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Productivity and carbon accumulation potential of transferred biofilms in reclaimed oil sands-affected wetlands

by

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For my mother and father

and for all parents who possess such

unflinching and unwavering faith in their children.

For my brother, who has chosen a

life of great honour, responsibility and risk

as a police officer.

For God, who has always had such

a strong positive presence in my life of science.

Abstract

Biofilms are significant contributors to primary production, nutrient cycling, bio-stabilization and the food web of wetland ecosystems. Photoautotrophic biomass (PB) and primary production (PP) were determined for biofilms exposed to various treatments and materials in wetlands near Fort McMurray. Biofilm additions and oil sands process-affected materials were expected to increase the microbial colonization rates on treated substrates and subsequently PB and PP of biofilms over time as compared to controls and unaffected materials. Biofilms survived the transfers and colonized new substrates immediately. Oil sands process affected materials were found to increase PB and PP throughout the first year. A strong decreasing trend for both PB and PP in treatment microcosms occurred in year two, eventually coalescing with control conditions at a lower equilibrium. Transferred biofilms and treatment materials, therefore, increased overall wetland productivity during the initial stages of wetland development when growing conditions are most limiting.

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Well, I guess this is the end. I never thought I would get to this point and still be living to talk about it. Amazingly, I am alive and doing quite well. How, you may ask. Well the only possible explanation for this phenomenon would be the people standing on the side lines always ready to lend a hand or in my case many hands. I have been blessed with great supervisors, committee members, colleagues, family and friends and the completion of this degree is in lage part due to their invaluable support along the way. The following is a small way of showing my gratitude towards these people who played such a significant role during my education.

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List of Abbreviations

OSPM = Oil sands process-affected materials

OSPW = Oil sands process-affected water

FFT = Fluid fine tailings (tailings material without gypsum)

CT = Consolidated tailings (tailings material with gypsum to accelerate consolidation)

GPP = Gross primary productivity

NPP = Net primary productivity

FW = Fresh water

SoSo = layered substrate with soil overlaying soil

SoSa = layered substrate with soil overlaying sand

SoCT = layered substrate with soil overlaying CT

PRS = Plant root simulator probes (ion exchange membrane devices use to measure substrate nutrient supply rates)

TDW = Total dry weight (biomass samples oven dried and weighed)

1 INTRODUCTION AND LITERATURE REVIEW

1.1 Benthic Microbial Mats (Biofilms)

Benthic microbial mats (biofilms) have been gaining recognition among the scientific community as researchers discover their ecological importance and valuable industrial applications (Bender et al. 2004; Garcia de Oteyza et al. 2006; Roeselers et al. 2008). Biofilms are described here as mixed microbial communities that colonize benthic substrates. Their formation and configuration are the result of light, chemical and redox gradients. Biofilms can develop from single cellular microbial communities into complex structures containing distinct stratified layers. In the latter case, surface layers exposed to light are dominated by photoautotrophic cyanobacteria, desmids and diatoms; deeper layers are colonized by anaerobic phototrophs and heterotrophs such as purple sulfur bacteria, sulfate reducing bacteria and methanogens (Madigan et al. 2000; Van Gemerden 1993). Biofilms are encased in a film known as the extracellular polymeric substance (EPS). The EPS is secreted by the inhabiting bacterial communities (Davey and O'toole 2000), helps preserve the vertically stratified structure, allows for efficient metabolic activity and interspecific interactions and provides protection from UV radiation, desiccation and sudden pH shifts (Flemming 1993). It also increases attachment capability for the encased microbial community. Spatial distribution and survival of each individual microbial layer is highly dependent on multiple environmental variables including light, temperature, and oxygen availability. With their basic requirements satisfied, biofilms thrive in a wide range of aquatic habitats such as freshwater wetlands, dune slacks, hot springs, estuaries, hydrothermal vents, hypersaline coastal lagoons and sea ice (Adema et al. 2004; Hawes and Schwarz 2000; Paerl et al. 2003; Roeselers et al. 2007a).

Biofilms are highly resilient to environmental and anthropogenic stressors and, in most cases, are the first organisms to colonize new wetland substrates (Sim et al. 2006). Colonization in wetlands can take place on a wide range of surfaces including wood, rock, sand, organic sediment and macrophytes. Species

dominance depends on the chemical and physical properties within the substrate. Once colonized, the microbial communities within the biofilm contribute many beneficial functions to the wetland ecosystem regardless of wetland age.

Biofilms are currently receiving a great deal of attention both in the field of wetland ecology and for their potential industrial applications including wastewater treatment, aquaculture, biofuels and agriculture. My thesis considers the potential role biofilms have in wetland reclamation and the possibility of incorporating biofilms as a key component in early wetland reclamation.

1.1.1 Colonization and Development

Initial colonization and subsequent matrix development is highly influenced by the following physical and chemical environmental parameters: light, temperature, substrate quality, density, O_2 supply, redox potential, pH and salinity (Franks and Stolz 2009; Roeselers et al. 2007b). Photoautotrophs such as green algae and cyanobacteria are usually the initial colonizers of new substrates, though under low light conditions, heterotrophic bacteria have been found to be the primary colonizers facilitating further biofilm development (Roeselers et al. 2007b). Heterotrophic communities such as sulfate-reducing bacteria begin growing beneath the initial layers as they are able to utilize the waste products produced from the photosynthesizers above as their carbon source (Roeselers et al., 2008; Van Germerden 1993). Colorless and purple sulfur bacteria, capable of re-oxidizing sulfide to sulfate, typically colonize at the lowest layers of the mat. Due to these syntrophic relationships occurring between layers, microbial mats are seen as interlinked, highly structured and somewhat self-sufficient ecosystems. Their existence during the Precambrian era (3.5 bya) suggests they were one of the first life forms to organize and colonize the once hostile planet and reaffirms their capacity to adapt and survive in new and unforgiving environments (Marais 1990).

In their studies of substrate effects on productivity, Vadeboncoeur et al. (2006) found relatively high levels of biofilm production on soils, sand, rock, wood and plants and more specifically identified that biofilms on rocks and wood

relied on the water column for nutrients while biofilms living on porous sediments obtained their nutrients from beneath. These findings suggest that resource partitioning may be alleviating competition between biofilm communities. A wetland with high substrate heterogeneity may still be completely colonized by biofilm. Their results also show the importance of all classes of biofilms and their contributions to total primary production of a wetland ecosystem. For the remainder of this thesis *biofilm* will only refer to sediment attached (benthic) microbial communities ranging from single cellular- to multi-layered microbial mats unless otherwise specified.

Grazing of biofilms by scrapers, such as snails, and competition with submersed and emergent vegetation are critical factors that may affect colonization success. Grazing by micro-, meio- and macrofauna has a detrimental impact on biofilm establishment and persistence. According to the investigations by Fenchel (1998), 1 to 2 weeks were required for complete decimation of a biofilm from consumption and bioturbation. Conversely, Skov et al. (2010) found grazing was beneficial to biofilm growth since autotroph presence in the encased microbial community was found to increase in relative abundance as a compensation response to grazing pressure. Grazing was suggested to also increase light availability by removing the top dead layers of biofilm (Skov et al. 2010). The presence of a healthy invertebrate community typically depends on the amount of stress exerted on the system whereas biofilms are highly resilient to stressors and will survive in some of the most extreme and highly disturbed environments (Castenholz 1976; Cronk and Mitsch 1994; Pinckney and Paerl 1997; Sorkhoh et al. 1992). Such studies highlight the importance of context and conditions under which grazing is a factor and may vaguely suggest what is to be expected from grazer effects among the general population of wetland ecosystems.

Rapid colonization is an important feature in anthropogenically altered ecosystems, highly variable natural systems or disturbed systems for which reclamation or restoration is an objective. Biofilms are among the first colonizers of newly exposed wetland substrates (Adema et al. 2004; Fenchel 1998;

Underwood 1997) and withstand and adapt to successional change including the colonization of grazing invertebrates and the germination and growth of wetland vegetation (Sim et al. 2006; Zheng and Stevenson 2006). If a viable seed bank exists however and salinity levels are tolerable, submerged and emergent macrophytes will become dominant, regardless of whether there is a well established benthic or pelagic microbial community (Sim et al. 2006). Biofilms, however, may slow macrophyte colonization by blocking a seed's access to the sediment or creating anoxic conditions in the sediment (Adema et al. 2004; Sim et al. 2006). Adema et al. (2004) also concluded that the presence of a biofilm may support the germination and subsequent growth of both early and intermediate successional macrophyte species. They attributed this to the increase in bioavailable nitrogen (N_2) caused by nitrogen fixing cyanobacteria. Benthic biofilms have also been found to play a significant role in phosphorous deposition and thereby helping to prevent eutrophic conditions (Dodds 2003). Excess phosphorous in the water may cause plankton blooms, which will compete with both macrophyte and biofilm colonization and growth.

Though macrophyte dominance is most often inevitable in wetlands with low salinities and disturbance, biofilm production can continue to exist and even thrive. Submerged and emergent macrophytes create shade and cause displacement, both of which are detrimental to biofilm development (Sim et al. 2006). In some cases, however, shade provides autotrophs relief from extreme sunlight exposure during mid-summer months. Macrophytes also prevent the re-suspension of particles and phytoplankton into the water column while their associated fauna help further clarify the water column by grazing on suspended plankton (Lassen et al. 1997). Generally, benthic biofilms will colonize a wetted substrate and may develop into a complex layered microbial community or remain a detached single cell algal community. Because they are usually among the first to colonize wet surfaces, the species composition and structure will be modified as the wetland matures and evolves seasonally and annually (Spackova et al. 2009; Underwood 1997).

1.1.2 Functions and Applications

Biofilms occurring in wetlands or on shorelines along lakes and oceans can regulate the cycling of nutrients across the sediment-water interface, provide protection for substrates against erosion, deplete dissolved CO_2 concentrations and contribute to net primary production (Dodds 2003; Van Gemerden et al. 1989; Velasco et al. 2003; Yallop et al. 1994). Denitrification rates stimulate benthic microbial community development and are further accelerated with high proportions of diatoms present within the microbial consortium (Ishida et al. 2008). Cyanobacteria commonly associated with the top layers of a biofilm are able to fix nitrogen thereby facilitating colonization and growth of early and intermediate successional wetland vegetation (Adema et al. 2004). Bacterial nitrification is also supported within the biofilm community as the upper level phototrophs fuel this process by providing oxygen to the system (Roeselers et al. 2008). Aerobic and anaerobic processes may be carried out simultaneously within a biofilm due to the formation of steep vertical oxygen, light and redox gradients (Pinckney and Paerl 1997; Revsbech et al. 1983). Additionally, phosphorous, sulfur metals and metalloids are efficiently cycled within benthic biofilms by sulfate reduction, phosphorous assimilation and immobilization and metal uptake, removal and transformation (Bender et al. 1995; Canfield and Des Marais 1993; Dodds 2003; Jarvie et al. 2002).

Besides nutrient transport and removal, biofilms are critical sources of energy for higher trophic organisms such as detritivores and scrapers (Coesel, 1997; Moulten et al., 2004). In some ecosystems, biofilms are the preferred carbon supply for consumers due to their high digestibility compared to macrophytic vegetation (Hart and Lovvorn 2003). Small particle size facilitates handling and ingestion by many sizes of organisms and surface area dynamics are optimal for nutrient absorption (Hillebrand 2005; Poulickova et al. 2008). Due to high turnover rates, biofilms maintain relatively high production levels that compete with macrophytes and dominate neighboring microbial communities including epiphytes and planktonic species (Hart and Lovvorn 2000; Liboriussen and Jeppesen 2003; Whalen et al. 2008).

Biofilm applications in industries, including aquaculture, agriculture, biofuels, and wastewater treatment, have been topics of great commercial interest for some time (Bender and Phillips, 2004; Roeselers et al., 2008). Wastewater treatment systems benefit from biofilm colonization due to their highly efficient sorption abilities. Biofilm communities rapidly digest and metabolize a diverse collection of organic, inorganic and macroscopic pollutants from both settled materials and free-floating components in the water column (Mehta and Gaur 2005). In commercial applications, biofilms can be efficient fertilizers (via N_2 fixation) and photo-hydrogen producers due to internal metabolic processes (Ariosa et al. 2004; Sasikala et al. 1993). Lastly, biofilms can increase the efficiency of fish farms because they attenuate levels of potentially toxic ammonia and nitrate concentrations by rapidly processing these compounds simultaneously through the stages of nitrification and denitrification into nitrogen gas. The additional benefit of having benthic biofilms treat wastewater is they can be harvested and subsequently used as a fish feed alternative and a crop fertilizer (Bender et al. 2004; Eslayed and Teshima 1991). Planktonic species capable of performing similar water treatment functions must be extracted through a time-consuming filtration processes. Regardless of the type of treatment the algae must be harvested to prevent release of the captured nutrients during decomposition.

1.1.3 Methods for Estimating Production

Primary production is defined as the creation of organic carbon from the reaction between carbon dioxide and radiant energy (photosynthesis) or other inorganic chemicals (chemosynthesis). This phenomenon takes place ubiquitously in photo- and chemolitho-autotrophs (primary producers) around the globe. Primary producers are the base of the food web and the driver for all life on the planet. It is, therefore, critical that we acknowledge their significance while conducting reclamation and restoration practices, creating adaptive management plans and performing ecosystem health assessments.

Primary production can be measured in terms of net primary production (NPP) and gross primary production (GPP) components. NPP represents the

accumulation of biomass after allowing for the loss of carbon via respiration for growth and maintenance. Respiration represents O_2 lost from the system and therefore is a negative value. GPP represents total amount of carbon produced by photosynthesis. GPP is extremely difficult to measure directly in the field and is therefore usually calculated using the following modified equation from Wetzel and Likens (1979):

$$GPP = NPP - respiration$$

There are various ways of measuring levels of production. One such method includes the incorporation of Carbon-14 (^{14}C) where the radioactive compound is taken up as organic material is being produced (Bender et al. 1987; Liboriussen and Jeppesen 2003; Whalen et al. 2008). Both NPP and GPP can be estimated using this technique. Using short time intervals researchers may reduce the chances of any carbon loss due to respiration and therefore will allow the approximation of GPP. Long time intervals provide estimates of NPP.

The light versus dark bottle method is another popular production-estimating technique involving measurements of dissolved oxygen (DO) produced from sediments over time under two treatments. A posititive relationship is assumed between DO and primary production. Increasing DO evolution in the water column suggests high photosynthetic activity which leads to the synthesis of organic carbon and, therefore, high productivity. The first treatment involves transparent chambers to allow light penetration during measurements and the second treatment utilizes opaque chambers to inhibit light penetration (Bender et al. 1987; Reeder and Binion, 2001; Ryder and Miller 2005). Samples placed in the light chamber will photosynthesize and respire. Measuring DO rate of change within the light chamber gives the NPP. If NPP is positive more DO is being produced via photosynthesis versus the amount lost due to respiration, therefore, the system would be considered net autotrophic. If NPP is negative more DO is lost than produced and the system would be considered net heterotrophic. Samples in the dark chamber are only respiring and therefore measuring the DO

rate of change provides the amount of respiration occurring within the specified time interval. These two variables, NPP and respiration, can be used to calculate GPP using the equation above.

Biomass of primary producers can be measured directly as wet and dry weights, through the process of ashing to obtain carbon content, chlorophyll *a* analyses to obtain photoautotrophic biomass and oven drying to determine total dry weight biomass (Cano et al. 2008; Casco et al. 2009; Liboriussen and Jeppesen 2003). Chlorophyll *a* is most often used in studies as a surrogate for photoautotrophic biomass. Chlorophyll *a* is green pigment found in photoautotrophs and when analyzed, provides an indicator of the amount of living autotrophic material in the sample excluding all other particulate matter. This helps to reduce variability caused by contaminating material such as decomposing organic debris, mineral soils and microorganisms.

1.2 Wetlands

Wetlands are host to many ecological functions including water retention and recharge, flood control, shore stabilization, carbon storage, critical habitat and refugia for aquatic and terrestrial mammals, birds and insects, and preserving biodiversity (Kennedy and Mayer 2002; Zedler 2000). They also have high socio-economic value as hunting, trapping and fishing grounds, tourism and recreation areas, scientific research sites and carbon sinks (Alberta Environment 2008; Roulet 2000). Wetland values are becoming even more promising in the industry sector including agriculture, aquaculture, and waste water treatment facilities (Kennedy and Mayer 2002; Tanner 1996). Knowledge of such functions and societal benefits have only recently become commonly understood and discussed in the public forum.

In the last two centuries Canada has lost approximately 20 million ha of wetlands, which is one seventh of Canada's total wetland area (127 million ha) (Government of Canada 1991). In the last few decades, however, it is estimated that 50% of the world's wetlands have been lost (Geense 2004). Causes of wetland degradation or complete destruction include agriculture, urban sprawl,

climate change, forestry and peat harvesting, open pit mining, recreational development and pollution (Detenbeck et al. 1999; Holland et al. 1995; Kennedy and Mayer 2002; McDonnell and Pickett 1990). A new found respect for wetland ecosystems is causing society to slowly address wetland loss through sustainable practices, placing more effort on conservation and preservation, and undertaking reclamation and restoration projects that reduce loss, mitigate potential degradation and in some areas increase wetland quality or abundance (Detenbeck et al. 1999; Holland et al. 1995; McDonnell and Pickett 1990).

1.2.1 Wetland Reclamation and Restoration

Reclamation is defined as the act of rebuilding new landscapes where complete degradation has occurred or where they have not formerly existed, with the intent of returning some significant proportion of the ecological function that existed within the original landscape, recognizing that the configuration components and pathways to attaining functions may be vastly different from original conditions. *Restoration* is defined as the process of recapturing lost ecological functions within an existing but partially disturbed ecosystem and managing it back to prior disturbance conditions (Alberta Environment 2008). Reclamation and restoration processes are occurring extensively throughout North America as the US and Canada implement their respective wetland policies, each of which are heavily focused on no-net-loss of wetland area and function (Rubec 1994; Zedler 2000). Reclamation efforts range from simple flooding procedures, as done with the restoration of prairie potholes, to the recreation of entire landscapes beginning with below ground hydrology (Galatowitsch and Van der Valk 1996; Seabloom and Van der Valk 2003). Much human involvement is required in the reclamation of mine sites where entire landscapes are constructed (Johnson and Miyanishi 2008). The initial construction activities are geomorphic and include slope creation, land feature design and the placing of appropriate substrates to support the required below and above ground hydrology (Mitsch and Wilson 1996; Zedler 2000). Physical configuration sets the stage for natural wetland development but reclamation scientists seek ways to accelerate

colonization and succession processes by following through with simple tasks such as supplying seed sources, manually planting wetland vegetation, supplying topsoil and fertilizing or augmenting the substrate with organic matter (Erwin and Best 1985; Mitsch and Wilson 1996; Waddington et al. 2003). The focus of this thesis is to assess the potential of using benthic biofilms to speed successional development in reclaimed wetlands affected by oil sands materials and conditions.

1.3 Athabasca Oil Sands Industry

The Athabasca, Peace River and Cold Lake regions of Northern Alberta make up the largest bitumen deposit and the second largest proven crude oil reserve in the world (Government of Alberta 2009; Shuqing et al. 2008). Together they cover an area of 140,000 km^2 and are located within the boreal forest ecosystem (Government of Alberta 2009). The Athabasca oil sands region, the largest of the three deposits, is estimated to hold 1.7 trillion barrels of oil within an area of 75,000 km^2 (Holowenko et al. 2002; Shuqing et al. 2008). Oil sand or bitumen is viscous oil mixed with sand and water and lies at a depth ranging from 0 to 2000 m (Government of Alberta 2009; Shuqing et al. 2008).

1.3.1 Operations and Production

The oil sands industry is being led by Suncor Energy Inc. and Syncrude Canada Ltd. in terms of both production and now reclamation. Extraction is conducted by one of two methods 1) Open pit mining coupled with Clark Hot Water Extraction and 2) Steam-assisted gravity drainage (SAG-D) (Johnson and Miyanishi 2008; Romanova et al. 2004). I will focus on open pit mining as it is most relevant to my thesis. Approximately 20% of the Athabasca oil sands can be extracted by open pit mining due to its proximity to the surface (Shuqing et al. 2008). The remaining reserves are too deep and must be extracted by *in situ* methods such as SAG-D. Once the oil is separated from the sand by mixing in hot water and caustic soda (NaOH), it is transferred to an upgrading facility. In 2008 Alberta's oil sands industry production was averaging 1.3 million barrels of crude

oil per day and to date the oil sands industry has disturbed approximately 602 km^2 via mining processes (Government of Alberta 2009).

Tailings, a byproduct of the extraction process, consist of sand, fine clays, silt, residual bitumen, water, naphthenic acids, and high salts and ammonia concentrations. For every cubic meter of oil sands processed 3 $m^3$of water are required, which means 4 m^3 of tailings (water + fine clays + residual tailings sand + additional gypsum + unrecovered petroleum products) are produced (Allen 2008), though companies recycle and re-use substantial amounts of the contaminated water. For this thesis tailings will be referred to as oil sands process-affected material (OSPM). This can be divided into the following categories: fluid fine tailings (FFT), consolidated tailings (CT) and oil sands process-affected water (OSPW). The oil sands companies are obligated by law to contain all tailings on site. Thus, they have constructed large holding facilities known as tailing ponds or settling basins (Holowenko et al. 2002). The containment structures allow for deposition and consolidation of tailing constituents and at this point the material is referred to as FFT. To accelerate the consolidation and dewatering process gypsum ($CaSO_4.2H_20$) is added as a coagulant. The resulting product is known as CT (Matthews et al. 2002).

1.3.2 Reclamation

The large volumes of tailings produced are eventually incorporated into on-site reclamation efforts. Reclamation is now becoming an important part of the oil sands industry as some of the largest companies, including Suncor Energy Inc. and Syncrude Canada Ltd., are completing landform construction and approaching closure of portions of the original mines. Open pit mines can exceed 100 km^2 of surface coverage with depths of 100 m (Johnson and Miyanishi 2008) and the industry is required by the Alberta Government, under the Alberta Environment Protection and Enhancement Act (AEPEA), to create landscapes that are equivalent in land capability, if not configuration, to the original landscape (AEPEA, section 32, 1993). *Equivalent land capability* is defined as the land use available after reclamation is similar, though not always identical, to that

which was there before disturbance (Alberta Environment 2008). Due to the sheer size of open pit mines, reclamation efforts in the oil sands industry are conducted at a landscape scale, which differentiates them from most other reclamation projects occurring in North America and around the globe. The Athabasca oil sands industry is located within the relatively undisturbed northern boreal forest where wetlands make up approximately 50% of the landscape. Disturbance to the boreal ecosystem, due to oil sands development, forces the industry to reclaim a wide range of habitat types including end pit lakes, grazing land, forested terrestrial grounds and wetlands. Wetlands are expected to cover between 20-40% of the final reclaimed landscape (Alberta Environment 2008).

1.3.3 Wetland Reclamation

Wetland reclamation in the Athabasca oil sands is fraught with many complications. The first arises with peatlands and fens, the main type of wetland existing in the boreal ecosystem. Peat forming wetlands are nearly impossible to construct as research tells us centuries are required for the associated functions and characteristics to form (Harden et al. 1992). The second complication occurs with the incorporation of tailings, CT and OSPW. Their associated high salinities and ammonia and naphthenic acid concentrations are detrimental as they negatively affect fish reproduction, invertebrate colonization and survival and impede wetland development and biodiversity (Daly 2007; Hornung 2007; Leonhardt, 2003; MacKinnon et al., 2001; Scott, 2007). Growth of some macrophyte species can increase in OSPW, but relative biodiversity was suppressed to a few tolerant species (Hornung 2007).

Process materials, including CT and OSPW, are virtually sterile when removed from the extraction facility and placed within reclamation landscapes. Therefore constructed wetlands capped with CT as a substrate and OSPW undergo primary succession, characterized by extended periods of low colonization rates. Good quality organic substrates, however, can facilitate the establishment of microbial, macrophyte and invertebrate communities (Erwin and Best 1985). The oil sands companies have adopted a reclamation strategy that

involves the transfer of peat to constructed wetlands. In this particular case peat is stockpiled as the overburden is removed to expose the oil sands beneath and later transferred to newly reclaimed wetlands. This is expected to accelerate colonization and subsequently biodegradation of the tailings constituents (Quinty and Rochefort 2003). Peat quantity, however, is limited and stockpiling for later use reduces its viability as a reclamation resource.

This thesis is part of a larger wetland reclamation research initiative titled: Carbon flow, Food web dynamics & Reclamation strategies in Athabasca oil sands Wetlands (CFRAW; Ciborowski et al. 2006). CFRAW is a multi university (University of Alberta, University of Guelph, University of Saskatchewan, University of Waterloo and University of Windsor) multi industry partner (Suncor Energy Ltd., Syncrude Canada Ltd., Albian Sands and Canadian Natural Resources Ltd.) collaboration established to study the impacts of mine process affected materials and water on the development and carbon dynamics of reclaimed wetland ecosystems.

1.4 Thesis Objectives and Final Statement

1.4.1 Objectives

The goal of my research was to assess and quantify the contributions of benthic biofilms to total primary productivity within newly constructed wetlands. Concurrently, I evaluated the potential use of biofilms as a reclamation application that will facilitate and accelerate colonization and succession. I determined the effects of two biofilm transfer methods, four substrate mixtures and OSPW on the productivity of benthic biofilms. This research in a general sense will help demonstrate the utility of benthic biofilms for initiating carbon sequestration during the primary stages of reclaimed wetland development as well as provide insight on the unique oil sands reclamation materials and conditions and their associated effects on colonizing microbial communities.

1.4.2 Final Statement

This thesis addresses a larger, more conflicted picture and although this is not discussed any further, the reader should note this as it provides some insight regarding the context in which this thesis is based. The oil sands industries are situated in the middle of a global tug-of-war as politicians, scientists, industrialists, stakeholders and environmentalists all take sides and argue over the most effective, sustainable and economical ways of utilizing such a valuable resource. Internationally, the public observes the destruction of large expanses of wilderness while the value of oil in today's global economy elicits strong political support for speeding production. Controversies are found in every aspect of the industry including the extraction and certification processes and the effectiveness of reclamation and associated approvals. There is national and international demand for implementation of more sustainable practices and renewable energy production. This is, however, matched by motivating factors such as the billions of dollars invested by the private sector in infrastructure or the tens of billions that have contributed to the GDP of Canada (Government of Alberta 2009).

Most reclamation practices conducted on site are unseen and unaffected by the media. Motivation to do respectable, unbiased research lies in the hands of the scientist. Researchers will not release their findings until the appropriate conclusions and interpretations have been made, with the hopes that they will positively influence science and reclamation efforts. Unfortunately, research results can sometimes find their way into the political domain where the official application of such knowledge becomes a byproduct of negligence and misinterpretation.

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2 IN THE BEGINNING: MANAGING INITIAL COLONIZATION OF WETLAND SUBSTRATES

2.1 Introduction

This study examines the productivity of microbial mats placed on new substrates in young wetlands to assess response and feasibility of the technique for reclamation of areas previously mined for the extraction of bitumen from oil sands.

2.1.1 Biofilms

Phototrophic microbial mats (biofilms) are surface-associated microbial communities that constitute single cellular algal communities or evolve into a complex, stratified layered, microbial consortium encased within a protective outer membrane (Roeselers et al. 2008; Wimpenny et al. 2000). For this study, 'biofilm' is operationally defined as microbial mats attached to wetland sediments with or without stratified layering. Multi-layered biofilm development usually originates with the colonization of photosynthesizing cyanobacteria and/or algae. Sulfate-reducing bacteria and other heterotrophs are found below the mat surface, where they consume waste products produced by photosynthesizers, such as cyanobacteria, above (Bruckner et al. 2008; Murray et al. 1986). Consequent production of sulfide facilitates the growth of colorless and purple sulfur bacteria (Van Gemerden 1993). The varied species composition and functional diversity of mats results in formation of steep, vertical physical and chemical gradients, including oxygen, redox and pH, which help maintain microbial stratification within the biofilm (Jorgensen et al. 1983; Revsbech et al. 1983). Light and oxygen availability decrease drastically with depth allowing oxygenic phototrophs to dominate in the top layers and relegating anaerobic chemotrophs to the lower strata (Franks and Stolz 2009). The protective outer membrane is referred to as the extracellular polymeric substance (EPS), which also acts as a stronghold between the biofilm and substrate (Wimpenny et al., 2000).

Great interest in biofilms exists within the scientific community due to the critical functions they provide to the system in which they inhabit. Biofilms stabilize substrates, cycle nutrients at the sediment-water interface, treat wastewater through bioaccumulation, contribute significantly to gross primary production and carbon storage, facilitate macrophyte colonization via N_2 fixation, and outcompete macrophytes in supplying nutrients and energy to the local food web (Adema et al. 2004; Canfield and Des Marais 1993; Domozych and Domozych 2008; Gerbersdorf et al. 2009; Hart and Lovvorn 2003; Ishida et al. 2008; Yallop et al. 1994). This is of great interest to many industries including agriculture, aquaculture, water treatment, bioremediation and biohydrogen production, as they learn to incorporate biofilms and their associated functions into more sustainable and effective practices (Bender et al. 1995; Bender et al. 2004; Bender and Philips 2004; Roeselers et al. 2008). The application of biofilms in an industrial context is growing exponentially as methods of cultivation and species composition manipulation continue to improve (Roeselers et al. 2008).

Biofilms are among the first colonizers of water-saturated substrates (Adema et al. 2004; Sim et al. 2006). They are resilient to stress and are able to grow rapidly and accumulate biomass in highly disturbed ecosystems (Janousek et al. 2007; Moseman et al. 2004; Sim et al. 2006). These characteristics make them a potentially valuable resource for wetland reclamation practices and, therefore, for the Athabasca oil sands industry of northeastern Alberta.

Here I will explore the potential for successfully transferring established biofilms from natural wetland habitats to newly constructed wetlands by following and quantifying the fate of these transplanted microbial communities on a variety of reclamation substrates and treatment conditions.

2.1.2 Wetland Reclamation

Wetlands are being reclaimed and restored on a global scale to offset losses to natural and anthropogenic degradation (Geense 2004; Mitsch and Wilson 1996). Wetlands also store carbon, preserve biodiversity, control flooding and treat waste water (Geense 2004; Kennedy and Mayer 2002; Whiting and Chanton

2001). The majority of wetland losses are due to agriculture, urban sprawl, and mining developments (Bartzen et al. 2010; Holland et al. 1995; Peng et al. 2010). These disturbances can also cause partial degradation where functions are lost to overwhelming pollution, changes in hydrology or physical disruption (Cooke et al. 1990; Peng et al. 2010; Tiner 2005).

