S_{(aq)}$ was < 2.2 umol and indistinguishable from abiotic $\Sigma H_2S_{(aq)}$ generation in the sterile treatments. The removal of fines appears to have inhibited $\Sigma H_2S_{(aq)}$ generation. While it is difficult to constrain the effect of augmenting the endemic microbial community with microorganisms from elsewhere in the deposit, treatments with microbially-enriched filter additions from different source waters appeared to out-perform endemic communities in terms of observed $\Sigma H_2S_{(aq)}$ values. This trend may be linked to increased biomass or microbial diversity in filter-augmented treatments. Notably, wetland-augmented treatments showed a significant increase in $\Sigma H_2S_{(aq)}$ concentrations at t=53. The magnitude of produced $\Sigma H_2S_{(aq)}$ matches the peak values obtained in unfiltered CT and sand cap samples. The

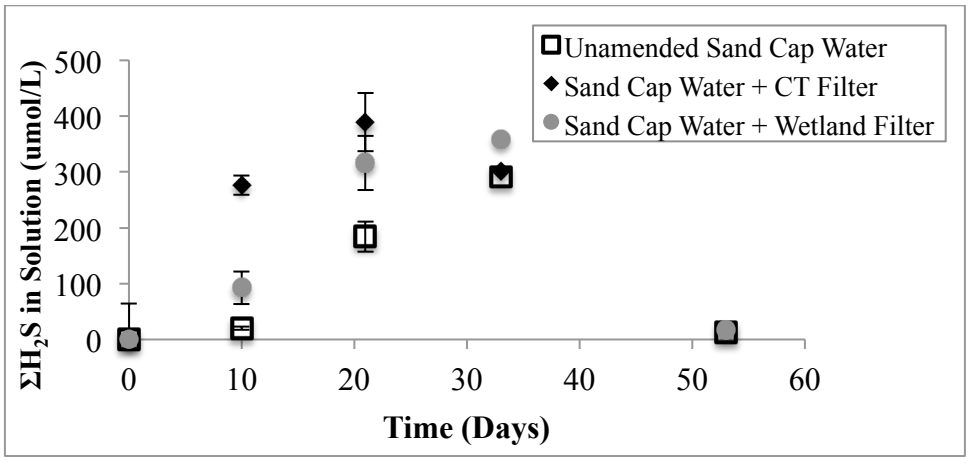
magnitude of $\Sigma H_2S_{(aq)}$ accumulation in only the wetland-augmented filtered samples suggests that the wetland microbial communities likely utilized dissolved sulfate for sulfur reduction, which was not the case for the other filtered treatments.



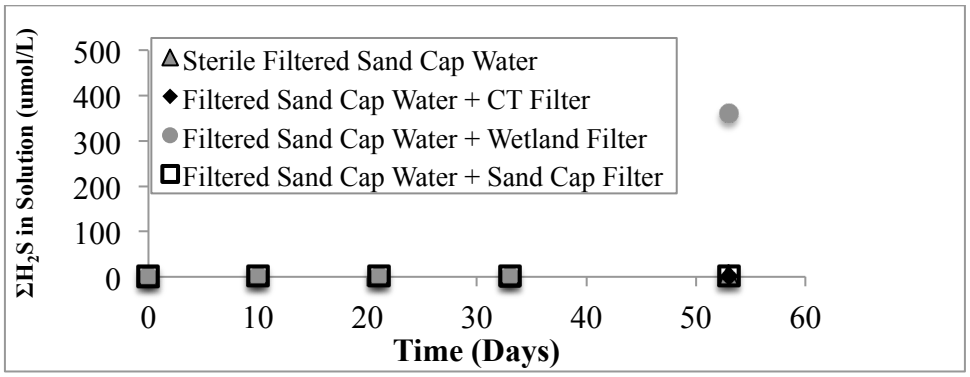
A. Unfiltered CT Treatments



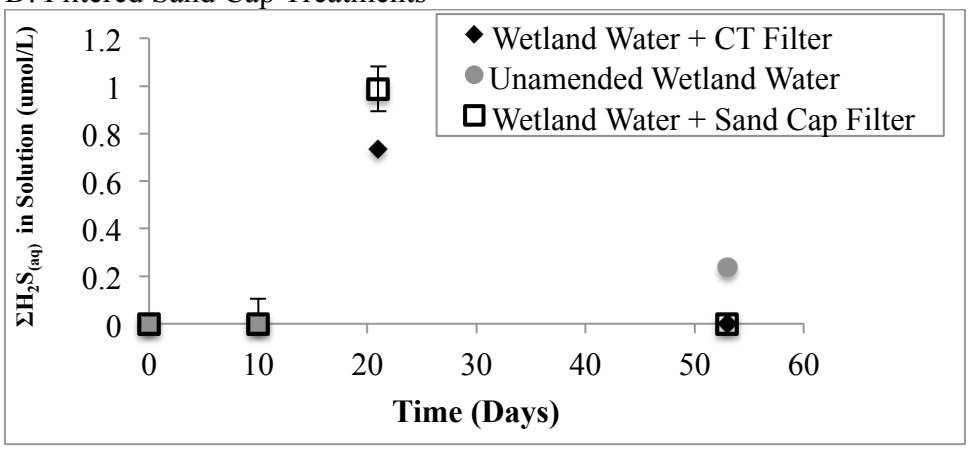
B. Filtered CT Treatments



C. Unfiltered Sand Cap Treatments



D. Filtered Sand Cap Treatments



E. Unfiltered Wetland Treatments

Figure 5.1: $\Sigma H_2S_{(aq)}$ concentrations in all microcosm treatments over t=53 days. Filtered wetland treatments showed no detectable $\Sigma H_2S_{(aq)}$. Error bars represent 1 standard error of the mean, with replicates of n=4.

Table 5.2: $\Sigma H_2S_{(aq)}$ concentrations in each microcosm treatment. ND indicates that $\Sigma H_2S_{(aq)}$ was not detected. NS indicates parameters that were not sampled.

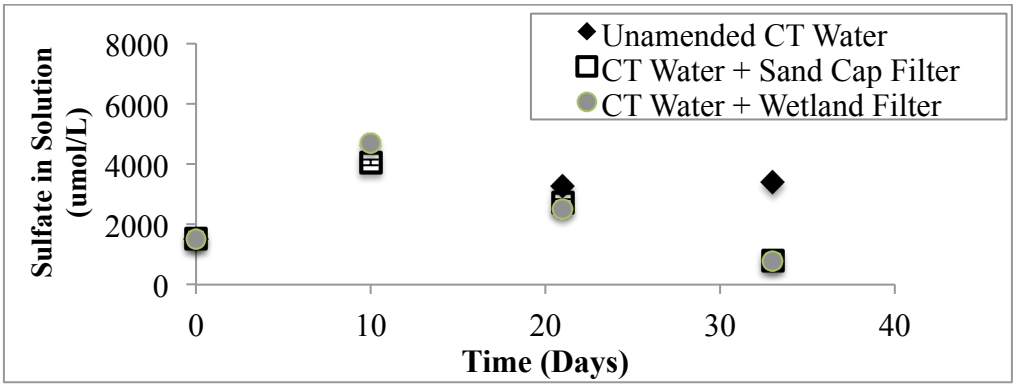
Treatment Name	ΣH_2S in Solution (µmol/L)									
	t=0		t=10		t=21		t=33		t=53	
	Mean	± 1 Std. Err.	Mean	± 1 Std. Err.	Mean	± 1 Std. Err.	Mean	± 1 Std. Err.	Mean	± 1 Std. Err.
Unfiltered CT Water	ND	-	19.9	0.3	156.3	33.2	22.6	1.4	2.0	0.9
Unfiltered CT Water + Sand Cap Filter	ND	-	47.4	2.8	132.2	2.4	115.4	4.5	44.9	2.3
Unfiltered CT Water + Wetland Filter	ND	-	52.4	2.6	399.4	6.3	140.6	14.3	77.1	5.3
Sterile Filtered CT Water	ND	-	1.7	<0.1	2.1	0.3	0.5	<0.1	2.7	0.3
Filtered CT Water + CT filter	ND	-	1.8	0.1	1.1	0.1	1.2	0.3	1.7	0.1
Filtered CT Water + Wetland Filter	ND	-	2.1	0.1	1.0	0.1	1.1	0.1	360.5	6.9
Filtered CT Water + Sand Cap Filter	ND	-	1.9	<0.1	1.1	0.1	1.4	0.2	1.7	0.2
Unfiltered Sand Cap Water	ND	-	20.0	5.5	184.2	2.7	291.0	27.1	12.9	1.4
Unfiltered Sand Cap Water + CT Filter	ND	-	275.9	64.0	389.0	17.2	301.6	51.9	-	-
Unfiltered Sand Cap Water + Wetland Filter	ND	-	92.5	8.2	316.1	29.0	358.9	48.6	17.5	0.4
Sterile Filtered Sand Cap Water	ND	-	0.2	<0.1	0.3	0.3	6.1	5.6	0.0	<0.1
Filtered Sand Cap Water + CT Filter	ND	-	0.4	0.1	0.0	<0.1	0.7	0.4	0.8	<0.1
Filtered Sand Cap Water + Wetland Filter	ND	-	0.2	<0.1	0.1	0.1	1.4	0.2	153.6	3.9
Filtered Sand Cap Water + Sand Cap Filter	ND	-	0.2	<0.1	1.4	1.0	1.2	0.1	0.8	<0.1
Unfiltered Wetland Water + CT Filter	ND	-	ND	-	0.7	0.1	NS	-	ND	-
Unfiltered Wetland Water	ND	-	ND	-	ND	-	NS	-	0.2	0.1
Unfiltered Wetland Water + Sand Cap Filter	ND	-	ND	-	1.0	0.1	NS	-	ND	-
Sterile Filtered Wetland Water	ND	-	ND	-	ND	-	NS	-	ND	-
Filtered Wetland Water + CT Filter	ND	-	ND	-	ND	-	NS	-	ND	-
Filtered Wetland Water + Wetland Filter	ND	-	0.2	0.1	ND	-	NS	-	ND	-
Filtered Wetland Water + Sand Cap Filter	ND	-	ND	-	ND	-	NS	-	ND	-

5.2 Sulfate Consumption

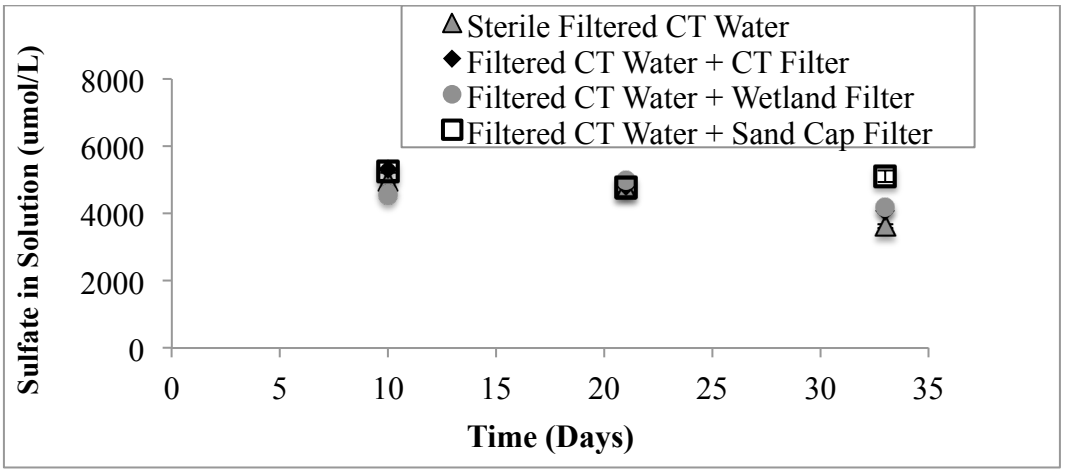
In the unfiltered CT and sand cap treatments, SO_4^{2-} decreased linearly with time after t=10, suggesting a constant rate of sulfur reduction was occurring in each treatment (Figure 5.2, Table 5.3). SO_4^{2-} values in the CT and sand cap were very similar, as they ranged from 781-4674 uM in the CT unfiltered treatments, and 865.7-5110.7 uM in the sand cap treatments. Sulfate concentrations in the unfiltered wetland increased over time, suggesting that sulfur oxidation rates exceeded those of sulfur reduction. Filtered samples did not display the same degree of sulfate decrease over time, indicating that sulfur reduction is not nearly as active without the presence of fines.