Wetland reclamation and restoration are some of the few main efforts combating such large scale environmentally destructive forces. Reclamation is the act of constructing and restoring lost ecosystems and their associated ecological functions (Zeddler 2000). Restoration is similar to reclamation in most respects except that it pertains to ecosystems that have been partially degraded, therefore remnants of the original ecosystem still exist (Alberta Environment 2008). Wetlands are now being reclaimed and restored all over North America, including coastal areas where berms and dykes are removed to restore the required hydrology or in the prairies where farmland drainage tiles are broken and the depressions in the landscape are re-wetted (Seabloom and Van der Valk 2003). Such methods sometimes lead to viable (though typically compromised) wetland ecosystems via natural succession processes. The restoration of peatlands after peat extraction activities incorporates the planting of sphagnum moss or transfers of organic matter (Graf and Rochefort 2008; Waddington et al. 2003). To ensure native vegetation outcompete invasive species may require seed bank replenishment or manual plantings (Erwin and Best 1985; Zeddler 2000). Though wetland reclamation is a challenging task regardless of the type of wetland and the degree of degradation, the Athabasca oil sands industries of northeastern Alberta are an extraordinary case simply due to the sheer size and rate of their mining developments (Johnson and Miyanishi 2008).

2.1.3 Oil Sands Operations and Reclamation

Companies actively mining in the Athabasca oil sands near Fort McMurray, Alberta are required to reclaim entire landscapes following their creation, and later, closure of open pit mines, which can cover an area of up to 100 km^2 (Johnson and Miyanishi 2008). Currently, 602 km^2 have been disturbed

via mining processes. As of early 2009, 1,352 km^2 of the total mineable area (4,800 km^2) within the oil sands deposit has been approved for surface mining projects (Government of Alberta 2009). This does not include the area disturbed by in situ extraction. According to the Alberta Environmental Protection and Enhancement Act (AEPEA), oil companies must reclaim surface-mined areas to systems equivalent in capability, if not in configuration, to the original landscape (AEPEA, section 32, 1993). Wetlands are expected to comprise between 20 to 40% of the reclaimed land, and many are constructed using oil sands process materials (OSPM), byproducts of the bitumen extraction process (Alberta Environment 2008). Primary constituents of OSPM include oil sands process-affected water (OSPW) and composite tailings (CT; soft clays, sand, gypsum and bitumen) and will be used as wetland reclamation amendments (Matthews et al. 2002). Each product has elevated levels of salinity and high concentrations of naphthenic acids and ammonia, making them toxic to fish and causing chronic stress on some aquatic vegetation until degraded into less bioreactive products (Daly 2007; MacKinnon et al. 2001; Scott 2007). Reclaimed wetlands constructed in the oil sands and classified as OSPM-affected are essentially excavation pits filled with CT and capped with either fresh water or OSPW. These reclamation materials emerge from caustic, pressurized processes held above the boiling temperature. Therefore, the landscape produced from these near-sterile, carbon-poor materials approach primary succession conditions. These wetlands provide a unique research opportunity, allowing one to safely examine, manipulate and evaluate near-primary succession wetland processes on a large scale.

Wetlands constructed with OPSM have reduced microbial activity beneath and at the sediment water interface (Slama 2010; Gardner Costa 2010), and require more time to acquire main wetland functions than equivalent wetlands constructed with organic soils and fresh water. Leonhardt (2003) used the relative colonization rates of invertebrates as an indicator of wetland maturity. She concluded that OSPM-affected wetlands accumulated asymptotic richness of invertebrate families more slowly (7 years) than reference wetlands (achieved in 3-4 years). Emergent macrophytes, *Typha* and *Scirpus,* usually become

established quickly when hand planted or when they are able to develop from seed banks associated with wetlands soils placed during the reclamation process (Hornung 2007). Seed collection, however, is extremely time consuming and diversity is constrained due to the aggressive and resilient nature of cattail and bulrush. In pilot reclamation studies, living peat turfs have been cut from existing wetlands and transferred to reclaimed wetlands to initiate critical wetland functions including carbon capture and storage, and nutrient cycling via microbial activity (Wytrykush 2010). Peat is stockpiled during the land-clearing phase of open pit mine construction or transferred directly from existing peatlands that will soon be drained for mining activities. Typical peat transfer depths can reach a meter in depth and cover large surface areas. Consequently, the demand for peat is high in reclamation practices.

2.1.4 Objectives

The objective of this study is to evaluate and propose an alternative reclamation approach using benthic microbial flora that can be applied in an industrial setting. I investigated the effectiveness of manually transferring benthic biofilms as a means to initiate and promote increased rates of colonization, productivity and carbon accumulation in both constructed OSPM and non-OSPM affected wetlands. This study examines the productivity of transferred biofilms to assess microbial response and evaluate the feasibility of the technique for reclamation. I also determined the relative efficacy of several transfer methods and reclamation materials to accumulate the greatest rates of colonization and growth.

I hypothesized the following:

1) Productivity and photoautotrophic biomass will increase over time,

2) Biofilms exposed to OSPM will have higher levels of production and accumulate more biomass than biofilms exposed to reference substrates and water, due to nutrient enrichment from mine tailing materials,

3) Biofilms or their components will survive the transfer process

4) Microcosms into which biofilm has been transferred will have greater biomass and productivity than control microcosms that have not received biofilm inoculations,

5) Procedures that retain the biofilm's physical integrity will be more productive and accumulate more biomass than biofilm that has been homogenized before application. Both types of treatments will be more productive than those involving no inoculation of biofilm.

This study also allows examination of the resistant and adaptive characteristics of biofilms exposed to extreme conditions.

2.1.5 Study Site: Athabasca Oil Sands Region of Alberta

Suncor Energy Inc. and Syncrude Canada Ltd., are located in northeastern Alberta, approximately 25 km north of Fort McMurray, Alberta (Figure 2.1). Suncor and Syncrude operations are the oldest and largest of five active mines and have undertaken the most advanced reclamation efforts. With mining activities and bitumen production increasing over time and projects expanding in scale and in quantity, cumulative environmental degradation is occurring. Cost effective, well-planned reclamation research and subsequent application is required to maintain commensurate rates of progress. In recognition of this, Suncor and Syncrude support and fund numerous research projects from Universities across Canada.

All Athabasca oil sands operations are located within the mixed wood region of the Boreal Forest (Vitt et al. 1996). Tree species are white and black spruce, trembling aspen, balsam poplar and tamarack. The landscape is generally flat with deep valleys eroded by river and stream beds including the Athabasca and Clearwater rivers. The landscape surrounding Fort McMurray contains a high density of wetlands including fens, bogs and open water marshes (Alberta Environment 2008). Winters are long and cold with an average temperature of -19 °C and summers are short, hot and dry with temperatures averaging around 17 °C. Annual precipitation can reach up to 450 mm, with highest volumes of rainfall

occurring during the months of May to Sept. The winter months, Oct. to April, produce an average of 155 cm of snowfall (NCDIA 2010).

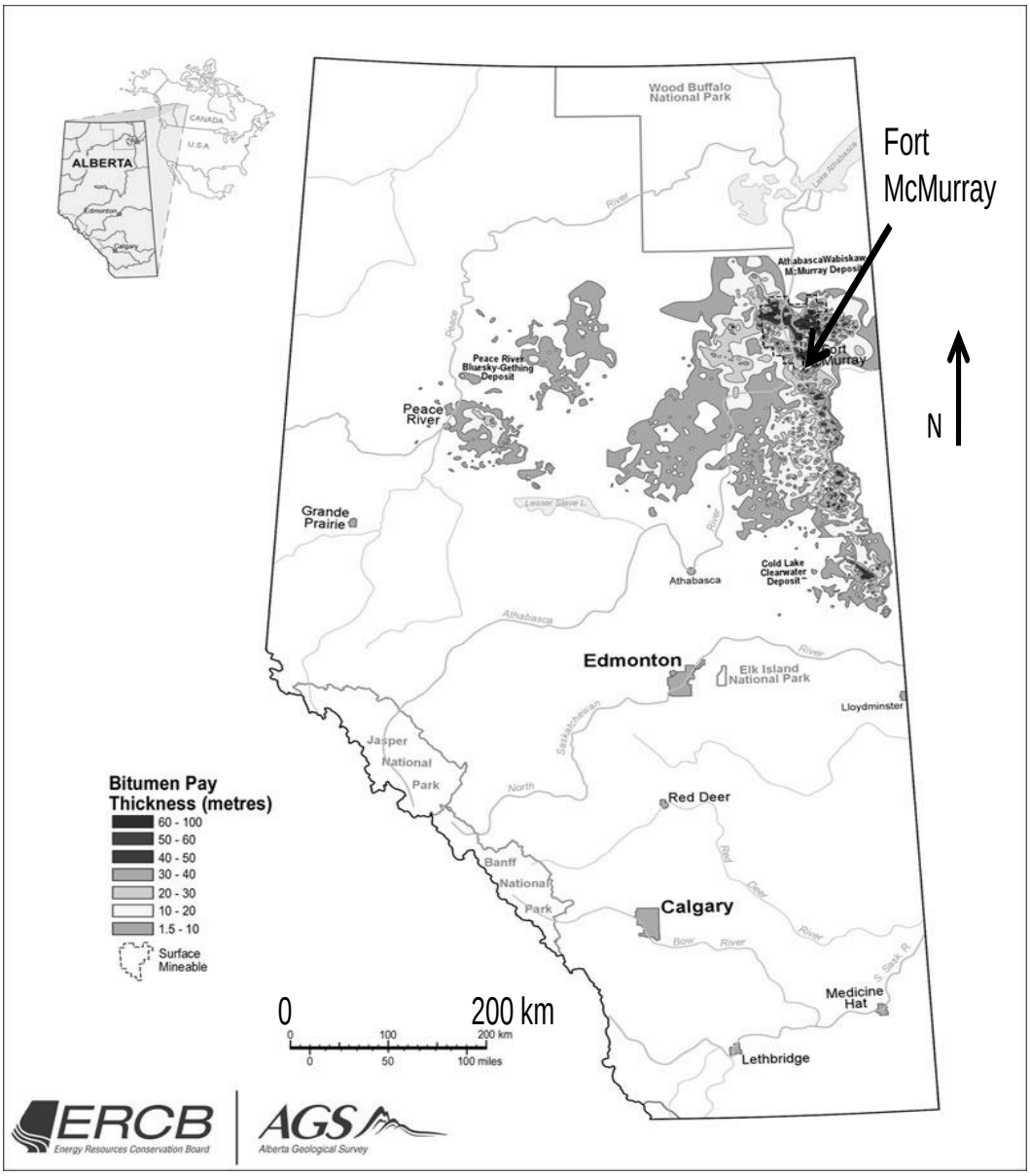


Figure 2.1 - Map of the location of the Athabasca oil sands deposit surrounding Fort McMurray, AB (modified from http://www.ags.gov.ab.ca/energy/oilsands/alberta_oil_sands.html).

2.2 Materials and Methods

2.2.1 Experiment 1: Design

To examine biofilm transfer and colonization success, two trials of algal transfers were carried out over a 7-d period in an open field at Syncrude's environmental complex. I used 21-L aquaria as sample containers for maximum sunlight. Aquaria dimensions were 41 x 20.25 x 25.5 cm. Transfers followed the slurry method described in section 2.2.4. The three treatments of the first transfer were:

1) 2 L of fresh water + 1 L of homogenized substrate + 1 L of biofilm slurry,

2) 7 L of fresh water +1 L of biofilm slurry,

3) 3 L of fresh water +1 L of biofilm slurry (Figure 2.2).

Each treatment was randomly assigned into one of 21 aquaria (n=7 replicates). Deep and shallow treatments were given a water column depth of 7 and 3.5 cm, respectively. Chlorophyll *a* samples were collected from all treatment replicates after 24 and 96 h and were immediately preserved with magnesium carbonate ($MgCO_3$) and placed in the freezer until analyzed. Magnesium carbonate helps maintain basic pH levels in the sample. The first transfer focused on the potential effects of water depth and turbidity on initial colonization and growth. The second transfer followed the same design but in this case, the treatments were;

1) 2 L of fresh water + 1 L algal slurry + 1 L of fluid fine tails (FFT),

2) 2 L of fresh water + 1 L algal slurry + 1 L of CT,

3) 3 L of fresh water + 1 L algal slurry.

All treatments had a water column depth of 3.5 cm. Chlorophyll *a* samples were collected from all treatment subsamples after 24 and 96 h, preserved in $MgCO_3$ and frozen until analyzed. Since only a small amount of substrate was needed and time was limites I collected the substrates from established wetlands on site, "MFT North" and "4mCT". The second transfer focused on the effects of substrates on colonization and growth.

Observations from a preceding pilot scale study, which was carried out over 72 h, suggested that aquaria, without transferred biofilm slurry, were unable

to acquire an adequate quantity of biofilm for sampling (no benthic algae was visible). I, therefore, did not incorporate controls (aquaria without transferred biofilm slurry) into this experiment due to the short time scale and insufficient resources.

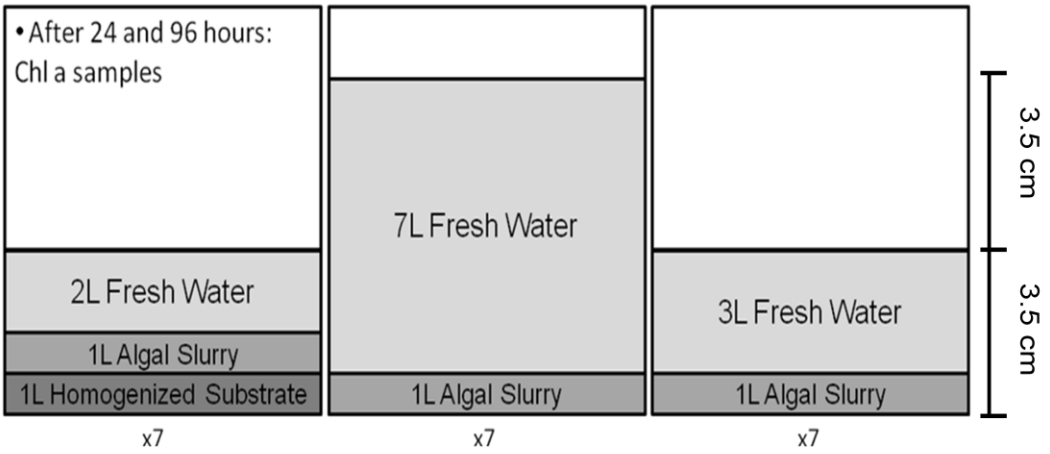


Figure 2.2 - Diagram of first transfer study and the three treatments.

2.2.2 Experiment 2: Design

The experimental field study site was located on Suncor's reclamation site where six parallel 10 x 50 m trenches had been constructed in 1995 (Figure 2.3A & B). Trench floors were lined with vinyl and covered with 10 cm of organic sediment, collected from on-site stockpiles, to isolate surface water from groundwater and to minimize water loss through percolation. Only since 2005 the trenches have been allowed to acclimate to wetland conditions due to consistent maintainence of a relatively stable water column depth for research projects. Trenches 1, 3 and 5 were filled with fresh water (FW) from a nearby natural lake and the remaining three (trenches 2, 4 and 6) received OSPW from a nearby tailings pond. Conductivity levels were maintained for FW and OSPW trenches at approximately 500 and 2,000 uS/cm^2, respectively. Conductivity was used as a surrogate for tailings water content. The trenches were filled to a depth of approximately 50 cm, which was maintained by the addition of water as necessary throughout the entire study. Depths were measured halfway along the length of the trench. Variability in depth measurements may occur from highly uneven substrates where the meter stick was placed. The main purpose for these trenches was to create a semi-natural setting outdoors, yet still permit control of potentially complicating environmental factors such as depth, shading effects from nearby aquatic and terrestrial vegetation and water chemistry.

2.2.3 Experiment 2: Microcosm Construction

Five sets of four microcosms were submersed within each trench (Figure 2.4). The microcosms were 13-L buckets with rows of holes drilled around the mid section then covered with screening to simultaneously contain sediments and allow water, ionic, and chemical exchanges between the substrates and water column. Each set consisted of four microcosms attached to each other to make a single row. Each microcosm in a set received one of four randomly-assigned substrates. The substrate treatments consisted of soil over soil (SoSo), CT over CT (CT), soil over sand (SoSa) and soil over CT (SoCT) (Figure 2.4). Each layer in a substrate was 8 cm in depth. Soil was a 70:30 vol./vol. mixture of peat and

mineral soil (clay). Substrates were taken from stockpiles on Suncor's lease site that were stored for reclamation purposes. For four of the five sets of microcosms, substrate placement followed a Latin Square Design where each substrate type was randomly assigned to a bucket within a set while never occupying the same position twice among the designated four sets in a single trench (Figure 2.3A). Three of the four sets received 1 L of algal slurry (described below) and the fourth set was designated a control and therefore did not receive any slurry. The set designated to receive control buckets was randomly chosen within each trench. A fifth set of microcosms received intact microbial mats and was randomly assigned a location among the other four sets within the trench.

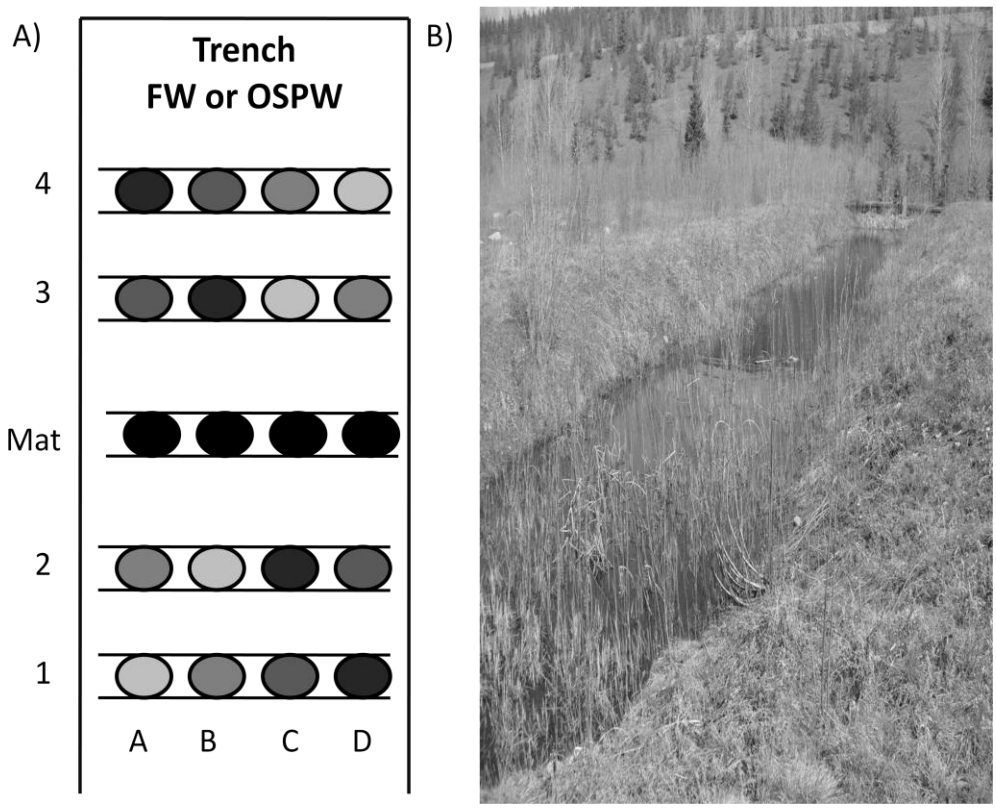


Figure 2.3 - A) Example of the tratified random sampling design found within each trench. Numbers represent the four sets following a latin square design. The "Mat" set was randomly placed among the other four sets. Different shades represent the 4 substrates and their possible locations within a trench. Substrate and set location varied among trenches. Substrates placed within microcosms of the "Mat" set were randomized. B) One of six trenches used in the study (Spring, 2009).

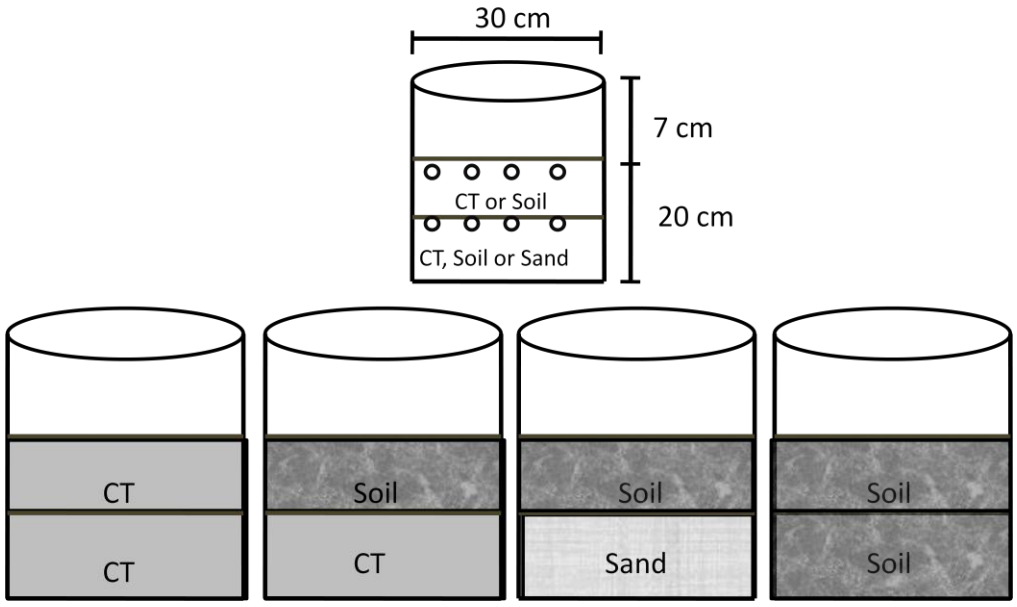


Figure 2.4 - Microcosm dimensions, construction and substrate mixtures.

2.2.4 Experiment 2: Biofilm Collection and Transfer

Slurry transfer materials consisted of a composite of biofilms collected from several wetlands (Test Pond 10, Seepage Control Pond, Shallow Wetland and Trench 6) during summer when water temperatures were high (late July - early August) and a well-defined mat was present. Biofilm material was collected into a single cooler and vigorously stirred to produce a relatively homogeneous slurry. This mixing was done to mimic the stress that may be expected from industrial practices during transport and to provide each microcosm with microbial representatives from all collection sites. One L of homogeneous slurry was poured into each treatment microcosm.

Biofilm mat transfers involved placing intact mats on the substrates. To maintain structural integrity, mats were collected from a single wetland (Trench 6) using a 25-cm diameter flat plastic plate while taking care to minimize disturbance to the layers. This produced a biofilm layer of standardized, repeatable dimensions. The plate was carefully slid under the mat, and gently lifted. The intact mat was then transported to the recipient microcosm and placed on the substrate. Maximum distance for mat transport was approximately 100 m.

Both the slurry and the microbial mats were transferred on the same day and all transfers took place outside of the trenches. After the transfer, each microcosm was allowed to sit for 30 min before being submersed in its experimental trench to allow materials to settle on the substrate. This reduced the likelihood of any pieces of sample becoming suspended during immersion and subsequently floating away.

2.2.5 Experiment 2: Sampling and Analyses

Biofilm samples for chlorophyll *a* determination were collected, and change in dissolved oxygen (DO) was measured to provide indications of photoautotrophic biomass and primary production, respectively (Reeder et al. 2001; Wasmund et al. 2006).

Biofilm chlorophyll *a* samples were collected from the substrate of each microcosm using the barrel of a 50-mL syringe (IA 5.6 cm) as a coring device. As

the syringe barrel was pressed into the substrate, the plunger was lifted at the same rate to maintain negative pressure on the sample. After the core had been collected, the plunger was removed from the top of the barrel, inserted upward from the bottom, and used to push the sample upward through the barrel until the biofilm layer rested above the barrel's top edge. The mass of biofilm was scraped from the substrate surface into a 20-mL scintillation vial. To minimize loss of substrate from the microcosm the remaining substrate core was placed back into the hole it was taken from. Four to six drops of saturated $MgCO_3$ solution were added as a preservative. Samples were placed in darkness on ice during transport and subsequently stored frozen at -20^{o} C until they were analyzed.

Chlorophyll *a* analyses were conducted by the Biogeochemical Analytical Laboratory at the University of Alberta. Chlorophyll a was extracted from biofilm in 95% ethanol, which was then passed through a 0.45 um filter. A Shimadzu RF-1501 spectrofluorophotometer and methods suggested by (Welschmeyer 1994) were used to measure relative chlorophyll fluorescence as an indicator of chlorophyll content. Minimum detection limit for all chlorophyll *a* samples was 1 μg/L for a 200-mL sample. Samples below the detection limit were assumed to be zero. Chlorophyll *a* samples were taken from each microcosm four times throughout the study; 1) Aug., 2008 (summer 2008), 2) May, 2009 (spring 2009), 3) Aug., 2009 (summer 2009), 4) May, 2010 (spring 2010).

Chlorophyll *a* is a representative measure of photoautotrophic microbial biomass in that it excludes other organic/inorganic debris that may have otherwise added to the overall biomass of microbes in the sample. Although this technique is frequently used as a measure of phytoplankton or periphyton biomass, it may or may not be a reliable indicator of total biomass in a sample (Cano et al. 2008; Casco et al. 2009; Liboriussen and Jeppesen 2003; Reeder and Binion 2001; Sim et al. 2006; Squires and Lesack 2001; Vadeboncoeur et al. 2006). I, therefore, used chlorophyll *a* concentrations as a surrogate measure of photoautotrophic biomass and also determined the relationship between chlorophyll *a* and total dry weight (TDW) biomass via regression analysis. To accomplish this I collected a parallel set of biofilm TDW biomass samples concurrently with the last set of

chlorophyll *a*. Biomass samples were collected from each microcosm following the same methods as for chlorophyll *a* except that samples were placed in a pre-weighed tin plate rather than a scintillation vial. Samples were brought back to lab and placed in a drying oven for 24 h at 50°C to constant mass among all samples. A regression analysis was performed to estimate the relationship between TDW biomass and chlorophyll *a* concentration.

Primary productivity within each microcosm was estimated by employing a modified in situ light-dark bottle DO assay (Squires and Lesack 2001; McCormick et al. 1998; Reeder and Binion 2001). A light or dark cap, consisting of a modified Nalgene® desiccation chamber (cat. No. 5309), was placed on the surface of the substrate within each microcosm (Slama 2010). Every cap had a hole drilled at the top and was sealed with a removable plug. Water column DO was recorded every 10 min for 1 h under the caps by temporarily removing the plug and inserting the probe of a YSI 30 portable dissolved oxygen meter. The light and dark caps were exchanged among pairs of microcosms for the next 1 h, providing a measure of DO rates of change under both light and dark chambers for each microcosm. This method enabled the calculation of a surrogate for autotrophic gross primary productivity (GPP; (McCormick et al. 1998; Reeder and Binion 2001; Wetzel and Likens 1979)). Light chambers allow both photosynthetic and respiration processes to occur thereby capturing net primary productivity (NPP; Gross Primary Production (GPP) minus Respiration), while dark chambers inhibit the light phase of photosynthesis and therefore provide an estimate of respiration rates. Gross primary production (GPP) could thus be estimated by adding the estimate of respiration to the NPP estimate for each microcosm. By comparing the rate of change in DO between each cap I was also able to determine the net trophic status of the system. Primary productivity was estimated by measuring DO dynamics on 4 dates over the course of the experiment; 1) Aug., 2008, 2) May, 2009, 3) July, 2009, and 4) Aug., 2009.

Select dissolved oxygen values were converted to units of carbon using the C:O molar mass ratio of 0.375. Photosynthetic and respiratory quotients, which are dimensionless, were assumed at 1.2 and 1.0, respectively (Wetzel and

Likens 1979). Conversions were done and presented for only a few all encompassing values to provide comparable measures of productivity amongst other studies.

Substrate nutrient supply rates were measured using ion exchange membrane technology in plant root simulator (PRS^{TM}) probes (Western Ag Innovations Inc., Saskatoon, SK, Canada). Each probe contains passive ion collector surfaces designed to equilibrate with ambient levels of nutrients over time. Lab analyses were subsequently used to measure materials collected. The probes are called 'plant root simulators' because they measure dissolved ions, which are presumably bioavailable. Probes contained either cation- or anion-exchange resin membranes. We placed three pairs of probes (1 sample) into the four microcosms of one treatment (slurry) set per trench. After a 4-d exposure period, the three replicate members of each pair were pooled and sent for analysis. Pooling served to reduce microscale variation among replicate microcosms within trenches to account for substrate heterogeneity. One pair of probes was placed in the control set of each trench. Based on previous pilot studies and to estimate in situ nutrient supply rates during the growing season, the duration of probe exposure was four days ((Aug. 9-13, 2009) Slama 2010). Probe deployment and extraction procedures followed the PRS^{TM} operations manual suggestions (Western Ag Innovations., Saskatoon, SK, Canada). Probes were analyzed by Western Ag Innovations for NO_3, NH_4, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al and Cd.

A YSI Model 30 portable meter was used to measure temperature, DO, conductivity and salinity at the middle ofeach of the six trenches throughout the experiment. The depth in which the YSI probe was place ranged from 20-30 cm from the surface. Temperature was also monitored every 2 h for the 2 y using submersible HOBO data loggers. Data loggers were attached to the second set in each trench at an approximate depth of 25 cm from the water surface. Water quality samples taken in 2008 were sent to and analyzed at Syncrude Canada Ltd. (See Appendix I for parameters measured).

2.2.6 Statistical Analyses

All statistical analyses were performed using STATISTICA version 7 software (Statsoft, Inc., Tulsa, OK). Any data not conforming to a normal distribution, as explained by graphical inspection or with the Kolmogorov-Smironov Test, were Log_{10} transformed where appropriate. All graphs present data with the untransformed values for interpretation purposes.

For the two aquarium studies, a planned-comparison two-way factorial analysis of variance (ANOVA) was used to test for statistical significance of differences for chlorophyll *a* concentrations among and within time trials and treatments. In the main study, a forward stepwise multiple regression analysis using dummy variables was used to test for statistical significance of differences in chlorophyll *a* and DO concentrations among the five independent variables: substrate, water type, transfer technique, trench number and time. Forward stepwise multiple regression was also conducted for TDW biomass analysis among four independent variables: substrate, water type, transfer technique and trench number. Substrate nutrient supply rate comparisons between substrate types and transfer techniques were analyzed via one-way ANOVAsfor each individual trench. Probability values obtained were combined for FW and OSPW trenches using the methods of combining probabilities from multiple tests described by Sokal and Rohlf (1981). The relationship between chlorophyll *a* and TDW biomass was determined by scatter plot and regression analyses. A one-way ANOVA was used to compare water chemistry parameters among individual trenches and between OSPW and FW treatments. The significance level for all tests was set at $p < 0.05$ and p-values were divided by half for all one tailed tests.

2.3 Results: Experiment 1

2.3.1 Aquarium Trial 1

Chlorophyll *a* concentrations, on day 4, were significantly higher than on day 1 ($n = 21$; $F_{(1, 36)} = 4.77$; $p < 0.05$; Figure 2.5 & 2.6). Excluding zeros, as a result of samples being below detection limit and to provide a more accurate representation of data distribution, concentrations on day 1 and 4 ranged from 6.7

to 38.6 μg/cm^2 and 6.79 to 31.96 μg/cm^2, respectively. Planned comparison analyses following the two-way ANOVA revealed the treatments that were most productive. Chlorophyll *a* concentrations of shallow water replicates significantly increased from day 1 to 4, and replicates receiving substrate additions supported higher chlorophyll *a* concentrations than treatments without ($n = 7$; $p < 0.05$ and $n = 7$; $p < 0.05$, respectively).