Peak SO_4^{2-} reduction rates of ~218 nmol mL^{-1} day^{-1} occurred in the unfiltered sand cap water, which greatly exceeds measured rates in active tailings ponds by Stasik et al., (90 nmol mL^{-1} day^{-1}, 2014) and Ramos-Padron (50 nmol mL^{-1} day^{-1}). Beyond t=10, sulfate decreased linearly in the unfiltered CT+Wetland filter treatment (R^2=0.99),

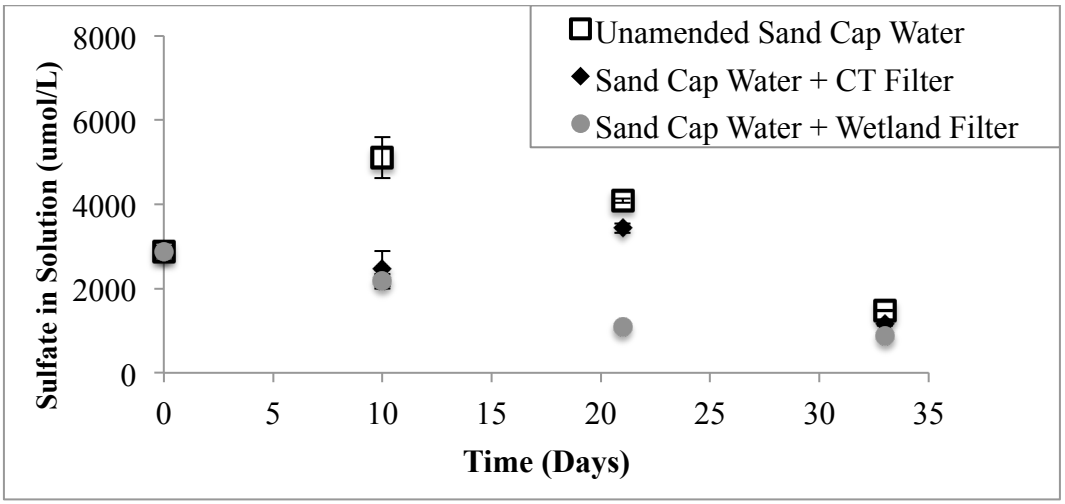
CT+Sand Cap filter treatment (R^2=0.99), Sand Cap+Wetland filter treatment (R^2=0.86) and the Unamended Sand Cap treatment (R^2=0.95). This linear decrease of sulfate is unexpected as it suggests that the rate of sulfate reduction occurred independently from the amount of available sulfate in solution. In comparison, peak $\Sigma H_2S_{(aq)}$ accumulation rates of ~ 0.9 umol mL^{-1} day^{-1} were observed in the sand cap samples from t=0 to t=10, representing $\Sigma H_2S_{(aq)}$ accumulation rates of approximately 28 nmol mL^{-1} day^{-1}. The rates of $\Sigma H_2S_{(aq)}$ accumulation and SO_4^{2-} reduction are decoupled, as the rates of change in SO_4^{2-} and $\Sigma H_2S_{(aq)}$ concentrations do not display a 1:1 relationship. Considering the sulfur mass balance between maximum and minimum observed sulfate values, >80% of CT sulfur and 86% of sand cap sulfur is unaccounted for by $\Sigma H_2S_{(aq)}$. Therefore, $\Sigma H_2S_{(aq)}$ cannot account for the observed linear decrease in SO_4^{2-} concentrations, indicating that there are other mechanisms regulating $\Sigma H_2S_{(aq)}$ accumulation in the microcosms. To account for the lost sulfate, one of three processes likely occurred: 1) $\Sigma H_2S_{(aq)}$ incorporation in amorphous FeS or FeS_2, 2) $H_2S_{(g)}$ loss from the microcosm; or 3) disproportionation of the reduced sulfur, with a significant portion of the total microcosm sulfur pool consisting of sulfur oxidation intermediates.



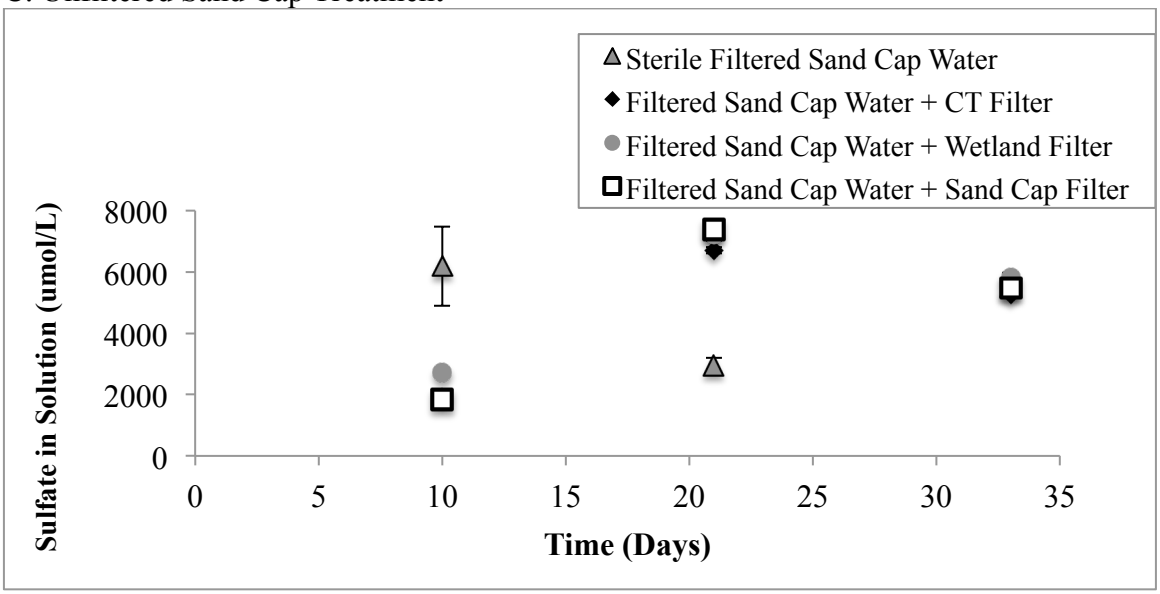
A. Unfiltered CT Treatments



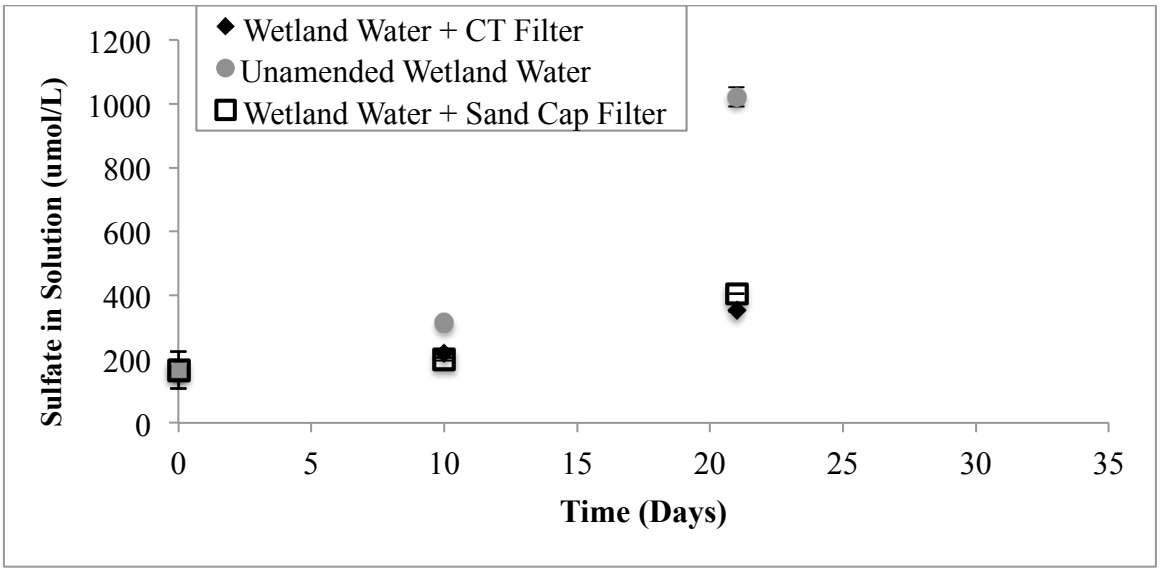
B. Filtered CT Treatment



C. Unfiltered Sand Cap Treatment



D. Filtered Sand Cap Treatment



E. Unfiltered Wetland Treatment. T=33 was not sampled for the wetland microcosms.
Figure 5.2: Sulfate concentrations in each treatment over t=31 days

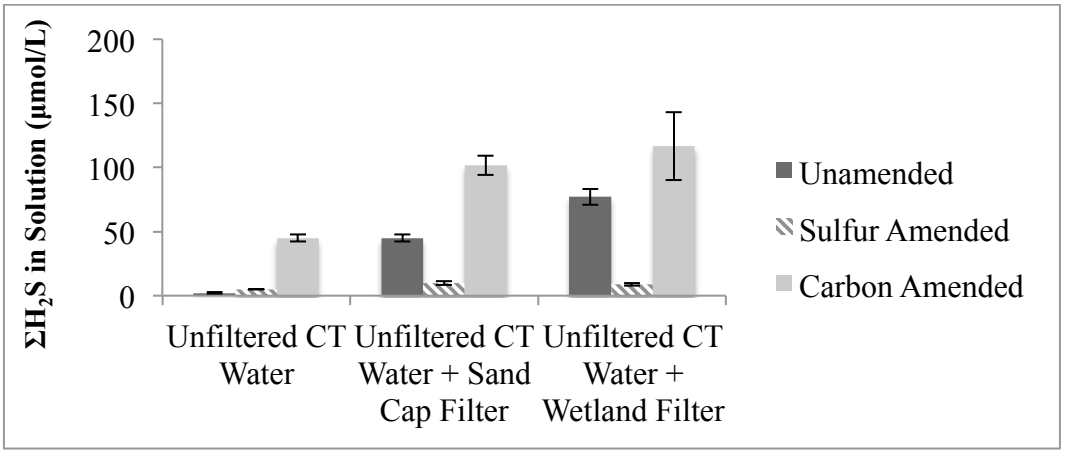
Table 5.3: Sulfate concentrations in each microcosm treatment

Treatment Name	Sulfate in Solution (µmol/L)							
	t=0		t=10		t=21		t=33	
	Mean	± 1 Std. Err.	Mean	± 1 Std. Err.	Mean	± 1 Std. Err.	Mean	± 1 Std. Err.
Unfiltered CT Water	1525.9	91.8	-	-	3286.4	60.5	3384.3	73.8
Unfiltered CT Water + Sand Cap Filter	1525.9	91.8	4052.8	105.0	2717.9	117.3	800.0	35.5
Unfiltered CT Water + Wetland Filter	1525.9	91.8	4674.0	160.2	2519.7	90.8	781.2	14.7
Sterile Filtered CT Water	NS	-	4961.1	39.4	4739.3	109.6	3623.9	61.1
Filtered CT Water + CT filter	NS	-	5310.8	113.1	4808.2	161.1	4089.1	54.2
Filtered CT Water + Wetland Filter	NS	-	4553.8	33.2	5003.5	45.9	4187.7	47.7
Filtered CT Water + Sand Cap Filter	NS	-	5246.1	34.5	4785.3	181.0	5104.0	167.2
Unfiltered Sand Cap Water	2876.3	133.5	5110.7	488.8	4086.4	50.0	1476.4	14.1
Unfiltered Sand Cap Water + CT Filter	2876.3	133.5	2472.7	417.2	3432.0	109.3	1152.3	32.3
Unfiltered Sand Cap Water + Wetland Filter	2876.3	133.5	2172.4	172.9	1102.6	39.4	865.7	32.5
Sterile Filtered Sand Cap Water	NS	-	6184.1	1286.2	2958.0	248.8	5511.1	78.6
Filtered Sand Cap Water + CT Filter	NS	-	1913.2	38.2	6704.3	96.8	5266.9	217.0
Filtered Sand Cap Water + Wetland Filter	NS	-	2712.0	35.9	7233.9	211.4	5802.4	164.8
Filtered Sand Cap Water + Sand Cap Filter	NS	-	1841.2	29.9	7371.6	219.7	5468.9	136.9
Unfiltered Wetland Water + CT Filter	165.7	58.0	217.3	13.1	351.2	2.7	NS	-
Unfiltered Wetland Water	165.7	58.0	313.7	2.7	1022.0	30.2	NS	-
Unfiltered Wetland Water + Sand Cap Filter	165.7	58.0	199.9	5.5	404.5	1.2	NS	-
Sterile Filtered Wetland Water	NS	-	307.4	5.6	413.9	4.1	NS	-
Filtered Wetland Water + CT Filter	NS	-	136.5	0.8	407.6	<0.1	NS	-
Filtered Wetland Water + Wetland Filter	NS	-	238.5	0.0	443.7	4.0	NS	-
Filtered Wetland Water + Sand Cap Filter	NS	-	117.6	1.0	351.2	2.7	NS	-