2.3.2 Aquarium Trial 2

Chlorophyll *a* concentrations were significantly higher at day 1 than day 4 ($n = 21$; $F_{(1, 36)} = 4.79$; $p < 0.05$; see Figure 2.7). Excluding zeros again, concentrations ranged from 3.66 to 55.28 μg/cm^2 and 1.1 to 22.84 μg/cm^2, on days 1 and 4, respectively. At day 4 chlorophyll *a* concentrations dropped significantly for FFT and CNTRL treatments (planned comparison, $n = 7$; $p < 0.05$ and $n = 7$; $p < 0.01$, respectively). Replicates with FFT supported significantly lower chlorophyll a concentrations than the shallow and shallow + CT treatments (planned comparison, $n = 7$; $p < 0.01$).

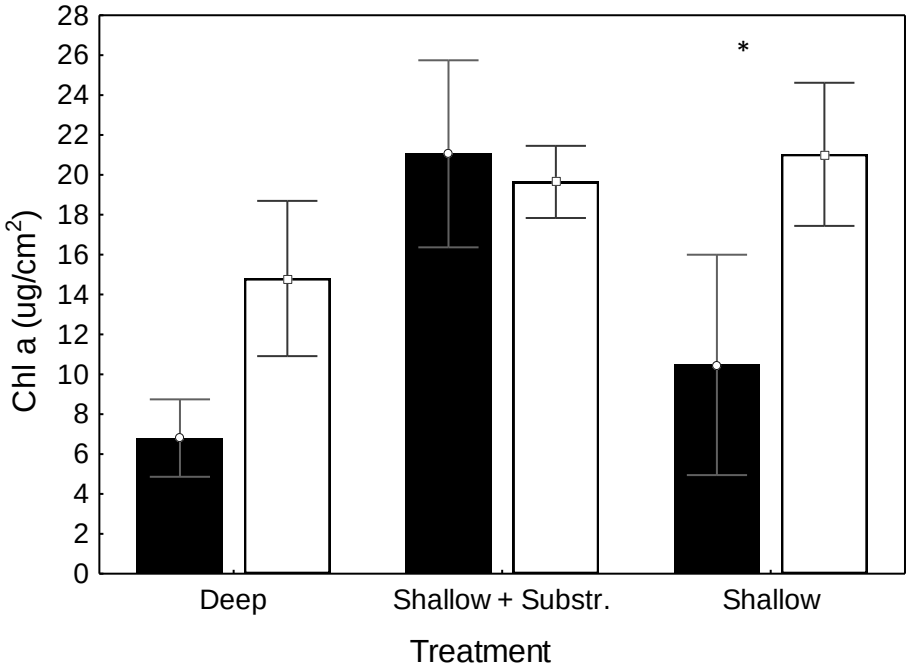


Figure 2.5 - Mean (± SE) chlorophyll *a* (Chl a) concentrations on days 1 (filled bars) and 4 (open bars) of the first aquarium study (n = 7). Treatments were deep: 7 cm of water, shallow + substr.: 3.5 cm of water with 1 L organic substrate, and shallow: 3.5 cm of water. A significant difference between days 1 and 4 is indicated with an asterisk.

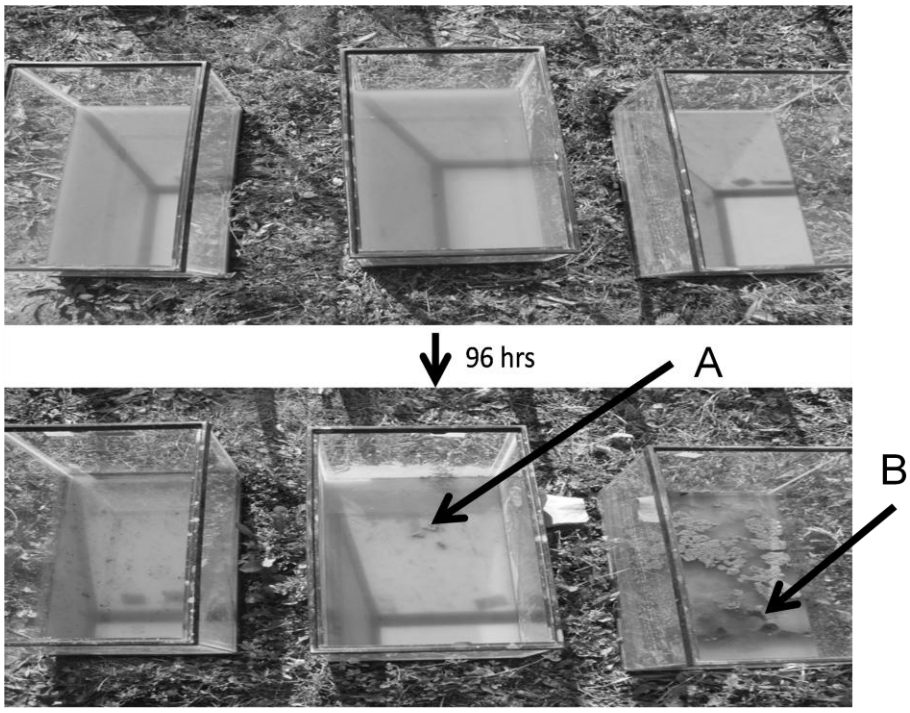


Figure 2.6 - Photo of the three treatments for trial 1 after 24 and 96 h. From left to right the treatments are: Shallow + substrate, deep and shallow. Arrows A & B are pointing to benthic algal biofilms that are beginning to detach from the substrate due to oxygen bubble accumulation within the recolonized intact mat.

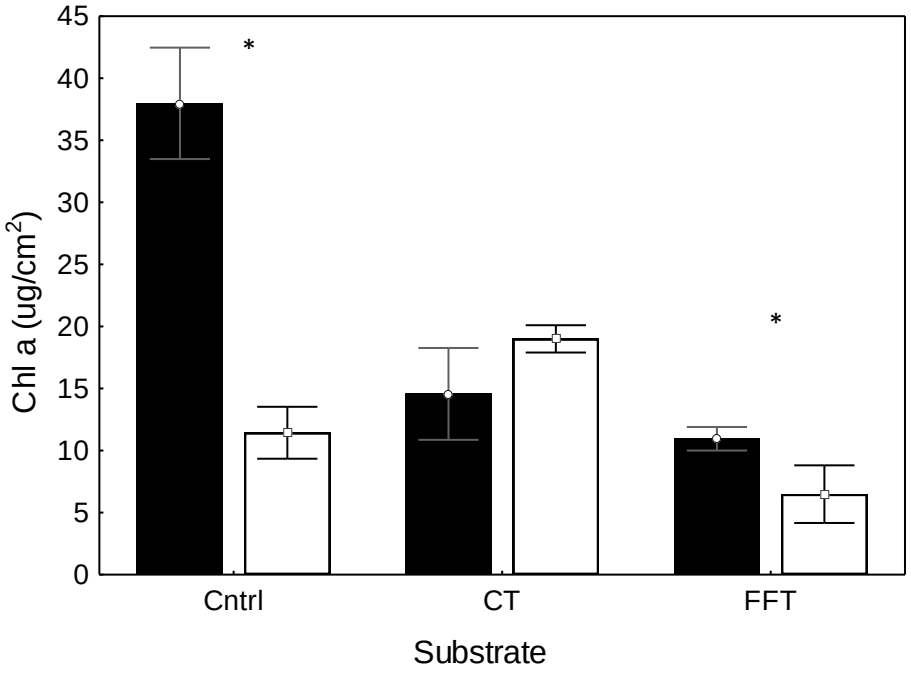


Figure 2.7 - Mean (± SE) chlorophyll *a* (Chl a) concentrations at day 1 (filled bars) and 4 (open bars) of the second aquarium study (n = 7). Treatments were cntrl: 3.5 cm of water, CT: 3.5 cm of water with 1 L CT and FFT: 3.5 cm of water with 1 L FFT. A significant difference between days 1 and 4 is indicated with an asterisk.

2.4 Results: Experiment 2

2.4.1 Trench Water Chemistry

Water chemistry parameters including conductivity, salinity and pH were significantly higher in OSPW trenches (one-way ANOVA; n = 42, $F_{(1, 82)}$ = 156.78, $p < 0.001$; n = 42, $F_{(1, 82)} = 187.86$, $p < 0.001$; n = 6, $F_{(1, 10)} = 10.083$, $p < 0.01$; respectively). Table 2.1 summarizes the mean (± SE) of physical and chemical parameters sampled in situ throughout the three field seasons: Temperature (°C), Conductivity (µs/cm^2), Salinity (ppt), Dissolved Oxygen (mg/L), pH and Depth (cm). Comparisons among individual trenches show significantly lower water column DO in trench 2 (planned comparison, n = 14, $p < 0.05$). No differences were found between individual trenches for temperature and pH. Depth was determined to be significantly higher in trenches 3 and 5 with mean (± SE) depth at 63.92 ± 2.38 and 60.21 ± 3.41 cm, respectively (planned comparison, n = 8, $p < 0.05$). Water column temperatures in winter 2008/2009 and 2009/2010 recorded from HOBO data loggers, reached below -1 and -5°C, respectively (see Figure 2.8). Naphthenic acid concentrations, as predicted, were much higher in OSPW trenches ranging from 16.5 to 19.3 mg/L. In freshwater trenches naphthenic acid concentrations ranged from 2.5 to 2.9 mg/L (See Appendix I for summary table of water quality analysis).

2.4.2 Microcosm Substrate Chemistry

Nutrient supply rates are summarized in Appendix II. All nutrient supply rates values are presented with the following units: µg/10 cm^2/4 d. As determined by Western Ag Innovations, absorption rate of ions by the PRS probe membrane is not linear and therefore I could not convert units to per day (Western Ag Innovations., Saskatoon, SK, Canada). No statistically significant differences were observed in nutrient supply rates for NO_3, NH_4, Fe, Mn, Cu, Zn, B, Pb, Al, and Cd between transfer treatments (slurry vs control only) or beween substrates (CT vs non-CT) since all resulting values of combined probabilities (-2Log(ΣLNp)) were lower than the critical value ($X^2_{[0.05, 6]} = 12.59$; Table 2.2). Table 2.2 summarizes the analyses to those nutrients that resulted in a significant

effect (See Appendix II for a complete list of nutrients analyzed). In freshwater calcium and magnesium were highest for non-CT substrates (mean ± SE; 2020.38 ± 53.14 and 406.95 ± 20.50, respectively) compared to CT containing substrates (mean ± SE; 1734.13 ± 146.79 and 350.03 ± 37.09, respectively). Potassium supply rates, for both freshwater and OSPW treatments, were greatest in CT substrates (mean ± SE; 57.57 ± 14.31 and 67.1 ± 9.35, respectively) relative to non-CT substrates (mean ± SE; 21.77 ± 2.67 and 40.77 ± 3.20, respectively). Sulfur rates were elevated in slurry treatments (mean ± SE; 91.43 ± 24.40) versus control treatments (mean ± SE; 63.97 ± 23.24) within freshwater trenches. Figure 2.9 depicts the comparisons of mean (± SE) supply rates for NO_3, NH_4 and P between transfer technique and substrates. These nutrients were of primary interest due to their known capacity to cause significant effects on microbial communities and their suspected prevalence in CT substrates.

Analyzing each trench alone also resulted in mostly nonsignificant differences between substrates and transfer treatments, except for trenches 5 and 6. Mean (± SE) iron supply rates in trench 5 increased in CT substrates (794.05 ± 120.53) compared to non-CT substrates (489.45 ± 155.46; one-way ANOVA, $n = 4$, $p < 0.05$). Mean (± SE) phosphorous supply rates in trench 6 increased in non-CT substrates (0.85 ± 0.32) compared to CT substrates (0.3 ± 0.06; one-way ANOVA, $n = 4$, $p < 0.01$).

Table 2.1 - Water chemistry parameters (mean ± SE) of experimental trenches. Samples were collected irregularly between May and September throughout the course of the study (Aug. 2008 – June 2010).

Trench	Water Type (n=3)	Temperature (°C) (n=14)	Conductivity (µs/cm^2) (n=14)	Salinity (ppt) (n=14)	Dissolved Oxygen (mg/L) (n=14)	pH (n=2)	Depth (cm) (n=8)
1	Freshwater	16.0 ± 1.8	496.2 ± 32.9	0.2 ± 0.0	5.5 ± 0.6	8.1 ± 0.2	50.7 ± 2.1
3		15.1 ± 1.6	491.1 ± 26.0	0.2 ± 0.0	4.8 ± 0.7	7.9 ± 0.2	63.9 ± 2.4
5		15.1 ± 1.6	561.4 ± 20.2	0.3 ± 0.0	5.0 ± 0.6	8.0 ± 0.2	60.2 ± 3.4
2	OSPW	15.7 ± 1.7	1338.2 ± 97.4	0.7 ± 0.1	3.3 ± 0.5	8.2 ± 0.1	53.2 ± 2.6
4		15.8 ± 1.7	1104.4 ± 363.7	0.6 ± 0.1	5.7 ± 1.1	8.4 ± 0.0	53.8 ± 3.1
6		15.6 ± 1.7	1484.8 ± 105.6	0.8 ± 0.1	4.0 ± 0.5	8.4 ± 0.0	47.2 ± 2.4

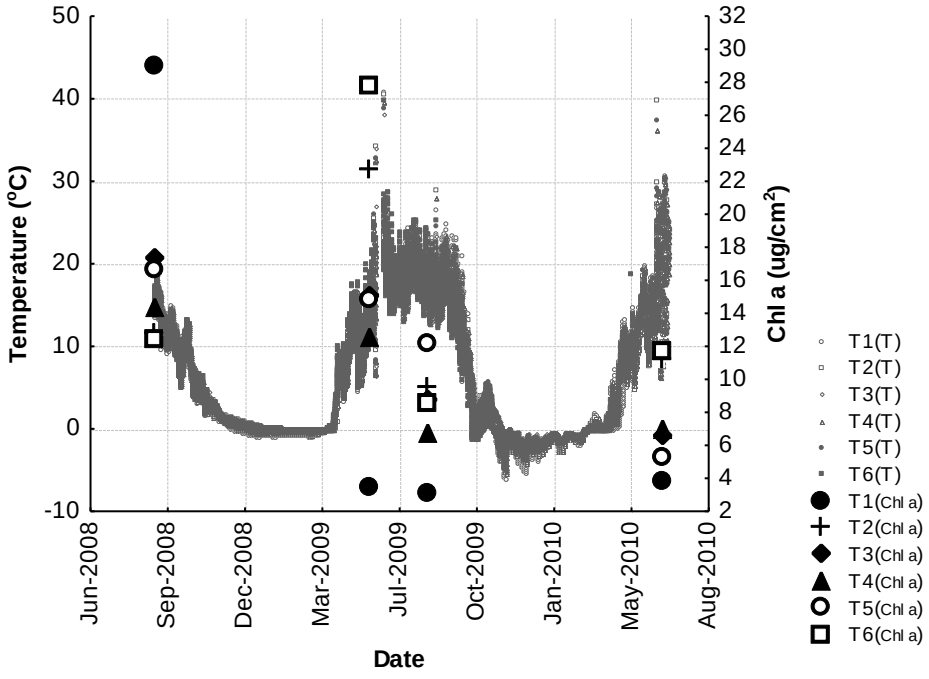


Figure 2.8 - Mean chlorophyll *a* (Chl a) concentration (right axis) and temperature (left axis) for each trench over the course of the study. Chlorophyll *a* concentrations were averaged amongst samples withing trenches. Temperature (shown for each trench) was recorded every 2 h throughout the 2-y study.

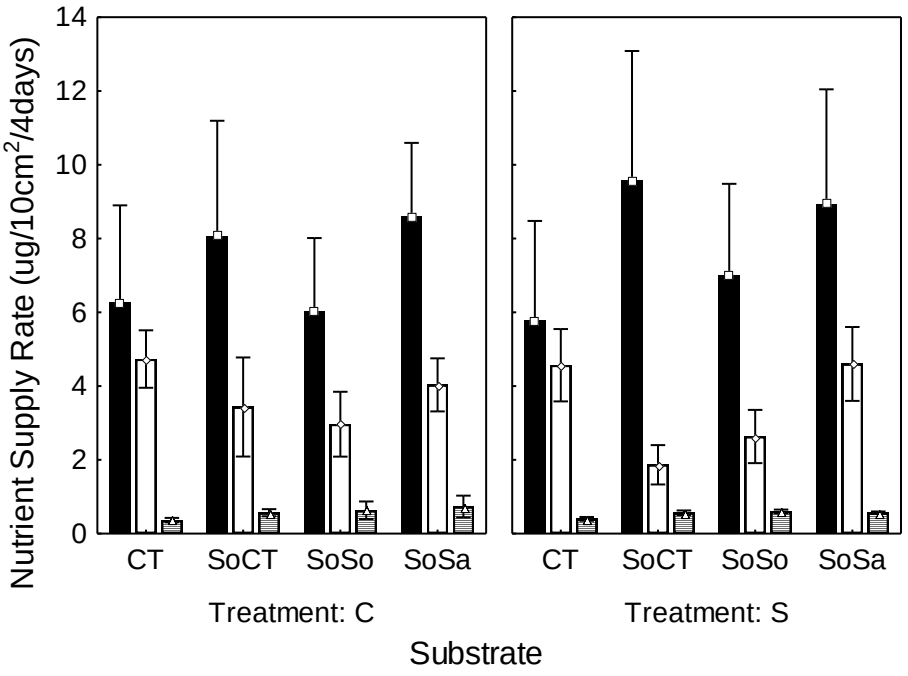


Figure 2.9 - Mean (± SE) nutrient supply rates with units (µg/10 cm^2/4 d) shown for all four substrates and comparing controls (C) versus slurry (S) treatments. Ions included are NO_3-N (filled bars), NH_4-N (open bars) and P (–). See Appendix II for a summary of nutrient supply rates.

Table 2.2 - Summary of nutrient supply rate comparisons between transfer techniques (cntrl (control) vs trtmnt (slurry)) and substrates (CT vs Non-CT) using one way ANOVA statistical analyses. P-value combinations were done for FW trenches and OSPW trenches and analyzed using a chi squared test. Only nutrients whose supply rate varied significantly between treatments in at least one test are shown.

Nutrient	Water Type	Test	Combined 'p' Values (-2∑LNp)	X^2 (.05, 6)	Significance of X^2 Test
Ca	FW	Cntrl vs Trtmnt	3.89	12.59	p > 0.05
Ca	OSPW	Cntrl vs Trtmnt	2.23	12.59	p > 0.05
Ca	FW	CT vs Non-CT	19.13	12.59	p < 0.05*
Ca	OSPW	CT vs Non-CT	9.18	12.59	p > 0.05
Mg	FW	Cntrl vs Trtmnt	4.85	12.59	p > 0.05
Mg	OSPW	Cntrl vs Trtmnt	3.14	12.59	p > 0.05
Mg	FW	CT vs Non-CT	13.99	12.59	p < 0.05*
Mg	OSPW	CT vs Non-CT	5.22	12.59	p > 0.05
K	FW	Cntrl vs Trtmnt	2.56	12.59	p > 0.05
K	OSPW	Cntrl vs Trtmnt	0.67	12.59	p > 0.05
K	FW	CT vs Non-CT	40.58	12.59	p < 0.05*
K	OSPW	CT vs Non-CT	20.5	12.59	p < 0.05*
S	FW	Cntrl vs Trtmnt	16.54	12.59	p < 0.05*
S	OSPW	Cntrl vs Trtmnt	3.04	12.59	p > 0.05
S	FW	CT vs Non-CT	6.56	12.59	p > 0.05
S	OSPW	CT vs Non-CT	2.07	12.59	p > 0.05

2.4.3 Chlorophyll a and TDW Biomass

There was a positive relationship between chlorophyll *a* concentration ($\mu g/cm^2$) and TDW biomass (g) (Regression, n = 115, $F_{(1,113)} = 5.045$, $p < 0.05$). Variation explained, however, was extremely low with $R^2 = 0.043$ (Figure 2.10). An increase in R^2, however, was observed when conducting regression analyses for individual substrates. A significant relationship was also found between chlorophyll a and TDW biomass for three of the four substrates: SoCT, SoSo and SoSa (Figure 2.11). Though a significant relationship exists, variability is too high for the relaitionship to be a predictive value, therefore, chlorophyll a concentrations discussed in this thesis were used only as a proxy for photoautotrophic biomass.

Table 2.3 summarizes the multiple regression analysis conducted using dummy variables to determine which treatments had a significant effect on chlorophyll *a*. Chlorophyll *a* concentrations declined over the course of the study. Peak chlorophyll *a* concentrations were found in spring 2009 (mean ± SE; 15.75 ± 1.44), followed by summer 2008 (mean ± SE; 14.66 ± 1.66). Summer 2008, though significantly lower in chlorophyll *a* concentration than spring 2009 (Multiple Regression, n = 120, $p < 0.001$), supported elevated concentrations compared to the last two sampling dates, summer 2009 and spring 2010. Mean (± SE) chlorophyll *a* concentrations for summer 2009 and spring 2010 were 8.12 ± 0.75 and 7.49 ± 0.509, respectively. The difference in concentrations between summer 2008 and summer 2009 was not significant ($p > 0.05$).

Chlorophyll *a* concentrations were similar among substrate treatments for sample sets collected in summer 2008, and spring 2010. Mean chlorophyll *a* concentrations were greater on CT substrate than on other sediments in spring and summer 2009 (Figure 2.12). Also, when data from all sample sets were combined, CT substrate supported significantly higher chlorophyll *a* concentrations than the other sediment treatments (multiple regression, n = 120, $p < 0.01$). Mean (± SE) chlorophyll *a* concentrations for CT ranged from 7.25 ± 1.03 to 20.86 ± 3.07 amongst the sample sets. The overall mean chlorophyll *a* concentrations for the four substrate treatments were (in descending order) 13.67 ± 1.34 (CT), 11.21 ±

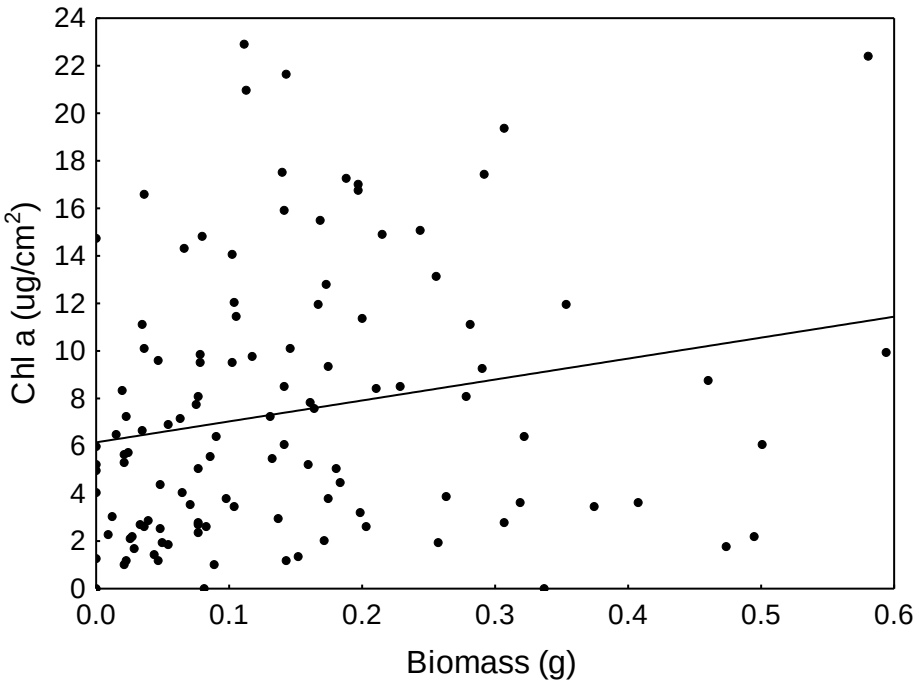
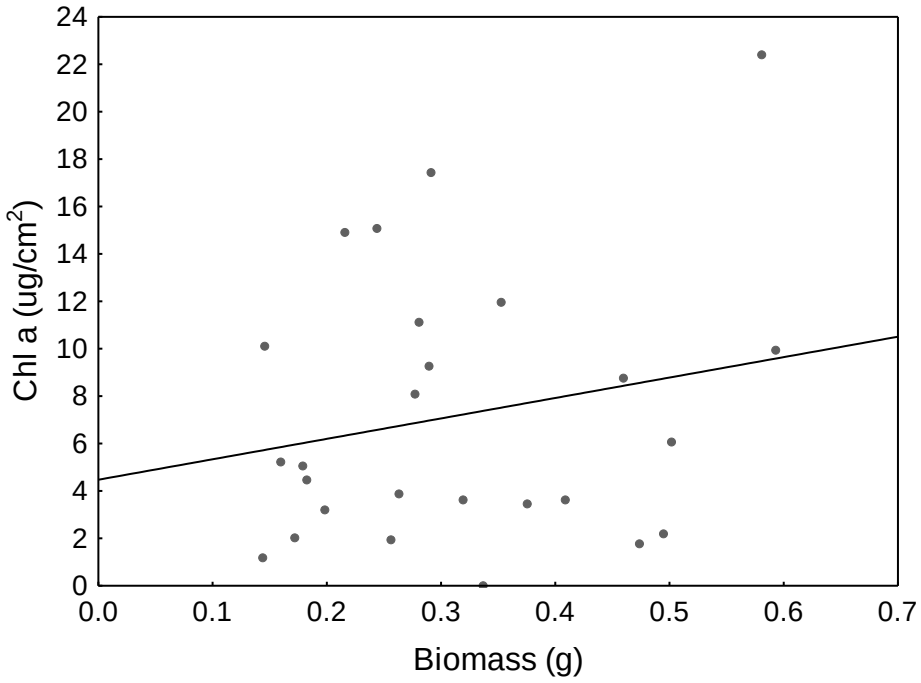


Figure 2.10 - Chlorophyll *a* (Chl a) sampled in 2010 as a function of TDW biomass. The slope of the relationship was significant but the variability is poorly explained by what was measured ($R^2 = 0.043$, $F_{(1,113)} = 5.045$, $p < 0.05$).

A)


Chl a (ug/cm2)
Biomass (g)
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2
4
6
8
10
12
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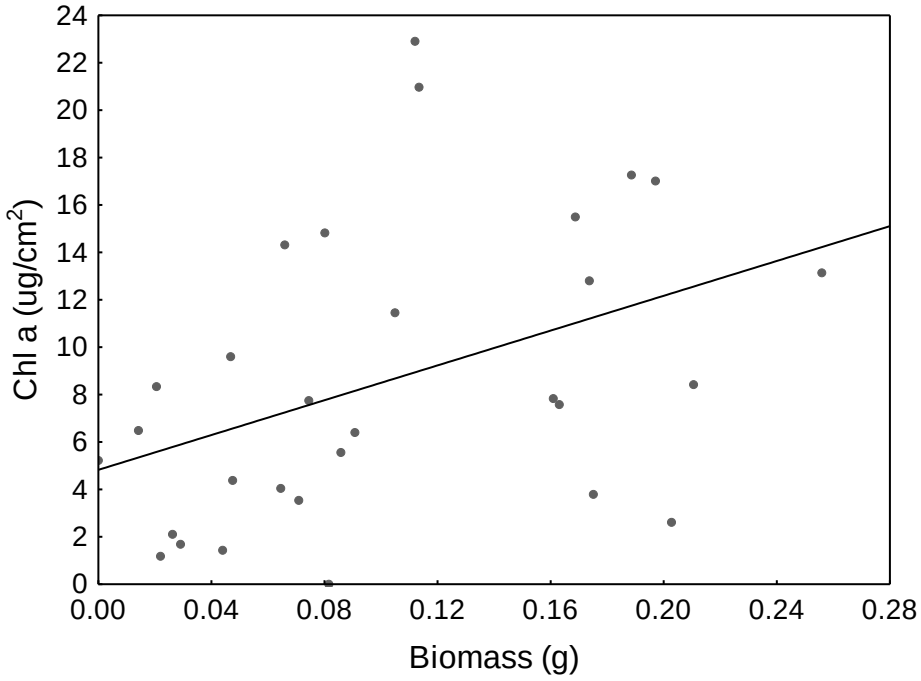
B)


Chl a (ug/cm2)
Biomass (g)
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2
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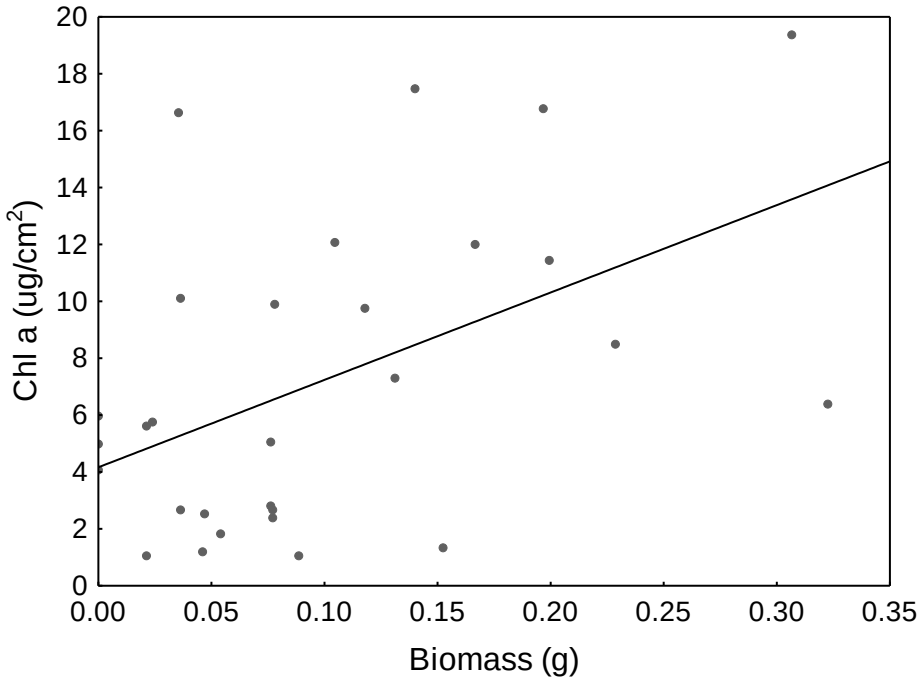
C)



D)

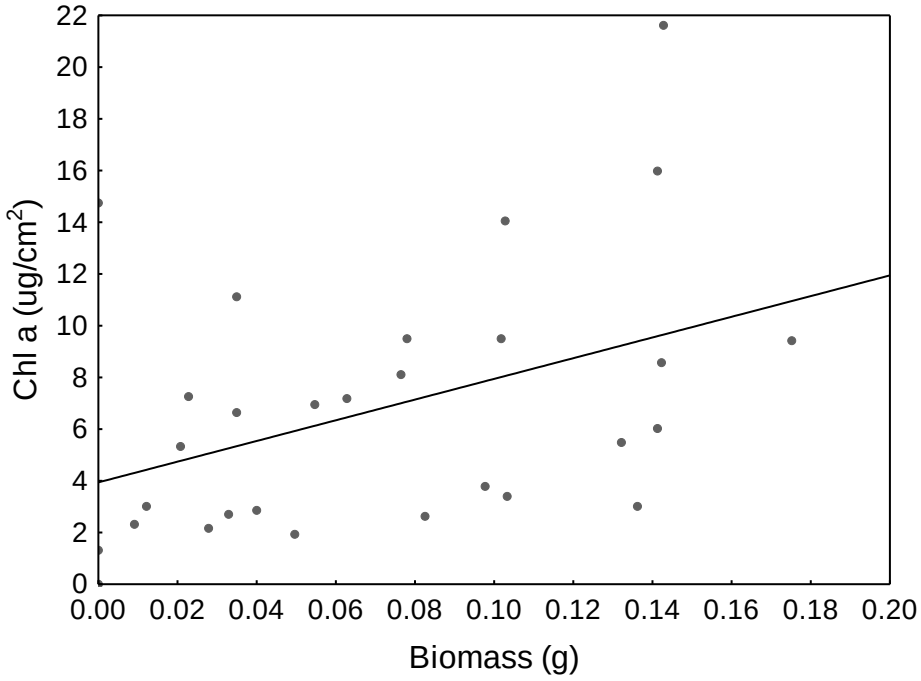


Figure 2.11 - Chlorophyll *a* (Chl a) as a function of TDW biomass for each substrate A) CT, B) SoCT, C) SoSa and D) SoSo. The slope of the relation was significant for SoCT, SoSa and SoSo ($R^2 = 0.18$, $F_{(1,28)} = 5.92$, $p < 0.05$; $R^2 = 0.24$, $F_{(1,27)} = 8.401$, $p < 0.01$; $R^2 = 0.18$, $F_{(1,28)} = 1.488$, $p < 0.05$, respectively).