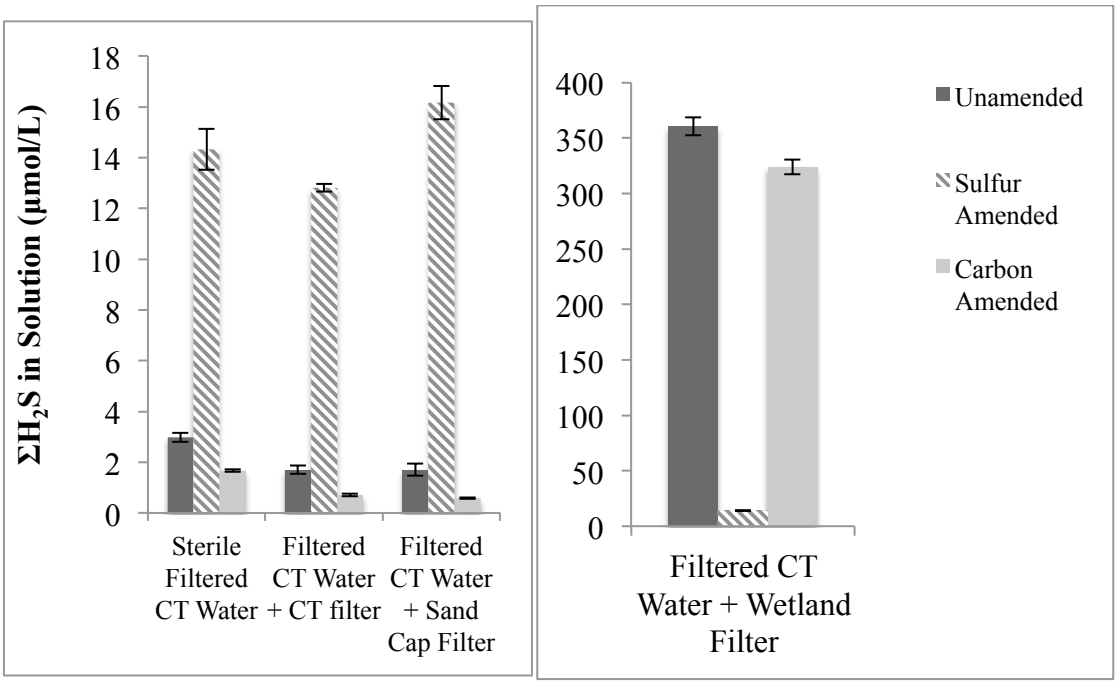
ND indicates that $\Sigma H_2S_{(aq)}$ was not detected. NS indicates parameters that were not sampled.

5.3 Microcosm carbon and sulfur amendments

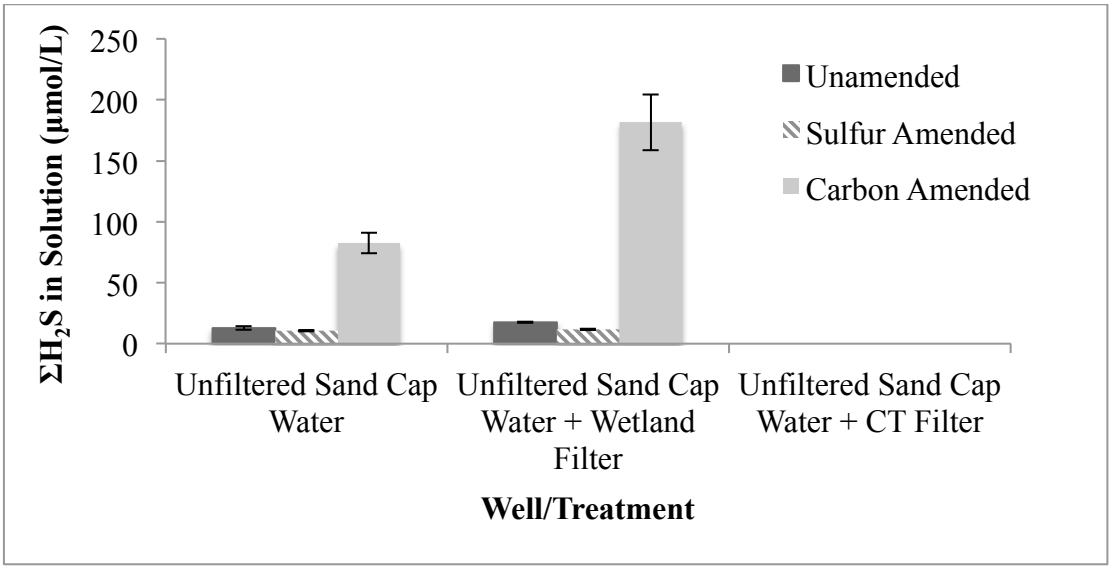
To assess which metabolic substrates were limiting $\Sigma H_2S_{(aq)}$ generation after t=33 days, labile organic carbon and SOI additions were added to sand cap and CT treatments. In all carbon-amended unfiltered treatments, resulting $\Sigma H_2S_{(aq)}$ concentrations on t=53 were significantly higher ($p<0.05$, Figure 5.2). The increase in $\Sigma H_2S_{(aq)}$ concentrations over unamended samples indicates that microbial sulfur reduction in the sand cap and CT microcosms was limited by the availability of labile organic carbon. Carbon did not stimulate $\Sigma H_2S_{(aq)}$ generation in filtered samples, suggesting that $\Sigma H_2S_{(aq)}$ generation rates were not controlled by carbon accessibility. The addition of thiosulfate and elemental sulfur increased $\Sigma H_2S_{(aq)}$ concentrations in filtered systems, but the observed increase was not significantly different from the increase in sterile controls.