Table 2.3 - Multiple Regression analysis using dummy variables for chlorophyll a and treatments. Variables chosen as base comparison factors, in which all other remaining treatments within each variable were compared to, were; time = summer 2008, substrate = SoSo, water = FW, transfer = control and trench = trench 1.

Variable	Slope	SE	T(393)	Partial R^2	Cumulative R^2	p - level
Intercept	0.415	0.063	6.542			0.000
Mat	0.329	0.060	5.528	0.0543	0.0543	0.000
OSPW	0.274	0.057	4.847	0.0104	0.0647	0.000
Slurry	0.273	0.049	5.622	0.0142	0.0789	0.000
Trench 5	0.216	0.065	3.317	0.0081	0.087	0.001
Trench 3	0.192	0.065	2.941	0.0154	0.1024	0.003
Spring 2009	0.183	0.046	3.972	0.038	0.1404	0.000
CT	0.105	0.044	2.409	0.0103	0.1507	0.008
Summer 2009	-0.053	0.046	-1.150	0.0024	0.1531	0.125
Trench 4	-0.140	0.057	-2.481	0.011	0.1641	0.007

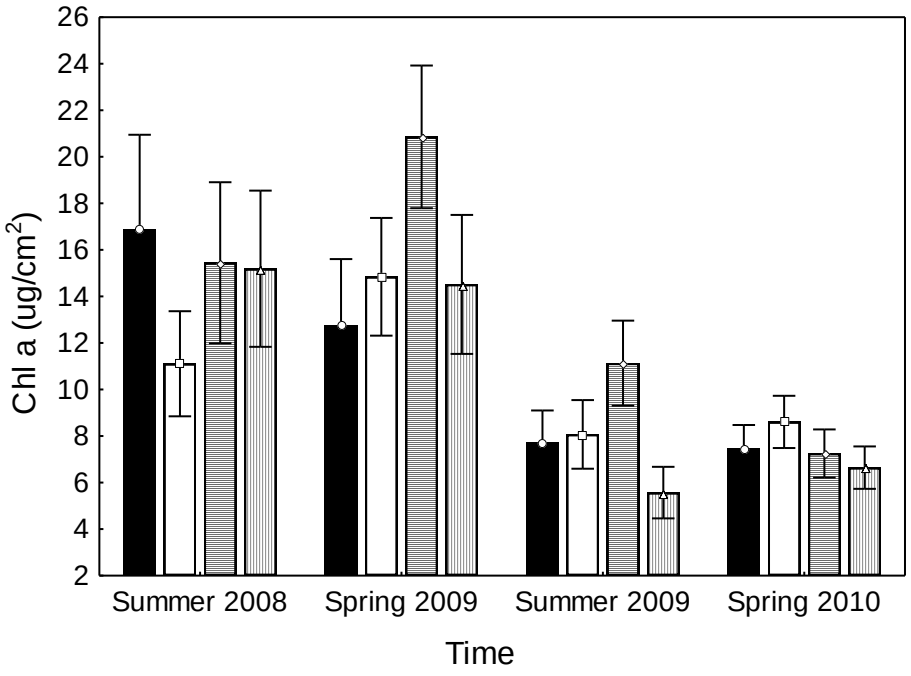


Figure 2.12 - Time and substrate-specific trends for chlorophyll *a* (Chl a) concentrations. Each bar represents mean (± SE) chlorophyll *a* concentrations averaged across trenches' substrate (SoSa (filled bars), SoCT (open bars), CT (–) and SoSo (|)).

1.34 (SoSa), 10.66 ± 0.98 (SoCT) and 10.48 ± 1.23 (SoSo).

Greater photoautotrophic biomass was observed in samples inoculated with the mat and slurry treatments than in control samples (multiple regression, n = 96, 288 & 96 respectively, $p < 0.001$; Figure 2.13). Water type also significantly influenced photoautotrophic biomass. Mean chlorophyll *a* concentrations were greater in trenches containing OSPW (mean ± SE, 12.37 ± 0.87) than those containing fresh water (mean ± SE, 10.64 ± 0.87; multiple regression, n = 240, $p < 0.001$; Figure 2.14). Significant effects were also found among individual trenches. Chlorophyll *a* concentrations were significantly higher in trenches 3 and 5 than in trench 1, all of which contained fresh water (multiple regression, n = 20, $p < 0.01$). Trench 1 (FW) maintained significantly higher concentrations than trench 4 (OSPW) (multiple regression, n = 20, $p < 0.01$).

Results for TDW biomass (g) were congruent with chlorophyll *a* concentrations during spring 2010 for water type and transfer technique comparisons. See Table 2.4 for a summary of the multiple regression analyses for TDW biomass. Mean TDW biomass (± SE; 0.172 ± 0.02; n = 118) was significantly higher in OSPW compared to FW (0.115 ± 0.02; n = 117; Figure 2.15). This trend occurred for chlorophyll a concentrations as well (Figure 2.14). It was also evident that biomass levels for SoCT, SoSa and SoSo were more dependent on water type than biomass exposed to CT, which was equally high in both FW and OSPW (Figure 2.15). Control microcosm biomass did not differ ($p > 0.05$) from that found in treatment microcosms for all four substrates. This was the second trend also found in chlorophyll *a* concentrations (Figure 2.13). A contrasting result existed between the two response variables when comparing CT amongst the other substrates. No significant difference was found in chlorophyll *a* concentrations amongst all four substrates (Figure 2.12), yet CT mean (± SE) TDW biomass (0.315 ± 0.02) was significantly higher than that determined for SoCT, SoSa and SoSo (0.103 ± 0.01, 0.100 ± 0.02, 0.079 ± 0.01, respectively; Figure 2.16). Biofilms were noticeably thicker on CT than the remaining three substrates throughout the study. Biofilm thickness on CT ranged from 5 to 10 mm and from 2 to 5 mm on soil containing substrates (pers. obs.)

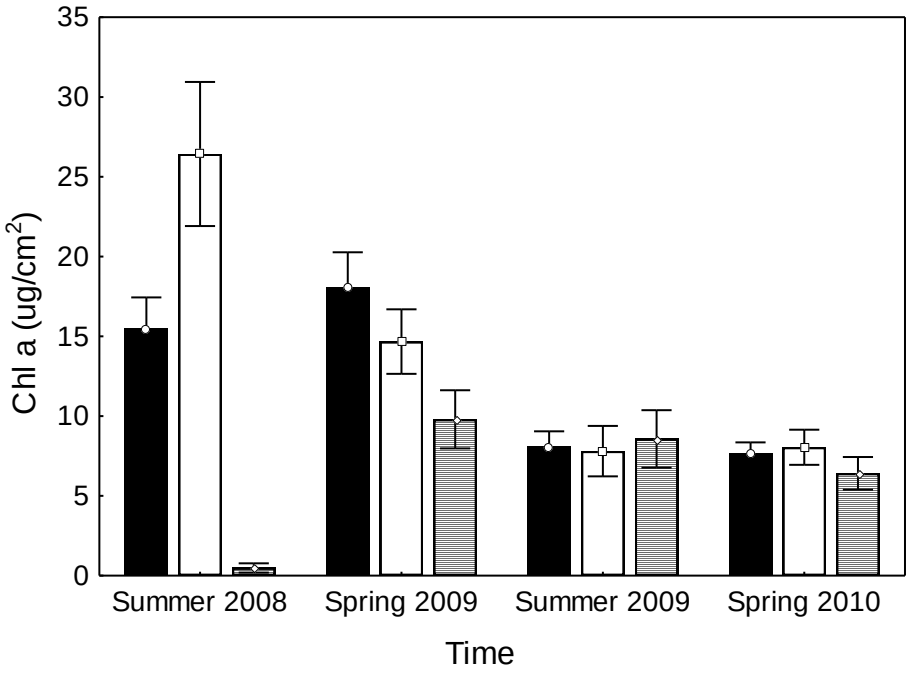


Figure 2.13 - Mean (± SE) chlorophyll *a* (Chl a) concentrations compared amongst sample sets and transfer technique (slurry (filled bars), mat (open bars) and control (–)).

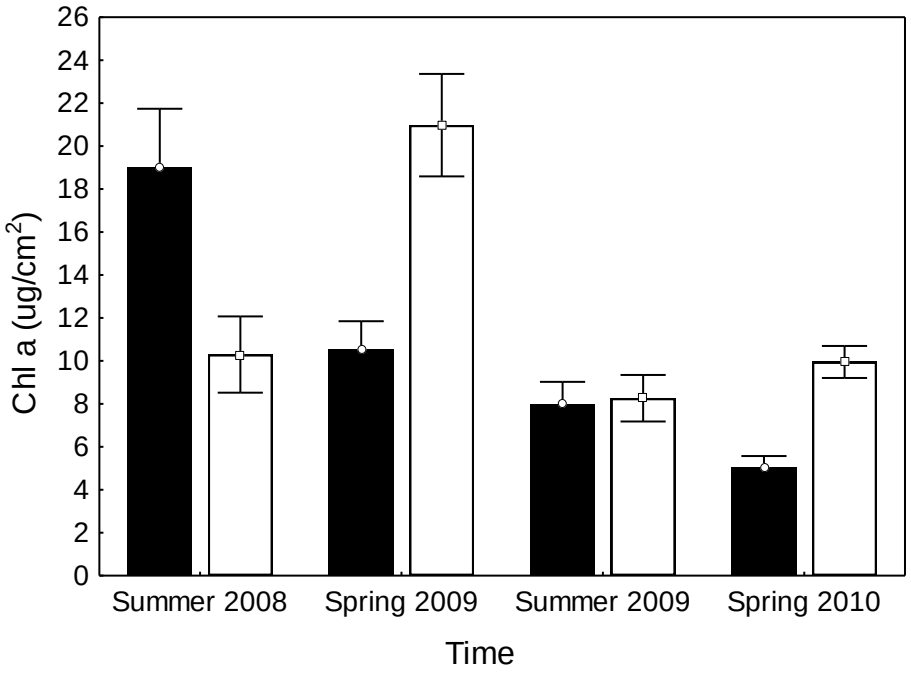


Figure 2.14 - Mean (± SE) chlorophyll *a* (Chl a) concentrations compared among sample sets and water type (FW (/) and OSPW (\ )).

Table 2.4 - Multiple regression analysis using dummy variables for TDW biomass and treatments. Variables chosen as base comparison factors, in which all other remaining treatments within each variable were compared to, were; substrate = SoSo, water = FW, transfer = control and trench = trench 1.

Variable	Slope	SE	T(393)	Partial R^2	Cumulative R^2	p - level
Intercept	0.027	0.0042	6.321			0.000
CT	0.078	0.0067	11.689	0.5106	0.5106	0.000
OSPW	0.03	0.0062	4.857	0.0587	0.5693	0.000
Trench 4	-0.024	0.0085	-2.841	0.0292	0.5985	0.005

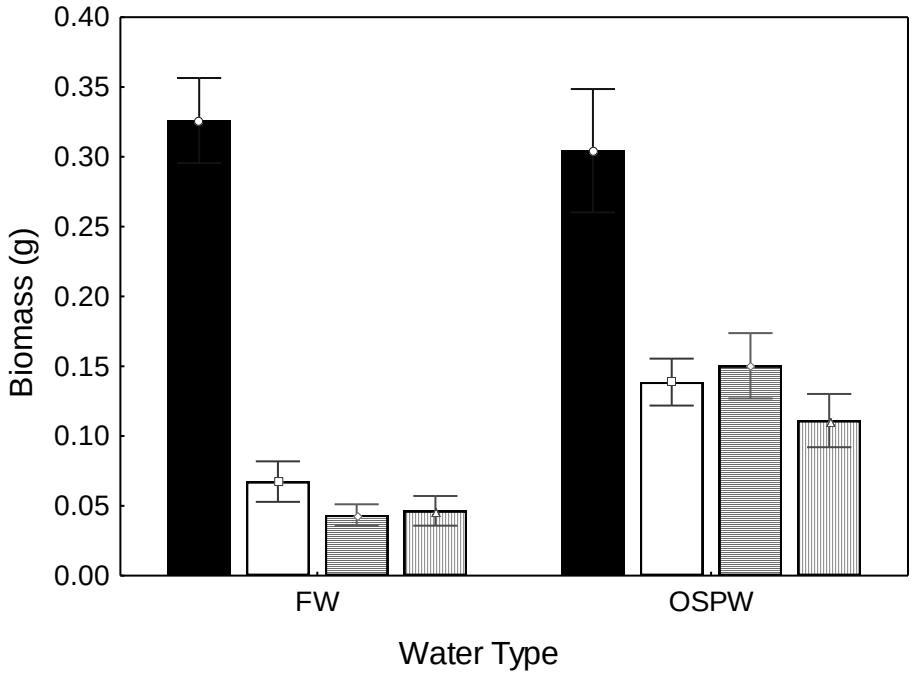


Figure 2.15 - Mean (± SE) TDW biomass (g) for the four substrates (CT (filled bars), SoCT (open bars), SoSa (–) and SoSo (|)) within each water treatment at the end of the study (Spring 2010).

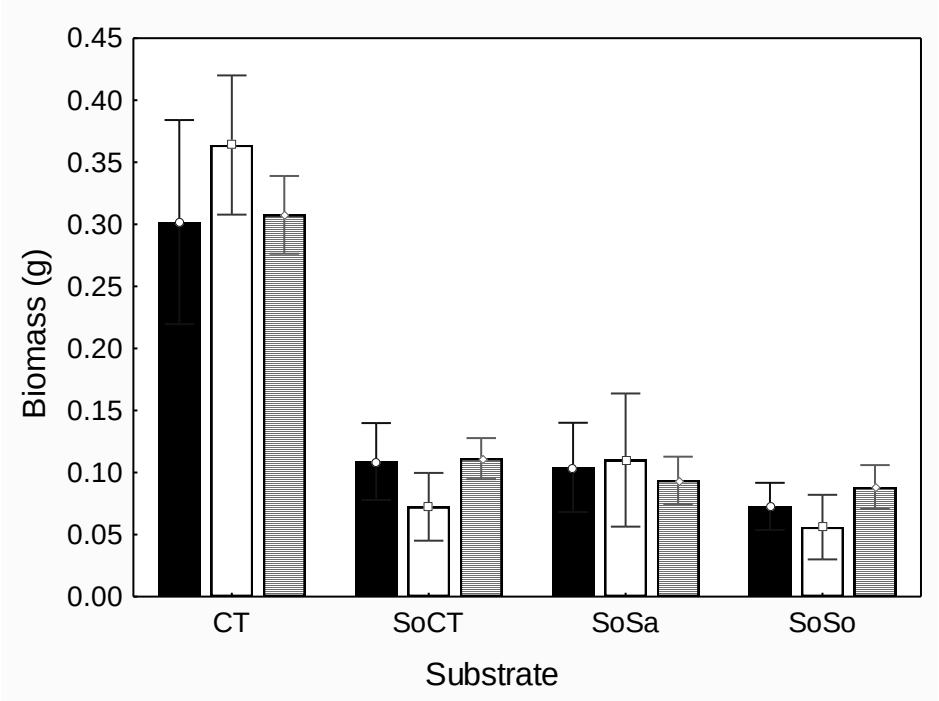


Figure 2.16 - Mean (± SE) TDW biomass (g) for transfer techniques (C (filled bars), M (open bars), S (–)) amongst each substrate at the end of the study (Spring 2010).

2.4.4 Dissolved Oxygen and Carbon Accumulation

Dissolved oxygen sampling was used here as a method for determining gross primary production (GPP). Gross primary production was obtained by subtracting respiration (DO change (negative rate) under dark chambers) from net primary production (DO change under light chambers) and has the units: g O_2/m^2/day. Table 2.5 summarizes results from the multiple regression analysis. GPP seemed to be most affected by the parameters within individual trenches. Trenches 6 (mean GPP ± SE; 0.162 ± 0.01), 2 (0.132 ± 0.03) and 5 (0.098 ± 0.01) supported the highest measures of GPP relative to trench 1 (0.059 ± 0.01) (multiple regression, n = 64, 64 & 72, $p < 0.01$, 0.01 & 0.05, respectively). Regardless of treatment, GPP was at its highest (0.143 ± 0.02; multiple regression, $p < 0.01$) during midsummer (July 2009) when respiration rates (-0.05 ± 0.01) were exceptionally low (Figure 2.17, 2.18 and 2.19). August 2009 supported the lowest GPP with a mean (± SE) of 0.0781 ± 0.01. The slurry transfers supported the highest GPP throughout the study compared to the mat and control treatments (multiple regression, $p < 0.05$, respectively). GPP rates for transfer techniques ranged from 0.112 ± 0.03 (slurry) to 0.086 ± 0.01 (mat) (Figure 2.18). However, the magnitude of this difference was hampered because slurry treatments experienced high respiration rates over the course of the study but nearly equivalent rates of NPP compared to controls and mat treatments. In order from earliest to latest, total GPP in units of g C/m^2/day for the four sampling dates were 0.028, 0.029, 0.045 and 0.024. Due to increased error and assumptions involved in C conversions from units of O_2, I have limited these calculations to the broader, all encompassing values.

No significant effect on GPP was found among substrates and water type (multiple regression, n = 100, $p > 0.05$). Respiration rates and NPP are shown in Figure 2.17 & 2.19 for water and substrate treatments across all four sampling dates. GPP (g O_2/m^2/day) for substrates ranged from 0.093 ± 0.01 (SoSa) to 0.120 ± 0.02 (SoSo). Figure 2.19 shows CT with increased gross primary production but low respiration rates and therefore lower GPP compared to SoSo and SoCT.

Finally, mean (± SE) GPP for water treatments ranged from 0.079 ± 0.01 (FW) to 0.126 ± 0.01 (OSPW).

Table 2.5 - Multiple regression analysis using dummy variables for gross primary production and treatments. Treatments chosen as base comparison factors, in which all other remaining treatments within each variable were compared to, were; time = Aug-2008, substrate = SoSo, water = FW, transfer = control and trench = trench 1.

Variable	Slope	± SE	t(393)	Partial R^2	Cumulative R^2	p - level
Intercept	0.016	0.005	3.021			0.003
Trench 6	0.036	0.007	5.224	0.0466	0.0466	0.000
Trench 2	0.022	0.007	3.138	0.0155	0.0621	0.001
July 2009	0.021	0.005	4.145	0.0383	0.1004	0.000
Trench 5	0.013	0.007	2.015	0.0063	0.1067	0.045
Slurry	0.008	0.005	1.699	0.0061	0.1128	0.045
Trench 4	0.008	0.007	1.127	0.0029	0.1157	0.129

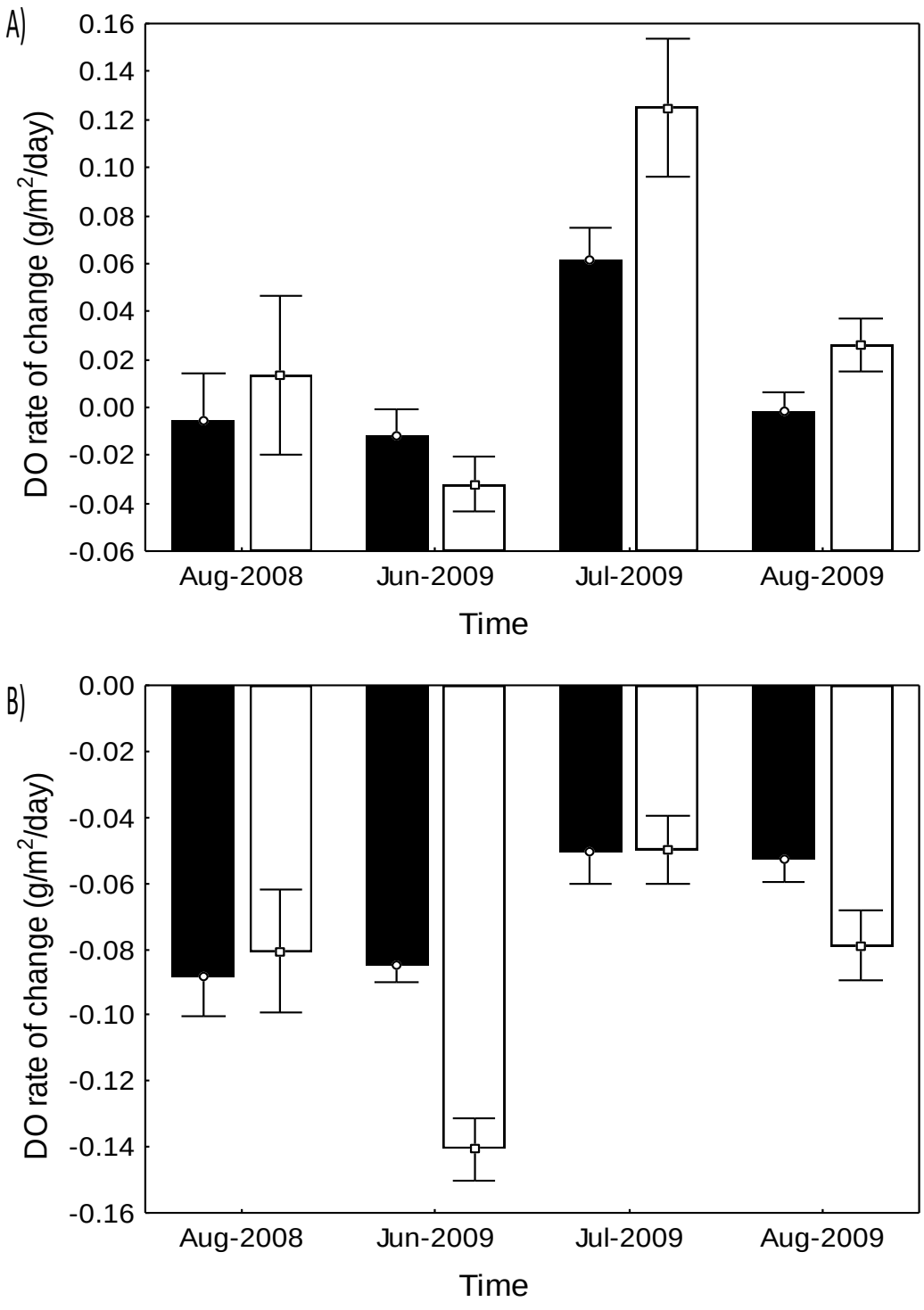


Figure 2.17 - Mean (± SE) DO rate of change compared among sample sets and water type (FW (filled bars) and OSPW (open bars)). Graph A: DO change under light chambers; Graph B: DO change under dark chambers.

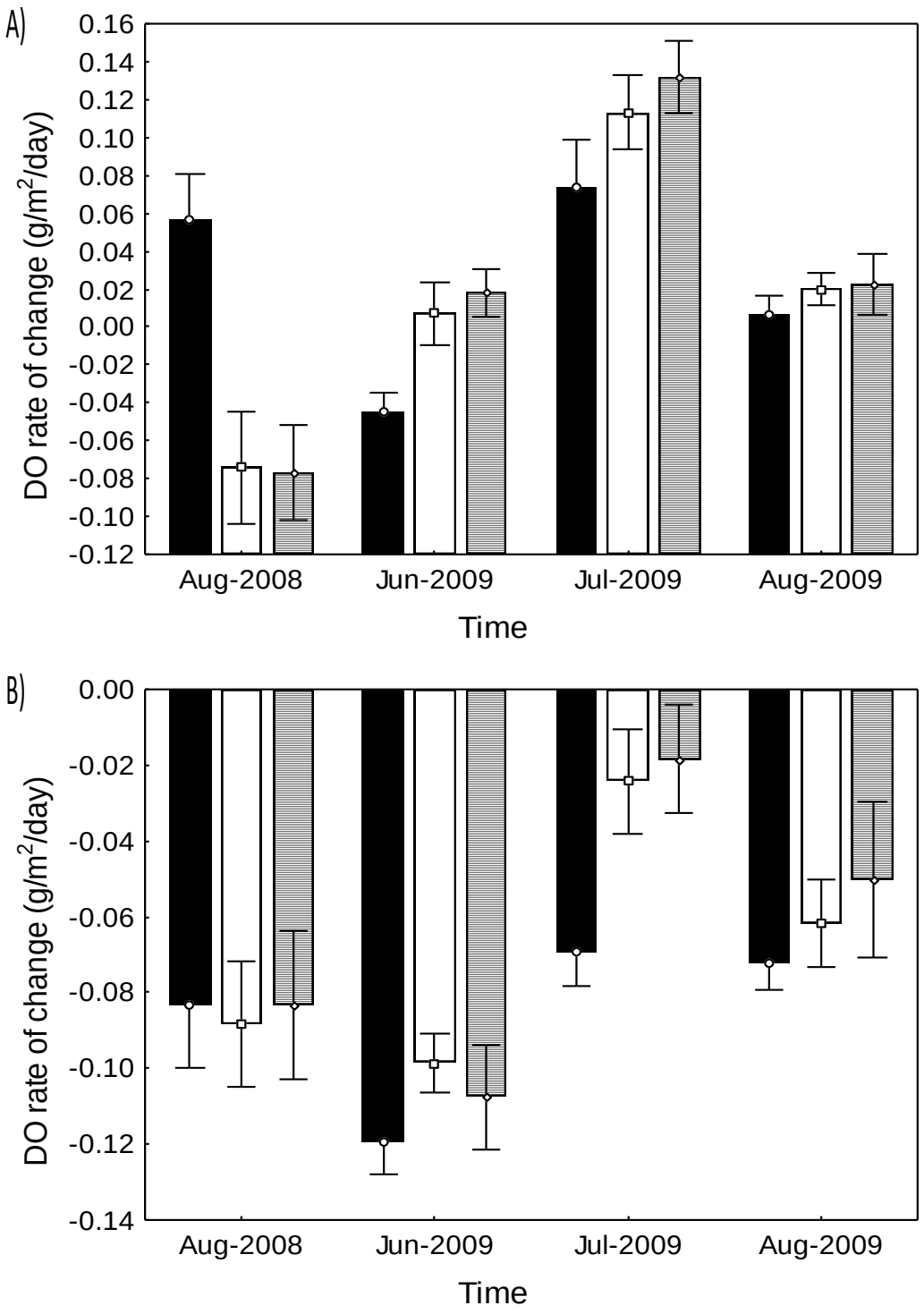


Figure 2.18 - Mean (± SE) DO rate of change compared among sample sets and transfer technique (S (filled bars), C (open bars) and M (–)). Graph A: DO change under light chambers; Graph B: DO change under dark chambers.

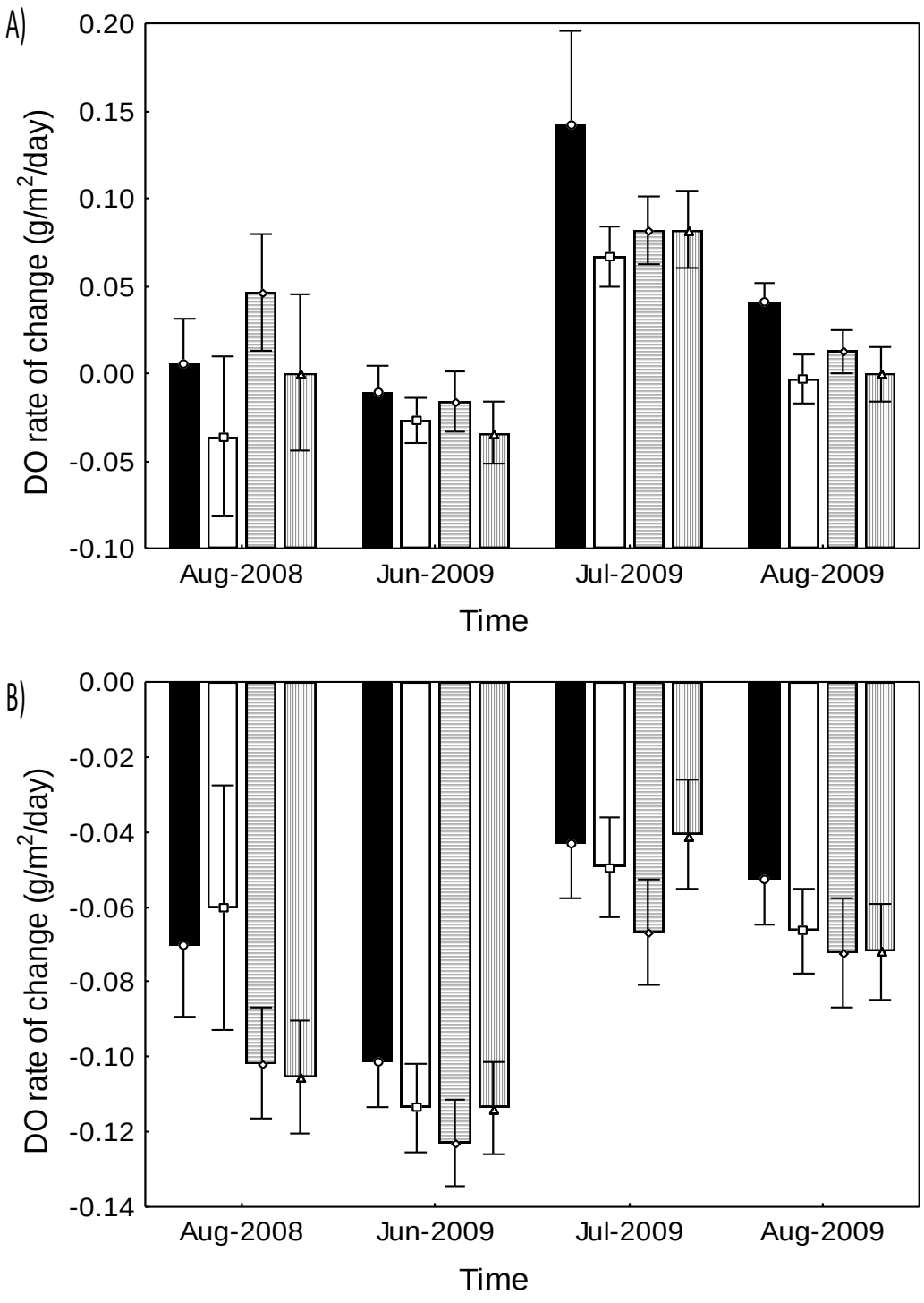


Figure 2.19 - Mean (± SE) DO rate of change compared amongst sample sets and substrate (CT (filled bars), SoSo (open bars), SoSa (–) and SoCT (|). Graph A: DO change under light chambers; Graph B: DO change under dark chambers.