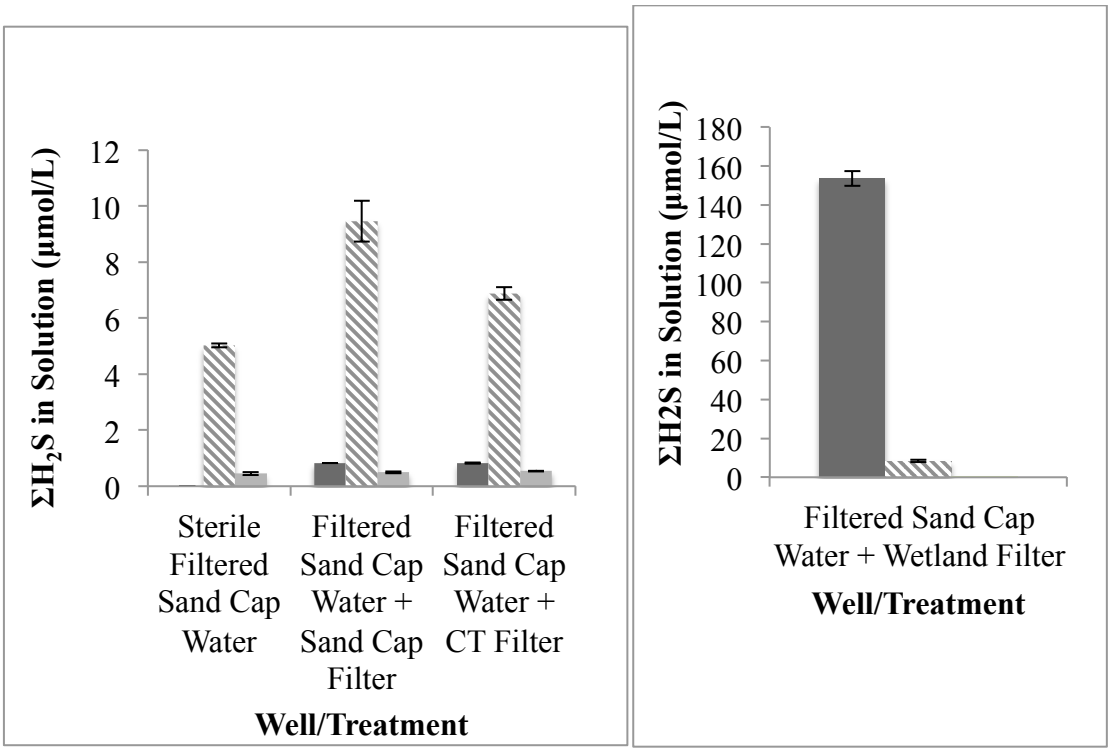
A. Unfiltered CT treatment amendments.



B. Filtered CT amendments



C. Unfiltered sand cap treatment amendments. Data for unfiltered sand cap + CT filter is unavailable.



D. Filtered sand cap Treatment amendments. Data for carbon-amended Filtered Sand Cap Water + Wetland Filter is unavailable.

Figure 5.3: Carbon and sulfur amendments for t=53 sand cap and CT treatments

5.4 Microcosm Experiment 2: Carbon and Sulfur Stimulation of Sand Cap $\Sigma H_2S_{(aq)}$ Generation

Over ten days, unfiltered sand cap water generated $\Sigma H_2S_{(aq)}$ at levels 1-2 magnitudes higher than all other treatments (Figure 5.4). The filtered sand cap water amended with its own filter displayed the third highest experimental $\Sigma H_2S_{(aq)}$. This suggests that the filtration step separating fines from solution disrupts the mechanisms associated with sulfur reduction. Thiosulfate stimulation displayed the highest $\Sigma H_2S_{(aq)}$ concentrations out of all sulfur and carbon amendments, suggesting that thiosulfate is likely a preferable substrate to sulfate for microbial sulfur reduction. In consideration with the results of the first microcosm experiment, amendment with labile carbon did not

generate significantly greater concentrations of $\Sigma H_2S_{(aq)}$ than sand cap fines on their own. This suggests that the fines contain an accessible source of organic carbon for sulfur reduction, and that carbon was not limiting sulfur reduction rates after 10 days.

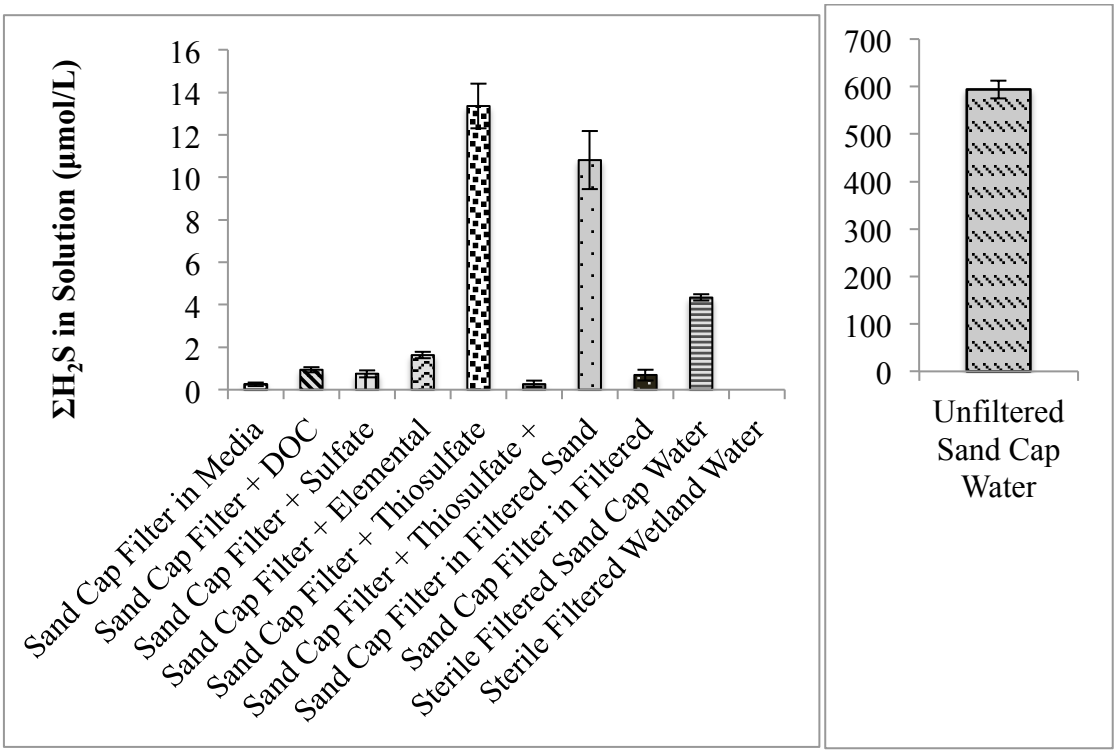


Figure 5.4: Microcosm amendments assessing $\Sigma H_2S_{(aq)}$ stimulation in sand cap fines

6.0 Interpreting the sulfur biogeochemistry of the Sandhill Fen

6.1 Microbial cycling of sulfur and carbon

Based on the basic equation for microbial sulfate reduction (Equation 9), we might expect the magnitude of sulfide to correlate with sulfate or DOC values.

$$(9)\ SO_4^{2-} + CH_2O \rightarrow H_2S + CO_2$$

Throughout the deposit as a whole, sulfide does not correlate to magnitude of observed sulfate. Abundant sulfate is present throughout the entire deposit at levels that are significantly higher than observed sulfide values. Sulfate availability is therefore not the dominant control on sulfur-reduction, as the microbial communities at all depths have readily accessible sulfate. Field samples from 2013 preserved for total sulfur show that an undefined fraction of total sulfur exists, which likely represents sulfur oxidation intermediates. At some depths, this fraction accounts for 30-50% of total sulfur, suggesting that sulfur intermediates may play a critical role in microbial sulfur metabolisms. Positive growth for sulfur reducing metabolisms on thiosulfate and elemental sulfur established the presence of sulfur disproportionating bacteria at all depths. Experimental microcosms demonstrated a microbial affinity for thiosulfate as a metabolite in sulfur reduction, showing greater generation of ΣH_2S in the presence of thiosulfate than sulfate. Therefore, the in situ microbial community may preferably metabolize thiosulfate or elemental sulfur, allowing the rate of sulfur reduction to be stimulated or constrained by sulfur intermediates rather than sulfate availability. This

means that the rate of sulfur cycling in the deposit cannot be fully constrained by measuring just the sulfur redox end-members of sulfide and sulfate. While the unamended bulk-water microcosm experiments indicated that sulfur reduction in the sand cap is largely heterotrophic, the potential exists for sulfur disproportionation to occur in situ regardless of the availability of labile organic carbon.

Dissolved organic carbon values are high throughout all layers, but the bioaccessibility and lability of carbon differs between the wetland and the sand/CT layers. Sand cap and CT DOC likely reflect more recalcitrant forms of carbon remaining from the extracted fine tailings, which contained residual bitumen (0.5-5% by mass) and naptha diluent (<1% by mass) (Penner & Foght, 2010). Recent analysis by Bradford et. al (in prep) has demonstrated that total organic carbon associated with the sand layer is significantly isotopically depleted in $\Delta^{14}C$ compared to the peat-associated carbon. This suggests that carbon in the sand and CT layers is petroleum-based, whereas the wetland carbon is in a younger, more labile form. When comparing biomass per gram of sediment in the sand layer to that of wetland peat, there was no correlation to concentration of total organic carbon. Biomass grouped more closely to the age of the carbon rather than the concentration, suggesting that the form of organic carbon is a more important control on microbial community growth than the availability of total organic carbon (Bradford et al. – in prep). $\Delta^{14}C$ values from microbial phospholipids showed that the bacterial community preferentially assimilated isotopically 'younger' carbon. While total biomass accounts for microorganisms that perform a variety of metabolisms, Bradford's results support the hypothesis that sulfur reducing microbial communities may be limited by the form, rather than the abundance, of organic carbon at depth. This hypothesis is further

supported by the microcosm results indicating increased ΣH_2S generation in the presence of abundant acetate and lactate. These results indicate that most wetland-associated organic carbon is bioaccessible and oxidized during sulfur reduction, whereas DOC in the sand cap may be more recalcitrant with only a small fraction is accessible to SRB. As particulate DOC is elevated in the sand cap and wetland with respect to the CT, the potential exists for downward migration of labile wetland carbon into the sand below. This reactive carbon would stimulate the carbon-limited heterotrophic sulfur reducers, potentially escalating sulfur reduction rates to levels of concern. Therefore, understanding water exchange between the sand cap and wetland is of critical importance for predicting possible implications for sulfur cycling in both layers as reclamation proceeds.

6.2 Sand cap as a dynamic mixing zone

The sand layer is a unique region in the deposit as both the wetland and the CT influence its porewater geochemistry. Designed to be an inert structural support component of the deposit, it is a highly dynamic layer receiving complex fluxes from above and below. While groundwater flow is unconstrained in this system, the movement of dissolved species through the pore matrix is not diffusive.

Underground drains situated between the wetland and the sand layers were installed to maintain the artificial water table required for the wetland plant species. When the drains are turned on, they prevent salty water in the sand from migrating up into the wetland, but they also allow porewater to move upwards and horizontally through the sand. Dewatering at the sump vault, where pumps at the eastern end of the property aid in the flushing of the CT layer, also facilitates horizontal movement across

the deposit. Flow in this system is further complicated by management practices, as the pumps at the research wetland are turned on and off at different intervals. The migration of fine particulates into the sand layer has inhibited hydraulic conductivity by reducing pore sizes. This has lead to slow recharge in sand cap wells and an over-pressurized CT well where the piezometric surface was nearly at the top of the well casing (1m above ground level). From these observations, it appears that water in the CT layer may be confined and under pressure in some areas, with unsaturated lenses in the sand cap. Unsaturated pore spaces may also have the potential to build up H_2S_g underground. Therefore, vertical and horizontal flow through this highly managed deposit is nearly impossible to model.