2.5 Discussion

Algal biofilms and/or the constituents survived the transfer process, colonized new substrates and bodies of water with varying chemical and physical composition and caused an immediate increase in productivity and biomass levels. A wide range of effects were observed throughout the study from the differing treatments. This allowed me to determine the optimal reclamation techniques and materials for enhanced biofilm colonization success and growth. Direct and indirect biofilm responses were observed for seasonal change, substrate nutrient dynamics and water chemistry parameters over the course of the study. This will enhance our understanding of benthic biofilms and their contributions to wetland production and provide insight into the carbon flow at the base of constructed wetlands exposed to reclamation materials.

Working within the trenches, though providing a more realistic setting than the laboratory, has presented its challenges. Trench vegetation, substrate heterogeneity, well established invertebrate communities and wildlife, all contributed to increased data variability. An intensive repeated sampling design was used to help mitigate these confounding variables.

2.5.1 Experiment 1

Experiment 1 was formulated based on a simple experimental design to effectively determine whether biofilms, or at least their constituents, could in fact be collected, disturbed and transferred to a new substrate without dying, and beyond this, colonize and grow. Photoautotrophic biomass, regardless of treatment, varied among sample sets (24 and 96 h) for trials 1 & 2 (see Figure 2.5 & 2.7). It is clear, however, that in most cases colonization and growth, or at least survival maintenance, occurred. This confirms that stress induced from the transfer was not the primary factor causing decreases in biomass but rather the treatments themselves or complicating factors such as temperature fluctuations and changing weather. This indicates that CT supports higher productivity than its reclamation substrate counterpart FFT. Photoautotrophic biomass production was similar between organic substrates and CT suggesting that CT may be able to

support a benthic microbial community of comparable productivity to that of natural organic substrates. The general increase in biomass production or growth during trial 1 and maintained or declining production in trial 2 may be the result of changing weather since the two trials were conducted sequentially. It should be noted that experiment 1 followed a pilot scale design and therefore all conclusions or suggestions interpreted from the data were pursued with the second larger more extensive experiment.

2.5.2 Experiment 2: Chlorophyll a and TDW Biomass

Contrary to my expectations, biomass production diminished over time (Figure 2.8), even though it appears as though the benthic biofilms survived the transfer with relatively high chlorophyll *a* concentrations during the summer 2008 sample set. The following spring and summer sample sets brought expected results with decreasing chlorophyll *a* concentrations from prime growing season to late summer when temperatures were drastically lower at night. Unexpectedly, biomass levels remained low for the following growing season. This observation was complemented by results for TDW biomass. An explanation for this may be the exceptionally cold winter that preceded spring 2010 sample set (Figure 2.8). Chlorophyll *a* concentrations generally decrease with decreasing temperatures (Liboriussen and Jeppesen 2003), however, a study researching the effects of freeze-thaw cycles on algae and cyanobacteria concluded that the effects of temperatures down to 5°C below zero were of no significance (Sabacka and Elster 2006). Negative impacts from low temperatures increased for algae communities not regularly exposed to these temperatures. Also, by the Spring 2010 sampling date, the microcosms have been exposed to high levels of disturbances from intensive sampling during the previous field seasons. The benthic microbial communities may not have had a sufficient amount of time to re-colonize between the last sample set of 2009 and the spring sample set of 2010. A contrasting argument for this result is that the transferred biofilms succumbed to the combined physico-chemical interactions such as light inhibition due to inhabiting plankton and macrophytes, grazing pressures resulting from an established

invertebrate community and a depleted sediment nutrient supply (Domozych and Domozych, 2008; Lassen et al., 1997; Stal, 2000). The decline to a lower stabilization point can be seen in Figure 2.13 where chlorophyll *a* concentrations for slurry and mat treatments eventually equilibrated back to control levels. Due to grazing pressures benthic microbial community development is strongly inhibited if there is a significant invertebrate grazer community (Awramik, 1984; Stal, 2000). Though snails were observed in a large proportion of microcosms while sampling, it is worth noting that 7 y, on average, are required for reclaimed OSPM-affected wetlands to become inhabited by a mature invertebrate community (Leonhardt 2003). Therefore, this effect may not be seen in newly constructed wetlands where invertebrate communities are underdeveloped.

Results for substrates and water type were as expected with CT and OSPW supporting significantly higher concentrations of chlorophyll *a*. Substrate nutrient supply rate analyses did not show any differences in ammonia, nitrates or phosphorous (Figure 2.9). I would suspect that excess ammonia, nitrate and phosphorous availability found within CT may have been depleted by the initial increased biofilm activity. All three are effectively cycled through biofilms with distinct heterotrophic and autotrophic communities (McCormick et al. 1998; Roeselers et al. 2008). For future studies I would recommend a before and after substrate nutrient analysis to determine the effects of biofilms on substrate nutrient availability.

Nitrogen in the form of ammonia will leach upwards to the oxic zone where nitrification is favored. Microbial communities within the biofilms will either consume and assimilate the nitrates or through denitrification release N_2 back into the system (Roeselers et al. 2008). Phosphorous is also efficiently assimilated by biofilms attached to the sediment. McCormick et al. (1998) found that epipelon accounted for the majority of stored phosphorous amongst the periphyton classes.

Nutrient biofilm interactions and subsequent nutrient depletion may account for the decreasing trend in chlorophyll *a* concentrations over time. As observed in Figure 2.14 the activities of individual or multiple taxa may have

been the cause for the peaks in chlorophyll *a* in FW and OSPW treatments during the two sampling intervals. High initial chlorophyll *a* concentrations in FW followed by high concentrations in OSPW during the following sample set suggests the possibility of different species responding to altered conditions. Subsequent nutrient depletion is evident with low biomass levels observed during the summer 2009 and spring 2010 sample sets.

High concentrations of potassium (K) found in CT substrates may also support increased algal growth. Anishchenko et al. (2010) correlated potassium, a nutrient that facilitates the creation and maintenance of starch, with a dominance of Chlorophyta, which is green algae. Starch was found to be the primary storage compound in green algae (Subbarao 2003, cited by: Anishchenko et al. 2010).

Increased sulfur prevalence within substrates exposed to the slurry treatment suggests the composition of the microbial layer possibly consists of heterotrophs including sulfate reducing bacteria as well as colorless and purple sulfur bacteria. These communities are known to cycle sulfur amongst each other but will also release it back into the sediments (Van Gemerden 1993). The presence of a benthic microbial biofilm will act as a potential barrier for any sulfur leaching into the water column from the sediments.

Water chemistry differed significantly between water types. OSPW had elevated levels of K, SO_4, salinity, conductivity and naphthenic acids (see Table 2.1 and Appendix I). K and SO_4 support algal growth and biofilm production (Anishchenko et al. 2010; Van Gemerden 1993). This may partially explain the overall trend of high chlorophyll *a* concentrations found in OSPW compared to FW trenches. Biofilms are known to persist in extreme environments including highly saline water bodies. High salinity has been found to stimulate microbial biofilm production through reduced competition and predation. A study conducted by Sim et al. (2006) found biofilms growing in water with salinities ranging from 15 ppt to 70 ppt. In this study, FW trenches were all below 0.5 ppt and OSPW trenches were between 0.5 and 1 ppt. Naphthenic acids, though high in concentration and acutely toxic to other wetland dwelling organisms, did not seem to have a significant detrimental effect on the transferred biofilms in OSPW.

Mat and slurry transfer techniques proved to be successful in that the biofilms survived and re-colonized new substrates. This is represented with significantly higher chlorophyll *a* concentrations in treatment microcosms than controls for the first two sample dates. Upon observing results from Experiment 1 (Figure 2.6), it is also evident that biofilms are capable of reforming a structured mat post collection and homogenization processes. As discussed above, Figure 2.13 shows chlorophyll *a* concentrations declined and equilibrated at approximately 8 $\mu g/cm^2$ for the last two sample dates. This value can be interpreted as a possible baseline for benthic photoautotrophic biomass production within the constructed trenches and may further be used to compare trench productivity with oil sands constructed wetlands.

Individual trench comparisons provided interesting results with biomass production significantly greater in trenches 3 and 5 compared to trench 1. All three trenches are FW and maintain similar physico-chemical characteristics. Trench 1, however, was overwhelmed with submerged macrophytes that were slowly colonizing the microcosms (pers. obs.). Coontail (*Ceratophyllum demersum*) was the most prevalent invader and were ultimately removed. The removal process, however, may have also caused disturbance to the substrate and subsequently to the colonized biofilms. In most cases, once an environment acquires suitable conditions for macrophyte establishment, biofilms will not be able to compete as the dominant producer (Sim et al. 2006). Biofilms will continue to persevere amidst the established macrophyte communities but will only dominate the deep water zones or substrata where vegetation establishment is inhibited.

Within this study chlorophyll *a* concentrations ($\mu g/cm^2$) achieved a maximum of 92.17 and a minimum of 0.1. Total mean (± SE) chlorophyll *a* concentration was 11.50 ± 0.62. These values are comparable to that determined by Domozych and Domozych (2008) who investigated biofilm development in freshwater wetlands of the Adirondack Region in New York. Minimum and maximum chlorophyll *a* concentrations were reported at 0.826 and 12.39 respectively, with a mean of 4.75, while Casco et al. (2009), studying a shallow

lake with a maximum depth of 2.5 m and Wu and Mitsch (1998), working in newly constructed freshwater wetlands, reported epipelic and periphyton chlorophyll *a* concentrations ranging from 2.9 to 25.3 and approximately 0 to 65, respectively. Generally, however, decreases in chlorophyll *a* concentrations from periphyton colonizing sediments in lakes to concentrations found here are sizeable. Chlorophyll *a* concentrations in oligotrophic lakes have been reported as high as 1,698 $\mu g/cm^2$ (Vadeboncoeur et al. 2006).

Results for TDW biomass are not interpreted as a reliable indicator of organic carbon content within the samples. The lack of relationship found beteen chlorophyll *a* and TDW biomass correlates with that determined by Vadebonceur et al. (2006) who reported a significant but weak relationship between organic matter and chlorophyll concentrations for epipelon. However, another study looking at periphyton in constructed freshwater wetlands reported a clear positive relationship between dry weight and chlorophyll a but a negative relationship between these two variables and organic content (Wu and Mitsch 1998). Also, methods for collection and analyses did not incorporate techniques required to eradicate the possibility of overestimating algal biomass due to inorganic content contamination. One can still extrapolate inferences made from the data about the conditions of treatments relative to each other. A significant trend can be seen in Figure 2.16 which shows no significance of differences in TDW biomass between controls and treatment for every substrate. This confirms the trend observed for chlorophyll *a* concentrations in that controls, by the conclusion of the study, became colonized by equal or possibly more productive biofilms than treatments. Due to high levels of TDW biomass and equivalent chlorophyll *a* concentrations relative to the other substrates, the data also infers that CT causes increased rates of algal death or the colonization of heterotrophic organisms (Figure 2.12 & 2.16).

2.5.3 Experiment 2: Primary Productivity – Dissolved Oxygen

Results for primary productivity were similar to those found for chlorophyll *a*. This measure was recorded to elucidate possible stressors. Stress

can be related to respiration rates where increased respiration, caused by an organism allocating its resources to survival rather than growth, indicates elevated stress and more importantly less carbon accumulation (Gardner Costa 2010). Increased respiration, however, may also be a strong indicator of a heterotrophic dominated system versus a net autotrophy system (Velasco et al. 2003). To be sustainable, net heterotrophic systems rely on allochthonous carbon sources and are not the endpoint goal for oil sands reclaimed wetlands. Ultimately, rates of DO change under light and dark chambers is a reliable measure of primary productivity and therefore an important tool for determining effects of different reclamation materials and techniques on the performance of transferred benthic biofilms and the overall state of the study system.

I expected similar results between primary production and chlorophyll *a* concentrations and in some cases this was true. Primary productivity increased significantly during mid-summer (July 2009) due to high water column temperatures and increased light intensity for longer periods of time. Light and turbidity have been determined as the primary limiting factors for biofilm production (Squires and Lesack 2001; Casco et al. 2009; Hart and Lovvorn 2000). The increased production observed during mid-summer suggests an optimal time in which a collection and transfer should take place if reclamation adopts this reclamation strategy. Figure 2.18 depicts this trend with low respiration rates and a high peak for NPP for July 2009, inferring increased levels of photosynthesis and relatively insignificant energy losses to respiration during that period. Fortunately, the system (generalization of all the microcosms), also appears to be dominated by autotrophic processes and therefore is accumulating C. A significant proportion of NPP values reported throughout the study are above zero suggesting that photosynthsis is outcompeting respiration (Figure 2.17, 2.18 and 2.19).

No significant effects on DO rate of change were observed from substrates. CT, however, does appear, in Figure 2.19, to have elevated NPP and low respiration rates relative to the others, signifying lower stress and increased rates of photosynthesis, a trend comparable to that found for chlorophyll *a*

concentrations indicating that CT is supporting a more developed benthic microbial community. Relatively high levels of TDW biomass may, therefore, be the product of enhanced microbial activity rather than a prolific source of decomposing biofilm. Interestingly, respiration rates increased in all three remaining substrates with the highest in SoSa. Two possible explanations for this are, first, increased algal and bacterial death rates and subsequent decomposition as these substrates were also supporting lower concentrations of chlorophyll *a* and secondly, the consequence of other organic matter present in the treatment soils decomposing at accelerated rates due to soil microbial activity. Sediment oxygen demand of organic sediments of constructed and natural wetlands in the oil sands region of northern Alberta has been found to be dominated by microbial respiration (Gardner Costa 2010).

Results for chlorophyll *a* concentrations were inconsistent with primary production for transfer techniques. Figure 2.18 shows a peak in NPP for slurry treatments during the Aug. 2008 sample set with relatively low levels of NPP and increased rates of respiration for the remainder of the study. Slurry chlorophyll *a* concentrations were low for the first sample set, increased at the beginning of the following season and then converged with mat and controls concentrations (Figure 2.13). Mat treatments and controls dominate GPP with high levels of NPP and low respiration rates during the last three sample sets. Biofilms transferred as slurries are in fact photosynthesizing and producing biomass but, possibly due to the homogenization process, are also experiencing elevated levels of stress and cell death leading to increased respiration from decomposition. This may also account for the difference in respiration rates between mat and slurry treatments.

According to the multiple regression results water type did not have an effect on primary production. The nonsignificant test results, due to high variability within OPSW treatments, are questionable as the mean GPP for OSPW was nearly two fold that found in FW. The same analysis also indicates that trenches 2 (OSPW), 6 (OSPW) and 5 (FW) were significantly higher in primary production than trench 1(FW). The differences in respiration and NPP between OSPW and FW can be observed in Figure 2.17. Respiration rates and NPP are

greater in OSPW which suggests increased levels of photosynthesis as well as stress and decomposition. The increased stress levels may also be the result of reduced compounds such as ammonium binding available oxygen in the substrates and water column (Gardner Costa 2010). It should also be noted again that CT and OSPW were found to support elevated concentrations of chlorophyll *a* than soil (peat mineral mix) containing substrates and freshwater in the first year. Elevated chlorophyll *a* concentrations suggest early bioiflm colonization on CT which could have accelerated grazing invertebrate colonization. Increased respiration may be the result of an established invertebrate community on CT substrates within OSPW trenches.

As mentioned by Wetzel and Likens (1979) there are many assumptions and associated errors with this measure (DO rate of change) of primary production. Respiration rates measured under dark chambers can be affected by bacterial respiration, decomposition and primary consumers. Also, changes in light intensity, pH, DO and CO_2 are also unaccounted for but will most certainly affect rates of photosynthesis.

2.5.4 Synthesis and Reclamation

Biofilms survived both transfer methods via immediate colonization and establishment. Levels of stress increased in slurry treatments but this was expected and ultimately, had no short term negative effects on photoautotrophic biomass (Figure 2.13). The explanation for the gradual decrease in biomass over time is unclear and requires further research in a more controlled setting. If in fact the decline until eventual convergence with control levels was due to uncontrollable environmental factors then it is safe to presume that chlorophyll *a* concentrations presented for the last two sample sets represent an approximate baseline for photoautotrophic biomass in trench inhabiting biofilms. Extrapolating this approximate value of base biofilm photoautotrophic biomass, one can make inferences to other reclaimed wetlands affected by reclamation materials in the oil sands. Additionally, by converting total GPP found for the four sampling dates into units of C, I was able to determine the overall minimum (0.024 g C/m^2/day)

and maximum (0.045 g C/m^2/day) rates of C production throughout the study. This will help quantify the impacts biofilms have on carbon flow in oil sands reclaimed wetlands. As expected CT and OSPW trenches maintained increased photoautotrophic biomass and productivity. Ammonia, nitrate and phosphorous levels were low in both CT and OSPW suggesting nutrient depletion from initial increased biofilm activity. Possible explanantions for increased biofilms biomass and productivity on process affected materials include higher potassium and sulfate concentrations which may have acted as nutrients and high salinity in process affected materials can reduce predation thereby indirectly having a positive effect on biomass and production levels. Overall the results indicate that the usual impacts caused from using CT and OSPW in wetland reclamation practices are not detrimental to benthic biofilm survival and growth.

In a reclamation context, there is great potential for microbial biofilms and this study helps elucidate the possibilities of incorporating biofilm transfers into oil sands reclamation processes. OSPM is known to have detrimental impacts on wetland development including macrophyte and invertebrate colonization and fish survival (Daly 2007; Leonhardt 2003; MacKinnon et al. 2001; Scott 2007). It is shown here, however, that reclamation materials such as CT and OSPW will support what would be a quick, cheap and effective first contribution to site reclamation and a technique that can be implemented immediately following wetland construction. Further studies are required to eliminate confounding factors like grazing invertebrates, water column production and disturbance via repeated sampling but this study is a good starting point and an indication that microbial biofilms will benefit newly constructed wetlands and through increased rates of carbon accrual, potentially help accelerate the process of macrophyte and invertebrate colonization. It is important to note that I do not expect this to take the place of macrophyte plantings or peat transfers but rather to reduce the required amounts and compliment nutrient management and production of the base of the trophic chain for subsequent arrivals of invertebrates. This could be accomplished by conducting a biofilm transfer immediately following substrate

placement and water capping. Based on success in the following season it can be determined whether plantings or peat transfers are necessary and to what degree.

The long term results and observations obtained from this study suggest that such a biofilm transfer may be an unnecessary approach towards wetland reclamation. Though, net autotrophy was found throughout the system, photoautotrophic biomass converged and stabilized at control levels. I would argue, however, that the beneficial functions associated with biofilms are most effective immediately following reclamation when substrates are most exposed to various disturbances and nutrients are unavailable to other forms of vegetation and higher trophic organisms. This study warrants further investigations into the application of biofilms in a reclamation context but under more controlled settings that are more representative of primary succession conditions.

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3 GENERAL DISCUSSION AND CONCLUSION

3.1 Oil Sands Reclamation and Biofilms

Transferred biofilms, as shown in the second chapter, are able to endure the stresses caused during collection and placement. Relatively new, bare substrates were rapidly colonized and increased levels of photoautotrophic biomass and productivity were sustained within all treatment microcosms through the first half of the study. The entire experimental system was also determined to be net autotrophic. During the second half of the study, however, photoautotrophic biomass declined in treatment microcosms and became indistinguishable from controls. TDW biomass exhibited a similar pattern at the conclusion of the study. Natural colonization occurred within 2 y of substrate placement and submersion into the trenches. Benefits towards wetland development, obtained from transferred biofilms, were therefore lost after 2 y in the trench study system. I would suspect, however, that natural colonization would be delayed significantly in newly constructed wetlands thereby extending the time in which transferred biofilms are beneficial for the system (Figure 3.1). The trenches were established wetlands with mature invertebrate communities and developed plankton and epiphyton communities. Over time grazing invertebrates and my intensive sampling design may have had significant detrimental effects on the transferred biofilm. The established algal communities present within the trenches may have contributed to the unexpected colonization of the control microcosms. For the following discussion I will assume that similar results as shown in this study would appear in newly reclaimed oil sands wetlands where one to two years are required for natural microbial colonization.

Biofilm functions are of critical importance during the initial stages of wetland development (Figure 3.1). Wetland conditions, at the conclusion of substrate and water capping, are at the very cusp of primary succession. Substrates are exposed and therefore are sensitive to low impact but consistent disturbances such as currents, waves and inhabiting organisms. High chemical oxygen demand found in CT (Gardner Costa 2010), due to elevated

concentrations of ammonia and sulfides, reduces dissolved oxygen availability for inhabiting organisms at the sediment water interface. High levels of ammonia in process-affected aquatic systems inhibit plant growth and photosynthetic activity and cause oxidative stress in colonizing macrophytes (Best 1980; Nimptsch and Pflugmacher 2007). Transferring biofilms would result in immediate ameliorating effects on the factors stated above by reducing bioturbation post colonization, assimilating and cycling nutrients from the sediments and water column increasing their bioavailability and producing oxygen at the sediment water interface (Dodds 2003; McCormick et al. 1998; Roeselers 2008; Yallop et al. 1994). Lastly, transferring biofilms will also increase overall organic carbon content of the system, thereby having similar effects to an organic substrate placement.

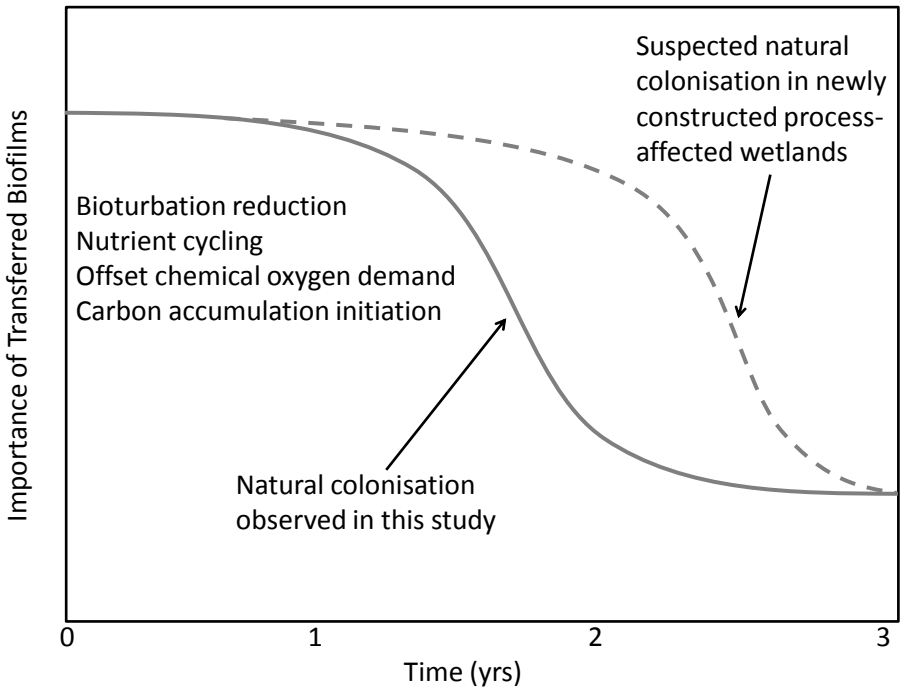

Figure 3.1 - Relationship between time and the importance of transferred biofilms. Importance is based on the benefits of biofilm functions towards wetland development. Time 0 is the point at which the constructed wetland is capped or naturally filled with water.

A reclamation technique using microbial biofilms may be an economically viable and effective technique as accelerated biofilm establishment can play a significant role in the very early stages of development of newly constructed wetlands. Here I will review and compare current reclamation strategies with a technique I propose using the synthesized information gathered from chapter 2.

3.1.1 Reclamation Techniques

Wetland reclamation and restoration is occurring on a global scale as rates of wetland loss increase due to development, agriculture, recreational activities etc. (Zedler 2006). I will only consider reclamation techniques directly used within oil sands operations or having some relevance therein, thereby eliminating biases due to differences in scale, degree of degradation and landscape type. Lastly, the boreal forest in which the oil sands are situated is an ecosystem that supports the existence of multiple wetland types, including marshes, ponds (shallow open water wetlands), swamps, bogs and fens (NWWG 1997). Techniques discussed are limited to these wetland classes and focus on the stages of reclamation between substrate capping and primary colonization.

There is a wealth of information in the literature on wetland restoration techniques that focus on soil reintroduction and manual seeding to assist with vegetation establishment and the eventual achievement of carbon accrual (Brown and Bedford 1997; Bruland et al. 2009; Budelsky and Galatowitsch 2004; De Steven and Sharitz 2007; Graf and Rochefort 2008). Once the hydrology is restored to the system, primary colonization becomes the immediate concern. Where reclaimed wetlands have many neighboring natural wetlands, initial colonization may be assisted by wind and water-driven seed dispersal (Soons 2006). Acquiring a viable seed bank and initiating primary colonization is a challenge when wetlands are reclaimed in isolation from a natural seed source (Morzaria-Luna and Zedler 2007; Mulhouse and Galatowitsch 2003; Soons 2006). For peat-forming wetlands and marshes to an extentt, carbon accumulation is an additional function that must be restored. Some attempts to hasten this

accumulation process include: organic soil (peat), mulch and fragmented plant transfers, direct seeding and vegetation transplants.

Organic soils placed into reclaimed wetlands may contain a viable seed bank, thus initiating macrophyte colonization. A study conducted by Brown and Bedford (1997) suggests that by transplanting soils from donor wetlands about to be disturbed or completely removed to newly reclaimed wetlands, could result in increased vegetation colonization and diversity. These results are consistent with the findings of Vivian-Smith and Handel (1996) who determined that soils imported from an undisturbed wetland facilitate higher species richness and plant density on restored sites. Transplanting soils with a viable native vegetation seed bank will impede the invasion of non-native, but highly resilient, plants including species of *Typha* (Brown and Bedford 1997). As suggested by Alberta Environment (2008) transplanted soils should, however, be analyzed prior to transfer to ensure the embedded seeds are of native wetland vegetation. To nurture the germination and successful colonization of the imported seed bank Stauffer and Brooks (1997) reported that adding a thin layer of leaf litter compost on top of the transplanted soil will provide suitable conditions for macrophytes such as *Carex* by increasing the availability of organic matter, NO_3 and NH_4 and retaining soil moisture.

Straw mulch facilitates vegetation germination on bare peat by reducing temperatures at the peat surface, supporting a higher water table and minimizing the potential for desiccation of the vegetation germinating layer beneath. The method consists of a thin layer of straw mulch spread over top of either bare peat or a layer of plant fragments covering the peat substrate (Quinty and Rochefort 2003). The use of straw mulch to facilitate vegetation establishment on peat substrates may also, however, offset wetland carbon sequestration via increased decomposition and subsequent CO_2 release (Waddington et al. 2003).

Fragmented Sphagnum moss spread over bare peat is an efficient plant establishment technique for peat-accumulating wetlands. Bare peat surfaces are usually all that remains upon the closure of peat mining activities. Spreading a layer of shredded fragments of *Sphagnum* moss over top will accelerate

colonization of *Sphagnum* but also other bryophyte species including *Carex* (Graf and Rochefort, 2008). The layers must, however, be a certain thickness because a layer too thick will result in the top layers succumbing to desiccation and burying the bottom layers, thereby reducing light penetration. The amount of vegetation spread must also be sufficient enough to cover the entire peat surface because it may take years for the established vegetation to colonize neighboring bare spots (Quinty and Rochefort 2003). This technique is effective in that it does not cause permanent damage to the donor site and relies on the diaspores of shredded vegetation to initiate primary colonization in the recipient site. All parts of *Sphagnum*, besides their leaves, are considered diaspores, which means new plants can arise from *Sphagnum* roots, stems, spores and branches (Quinty and Rochefort 2003).

Direct seeding and vegetation transplants are usually associated with sites that have been restored by flooding and have nonviable seed banks due to long term desiccation from extensive agricultural or mining practices. Seeding, where seeds are scattered onto the soil, is a relatively inexpensive restoration method but also not as effective as techniques such as seedling transplants (Alberta Environment 2008). Numerous studies have determined that to obtain a healthy and diverse community of native wetland species passive re-vegetation processes may not be sufficient. Reasons for this include reduced or non-existent seed banks, infrequent flooding, competition from invasive species and hydrological and geographical isolation (Aronson and Galatowitsch 2008; Campbell et al. 2003; Morzaria-Luna and Zedler 2007; Mulhouse and Galatowitsch 2003). Restoring vegetation by seeding has had mixed results and is highly dependent on the species. Reinartz and Warne (1993) planted seeds from 22 different species and after two years 17 species germinated and became established. It was also reported that cattail abundance was lower on seeded sites compared to reference sites. Cooper and MacDonald (2000) did not find as much promise in this technique as they only observed one out of eight seeded species germinate and establish seedlings. Like seeding, rhizome and seedling transplant success is also highly dependent on species but germination success rates are significantly higher

once the tolerant species are determined. *Carex stricta*, as reported in Yetka and Galatowitsch (1999), was not a successful candidate for rhizome transplants, however, Budelsky and Galatowitsch (2004) show *Carex stricta* successfully germinating and establishing from seedling transplants. Transplanted rhizomes of *Carex aquatilis*, however, were reported to have a higher tolerance in deeper waters relative to transplanted seedlings thereby giving preference to seedling transplants (Cooper and MacDonald 2000). Unfortunately, not only are rhizome and seedling transplants species specific, rhizome collection results in considerable donor site disturbance and seedlings are costly to culture in lab or greenhouse settings (Alberta Environment 2008).

3.2 Proposed surrogate reclamation technique

The microbial biofilm transfer conducted for this study consisted of the following steps and are framed here as recommendations;

1. A renewable source of biofilms should be located on site. This may be an extant constructed wetland or a natural wetland about to be destroyed for mining purposes. Another possibility is the construction of large scale cultures, should further research find biofilms useful and economically viable reclamation tools. Although one productive wetland would be sufficient in providing biofilm material, it would be in the oil sands companies' best interest to locate multiple sources with differing composition. This may increase the likelihood of colonization success.
2. Biofilms in this study were extracted using a net and pail. Benthic biofilms were simply scraped off the wetland substrates and pooled in coolers. Scaling this up for industry collection may require a submersible vacuum system. The substrate should be lightly skimmed to minimize the amount of inadvertently collected soil. The slurry method used in this study indicated that biofilms can re-colonize after severe disturbances.
3. Collected biofilms can be mixed with water to increase the transfer quantity and could be pumped into the reclaimed wetland using a modified

water or sewage disposal truck. A boat would be required to transfer slurries to wetland interiors if the wetland was large. The collection and transfer should take place during early summer when productivity is highest and there is still a considerable amount of time for the transferred biofilms to colonize and establish on new substrates before the onset of cooler autumn temperatures.

4. This biofilm transfer reclamation technique would be most beneficial immediately after capping the constructed wetland with water. The colonized microbes through natural metabolic processes may facilitate the subsequent natural colonization of macrophytes (Adema et al. 2004). This technique would also be most appropriate for wetlands containing bare CT substrate and oil sands process-affected water.

3.2.1 Advantages, Limitations and Future Directions

Biofilms play important roles in reclaimed wetland ecosystems, especially during the initial stages of wetland development (Figure 3.1). Their reduced occurrence in oil sands affected wetlands may be the result of several factors. First, reclamation materials such as CT and OSPW are exposed to extreme temperatures and pressures during the bitumen extraction process and are near sterile conditions when released. Wetlands capped with CT and OSPW experience primary succession conditions. Second, natural dispersal vectors such as birds and mammals are deterred from making contact with oil sands process affected wetlands. Lastly, since some biofilm forming microbes are ubiquitous in nature I would suspect the chemical and physical constituents of oil sands produced reclamation materials may be too overwhelming for benthic microbes to establish to the extent where biofilm formation is possible. Therefore, transferring biofilms to a reclaimed wetland site will help benthic microbial communities overcome these barriers.

These transfer techniques also pose many benefits compared to conventional reclamation techniques. Peat and organic soil is limited in supply, expensive to move and used in massive quantities to increase success rates.

Microbial biofilms are a renewable resource with a high turnover rate. It would therefore be possible to have one or multiple source wetlands provide sufficient biofilm quantities for multiple transfers in a single season. If the transferred biofilms do not survive, the proceeding processes will be similar to that of transferred leaf litter or mulch. The decomposing biofilms will simply be a source of organic material for colonizing invertebrates and a source of nutrients for germinating aquatic plants. Dead or alive, the transferred biofilms may also initiate the earliest accumulation of carbon within the wetland ecosystem.

The biofilm transfer technique described above can easily be adopted by industry due to its ease and efficiency. Collecting and planting seeds is time consuming and labor intensive. Collecting rhizomes causes considerable disturbance and culturing vegetation in greenhouses is extremely expensive (Alberta Environment 2008). Collecting biofilms does not require one to identify, clean or count such as with seed collection; little skill or care is required for biofilm placement and lastly, the entire process would only require days if not hours to complete. Depending on colonization success, industry can decide whether to follow through with seeding, planting or peat transfers

One limiting factor of this technique is locating a reliable source that provides sufficient quantities of biofilm. Biofilms used in chapter 2 experiments were all collected from wetlands on site. These sources can quickly become macrophyte dominated wetlands the following season. Therefore a new search may be required with every upcoming season. One possible method to mitigate this problem is to research the most effective methods for culturing biofilms in mass quantities as is being done in the waste water treatment, aquaculture and agriculture industries (Roeselers et al. 2008). Biofilm culturing wetlands are constructed shallow with expansive open water surface areas and high nutrient concentrations; conditions that are suitable for consistent biofilm growth (Roeselers et al. 2008).

To test the biofilm transfer technique at an industrial scale or in a more controlled setting was beyond the scope of this study but would be useful to evaluate potential limitations with collection and transfers. Lastly, for both

reclamation and the scientific community, future research should evaluate species composition within the biofilms before and after transfer as well as between the different reclamation materials. This would identify the species most adapted to the associated conditions of the materials used. Research can subsequently look at specific species efficient in removing contaminants through metabolic processes and colonizing reclamation substrates.

Using chlorophyll *a* and dissolved oxygen production as surrogate measures of photoautotrophic biomass and primary production, I was able to quantify the contributions, via carbon input, biofilms have on initial wetland development. These methods also, however, have limitations. Though chlorophyll *a* was shown here to correlate with biomass, variability is inherent with this limnological method. For example, under low light conditions chlorophyll *a* concentrations will increase as a compensational response while little change is observed in overall photoautotrophic biomass (Wassink 1959). The light/dark bottle method applied in this study also has its share of limitations due to many associated assumptions (Wetzel and Likens 1979). It remains a challenge to account for carbon loss due to decomposition and invertebrate respiration. Therefore, to increase accuracy I would suggest combining the results from two separate measures of production.

3.3 Implications and Conclusions

Biofilms are an invaluable component of wetland ecosystems. Reclamation efforts in the oil sands industry of Northern Alberta have provided me with the opportunity to further research their significance in new wetlands with different degrees of disturbance. According to this research biofilm response varies with reclamation material. To my knowledge no research has been conducted on biofilm colonization in wetlands within the oil sands region of Northern Alberta nor have I found any past work on testing the application of biofilms in a reclamation setting. Therefore this research has essentially provided new insight into the importance of biofilms within newly constructed wetlands and their potential application as a reclamation resource.

Tracking the productivity of biofilms for two years post transfer and colonization has allowed me to observe key functions and characteristics. Beyond the reclamation technique aspect of this study, key findings, including overall rates of C production, will support the goals of CFRAW and that of wetland reclamation management practices. The suite of researchers associated with CFRAW are attempting to map out the carbon dynamics of newly reclaimed oil sands wetlands by individually assessing specific sources, pathways and endpoints of the carbon flow model. Compartments of carbon flow dynamics previously or currently being studied include respiration, decomposition and production of the microbial and vegetation communities (H. Chen, University of Waterloo in prep.; C. Wytrykush, University of Windsor, in prep.; M.C. Roy, University of Alberta, in prep.; Gardner Costa 2010; Daly 2007; F. Mollard, University of Alberta, in prep) as well as secondary production (Ganshorn 2002; Leonhardt 2003; Martin 2010), and overall wetland gas (CO_2 and CH_4) flux (Gardner Costa 2010; Slama 2010). One of the chief wetland functions in which CFRAW is attempting to find or develop in reclaimed wetlands is carbon accrual. Carbon accrual is a process exhibited by most natural wetlands in the boreal landscape surrounding the oil sands and is required to be present in reclaimed wetlands to satisfy the 'equivalent capability' requirements. The oil sands must reclaim oil sands mining affected landscapes to equivalent capability as that of pre-mining conditions.

Specifically, this research provides CFRAW with A) baseline rates of biofilm carbon production for multiple reclamation materials, B) rates of colonization of new substrates over a two-year time period and C) a potential reclamation technique that can enhance initial carbon production in newly constructed wetlands affected or un-affected by oil sands process-affected materials. Components A and B can be tied directly in with the CFRAW carbon model under primary productivity, also keeping in mind that I conducted my research in semi-wetlands (trenches) rather than the wetlands designated within the CFRAW matrix. These values can be analyzed and compared to estimates of productivity for the plankton, other forms of periphyton and vegetation within oil

sands reclaimed wetlands to gain an understanding of the relative significance of each primary producer in terms of C production. Ultimately and on a broader scale, this research initiated the exploration of biofilm application for reclamation purposes but also contributed to the lack of knowledge pertaining to freshwater wetland biofilms and their contributions to primary production.

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4 APPENDICES

APPENDIX I – TRENCH WATER QUALITY DATA FOR 2008. (DATA COURTESY OF MIKE MACKINNON (SYNCRUDE CANADA LTD.))

Units: mg/L

Trench #	Date	Nap Acids	NH_4	Na	K	Mg	Ca	F	Cl	SO_4	CO_3	HCO_3	Alkalinity expressed as $CaCO_3$	Br	Total Cations	Total Anions	Ratio Cat/Ani	Na/Cl	(Ca+Mg) / HCO3	Na / (Ca+Mg)
1	19-Aug-08	2.5	<0.01	47.8	3.1	25.6	72.5	BDL	45	101.8	0.0	256	210	BDL	7.9	7.6	1.04	1.64	1.37	0.36
2	19-Aug-08	16.6	<0.01	392	12.2	20.3	33.8	0.3	160	185	8.7	630	531	BDL	20.7	19.0	1.09	3.78	0.32	5.04
3	19-Aug-08	2.8	<0.01	42.3	2.8	22.6	75.9	BDL	39	61.5	0.0	291	239	BDL	7.6	7.2	1.06	1.67	1.19	0.32
4	19-Aug-08	16.5	<0.01	338	9.1	21.6	29.5	BDL	140	212	11	508	435	BDL	18.2	17.1	1.07	3.73	0.38	4.49
5	19-Aug-08	2.9	<0.01	68.9	4.2	20.6	59.5	BDL	41	81.5	0.0	268	220	BDL	7.8	7.2	1.08	2.59	1.07	0.64
6	19-Aug-08	19.3	0.14	389	10.8	25.1	37.3	BDL	170	241	8.4	583	492	BDL	21.1	19.6	1.08	3.53	0.40	4.27

Trench #	Date	Al	B	Ba	Cd	Co	Cr	Cu	Fe	Li	Mn	Mo	Ni	p	Pb	Sb	Se	Si	Sr	Ti	V	Zn	Zr
1	19-Aug-08	BDL	0.10	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.4	0.3	BDL	BDL	BDL	BDL
2	19-Aug-08	BDL	2.01	BDL	BDL	BDL	BDL	BDL	0.1	BDL	0.0	BDL	BDL	BDL	BDL	BDL	BDL	1.7	0.4	BDL	BDL	BDL	BDL
3	19-Aug-08	BDL	0.20	0.0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.9	0.3	BDL	BDL	BDL	BDL
4	19-Aug-08	BDL	1.70	BDL	BDL	BDL	BDL	BDL	0.0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.4	0.3	BDL	BDL	BDL	BDL
5	19-Aug-08	BDL	0.34	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.9	0.3	BDL	BDL	BDL	BDL
6	19-Aug-08	BDL	1.83	BDL	BDL	BDL	BDL	BDL	0.2	BDL	0.0	BDL	BDL	BDL	BDL	BDL	BDL	2.1	0.4	BDL	BDL	BDL	BDL

BDL = Below detectable limits

APPENDIX II – RAW DATA FOR SUBSTRATE NUTRIENT SUPPLY RATES WITH INDEPENDENT VARIABLES

Units: μg/10cm^2/4 days; Date of exposure: Aug. 9-13, 2009; (#) under nutrient title = Detection Limit

Trench	Substrate	Water Type	Treatment	# Anion probes	# Cation probes	Total N (2)	NO3-N (2)	NH4-N (2)	Ca (2)	Mg (4)	K (4)	P (0.2)	Fe (0.4)	Mn (0.2)	Cu (0.2)	Zn (0.2)	B (0.2)	S (2)	Pb (0.2)	Al (0.4)	Cd (0.2)
1	SoSa	FW	Slurry	1	1	16.0	7.0	9.0	2356.0	487.6	26.8	0.6	649.0	93.4	0.0	0.6	1.0	218.8	0.2	50.2	0.0
1	SoSo	FW	Slurry	1	1	6.2	4.0	2.2	2032.0	391.2	14.6	0.6	676.4	65.8	0.0	0.4	0.8	184.2	0.2	42.6	0.0
1	CT	FW	Slurry	1	1	8.8	2.2	6.6	1024.4	220.0	69.2	0.4	786.2	35.2	0.0	0.6	1.2	96.8	0.2	31.6	0.0
1	SoCT	FW	Slurry	1	1	6.4	4.8	1.6	1815.4	375.4	15.4	0.4	708.2	59.8	0.0	0.6	0.4	262.0	0.0	39.6	0.0
1	SoCT	FW	Control	3	3	10.0	5.0	5.0	2058.0	414.6	21.0	1.0	640.2	72.6	0.0	0.4	0.4	252.4	0.2	44.8	0.0
1	SoSa	FW	Control	3	3	13.2	6.4	6.8	1787.2	324.4	19.6	0.4	855.8	104.2	0.0	0.6	0.4	83.4	0.2	35.2	0.0
1	SoSo	FW	Control	3	3	11.6	10.2	1.4	2008.0	437.6	13.8	0.4	1071.6	48.4	0.0	0.4	0.2	173.4	0.2	49.2	0.0
1	CT	FW	Control	3	3	10.2	4.4	5.8	989.8	200.6	69.6	0.4	607.4	40.8	0.0	0.6	1.4	111.4	0.6	30.8	0.0
2	CT	OSPW	Slurry	1	1	7.2	5.2	2.0	1204.8	343.2	84.4	0.6	278.2	33.8	0.0	0.4	1.2	626.4	0.0	43.8	0.0
2	SoSo	OSPW	Slurry	1	1	2.4	2.4	0.0	1679.4	509.6	40.0	0.4	154.4	26.4	0.0	0.4	1.0	690.8	0.0	39.8	0.0
2	SoSa	OSPW	Slurry	1	1	11.6	6.2	5.4	1416.0	439.6	36.6	0.6	151.0	51.0	0.0	0.4	1.2	857.2	0.0	48.4	0.0
2	SoCT	OSPW	Slurry	1	1	4.6	3.2	1.4	1613.0	472.2	34.0	0.6	164.4	39.8	0.0	0.4	1.0	746.0	0.0	44.0	0.0
2	SoSo	OSPW	Control	3	3	11.0	7.0	4.0	2118.0	628.2	37.0	0.4	195.4	54.4	0.0	0.2	1.6	625.0	0.0	57.0	0.0
2	CT	OSPW	Control	3	3	8.0	6.0	2.0	1081.4	327.6	83.4	0.4	82.6	26.2	0.0	0.4	1.0	877.4	0.0	51.6	0.2
2	SoCT	OSPW	Control	3	3	8.4	8.4	0.0	1662.0	512.6	38.4	0.4	40.0	10.8	0.0	0.4	0.8	1002.2	0.0	51.0	0.0
2	SoSa	OSPW	Control	3	3	15.2	13.2	2.0	1485.8	452.8	30.0	0.4	509.4	44.4	0.0	0.4	1.2	272.8	0.2	42.4	0.0
3	SoSo	FW	Control	1	1	12.2	8.4	3.8	2020.0	368.2	9.6	0.6	592.2	45.2	0.0	0.8	2.2	80.8	0.0	40.4	0.0

3	CT	FW	Control	1	1	14.0	8.4	5.6	1018.0	179.6	71.6	0.4	558.6	36.8	0.0	0.6	0.4	10.2	0.4	38.2	0.0
3	SoCT	FW	Control	1	1	8.2	6.8	1.4	1980.0	315.8	8.4	0.4	850.4	59.0	0.0	1.0	2.0	6.4	0.2	47.4	0.0
3	SoSa	FW	Control	1	1	13.0	8.6	4.4	2126.0	414.4	16.2	0.4	869.0	100.4	0.0	1.0	0.8	7.8	0.2	42.0	0.4
3	SoCT	FW	Slurry	3	3	13.2	11.4	1.8	2102.0	379.4	11.8	0.6	380.8	43.6	0.0	1.0	1.4	81.0	0.0	43.4	0.0
3	SoSa	FW	Slurry	3	3	10.6	6.4	4.2	1739.0	289.4	21.4	0.4	690.0	54.6	0.0	0.6	0.8	25.4	0.2	33.6	0.0
3	SoSo	FW	Slurry	3	3	6.8	4.8	2.0	2116.0	340.0	12.6	0.6	590.6	62.0	0.0	0.8	1.4	73.4	0.0	41.0	0.0
3	CT	FW	Slurry	3	3	17.2	10.6	6.6	1796.0	283.0	58.2	0.4	547.8	52.6	0.0	1.4	1.4	17.0	0.2	46.0	0.0
4	CT	OSPW	Slurry	1	1	6.2	0.0	6.2	972.2	343.2	56.0	0.4	334.8	36.2	0.0	0.8	1.0	382.6	0.0	42.4	0.0
4	SoCT	OSPW	Slurry	1	1	2.0	0.0	2.0	1418.4	465.8	29.0	0.6	355.6	45.2	0.0	1.4	2.4	571.6	0.2	44.8	0.0
4	SoSa	OSPW	Slurry	1	1	4.2	0.0	4.2	1352.6	451.2	49.8	0.6	403.4	42.8	0.0	1.2	1.2	588.4	0.2	46.4	0.0
4	SoSo	OSPW	Slurry	1	1	14.6	12.2	2.4	1220.8	414.8	30.0	0.6	175.2	40.4	0.0	1.4	1.0	607.6	0.0	38.6	0.0
4	SoCT	OSPW	Control	3	3	8.8	0.0	8.8	1700.4	703.6	42.8	0.6	354.6	46.0	0.0	0.8	3.0	433.6	0.2	57.4	0.0
4	SoSa	OSPW	Control	3	3	16.4	12.0	4.4	1471.6	464.2	29.8	0.6	260.6	71.6	0.0	0.6	2.4	492.6	0.2	41.6	0.0
4	SoSo	OSPW	Control	3	3	0.8	0.0	0.8	1248.4	433.2	33.4	0.2	545.8	67.0	0.0	0.8	2.0	253.2	0.2	39.6	0.0
4	CT	OSPW	Control	3	3	4.8	1.0	3.8	1007.2	323.8	84.6	0.2	333.0	23.4	0.0	0.4	0.8	560.4	0.4	38.6	0.0
5	CT	FW	Slurry	1	1	21.4	16.6	4.8	1517.6	314.6	108.4	0.4	546.8	35.8	0.0	0.4	1.0	7.6	0.2	46.4	0.0
5	SoCT	FW	Slurry	1	1	15.0	14.8	0.2	1951.0	441.4	30.2	0.8	752.4	94.2	0.0	0.6	1.0	44.6	0.2	45.2	0.0
5	SoSa	FW	Slurry	1	1	25.2	22.6	2.6	1847.8	390.0	24.8	0.6	593.8	76.8	0.0	0.6	1.0	29.0	0.2	41.2	0.0
5	SoSo	FW	Slurry	1	1	6.0	1.8	4.2	2142.0	455.4	28.8	0.8	583.8	80.0	0.0	0.4	1.2	57.4	0.2	38.8	0.0
5	SoSa	FW	Control	3	3	15.8	11.4	4.4	2210.0	536.2	32.0	2.2	744.0	81.8	0.0	0.4	0.8	12.2	0.2	46.4	0.0
5	SoSo	FW	Control	3	3	12.0	10.6	1.4	1860.6	449.0	41.0	0.4	36.2	13.2	0.0	0.4	0.4	9.6	0.0	42.2	0.2

5	CT	FW	Control	3	3	7.4	0.0	7.4	1907.4	433.8	181.8	0.6	751.8	35.0	0.0	0.4	0.8	4.4	0.2	41.2	0.0
5	SoCT	FW	Control	3	3	10.0	5.6	4.4	2650.0	642.2	45.2	0.6	1125.2	122.4	0.0	0.6	0.4	15.6	0.2	45.4	0.0
6	SoSa	OSPW	Slurry	1	1	13.6	11.4	2.2	1932.0	767.4	45.6	0.6	318.2	68.4	0.0	0.4	1.4	662.4	0.2	41.8	0.0
6	SoSo	OSPW	Slurry	1	1	21.8	16.8	5.0	2338.0	925.6	52.4	0.6	123.4	42.0	0.0	0.4	1.4	774.4	0.0	49.6	0.0
6	CT	OSPW	Slurry	1	1	1.2	0.0	1.2	1715.2	703.4	120.2	0.2	178.2	25.6	0.0	0.4	1.0	402.4	0.2	46.2	0.0
6	SoCT	OSPW	Slurry	1	1	27.4	23.2	4.2	2138.0	879.8	52.6	0.4	223.0	65.2	0.0	0.6	1.2	543.4	0.2	44.2	0.0
6	CT	OSPW	Control	3	3	21.6	17.8	3.8	2016.0	821.8	54.4	0.2	366.0	30.4	0.0	0.4	1.2	843.4	0.2	35.8	0.0
6	SoCT	OSPW	Control	3	3	23.6	22.6	1.0	1555.2	637.2	125.4	0.4	709.2	62.6	0.0	0.4	1.2	936.8	0.2	50.2	0.0
6	SoSa	OSPW	Control	3	3	2.2	0.0	2.2	2220.0	940.2	66.6	0.4	74.8	25.8	0.0	0.6	2.2	1020.8	0.2	53.0	0.0
6	SoSo	OSPW	Control	3	3	6.4	0.0	6.4	1822.4	578.0	38.0	1.8	485.2	112.6	0.0	1.0	1.2	380.8	0.2	35.0	0.0

APPENDIX III – RAW DATA FOR CHLOROPHYLL A CONCENTRATIONS WITH INDEPENDENT VARIABLES

Time	Transfer	Water Type	Substrate	Chlorophyll a (µg/cm^2)	Biomass (g) for Spring 2010
Summer 2008	Control	FW	CT	0.37	
Summer 2008	Control	FW	CT	0.00	
Summer 2008	Control	FW	CT	0.52	
Summer 2008	Control	FW	SoCT	1.27	
Summer 2008	Control	FW	SoCT	0.00	
Summer 2008	Control	FW	SoCT	0.00	
Summer 2008	Control	FW	SoSa	0.53	
Summer 2008	Control	FW	SoSa	0.00	
Summer 2008	Control	FW	SoSa	0.00	
Summer 2008	Control	FW	SoSo	0.64	
Summer 2008	Control	FW	SoSo	7.02	
Summer 2008	Control	FW	SoSo	0.50	
Summer 2008	Control	OSPW	CT	0.46	
Summer 2008	Control	OSPW	CT	0.00	
Summer 2008	Control	OSPW	CT	0.00	
Summer 2008	Control	OSPW	SoCT	0.00	
Summer 2008	Control	OSPW	SoCT	0.00	
Summer 2008	Control	OSPW	SoSa	0.00	
Summer 2008	Control	OSPW	SoSa	0.00	
Summer 2008	Control	OSPW	SoSa	0.35	
Summer 2008	Control	OSPW	SoSo	0.00	
Summer 2008	Control	OSPW	SoSo	0.00	
Summer 2008	Control	OSPW	SoSo	0.00	
Summer 2008	Mat	FW	CT	45.30	
Summer 2008	Mat	FW	CT	81.73	
Summer 2008	Mat	FW	CT	55.35	
Summer 2008	Mat	FW	SoCT	18.54	
Summer 2008	Mat	FW	SoCT	11.04	
Summer 2008	Mat	FW	SoCT	5.62	
Summer 2008	Mat	FW	SoSa	35.22	
Summer 2008	Mat	FW	SoSa	46.84	
Summer 2008	Mat	FW	SoSa	32.05	
Summer 2008	Mat	FW	SoSo	41.76	
Summer 2008	Mat	FW	SoSo	1.11	
Summer 2008	Mat	FW	SoSo	22.96	
Summer 2008	Mat	OSPW	CT	0.92	

Summer 2008	Mat	OSPW	CT	30.20	
Summer 2008	Mat	OSPW	CT	0.34	
Summer 2008	Mat	OSPW	SoCT	30.06	
Summer 2008	Mat	OSPW	SoCT	23.56	
Summer 2008	Mat	OSPW	SoCT	10.43	
Summer 2008	Mat	OSPW	SoSa	66.66	
Summer 2008	Mat	OSPW	SoSa	38.83	
Summer 2008	Mat	OSPW	SoSa	0.00	
Summer 2008	Mat	OSPW	SoSo	2.37	
Summer 2008	Mat	OSPW	SoSo	17.63	
Summer 2008	Mat	OSPW	SoSo	15.80	
Summer 2008	Slurry	FW	CT	33.59	
Summer 2008	Slurry	FW	CT	28.15	
Summer 2008	Slurry	FW	CT	27.60	
Summer 2008	Slurry	FW	CT	10.73	
Summer 2008	Slurry	FW	CT	18.50	
Summer 2008	Slurry	FW	CT	19.28	
Summer 2008	Slurry	FW	CT	0.00	
Summer 2008	Slurry	FW	CT	18.25	
Summer 2008	Slurry	FW	CT	11.37	
Summer 2008	Slurry	FW	SoCT	17.62	
Summer 2008	Slurry	FW	SoCT	30.78	
Summer 2008	Slurry	FW	SoCT	30.22	
Summer 2008	Slurry	FW	SoCT	8.73	
Summer 2008	Slurry	FW	SoCT	12.26	
Summer 2008	Slurry	FW	SoCT	13.48	
Summer 2008	Slurry	FW	SoCT	0.00	
Summer 2008	Slurry	FW	SoCT	10.35	
Summer 2008	Slurry	FW	SoCT	8.15	
Summer 2008	Slurry	FW	SoSa	22.38	
Summer 2008	Slurry	FW	SoSa	40.98	
Summer 2008	Slurry	FW	SoSa	92.17	
Summer 2008	Slurry	FW	SoSa	20.99	
Summer 2008	Slurry	FW	SoSa	4.96	
Summer 2008	Slurry	FW	SoSa	6.52	
Summer 2008	Slurry	FW	SoSa	1.25	
Summer 2008	Slurry	FW	SoSa	1.65	
Summer 2008	Slurry	FW	SoSa	13.65	
Summer 2008	Slurry	FW	SoSo	77.36	
Summer 2008	Slurry	FW	SoSo	8.81	
Summer 2008	Slurry	FW	SoSo	26.84	
Summer 2008	Slurry	FW	SoSo	9.21	

Summer 2008	Slurry	FW	SoSo	19.31	
Summer 2008	Slurry	FW	SoSo	2.90	
Summer 2008	Slurry	FW	SoSo	47.74	
Summer 2008	Slurry	FW	SoSo	31.73	
Summer 2008	Slurry	FW	SoSo	5.74	
Summer 2008	Slurry	OSPW	CT	10.06	
Summer 2008	Slurry	OSPW	CT	4.31	
Summer 2008	Slurry	OSPW	CT	18.15	
Summer 2008	Slurry	OSPW	CT	8.40	
Summer 2008	Slurry	OSPW	CT	12.96	
Summer 2008	Slurry	OSPW	CT	7.19	
Summer 2008	Slurry	OSPW	CT	10.98	
Summer 2008	Slurry	OSPW	CT	3.77	
Summer 2008	Slurry	OSPW	CT	4.95	
Summer 2008	Slurry	OSPW	SoCT	2.53	
Summer 2008	Slurry	OSPW	SoCT	0.00	
Summer 2008	Slurry	OSPW	SoCT	4.50	
Summer 2008	Slurry	OSPW	SoCT	14.38	
Summer 2008	Slurry	OSPW	SoCT	7.60	
Summer 2008	Slurry	OSPW	SoCT	5.62	
Summer 2008	Slurry	OSPW	SoCT	5.73	
Summer 2008	Slurry	OSPW	SoCT	6.78	
Summer 2008	Slurry	OSPW	SoCT	54.02	
Summer 2008	Slurry	OSPW	SoSa	8.40	
Summer 2008	Slurry	OSPW	SoSa	7.83	
Summer 2008	Slurry	OSPW	SoSa	1.95	
Summer 2008	Slurry	OSPW	SoSa	12.17	
Summer 2008	Slurry	OSPW	SoSa	10.34	
Summer 2008	Slurry	OSPW	SoSa	18.87	
Summer 2008	Slurry	OSPW	SoSa	1.95	
Summer 2008	Slurry	OSPW	SoSa	16.89	
Summer 2008	Slurry	OSPW	SoSa	3.50	
Summer 2008	Slurry	OSPW	SoSo	5.24	
Summer 2008	Slurry	OSPW	SoSo	11.70	
Summer 2008	Slurry	OSPW	SoSo	29.44	
Summer 2008	Slurry	OSPW	SoSo	2.34	
Summer 2008	Slurry	OSPW	SoSo	4.81	
Summer 2008	Slurry	OSPW	SoSo	0.00	
Summer 2008	Slurry	OSPW	SoSo	8.46	
Summer 2008	Slurry	OSPW	SoSo	45.50	
Summer 2008	Slurry	OSPW	SoSo	8.81	
Spring 2009	Control	FW	CT	2.37	

Spring 2009	Control	FW	CT	4.42	
Spring 2009	Control	FW	CT	3.69	
Spring 2009	Control	FW	SoCT	2.72	
Spring 2009	Control	FW	SoCT	4.92	
Spring 2009	Control	FW	SoCT	5.25	
Spring 2009	Control	FW	SoSa	5.35	
Spring 2009	Control	FW	SoSa	5.96	
Spring 2009	Control	FW	SoSa	27.75	
Spring 2009	Control	FW	SoSo	1.50	
Spring 2009	Control	FW	SoSo	14.27	
Spring 2009	Control	FW	SoSo	10.41	
Spring 2009	Control	OSPW	CT	40.59	
Spring 2009	Control	OSPW	CT	4.32	
Spring 2009	Control	OSPW	CT	18.30	
Spring 2009	Control	OSPW	SoCT	12.91	
Spring 2009	Control	OSPW	SoCT	4.46	
Spring 2009	Control	OSPW	SoCT	14.73	
Spring 2009	Control	OSPW	SoSa	7.30	
Spring 2009	Control	OSPW	SoSa	4.75	
Spring 2009	Control	OSPW	SoSa	5.27	
Spring 2009	Control	OSPW	SoSo	11.67	
Spring 2009	Control	OSPW	SoSo	10.24	
Spring 2009	Control	OSPW	SoSo	11.99	
Spring 2009	Mat	FW	CT	2.68	
Spring 2009	Mat	FW	CT	27.64	
Spring 2009	Mat	FW	CT	24.84	
Spring 2009	Mat	FW	SoCT	2.41	
Spring 2009	Mat	FW	SoCT	20.91	
Spring 2009	Mat	FW	SoCT	21.27	
Spring 2009	Mat	FW	SoSa	2.08	
Spring 2009	Mat	FW	SoSa	18.14	
Spring 2009	Mat	FW	SoSa	18.11	
Spring 2009	Mat	FW	SoSo	0.01	
Spring 2009	Mat	FW	SoSo	7.46	
Spring 2009	Mat	FW	SoSo	6.00	
Spring 2009	Mat	OSPW	CT	37.92	
Spring 2009	Mat	OSPW	CT	15.18	
Spring 2009	Mat	OSPW	CT	13.61	
Spring 2009	Mat	OSPW	SoCT	18.96	
Spring 2009	Mat	OSPW	SoCT	8.22	
Spring 2009	Mat	OSPW	SoCT	28.74	
Spring 2009	Mat	OSPW	SoSa	9.98	