The use of conservative tracer salts has shown that sand cap water is highly chemically similar to CT water, supporting the model of water exchange between these two layers. Simple mixing scenarios based on porewater and fines contributions from the wetland and CT could not replicate the AVS or carbon concentrations observed in the sand layer. In addition to aqueous sulfide and ferrous iron, solid-associated AVS and values were higher per litre of sand cap porewater than either in the CT or wetland. Particulate organic carbon (mg/L) values were elevated above CT levels, suggesting that fines-associated carbon from the wetland is likely moving down into the sand layer. This zonation indicates that microbial transformations of sulfur, iron, and carbon occurred as porewater entered the sand cap.

For all sampled wells and seasons, sulfide was consistently highest in the sand cap. Despite a highly managed water regime in the constructed wetland and CT layer,

chemical zonation of sulfide persists. To achieve such an elevated level of sulfide in this zone, one of two processes must have occurred:

1) Generation of ΣH_2S *in situ* through bacterial sulfur reduction. Microbial sulfate reduction rates could be enhanced in the sand cap due to high biomass, optimal growth conditions, or high availability of labile carbon and preferred sulfur sources.
2) Generation of ΣH_2S in the *CT layer*, which then migrated upwards and accumulated in the sand layer.

As discussed above, downward fluxes of labile carbon from the wetland may have stimulated the growth of sulfur reducing bacteria in the sand layer. Despite having a lower total biomass than the peat layer, a large percentage of the bacterial community in the sand might be able to metabolize sulfur (Bradford et al, in prep). In replicate enrichments for sulfur reducing bacteria in the porewater, sand cap samples showed positive growth much faster than wetland and CT samples. In the Mildred Lake Settling Basin, cell counts for sulfur reducing and sulfur oxidizing bacteria were significantly elevated at a depth of 5.5-7.5 m, correlating to peak values of sulfate reduction and thiosulfate oxidation potentials (Stasik et al, 2014). Thiosulfate oxidation rates were approximately 6x higher than sulfate reduction rates in this zone, demonstrating very active sulfur cycling at a depth similar to our sand cap wells. Perhaps fueled by a more accessible labile carbon source, sulfate reduction rates could increase as heterotrophic sulfur bacteria accessed a variety of sulfur redox species.

Alternatively, the elevated levels of sulfide in the sand cap may represent an accumulation of sulfidic compounds that were reduced in the CT layer. While the fines content of the well water in the sand cap increased over time, very low fines concentrations were observed in the CT water over multiple sampling trips. Fines moving upwards into the sand cap could act as transport vectors for reduced sulfur (AVS) and organic carbon. If we consider that sand cap fines likely originated as CT fines, then there was a loss of both solid-associated AVS and organic carbon as these fines moved upwards. As acid volatile sulfide generally consists of dissolved S(-II), iron sulfide nanoparticles, and amorphous FeS clusters (mackinawite, greigite), the increased sulfide and ferrous iron in solution might partly be a product of iron sulfide dissolution (Rickard and Morse, 2005).

As the solid content of porewater is highest where sulfide is elevated, it appears that the presence of CT fines is an important control on sulfur reduction in this system. As demonstrated in the experimental microcosms, removal of fines by filtration disrupted the ability of the endemic community to reduce sulfur, even when the fines were returned to the filtrate. This reliance on CT fines to stimulate $\Sigma H_2S_{(aq)}$ generation suggests that the fines-microbial complex is integral to sulfur cycling in this deposit, and that heterotrophic sulfur reducers may preferably accumulate on the fines. Fines in this system may act as colonization sites for microbial communities, allowing the bacteria to easily access organic carbon or sulfur compounds adhered to the particle's surface (Dawson et al, 1981). Adhesion to fines would facilitate the dispersion of sulfur cycling communities through the porous deposit, and the microbial-fines aggregates move upwards to enhance sulfur reduction rates near the wetland surface. Further work should explore the

relationship between oil sands fines and endemic microbial communities to elucidate the role of fine particulates in microbial sulfur reduction.

7.0 Conclusion

This study establishes the widespread presence of reduced sulfur compounds throughout the composite tailings, with biogeochemical sulfur cycling occurring in all layers of the deposit. Despite a highly managed water management regime, peak sulfide was consistently localized within the sand layer. The presence of high sulfide in this layer can be explained either by enhanced microbial sulfate reduction in situ due to preferred substrate availability, or upward mobilization of sulfide into the sand layer from the CT. The distribution of sulfate did not predict the elevated levels of sulfide in the sand cap, indicating that sulfur oxidation intermediates or more labile carbon sources are likely controlling sulfur reduction rates in the sand layer. In addition to experimental microcosm results, a strong positive correlation between sand cap organic carbon and sulfide is consistent with the hypothesis of organic carbon lability constraining microbial $\Sigma H_2S_{(aq)}$ generation. Conservative salt tracer concentrations showed that sand and CT porewaters are definitively linked, with clear upward movement of sodium and chloride into the sand cap. This vertical flux also influenced fine sediment transport, as CT-derived fines accumulated in the sand layer. These fines are transport vectors for reduced sulfur and organic carbon, as they facilitate redistribution of tailings-derived sulfur and carbon compounds to upper layers of the deposit. As sulfur-reducing microbial enrichments demonstrated a rapid growth response in samples with high CT-fines

contents, fines in the porewater might act as integral growth support structures for microbial communities. Further work should investigate the association between porewater fines and sulfide generation.

As dry reclamation landscapes are set to increase in the oil sands region, an understanding of sulfur dynamics in these novel, highly altered settings is critical for effective tailings management. This research stresses the importance of investigating sulfide generation mechanisms in CT, as the potential for outgassing exists if $\Sigma H_2S_{(aq)}$ concentrations exceed the capacity of dissolved iron to strip it from solution . As the reclamation wetland evolves and drain operations are set to end, free water exchange will occur between the wetland and the sand layer. Carbon transport between the wetland and sand layer has the potential to greatly stimulate sulfur reduction. To mitigate the generation of H_2S and ensure the long-term sustainability of these new reclamation landscapes, it is critical to understand the potential microbial dynamics in the system and their possible effects on sulfur biogeochemical cycling.

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