Spring 2009	Mat	OSPW	SoSa	11.58	
Spring 2009	Mat	OSPW	SoSa	25.34	
Spring 2009	Mat	OSPW	SoSo	3.97	
Spring 2009	Mat	OSPW	SoSo	17.11	
Spring 2009	Mat	OSPW	SoSo	9.93	
Spring 2009	Slurry	FW	CT	3.92	
Spring 2009	Slurry	FW	CT	11.64	
Spring 2009	Slurry	FW	CT	10.63	
Spring 2009	Slurry	FW	CT	17.05	
Spring 2009	Slurry	FW	CT	21.12	
Spring 2009	Slurry	FW	CT	7.40	
Spring 2009	Slurry	FW	CT	16.53	
Spring 2009	Slurry	FW	CT	28.87	
Spring 2009	Slurry	FW	CT	14.33	
Spring 2009	Slurry	FW	SoCT	0.42	
Spring 2009	Slurry	FW	SoCT	5.02	
Spring 2009	Slurry	FW	SoCT	5.06	
Spring 2009	Slurry	FW	SoCT	4.10	
Spring 2009	Slurry	FW	SoCT	7.00	
Spring 2009	Slurry	FW	SoCT	55.08	
Spring 2009	Slurry	FW	SoCT	18.43	
Spring 2009	Slurry	FW	SoCT	10.35	
Spring 2009	Slurry	FW	SoCT	20.23	
Spring 2009	Slurry	FW	SoSa	0.00	
Spring 2009	Slurry	FW	SoSa	2.34	
Spring 2009	Slurry	FW	SoSa	0.69	
Spring 2009	Slurry	FW	SoSa	0.00	
Spring 2009	Slurry	FW	SoSa	10.78	
Spring 2009	Slurry	FW	SoSa	9.79	
Spring 2009	Slurry	FW	SoSa	12.54	
Spring 2009	Slurry	FW	SoSa	2.46	
Spring 2009	Slurry	FW	SoSa	0.00	
Spring 2009	Slurry	FW	SoSo	0.72	
Spring 2009	Slurry	FW	SoSo	0.00	
Spring 2009	Slurry	FW	SoSo	2.75	
Spring 2009	Slurry	FW	SoSo	30.25	
Spring 2009	Slurry	FW	SoSo	12.26	
Spring 2009	Slurry	FW	SoSo	8.79	
Spring 2009	Slurry	FW	SoSo	21.69	
Spring 2009	Slurry	FW	SoSo	14.76	
Spring 2009	Slurry	FW	SoSo	3.93	
Spring 2009	Slurry	OSPW	CT	21.72	

Spring 2009	Slurry	OSPW	CT	19.26	
Spring 2009	Slurry	OSPW	CT	25.72	
Spring 2009	Slurry	OSPW	CT	16.23	
Spring 2009	Slurry	OSPW	CT	7.61	
Spring 2009	Slurry	OSPW	CT	36.29	
Spring 2009	Slurry	OSPW	CT	67.22	
Spring 2009	Slurry	OSPW	CT	67.33	
Spring 2009	Slurry	OSPW	CT	37.37	
Spring 2009	Slurry	OSPW	SoCT	19.24	
Spring 2009	Slurry	OSPW	SoCT	28.97	
Spring 2009	Slurry	OSPW	SoCT	23.85	
Spring 2009	Slurry	OSPW	SoCT	9.41	
Spring 2009	Slurry	OSPW	SoCT	3.90	
Spring 2009	Slurry	OSPW	SoCT	5.02	
Spring 2009	Slurry	OSPW	SoCT	1.53	
Spring 2009	Slurry	OSPW	SoCT	29.68	
Spring 2009	Slurry	OSPW	SoCT	52.56	
Spring 2009	Slurry	OSPW	SoSa	10.58	
Spring 2009	Slurry	OSPW	SoSa	65.46	
Spring 2009	Slurry	OSPW	SoSa	10.26	
Spring 2009	Slurry	OSPW	SoSa	61.00	
Spring 2009	Slurry	OSPW	SoSa	0.30	
Spring 2009	Slurry	OSPW	SoSa	8.71	
Spring 2009	Slurry	OSPW	SoSa	9.51	
Spring 2009	Slurry	OSPW	SoSa	19.87	
Spring 2009	Slurry	OSPW	SoSa	16.94	
Spring 2009	Slurry	OSPW	SoSo	10.09	
Spring 2009	Slurry	OSPW	SoSo	53.22	
Spring 2009	Slurry	OSPW	SoSo	21.44	
Spring 2009	Slurry	OSPW	SoSo	3.60	
Spring 2009	Slurry	OSPW	SoSo	16.38	
Spring 2009	Slurry	OSPW	SoSo	6.35	
Spring 2009	Slurry	OSPW	SoSo	49.63	
Spring 2009	Slurry	OSPW	SoSo	69.41	
Spring 2009	Slurry	OSPW	SoSo	5.69	
Summer 2009	Control	FW	CT	1.38	
Summer 2009	Control	FW	CT	33.53	
Summer 2009	Control	FW	CT	3.96	
Summer 2009	Control	FW	SoCT	1.12	
Summer 2009	Control	FW	SoCT	11.31	
Summer 2009	Control	FW	SoCT	15.11	
Summer 2009	Control	FW	SoSa	3.05	

Summer 2009	Control	FW	SoSa	19.94	
Summer 2009	Control	FW	SoSa	5.96	
Summer 2009	Control	FW	SoSo	0.44	
Summer 2009	Control	FW	SoSo	18.62	
Summer 2009	Control	FW	SoSo	5.98	
Summer 2009	Control	OSPW	CT	3.09	
Summer 2009	Control	OSPW	CT	4.90	
Summer 2009	Control	OSPW	CT	0.57	
Summer 2009	Control	OSPW	SoCT	0.81	
Summer 2009	Control	OSPW	SoCT	12.39	
Summer 2009	Control	OSPW	SoCT	27.41	
Summer 2009	Control	OSPW	SoSa	3.45	
Summer 2009	Control	OSPW	SoSa	12.02	
Summer 2009	Control	OSPW	SoSa	3.87	
Summer 2009	Control	OSPW	SoSo	8.74	
Summer 2009	Control	OSPW	SoSo	1.93	
Summer 2009	Control	OSPW	SoSo	6.10	
Summer 2009	Mat	FW	CT	2.91	
Summer 2009	Mat	FW	CT	1.28	
Summer 2009	Mat	FW	CT	21.52	
Summer 2009	Mat	FW	SoCT	1.54	
Summer 2009	Mat	FW	SoCT	1.57	
Summer 2009	Mat	FW	SoCT	7.69	
Summer 2009	Mat	FW	SoSa	2.71	
Summer 2009	Mat	FW	SoSa	3.06	
Summer 2009	Mat	FW	SoSa	11.45	
Summer 2009	Mat	FW	SoSo	0.00	
Summer 2009	Mat	FW	SoSo	2.32	
Summer 2009	Mat	FW	SoSo	5.26	
Summer 2009	Mat	OSPW	CT	11.65	
Summer 2009	Mat	OSPW	CT	3.99	
Summer 2009	Mat	OSPW	CT	17.38	
Summer 2009	Mat	OSPW	SoCT	25.11	
Summer 2009	Mat	OSPW	SoCT	5.00	
Summer 2009	Mat	OSPW	SoCT	5.43	
Summer 2009	Mat	OSPW	SoSa	2.41	
Summer 2009	Mat	OSPW	SoSa	21.25	
Summer 2009	Mat	OSPW	SoSa	21.73	
Summer 2009	Mat	OSPW	SoSo	3.08	
Summer 2009	Mat	OSPW	SoSo	5.79	
Summer 2009	Mat	OSPW	SoSo	3.26	
Summer 2009	Slurry	FW	CT	2.58	

Summer 2009	Slurry	FW	CT	14.21	
Summer 2009	Slurry	FW	CT	3.37	
Summer 2009	Slurry	FW	CT	5.81	
Summer 2009	Slurry	FW	CT	7.57	
Summer 2009	Slurry	FW	CT	36.32	
Summer 2009	Slurry	FW	CT	16.18	
Summer 2009	Slurry	FW	CT	10.69	
Summer 2009	Slurry	FW	CT	14.58	
Summer 2009	Slurry	FW	SoCT	2.68	
Summer 2009	Slurry	FW	SoCT	5.99	
Summer 2009	Slurry	FW	SoCT	2.80	
Summer 2009	Slurry	FW	SoCT	1.09	
Summer 2009	Slurry	FW	SoCT	2.11	
Summer 2009	Slurry	FW	SoCT	10.80	
Summer 2009	Slurry	FW	SoCT	19.77	
Summer 2009	Slurry	FW	SoCT	4.11	
Summer 2009	Slurry	FW	SoCT	9.82	
Summer 2009	Slurry	FW	SoSa	2.34	
Summer 2009	Slurry	FW	SoSa	2.16	
Summer 2009	Slurry	FW	SoSa	2.02	
Summer 2009	Slurry	FW	SoSa	2.86	
Summer 2009	Slurry	FW	SoSa	5.00	
Summer 2009	Slurry	FW	SoSa	4.48	
Summer 2009	Slurry	FW	SoSa	17.45	
Summer 2009	Slurry	FW	SoSa	8.20	
Summer 2009	Slurry	FW	SoSa	7.72	
Summer 2009	Slurry	FW	SoSo	3.85	
Summer 2009	Slurry	FW	SoSo	1.91	
Summer 2009	Slurry	FW	SoSo	1.51	
Summer 2009	Slurry	FW	SoSo	4.77	
Summer 2009	Slurry	FW	SoSo	3.49	
Summer 2009	Slurry	FW	SoSo	0.12	
Summer 2009	Slurry	FW	SoSo	16.06	
Summer 2009	Slurry	FW	SoSo	22.79	
Summer 2009	Slurry	FW	SoSo	19.84	
Summer 2009	Slurry	OSPW	CT	10.13	
Summer 2009	Slurry	OSPW	CT	14.86	
Summer 2009	Slurry	OSPW	CT	6.33	
Summer 2009	Slurry	OSPW	CT	6.39	
Summer 2009	Slurry	OSPW	CT	5.09	
Summer 2009	Slurry	OSPW	CT	11.48	
Summer 2009	Slurry	OSPW	CT	31.94	

Summer 2009	Slurry	OSPW	CT	3.84	
Summer 2009	Slurry	OSPW	CT	26.49	
Summer 2009	Slurry	OSPW	SoCT	25.41	
Summer 2009	Slurry	OSPW	SoCT	3.09	
Summer 2009	Slurry	OSPW	SoCT	19.97	
Summer 2009	Slurry	OSPW	SoCT	3.64	
Summer 2009	Slurry	OSPW	SoCT	0.31	
Summer 2009	Slurry	OSPW	SoCT	7.66	
Summer 2009	Slurry	OSPW	SoCT	1.02	
Summer 2009	Slurry	OSPW	SoCT	4.80	
Summer 2009	Slurry	OSPW	SoCT	2.53	
Summer 2009	Slurry	OSPW	SoSa	6.71	
Summer 2009	Slurry	OSPW	SoSa	25.29	
Summer 2009	Slurry	OSPW	SoSa	4.07	
Summer 2009	Slurry	OSPW	SoSa	23.42	
Summer 2009	Slurry	OSPW	SoSa	0.22	
Summer 2009	Slurry	OSPW	SoSa	0.47	
Summer 2009	Slurry	OSPW	SoSa	2.43	
Summer 2009	Slurry	OSPW	SoSa	4.08	
Summer 2009	Slurry	OSPW	SoSa	1.43	
Summer 2009	Slurry	OSPW	SoSo	9.56	
Summer 2009	Slurry	OSPW	SoSo	4.50	
Summer 2009	Slurry	OSPW	SoSo	1.23	
Summer 2009	Slurry	OSPW	SoSo	1.12	
Summer 2009	Slurry	OSPW	SoSo	6.10	
Summer 2009	Slurry	OSPW	SoSo	1.32	
Summer 2009	Slurry	OSPW	SoSo	4.96	
Summer 2009	Slurry	OSPW	SoSo	1.62	
Summer 2009	Slurry	OSPW	SoSo	0.84	
Spring 2010	Control	FW	CT	3.22	0.20
Spring 2010	Control	FW	CT	3.48	0.37
Spring 2010	Control	FW	CT	5.26	0.16
Spring 2010	Control	FW	SoCT	1.73	0.03
Spring 2010	Control	FW	SoCT	15.53	0.17
Spring 2010	Control	FW	SoCT	3.56	0.07
Spring 2010	Control	FW	SoSa	1.05	0.02
Spring 2010	Control	FW	SoSa	2.40	0.08
Spring 2010	Control	FW	SoSa	5.76	0.02
Spring 2010	Control	FW	SoSo	2.67	0.03
Spring 2010	Control	FW	SoSo	6.95	0.05
Spring 2010	Control	FW	SoSo	3.03	0.01
Spring 2010	Control	OSPW	CT	9.94	0.59

Spring 2010	Control	OSPW	CT	1.03	0.72
Spring 2010	Control	OSPW	CT	4.44	0.18
Spring 2010	Control	OSPW	SoCT	7.83	0.16
Spring 2010	Control	OSPW	SoCT	2.12	0.03
Spring 2010	Control	OSPW	SoCT	17.00	0.20
Spring 2010	Control	OSPW	SoSa	8.52	0.23
Spring 2010	Control	OSPW	SoSa	2.67	0.08
Spring 2010	Control	OSPW	SoSa	16.76	0.20
Spring 2010	Control	OSPW	SoSo	9.49	0.10
Spring 2010	Control	OSPW	SoSo	5.46	0.13
Spring 2010	Control	OSPW	SoSo	14.07	0.10
Spring 2010	Mat	FW	CT	3.63	0.41
Spring 2010	Mat	FW	CT	3.03	0.03
Spring 2010	Mat	FW	CT	1.95	0.26
Spring 2010	Mat	FW	SoCT	5.21	0.00
Spring 2010	Mat	FW	SoCT	6.47	0.01
Spring 2010	Mat	FW	SoCT	14.31	0.07
Spring 2010	Mat	FW	SoSa	4.06	0.00
Spring 2010	Mat	FW	SoSa	15.17	0.93
Spring 2010	Mat	FW	SoSa	2.66	0.04
Spring 2010	Mat	FW	SoSo	0.00	0.00
Spring 2010	Mat	FW	SoSo	2.20	0.03
Spring 2010	Mat	FW	SoSo	5.33	0.02
Spring 2010	Mat	OSPW	CT	9.29	0.29
Spring 2010	Mat	OSPW	CT	14.35	0.00
Spring 2010	Mat	OSPW	CT	6.08	0.50
Spring 2010	Mat	OSPW	SoCT	5.56	0.09
Spring 2010	Mat	OSPW	SoCT	14.81	0.08
Spring 2010	Mat	OSPW	SoCT	17.29	0.19
Spring 2010	Mat	OSPW	SoSa	5.05	0.08
Spring 2010	Mat	OSPW	SoSa	19.39	0.31
Spring 2010	Mat	OSPW	SoSa	7.28	0.13
Spring 2010	Mat	OSPW	SoSo	9.40	0.18
Spring 2010	Mat	OSPW	SoSo	11.15	0.03
Spring 2010	Mat	OSPW	SoSo	9.49	0.08
Spring 2010	Slurry	FW	CT	0.00	0.34
Spring 2010	Slurry	FW	CT	1.78	0.47
Spring 2010	Slurry	FW	CT	8.79	0.46
Spring 2010	Slurry	FW	CT	12.18	0.79
Spring 2010	Slurry	FW	CT	2.19	0.49
Spring 2010	Slurry	FW	CT	14.92	0.22
Spring 2010	Slurry	FW	CT	3.66	0.32

Spring 2010	Slurry	FW	CT	8.08	0.28
Spring 2010	Slurry	FW	CT	3.87	0.26
Spring 2010	Slurry	FW	SoCT	0.00	0.08
Spring 2010	Slurry	FW	SoCT	4.35	0.05
Spring 2010	Slurry	FW	SoCT	2.64	0.20
Spring 2010	Slurry	FW	SoCT	1.22	0.02
Spring 2010	Slurry	FW	SoCT	4.05	0.06
Spring 2010	Slurry	FW	SoCT	8.34	0.02
Spring 2010	Slurry	FW	SoCT	7.74	0.07
Spring 2010	Slurry	FW	SoCT	1.41	0.04
Spring 2010	Slurry	FW	SoCT	11.47	0.10
Spring 2010	Slurry	FW	SoSa	1.22	0.05
Spring 2010	Slurry	FW	SoSa	5.64	0.02
Spring 2010	Slurry	FW	SoSa	5.97	0.00
Spring 2010	Slurry	FW	SoSa	16.61	0.04
Spring 2010	Slurry	FW	SoSa	2.53	0.05
Spring 2010	Slurry	FW	SoSa	2.79	0.08
Spring 2010	Slurry	FW	SoSa	1.86	0.05
Spring 2010	Slurry	FW	SoSa	9.89	0.08
Spring 2010	Slurry	FW	SoSa	1.06	0.09
Spring 2010	Slurry	FW	SoSo	2.84	0.04
Spring 2010	Slurry	FW	SoSo	8.10	0.08
Spring 2010	Slurry	FW	SoSo	1.31	0.00
Spring 2010	Slurry	FW	SoSo	7.15	0.06
Spring 2010	Slurry	FW	SoSo	2.29	0.01
Spring 2010	Slurry	FW	SoSo	3.77	0.10
Spring 2010	Slurry	FW	SoSo	2.99	0.14
Spring 2010	Slurry	FW	SoSo	7.23	0.02
Spring 2010	Slurry	FW	SoSo	3.43	0.10
Spring 2010	Slurry	OSPW	CT	22.39	0.58
Spring 2010	Slurry	OSPW	CT	15.07	0.24
Spring 2010	Slurry	OSPW	CT	11.15	0.28
Spring 2010	Slurry	OSPW	CT	2.02	0.17
Spring 2010	Slurry	OSPW	CT	5.04	0.18
Spring 2010	Slurry	OSPW	CT	1.21	0.14
Spring 2010	Slurry	OSPW	CT	11.98	0.35
Spring 2010	Slurry	OSPW	CT	10.11	0.15
Spring 2010	Slurry	OSPW	CT	17.44	0.29
Spring 2010	Slurry	OSPW	SoCT	22.90	0.11
Spring 2010	Slurry	OSPW	SoCT	13.15	0.26
Spring 2010	Slurry	OSPW	SoCT	12.78	0.17
Spring 2010	Slurry	OSPW	SoCT	9.58	0.05

Spring 2010	Slurry	OSPW	SoCT	8.41	0.21
Spring 2010	Slurry	OSPW	SoCT	6.45	0.09
Spring 2010	Slurry	OSPW	SoCT	20.99	0.11
Spring 2010	Slurry	OSPW	SoCT	3.79	0.17
Spring 2010	Slurry	OSPW	SoCT	7.60	0.16
Spring 2010	Slurry	OSPW	SoSa	12.00	0.17
Spring 2010	Slurry	OSPW	SoSa	10.14	0.04
Spring 2010	Slurry	OSPW	SoSa	9.75	0.12
Spring 2010	Slurry	OSPW	SoSa	1.35	0.15
Spring 2010	Slurry	OSPW	SoSa	4.99	0.00
Spring 2010	Slurry	OSPW	SoSa	6.40	0.32
Spring 2010	Slurry	OSPW	SoSa	17.50	0.14
Spring 2010	Slurry	OSPW	SoSa	11.41	0.20
Spring 2010	Slurry	OSPW	SoSa	12.06	0.10
Spring 2010	Slurry	OSPW	SoSo	2.64	0.08
Spring 2010	Slurry	OSPW	SoSo	21.62	0.14
Spring 2010	Slurry	OSPW	SoSo	6.05	0.14
Spring 2010	Slurry	OSPW	SoSo	14.76	0.00
Spring 2010	Slurry	OSPW	SoSo	1.97	0.05
Spring 2010	Slurry	OSPW	SoSo	6.62	0.04
Spring 2010	Slurry	OSPW	SoSo	2.76	0.31
Spring 2010	Slurry	OSPW	SoSo	15.96	0.14
Spring 2010	Slurry	OSPW	SoSo	8.55	0.14

APPENDIX IV – RAW DATA FOR DISSOLVED OXYGEN MEASUREMENTS WITH INDEPENDENT VARIABLES

Time	Treatment	Transfer	Water Type	Substrate	DO rate of change ($g/m^2/day$)
Aug-2008	Dark	Control	FW	CT	-0.0448
Aug-2008	Dark	Control	FW	SoCT	-0.0936
Aug-2008	Dark	Control	FW	SoSa	-0.0969
Aug-2008	Dark	Control	FW	SoSo	-0.1067
Aug-2008	Dark	Control	OSPW	CT	-0.0024
Aug-2008	Dark	Control	OSPW	SoCT	-0.1539
Aug-2008	Dark	Control	OSPW	SoSa	-0.1270
Aug-2008	Dark	Control	OSPW	SoSo	-0.0814
Aug-2008	Dark	Mat	FW	CT	-0.1156
Aug-2008	Dark	Mat	FW	SoCT	-0.0529
Aug-2008	Dark	Mat	FW	SoSa	-0.1238
Aug-2008	Dark	Mat	FW	SoSo	-0.0928
Aug-2008	Dark	Mat	OSPW	CT	-0.0945
Aug-2008	Dark	Mat	OSPW	SoCT	-0.1254
Aug-2008	Dark	Mat	OSPW	SoSa	-0.1018
Aug-2008	Dark	Mat	OSPW	SoSo	0.0415
Aug-2008	Dark	Slurry	FW	CT	0.0366
Aug-2008	Dark	Slurry	FW	CT	-0.0464
Aug-2008	Dark	Slurry	FW	CT	-0.1563
Aug-2008	Dark	Slurry	FW	SoCT	-0.0480
Aug-2008	Dark	Slurry	FW	SoCT	-0.0717
Aug-2008	Dark	Slurry	FW	SoCT	-0.1433
Aug-2008	Dark	Slurry	FW	SoSa	-0.0106
Aug-2008	Dark	Slurry	FW	SoSa	-0.0733
Aug-2008	Dark	Slurry	FW	SoSa	-0.1653
Aug-2008	Dark	Slurry	FW	SoSo	-0.0480
Aug-2008	Dark	Slurry	FW	SoSo	-0.1352
Aug-2008	Dark	Slurry	FW	SoSo	-0.1751
Aug-2008	Dark	Slurry	OSPW	CT	-0.0578
Aug-2008	Dark	Slurry	OSPW	CT	-0.1490
Aug-2008	Dark	Slurry	OSPW	CT	-0.0717
Aug-2008	Dark	Slurry	OSPW	SoCT	-0.0749
Aug-2008	Dark	Slurry	OSPW	SoCT	-0.1946
Aug-2008	Dark	Slurry	OSPW	SoCT	-0.0969
Aug-2008	Dark	Slurry	OSPW	SoSa	-0.0635
Aug-2008	Dark	Slurry	OSPW	SoSa	-0.1653
Aug-2008	Dark	Slurry	OSPW	SoSa	-0.0896
Aug-2008	Dark	Slurry	OSPW	SoSo	-0.1164

Aug-2008	Dark	Slurry	OSPW	SoSo	0.1848
Aug-2008	Dark	Slurry	OSPW	SoSo	-0.0725
Aug-2008	Light	Control	FW	CT	-0.0027
Aug-2008	Light	Control	FW	SoCT	-0.0245
Aug-2008	Light	Control	FW	SoSa	-0.0282
Aug-2008	Light	Control	FW	SoSo	-0.0536
Aug-2008	Light	Control	OSPW	CT	-0.0445
Aug-2008	Light	Control	OSPW	SoCT	-0.1899
Aug-2008	Light	Control	OSPW	SoSa	-0.0254
Aug-2008	Light	Control	OSPW	SoSo	-0.2226
Aug-2008	Light	Mat	FW	CT	-0.0309
Aug-2008	Light	Mat	FW	SoCT	-0.0899
Aug-2008	Light	Mat	FW	SoSa	-0.0244
Aug-2008	Light	Mat	FW	SoSo	-0.1445
Aug-2008	Light	Mat	OSPW	CT	-0.1626
Aug-2008	Light	Mat	OSPW	SoCT	-0.0273
Aug-2008	Light	Mat	OSPW	SoSa	0.0209
Aug-2008	Light	Mat	OSPW	SoSo	-0.1581
Aug-2008	Light	Slurry	FW	CT	0.0472
Aug-2008	Light	Slurry	FW	CT	0.0727
Aug-2008	Light	Slurry	FW	CT	0.0781
Aug-2008	Light	Slurry	FW	SoCT	-0.1345
Aug-2008	Light	Slurry	FW	SoCT	0.1617
Aug-2008	Light	Slurry	FW	SoCT	0.0254
Aug-2008	Light	Slurry	FW	SoSa	0.0000
Aug-2008	Light	Slurry	FW	SoSa	-0.0127
Aug-2008	Light	Slurry	FW	SoSa	0.0636
Aug-2008	Light	Slurry	FW	SoSo	0.1154
Aug-2008	Light	Slurry	FW	SoSo	0.0527
Aug-2008	Light	Slurry	FW	SoSo	-0.1763
Aug-2008	Light	Slurry	OSPW	CT	-0.0463
Aug-2008	Light	Slurry	OSPW	CT	0.0500
Aug-2008	Light	Slurry	OSPW	CT	0.0963
Aug-2008	Light	Slurry	OSPW	SoCT	0.1781
Aug-2008	Light	Slurry	OSPW	SoCT	-0.1108
Aug-2008	Light	Slurry	OSPW	SoCT	0.2171
Aug-2008	Light	Slurry	OSPW	SoSa	0.0418
Aug-2008	Light	Slurry	OSPW	SoSa	0.3225
Aug-2008	Light	Slurry	OSPW	SoSa	0.1054
Aug-2008	Light	Slurry	OSPW	SoSo	0.0790
Aug-2008	Light	Slurry	OSPW	SoSo	-0.0645
Aug-2008	Light	Slurry	OSPW	SoSo	0.2099

Jun-2009	Dark	Control	FW	CT	-0.1523
Jun-2009	Dark	Control	FW	CT	-0.1197
Jun-2009	Dark	Control	FW	CT	-0.0700
Jun-2009	Dark	Control	FW	SoCT	-0.0627
Jun-2009	Dark	Control	FW	SoCT	-0.1034
Jun-2009	Dark	Control	FW	SoCT	-0.0936
Jun-2009	Dark	Control	FW	SoSa	-0.0993
Jun-2009	Dark	Control	FW	SoSa	-0.1173
Jun-2009	Dark	Control	FW	SoSa	-0.1164
Jun-2009	Dark	Control	FW	SoSo	-0.1189
Jun-2009	Dark	Control	FW	SoSo	-0.0399
Jun-2009	Dark	Control	FW	SoSo	-0.0049
Jun-2009	Dark	Control	OSPW	CT	-0.0643
Jun-2009	Dark	Control	OSPW	CT	-0.0741
Jun-2009	Dark	Control	OSPW	CT	-0.1067
Jun-2009	Dark	Control	OSPW	SoCT	-0.0822
Jun-2009	Dark	Control	OSPW	SoCT	-0.1287
Jun-2009	Dark	Control	OSPW	SoCT	-0.1173
Jun-2009	Dark	Control	OSPW	SoSa	-0.0676
Jun-2009	Dark	Control	OSPW	SoSa	-0.1433
Jun-2009	Dark	Control	OSPW	SoSa	-0.0871
Jun-2009	Dark	Control	OSPW	SoSo	-0.0782
Jun-2009	Dark	Control	OSPW	SoSo	-0.1384
Jun-2009	Dark	Control	OSPW	SoSo	-0.1791
Jun-2009	Dark	Mat	FW	CT	-0.0757
Jun-2009	Dark	Mat	FW	CT	-0.0440
Jun-2009	Dark	Mat	FW	CT	-0.0489
Jun-2009	Dark	Mat	FW	SoCT	-0.1156
Jun-2009	Dark	Mat	FW	SoCT	-0.0399
Jun-2009	Dark	Mat	FW	SoCT	-0.0741
Jun-2009	Dark	Mat	FW	SoSa	-0.1107
Jun-2009	Dark	Mat	FW	SoSa	-0.1327
Jun-2009	Dark	Mat	FW	SoSa	-0.0831
Jun-2009	Dark	Mat	FW	SoSo	-0.1050
Jun-2009	Dark	Mat	FW	SoSo	-0.0871
Jun-2009	Dark	Mat	FW	SoSo	-0.0774
Jun-2009	Dark	Mat	OSPW	CT	-0.0985
Jun-2009	Dark	Mat	OSPW	CT	-0.2190
Jun-2009	Dark	Mat	OSPW	CT	-0.0163
Jun-2009	Dark	Mat	OSPW	SoCT	-0.1327
Jun-2009	Dark	Mat	OSPW	SoCT	-0.3273
Jun-2009	Dark	Mat	OSPW	SoCT	-0.0578

Jun-2009	Dark	Mat	OSPW	SoSa	-0.1116
Jun-2009	Dark	Mat	OSPW	SoSa	-0.1637
Jun-2009	Dark	Mat	OSPW	SoSa	-0.0782
Jun-2009	Dark	Mat	OSPW	SoSo	-0.0920
Jun-2009	Dark	Mat	OSPW	SoSo	-0.2068
Jun-2009	Dark	Mat	OSPW	SoSo	-0.0822
Jun-2009	Dark	Slurry	FW	CT	-0.0016
Jun-2009	Dark	Slurry	FW	CT	-0.1116
Jun-2009	Dark	Slurry	FW	CT	-0.0326
Jun-2009	Dark	Slurry	FW	CT	-0.0106
Jun-2009	Dark	Slurry	FW	CT	-0.1539
Jun-2009	Dark	Slurry	FW	CT	-0.1059
Jun-2009	Dark	Slurry	FW	CT	-0.0692
Jun-2009	Dark	Slurry	FW	CT	-0.0562
Jun-2009	Dark	Slurry	FW	CT	-0.0863
Jun-2009	Dark	Slurry	FW	SoCT	-0.0448
Jun-2009	Dark	Slurry	FW	SoCT	-0.0904
Jun-2009	Dark	Slurry	FW	SoCT	-0.0936
Jun-2009	Dark	Slurry	FW	SoCT	-0.0977
Jun-2009	Dark	Slurry	FW	SoCT	-0.1392
Jun-2009	Dark	Slurry	FW	SoCT	-0.0912

Jun-2009	Dark	Slurry	FW	SoCT	-0.0969
Jun-2009	Dark	Slurry	FW	SoCT	-0.1002
Jun-2009	Dark	Slurry	FW	SoCT	-0.0448
Jun-2009	Dark	Slurry	FW	SoSa	-0.0114
Jun-2009	Dark	Slurry	FW	SoSa	-0.1523
Jun-2009	Dark	Slurry	FW	SoSa	-0.0708
Jun-2009	Dark	Slurry	FW	SoSa	-0.1278
Jun-2009	Dark	Slurry	FW	SoSa	-0.1506
Jun-2009	Dark	Slurry	FW	SoSa	-0.0358
Jun-2009	Dark	Slurry	FW	SoSa	-0.1059
Jun-2009	Dark	Slurry	FW	SoSa	-0.1164
Jun-2009	Dark	Slurry	FW	SoSa	-0.0554
Jun-2009	Dark	Slurry	FW	SoSo	-0.0147
Jun-2009	Dark	Slurry	FW	SoSo	-0.1368
Jun-2009	Dark	Slurry	FW	SoSo	-0.1002
Jun-2009	Dark	Slurry	FW	SoSo	-0.1368
Jun-2009	Dark	Slurry	FW	SoSo	-0.0993
Jun-2009	Dark	Slurry	FW	SoSo	-0.0269
Jun-2009	Dark	Slurry	FW	SoSo	-0.1018
Jun-2009	Dark	Slurry	FW	SoSo	-0.0749
Jun-2009	Dark	Slurry	FW	SoSo	-0.0692

Jun-2009	Dark	Slurry	OSPW	CT	-0.2549
Jun-2009	Dark	Slurry	OSPW	CT	-0.1311
Jun-2009	Dark	Slurry	OSPW	CT	-0.0896
Jun-2009	Dark	Slurry	OSPW	CT	-0.0195
Jun-2009	Dark	Slurry	OSPW	CT	-0.2011
Jun-2009	Dark	Slurry	OSPW	CT	-0.1620
Jun-2009	Dark	Slurry	OSPW	CT	-0.0839
Jun-2009	Dark	Slurry	OSPW	CT	-0.1506
Jun-2009	Dark	Slurry	OSPW	CT	-0.2304
Jun-2009	Dark	Slurry	OSPW	SoCT	-0.1775
Jun-2009	Dark	Slurry	OSPW	SoCT	-0.1205
Jun-2009	Dark	Slurry	OSPW	SoCT	-0.0733
Jun-2009	Dark	Slurry	OSPW	SoCT	-0.0432
Jun-2009	Dark	Slurry	OSPW	SoCT	-0.2369
Jun-2009	Dark	Slurry	OSPW	SoCT	-0.1197
Jun-2009	Dark	Slurry	OSPW	SoCT	-0.2011
Jun-2009	Dark	Slurry	OSPW	SoCT	-0.2622
Jun-2009	Dark	Slurry	OSPW	SoCT	-0.0399
Jun-2009	Dark	Slurry	OSPW	SoSa	-0.1401
Jun-2009	Dark	Slurry	OSPW	SoSa	-0.1034
Jun-2009	Dark	Slurry	OSPW	SoSa	-0.0529
Jun-2009	Dark	Slurry	OSPW	SoSa	-0.0985
Jun-2009	Dark	Slurry	OSPW	SoSa	-0.3208
Jun-2009	Dark	Slurry	OSPW	SoSa	-0.2060
Jun-2009	Dark	Slurry	OSPW	SoSa	-0.2093
Jun-2009	Dark	Slurry	OSPW	SoSa	-0.2443
Jun-2009	Dark	Slurry	OSPW	SoSa	-0.1791
Jun-2009	Dark	Slurry	OSPW	SoSo	-0.1637
Jun-2009	Dark	Slurry	OSPW	SoSo	-0.0684
Jun-2009	Dark	Slurry	OSPW	SoSo	-0.0432
Jun-2009	Dark	Slurry	OSPW	SoSo	-0.1889
Jun-2009	Dark	Slurry	OSPW	SoSo	-0.2630
Jun-2009	Dark	Slurry	OSPW	SoSo	-0.0920
Jun-2009	Dark	Slurry	OSPW	SoSo	-0.1946
Jun-2009	Dark	Slurry	OSPW	SoSo	-0.2418
Jun-2009	Dark	Slurry	OSPW	SoSo	-0.1816
Jun-2009	Light	Control	FW	CT	0.0845
Jun-2009	Light	Control	FW	CT	0.0127
Jun-2009	Light	Control	FW	CT	-0.0636
Jun-2009	Light	Control	FW	SoCT	-0.1154
Jun-2009	Light	Control	FW	SoCT	0.0127
Jun-2009	Light	Control	FW	SoCT	-0.0881

Jun-2009	Light	Control	FW	SoSa	-0.1226
Jun-2009	Light	Control	FW	SoSa	0.0191
Jun-2009	Light	Control	FW	SoSa	-0.0327
Jun-2009	Light	Control	FW	SoSo	0.0336
Jun-2009	Light	Control	FW	SoSo	-0.0091
Jun-2009	Light	Control	FW	SoSo	-0.0690
Jun-2009	Light	Control	OSPW	CT	-0.0518
Jun-2009	Light	Control	OSPW	CT	0.1136
Jun-2009	Light	Control	OSPW	CT	-0.0354
Jun-2009	Light	Control	OSPW	SoCT	0.1508
Jun-2009	Light	Control	OSPW	SoCT	0.1644
Jun-2009	Light	Control	OSPW	SoCT	-0.1190
Jun-2009	Light	Control	OSPW	SoSa	0.0427
Jun-2009	Light	Control	OSPW	SoSa	0.0927
Jun-2009	Light	Control	OSPW	SoSa	0.0681
Jun-2009	Light	Control	OSPW	SoSo	0.0672
Jun-2009	Light	Control	OSPW	SoSo	-0.0027
Jun-2009	Light	Control	OSPW	SoSo	0.0173
Jun-2009	Light	Mat	FW	CT	0.0654
Jun-2009	Light	Mat	FW	CT	0.0427
Jun-2009	Light	Mat	FW	CT	-0.0082

Jun-2009	Light	Mat	FW	SoCT	0.1181
Jun-2009	Light	Mat	FW	SoCT	0.0309
Jun-2009	Light	Mat	FW	SoCT	0.0036
Jun-2009	Light	Mat	FW	SoSa	0.1308
Jun-2009	Light	Mat	FW	SoSa	0.1018
Jun-2009	Light	Mat	FW	SoSa	-0.0273
Jun-2009	Light	Mat	FW	SoSo	-0.0754
Jun-2009	Light	Mat	FW	SoSo	0.0554
Jun-2009	Light	Mat	FW	SoSo	-0.0300
Jun-2009	Light	Mat	OSPW	CT	-0.0472
Jun-2009	Light	Mat	OSPW	CT	-0.0518
Jun-2009	Light	Mat	OSPW	CT	0.0309
Jun-2009	Light	Mat	OSPW	SoCT	-0.0918
Jun-2009	Light	Mat	OSPW	SoCT	0.0027
Jun-2009	Light	Mat	OSPW	SoCT	0.0372
Jun-2009	Light	Mat	OSPW	SoSa	-0.0681
Jun-2009	Light	Mat	OSPW	SoSa	0.0672
Jun-2009	Light	Mat	OSPW	SoSa	0.0591
Jun-2009	Light	Mat	OSPW	SoSo	-0.0200
Jun-2009	Light	Mat	OSPW	SoSo	0.0645
Jun-2009	Light	Mat	OSPW	SoSo	0.0527

Jun-2009	Light	Slurry	FW	CT	-0.0881
Jun-2009	Light	Slurry	FW	CT	-0.1426
Jun-2009	Light	Slurry	FW	CT	0.0136
Jun-2009	Light	Slurry	FW	CT	-0.0082
Jun-2009	Light	Slurry	FW	CT	0.0990
Jun-2009	Light	Slurry	FW	CT	0.0591
Jun-2009	Light	Slurry	FW	CT	0.0282
Jun-2009	Light	Slurry	FW	CT	0.0318
Jun-2009	Light	Slurry	FW	CT	0.0809
Jun-2009	Light	Slurry	FW	SoCT	-0.1417
Jun-2009	Light	Slurry	FW	SoCT	-0.1381
Jun-2009	Light	Slurry	FW	SoCT	-0.0018
Jun-2009	Light	Slurry	FW	SoCT	-0.1090
Jun-2009	Light	Slurry	FW	SoCT	0.0881
Jun-2009	Light	Slurry	FW	SoCT	-0.0109
Jun-2009	Light	Slurry	FW	SoCT	-0.0282
Jun-2009	Light	Slurry	FW	SoCT	0.0263
Jun-2009	Light	Slurry	FW	SoCT	0.0427
Jun-2009	Light	Slurry	FW	SoSa	-0.1672
Jun-2009	Light	Slurry	FW	SoSa	-0.1535
Jun-2009	Light	Slurry	FW	SoSa	-0.0354
Jun-2009	Light	Slurry	FW	SoSa	-0.0945
Jun-2009	Light	Slurry	FW	SoSa	0.0545
Jun-2009	Light	Slurry	FW	SoSa	0.0145
Jun-2009	Light	Slurry	FW	SoSa	-0.0318
Jun-2009	Light	Slurry	FW	SoSa	0.0191
Jun-2009	Light	Slurry	FW	SoSa	0.1417
Jun-2009	Light	Slurry	FW	SoSo	-0.1681
Jun-2009	Light	Slurry	FW	SoSo	-0.1681
Jun-2009	Light	Slurry	FW	SoSo	-0.0300
Jun-2009	Light	Slurry	FW	SoSo	-0.0709
Jun-2009	Light	Slurry	FW	SoSo	0.0500
Jun-2009	Light	Slurry	FW	SoSo	-0.0236
Jun-2009	Light	Slurry	FW	SoSo	-0.0200
Jun-2009	Light	Slurry	FW	SoSo	0.0518
Jun-2009	Light	Slurry	FW	SoSo	0.0718
Jun-2009	Light	Slurry	OSPW	CT	-0.1590
Jun-2009	Light	Slurry	OSPW	CT	0.0354
Jun-2009	Light	Slurry	OSPW	CT	0.0318
Jun-2009	Light	Slurry	OSPW	CT	-0.0409
Jun-2009	Light	Slurry	OSPW	CT	-0.1263
Jun-2009	Light	Slurry	OSPW	CT	-0.1308

Jun-2009	Light	Slurry	OSPW	CT	0.1018
Jun-2009	Light	Slurry	OSPW	CT	-0.0790
Jun-2009	Light	Slurry	OSPW	CT	-0.1090
Jun-2009	Light	Slurry	OSPW	SoCT	-0.0672
Jun-2009	Light	Slurry	OSPW	SoCT	0.0036
Jun-2009	Light	Slurry	OSPW	SoCT	0.0263
Jun-2009	Light	Slurry	OSPW	SoCT	-0.0845
Jun-2009	Light	Slurry	OSPW	SoCT	-0.1517
Jun-2009	Light	Slurry	OSPW	SoCT	-0.1181
Jun-2009	Light	Slurry	OSPW	SoCT	-0.1099
Jun-2009	Light	Slurry	OSPW	SoCT	-0.0736
Jun-2009	Light	Slurry	OSPW	SoCT	-0.2735
Jun-2009	Light	Slurry	OSPW	SoSa	0.0191
Jun-2009	Light	Slurry	OSPW	SoSa	0.0354
Jun-2009	Light	Slurry	OSPW	SoSa	-0.0300
Jun-2009	Light	Slurry	OSPW	SoSa	-0.0899
Jun-2009	Light	Slurry	OSPW	SoSa	-0.2208
Jun-2009	Light	Slurry	OSPW	SoSa	-0.0618
Jun-2009	Light	Slurry	OSPW	SoSa	-0.0064
Jun-2009	Light	Slurry	OSPW	SoSa	-0.0382
Jun-2009	Light	Slurry	OSPW	SoSa	-0.1699
Jun-2009	Light	Slurry	OSPW	SoSo	-0.0709
Jun-2009	Light	Slurry	OSPW	SoSo	-0.0191
Jun-2009	Light	Slurry	OSPW	SoSo	0.0191
Jun-2009	Light	Slurry	OSPW	SoSo	-0.1726
Jun-2009	Light	Slurry	OSPW	SoSo	-0.1372
Jun-2009	Light	Slurry	OSPW	SoSo	-0.0836
Jun-2009	Light	Slurry	OSPW	SoSo	-0.0363
Jun-2009	Light	Slurry	OSPW	SoSo	-0.0100
Jun-2009	Light	Slurry	OSPW	SoSo	-0.0645
Jul-2009	Dark	Control	FW	CT	0.0700
Jul-2009	Dark	Control	FW	CT	-0.1083
Jul-2009	Dark	Control	FW	CT	0.0375
Jul-2009	Dark	Control	FW	SoCT	0.0432
Jul-2009	Dark	Control	FW	SoCT	0.0049
Jul-2009	Dark	Control	FW	SoCT	-0.1669
Jul-2009	Dark	Control	FW	SoSa	-0.0318
Jul-2009	Dark	Control	FW	SoSa	-0.0383
Jul-2009	Dark	Control	FW	SoSa	-0.1360
Jul-2009	Dark	Control	FW	SoSo	-0.0570
Jul-2009	Dark	Control	FW	SoSo	-0.0456
Jul-2009	Dark	Control	FW	SoSo	-0.1686

Jul-2009	Dark	Control	OSPW	CT	-0.0138
Jul-2009	Dark	Control	OSPW	CT	0.0497
Jul-2009	Dark	Control	OSPW	CT	-0.0269
Jul-2009	Dark	Control	OSPW	SoCT	0.0049
Jul-2009	Dark	Control	OSPW	SoCT	0.0375
Jul-2009	Dark	Control	OSPW	SoCT	0.0554
Jul-2009	Dark	Control	OSPW	SoSa	-0.0171
Jul-2009	Dark	Control	OSPW	SoSa	0.0236
Jul-2009	Dark	Control	OSPW	SoSa	-0.0456
Jul-2009	Dark	Control	OSPW	SoSo	-0.0399
Jul-2009	Dark	Control	OSPW	SoSo	0.0261
Jul-2009	Dark	Control	OSPW	SoSo	-0.0391
Jul-2009	Dark	Mat	FW	CT	-0.0578
Jul-2009	Dark	Mat	FW	CT	-0.0171
Jul-2009	Dark	Mat	FW	CT	-0.0244
Jul-2009	Dark	Mat	FW	SoCT	-0.0953
Jul-2009	Dark	Mat	FW	SoCT	0.0448
Jul-2009	Dark	Mat	FW	SoCT	-0.0041
Jul-2009	Dark	Mat	FW	SoSa	-0.1743
Jul-2009	Dark	Mat	FW	SoSa	-0.0619
Jul-2009	Dark	Mat	FW	SoSa	-0.0122

Jul-2009	Dark	Mat	FW	SoSo	0.0831
Jul-2009	Dark	Mat	FW	SoSo	-0.0244
Jul-2009	Dark	Mat	FW	SoSo	0.0261
Jul-2009	Dark	Mat	OSPW	CT	-0.1352
Jul-2009	Dark	Mat	OSPW	CT	0.0041
Jul-2009	Dark	Mat	OSPW	CT	0.0155
Jul-2009	Dark	Mat	OSPW	SoCT	-0.0627
Jul-2009	Dark	Mat	OSPW	SoCT	-0.0383
Jul-2009	Dark	Mat	OSPW	SoCT	0.0586
Jul-2009	Dark	Mat	OSPW	SoSa	-0.1319
Jul-2009	Dark	Mat	OSPW	SoSa	0.1059
Jul-2009	Dark	Mat	OSPW	SoSa	0.0399
Jul-2009	Dark	Mat	OSPW	SoSo	0.0318
Jul-2009	Dark	Mat	OSPW	SoSo	-0.0391
Jul-2009	Dark	Mat	OSPW	SoSo	0.0277
Jul-2009	Dark	Slurry	FW	CT	0.0790
Jul-2009	Dark	Slurry	FW	CT	0.0472
Jul-2009	Dark	Slurry	FW	CT	-0.2052
Jul-2009	Dark	Slurry	FW	CT	-0.0684
Jul-2009	Dark	Slurry	FW	CT	-0.0228
Jul-2009	Dark	Slurry	FW	CT	-0.0782

Jul-2009	Dark	Slurry	FW	CT	-0.0562
Jul-2009	Dark	Slurry	FW	CT	-0.0798
Jul-2009	Dark	Slurry	FW	CT	0.0073
Jul-2009	Dark	Slurry	FW	SoCT	0.0261
Jul-2009	Dark	Slurry	FW	SoCT	-0.1303
Jul-2009	Dark	Slurry	FW	SoCT	0.0334
Jul-2009	Dark	Slurry	FW	SoCT	-0.0961
Jul-2009	Dark	Slurry	FW	SoCT	0.0448
Jul-2009	Dark	Slurry	FW	SoCT	-0.0220
Jul-2009	Dark	Slurry	FW	SoCT	-0.0480
Jul-2009	Dark	Slurry	FW	SoCT	-0.1018
Jul-2009	Dark	Slurry	FW	SoCT	0.0261
Jul-2009	Dark	Slurry	FW	SoSa	0.0456
Jul-2009	Dark	Slurry	FW	SoSa	-0.1433
Jul-2009	Dark	Slurry	FW	SoSa	-0.1913
Jul-2009	Dark	Slurry	FW	SoSa	-0.1221
Jul-2009	Dark	Slurry	FW	SoSa	-0.0472
Jul-2009	Dark	Slurry	FW	SoSa	-0.1181
Jul-2009	Dark	Slurry	FW	SoSa	-0.1303
Jul-2009	Dark	Slurry	FW	SoSa	-0.1181
Jul-2009	Dark	Slurry	FW	SoSa	0.0570
Jul-2009	Dark	Slurry	FW	SoSo	0.0399
Jul-2009	Dark	Slurry	FW	SoSo	-0.1441
Jul-2009	Dark	Slurry	FW	SoSo	-0.0505
Jul-2009	Dark	Slurry	FW	SoSo	-0.1132
Jul-2009	Dark	Slurry	FW	SoSo	-0.0847
Jul-2009	Dark	Slurry	FW	SoSo	-0.1458
Jul-2009	Dark	Slurry	FW	SoSo	-0.1116
Jul-2009	Dark	Slurry	FW	SoSo	-0.1124
Jul-2009	Dark	Slurry	FW	SoSo	0.0326
Jul-2009	Dark	Slurry	OSPW	CT	-0.1213
Jul-2009	Dark	Slurry	OSPW	CT	-0.0822
Jul-2009	Dark	Slurry	OSPW	CT	-0.0375
Jul-2009	Dark	Slurry	OSPW	CT	0.0391
Jul-2009	Dark	Slurry	OSPW	CT	-0.0432
Jul-2009	Dark	Slurry	OSPW	CT	0.0057
Jul-2009	Dark	Slurry	OSPW	CT	-0.0472
Jul-2009	Dark	Slurry	OSPW	CT	-0.2540
Jul-2009	Dark	Slurry	OSPW	CT	-0.1669
Jul-2009	Dark	Slurry	OSPW	SoCT	-0.1620
Jul-2009	Dark	Slurry	OSPW	SoCT	-0.0521
Jul-2009	Dark	Slurry	OSPW	SoCT	-0.0440

Jul-2009	Dark	Slurry	OSPW	SoCT	-0.0041
Jul-2009	Dark	Slurry	OSPW	SoCT	-0.1523
Jul-2009	Dark	Slurry	OSPW	SoCT	-0.0334
Jul-2009	Dark	Slurry	OSPW	SoCT	-0.0049
Jul-2009	Dark	Slurry	OSPW	SoCT	-0.2492
Jul-2009	Dark	Slurry	OSPW	SoCT	-0.1376
Jul-2009	Dark	Slurry	OSPW	SoSa	0.0073
Jul-2009	Dark	Slurry	OSPW	SoSa	-0.0440
Jul-2009	Dark	Slurry	OSPW	SoSa	-0.0383
Jul-2009	Dark	Slurry	OSPW	SoSa	-0.0684
Jul-2009	Dark	Slurry	OSPW	SoSa	-0.0822
Jul-2009	Dark	Slurry	OSPW	SoSa	-0.1189
Jul-2009	Dark	Slurry	OSPW	SoSa	-0.1132
Jul-2009	Dark	Slurry	OSPW	SoSa	-0.2093
Jul-2009	Dark	Slurry	OSPW	SoSa	-0.0928
Jul-2009	Dark	Slurry	OSPW	SoSo	0.0717
Jul-2009	Dark	Slurry	OSPW	SoSo	-0.0505
Jul-2009	Dark	Slurry	OSPW	SoSo	-0.0366
Jul-2009	Dark	Slurry	OSPW	SoSo	0.0073
Jul-2009	Dark	Slurry	OSPW	SoSo	-0.0708
Jul-2009	Dark	Slurry	OSPW	SoSo	-0.0586
Jul-2009	Dark	Slurry	OSPW	SoSo	-0.0717
Jul-2009	Dark	Slurry	OSPW	SoSo	-0.2084
Jul-2009	Dark	Slurry	OSPW	SoSo	-0.1555
Jul-2009	Light	Control	FW	CT	0.0563
Jul-2009	Light	Control	FW	CT	0.2017
Jul-2009	Light	Control	FW	CT	0.1263
Jul-2009	Light	Control	FW	SoCT	0.0945
Jul-2009	Light	Control	FW	SoCT	0.2462
Jul-2009	Light	Control	FW	SoCT	0.0763
Jul-2009	Light	Control	FW	SoSa	0.0727
Jul-2009	Light	Control	FW	SoSa	0.1744
Jul-2009	Light	Control	FW	SoSa	0.1299
Jul-2009	Light	Control	FW	SoSo	-0.0045
Jul-2009	Light	Control	FW	SoSo	0.1535
Jul-2009	Light	Control	FW	SoSo	0.0536
Jul-2009	Light	Control	OSPW	CT	0.0754
Jul-2009	Light	Control	OSPW	CT	0.0645
Jul-2009	Light	Control	OSPW	CT	0.2053
Jul-2009	Light	Control	OSPW	SoCT	0.0236
Jul-2009	Light	Control	OSPW	SoCT	0.0672
Jul-2009	Light	Control	OSPW	SoCT	0.4152

Jul-2009	Light	Control	OSPW	SoSa	0.0109
Jul-2009	Light	Control	OSPW	SoSa	0.1008
Jul-2009	Light	Control	OSPW	SoSa	0.1563
Jul-2009	Light	Control	OSPW	SoSo	0.0000
Jul-2009	Light	Control	OSPW	SoSo	0.0236
Jul-2009	Light	Control	OSPW	SoSo	0.1908
Jul-2009	Light	Mat	FW	CT	0.1190
Jul-2009	Light	Mat	FW	CT	0.0827
Jul-2009	Light	Mat	FW	CT	0.0209
Jul-2009	Light	Mat	FW	SoCT	0.1290
Jul-2009	Light	Mat	FW	SoCT	0.0781
Jul-2009	Light	Mat	FW	SoCT	-0.0091
Jul-2009	Light	Mat	FW	SoSa	0.1308
Jul-2009	Light	Mat	FW	SoSa	0.1672
Jul-2009	Light	Mat	FW	SoSa	-0.0282
Jul-2009	Light	Mat	FW	SoSo	0.0927
Jul-2009	Light	Mat	FW	SoSo	0.0827
Jul-2009	Light	Mat	FW	SoSo	-0.0191
Jul-2009	Light	Mat	OSPW	CT	0.3053
Jul-2009	Light	Mat	OSPW	CT	0.1435
Jul-2009	Light	Mat	OSPW	CT	0.2044
Jul-2009	Light	Mat	OSPW	SoCT	0.1281
Jul-2009	Light	Mat	OSPW	SoCT	0.2026
Jul-2009	Light	Mat	OSPW	SoCT	0.0972
Jul-2009	Light	Mat	OSPW	SoSa	0.1254
Jul-2009	Light	Mat	OSPW	SoSa	0.1872
Jul-2009	Light	Mat	OSPW	SoSa	0.3371
Jul-2009	Light	Mat	OSPW	SoSo	0.2635
Jul-2009	Light	Mat	OSPW	SoSo	0.0927
Jul-2009	Light	Mat	OSPW	SoSo	0.2262
Jul-2009	Light	Slurry	FW	CT	0.1099
Jul-2009	Light	Slurry	FW	CT	0.0382
Jul-2009	Light	Slurry	FW	CT	0.0491
Jul-2009	Light	Slurry	FW	CT	-0.0445
Jul-2009	Light	Slurry	FW	CT	-0.0445
Jul-2009	Light	Slurry	FW	CT	-0.0754
Jul-2009	Light	Slurry	FW	CT	0.0045
Jul-2009	Light	Slurry	FW	CT	0.0872
Jul-2009	Light	Slurry	FW	CT	0.3071
Jul-2009	Light	Slurry	FW	SoCT	0.1063
Jul-2009	Light	Slurry	FW	SoCT	0.0136
Jul-2009	Light	Slurry	FW	SoCT	-0.1499

Jul-2009	Light	Slurry	FW	SoCT	0.0700
Jul-2009	Light	Slurry	FW	SoCT	-0.0999
Jul-2009	Light	Slurry	FW	SoCT	0.0209
Jul-2009	Light	Slurry	FW	SoCT	0.0091
Jul-2009	Light	Slurry	FW	SoCT	0.0981
Jul-2009	Light	Slurry	FW	SoCT	0.3262
Jul-2009	Light	Slurry	FW	SoSa	0.0636
Jul-2009	Light	Slurry	FW	SoSa	0.0291
Jul-2009	Light	Slurry	FW	SoSa	-0.1490
Jul-2009	Light	Slurry	FW	SoSa	0.0600
Jul-2009	Light	Slurry	FW	SoSa	-0.0536
Jul-2009	Light	Slurry	FW	SoSa	-0.0618
Jul-2009	Light	Slurry	FW	SoSa	-0.0164
Jul-2009	Light	Slurry	FW	SoSa	0.1181
Jul-2009	Light	Slurry	FW	SoSa	0.2771
Jul-2009	Light	Slurry	FW	SoSo	0.0418
Jul-2009	Light	Slurry	FW	SoSo	0.0173
Jul-2009	Light	Slurry	FW	SoSo	0.0045
Jul-2009	Light	Slurry	FW	SoSo	0.0118
Jul-2009	Light	Slurry	FW	SoSo	-0.0209
Jul-2009	Light	Slurry	FW	SoSo	-0.1381
Jul-2009	Light	Slurry	FW	SoSo	0.0627
Jul-2009	Light	Slurry	FW	SoSo	0.0854
Jul-2009	Light	Slurry	FW	SoSo	0.2953
Jul-2009	Light	Slurry	OSPW	CT	1.6081
Jul-2009	Light	Slurry	OSPW	CT	-0.0354
Jul-2009	Light	Slurry	OSPW	CT	-0.0400
Jul-2009	Light	Slurry	OSPW	CT	0.2071
Jul-2009	Light	Slurry	OSPW	CT	0.2671
Jul-2009	Light	Slurry	OSPW	CT	0.0354
Jul-2009	Light	Slurry	OSPW	CT	0.1554
Jul-2009	Light	Slurry	OSPW	CT	0.0018
Jul-2009	Light	Slurry	OSPW	CT	0.0091
Jul-2009	Light	Slurry	OSPW	SoCT	0.0690
Jul-2009	Light	Slurry	OSPW	SoCT	0.0209
Jul-2009	Light	Slurry	OSPW	SoCT	-0.0273
Jul-2009	Light	Slurry	OSPW	SoCT	0.2453
Jul-2009	Light	Slurry	OSPW	SoCT	0.2135
Jul-2009	Light	Slurry	OSPW	SoCT	-0.0282
Jul-2009	Light	Slurry	OSPW	SoCT	0.1154
Jul-2009	Light	Slurry	OSPW	SoCT	-0.0300
Jul-2009	Light	Slurry	OSPW	SoCT	-0.0527

Jul-2009	Light	Slurry	OSPW	SoSa	-0.0100
Jul-2009	Light	Slurry	OSPW	SoSa	0.0391
Jul-2009	Light	Slurry	OSPW	SoSa	0.0927
Jul-2009	Light	Slurry	OSPW	SoSa	0.2653
Jul-2009	Light	Slurry	OSPW	SoSa	0.0545
Jul-2009	Light	Slurry	OSPW	SoSa	0.0472
Jul-2009	Light	Slurry	OSPW	SoSa	0.1526
Jul-2009	Light	Slurry	OSPW	SoSa	-0.0282
Jul-2009	Light	Slurry	OSPW	SoSa	0.0100
Jul-2009	Light	Slurry	OSPW	SoSo	0.0681
Jul-2009	Light	Slurry	OSPW	SoSo	0.0064
Jul-2009	Light	Slurry	OSPW	SoSo	-0.0064
Jul-2009	Light	Slurry	OSPW	SoSo	0.2190
Jul-2009	Light	Slurry	OSPW	SoSo	0.0763
Jul-2009	Light	Slurry	OSPW	SoSo	0.0182
Jul-2009	Light	Slurry	OSPW	SoSo	0.1208
Jul-2009	Light	Slurry	OSPW	SoSo	-0.0027
Jul-2009	Light	Slurry	OSPW	SoSo	-0.0082
Aug-2010	Dark	Control	FW	CT	-0.0098
Aug-2010	Dark	Control	FW	CT	-0.0252
Aug-2010	Dark	Control	FW	CT	-0.0358
Aug-2010	Dark	Control	FW	SoCT	-0.0668
Aug-2010	Dark	Control	FW	SoCT	-0.0578
Aug-2010	Dark	Control	FW	SoCT	-0.0497
Aug-2010	Dark	Control	FW	SoSa	-0.0684
Aug-2010	Dark	Control	FW	SoSa	-0.0578
Aug-2010	Dark	Control	FW	SoSa	-0.0114
Aug-2010	Dark	Control	FW	SoSo	-0.0700
Aug-2010	Dark	Control	FW	SoSo	-0.0285
Aug-2010	Dark	Control	FW	SoSo	-0.0814
Aug-2010	Dark	Control	OSPW	CT	-0.1506
Aug-2010	Dark	Control	OSPW	CT	0.0106
Aug-2010	Dark	Control	OSPW	CT	-0.0513
Aug-2010	Dark	Control	OSPW	SoCT	-0.1620
Aug-2010	Dark	Control	OSPW	SoCT	-0.0024
Aug-2010	Dark	Control	OSPW	SoCT	-0.0839
Aug-2010	Dark	Control	OSPW	SoSa	-0.1970
Aug-2010	Dark	Control	OSPW	SoSa	0.0065
Aug-2010	Dark	Control	OSPW	SoSa	-0.0570
Aug-2010	Dark	Control	OSPW	SoSo	-0.1694
Aug-2010	Dark	Control	OSPW	SoSo	-0.0065
Aug-2010	Dark	Control	OSPW	SoSo	-0.0529

Aug-2010	Dark	Mat	FW	CT	0.0537
Aug-2010	Dark	Mat	FW	CT	-0.0122
Aug-2010	Dark	Mat	FW	CT	-0.0350
Aug-2010	Dark	Mat	FW	SoCT	-0.1010
Aug-2010	Dark	Mat	FW	SoCT	-0.0138
Aug-2010	Dark	Mat	FW	SoCT	-0.0822
Aug-2010	Dark	Mat	FW	SoSa	0.0822
Aug-2010	Dark	Mat	FW	SoSa	-0.0163
Aug-2010	Dark	Mat	FW	SoSa	-0.0839
Aug-2010	Dark	Mat	FW	SoSo	-0.0171
Aug-2010	Dark	Mat	FW	SoSo	-0.0252
Aug-2010	Dark	Mat	FW	SoSo	-0.0717
Aug-2010	Dark	Mat	OSPW	CT	-0.0187
Aug-2010	Dark	Mat	OSPW	CT	0.0578
Aug-2010	Dark	Mat	OSPW	CT	-0.2361
Aug-2010	Dark	Mat	OSPW	SoCT	-0.0440
Aug-2010	Dark	Mat	OSPW	SoCT	0.0049
Aug-2010	Dark	Mat	OSPW	SoCT	-0.2361
Aug-2010	Dark	Mat	OSPW	SoSa	-0.0277
Aug-2010	Dark	Mat	OSPW	SoSa	-0.0073
Aug-2010	Dark	Mat	OSPW	SoSa	-0.2988

Aug-2010	Dark	Mat	OSPW	SoSo	-0.0195
Aug-2010	Dark	Mat	OSPW	SoSo	0.1197
Aug-2010	Dark	Mat	OSPW	SoSo	-0.1783
Aug-2010	Dark	Slurry	FW	CT	0.0350
Aug-2010	Dark	Slurry	FW	CT	-0.0277
Aug-2010	Dark	Slurry	FW	CT	-0.0993
Aug-2010	Dark	Slurry	FW	CT	-0.0293
Aug-2010	Dark	Slurry	FW	CT	0.0212
Aug-2010	Dark	Slurry	FW	CT	-0.0806
Aug-2010	Dark	Slurry	FW	CT	-0.0024
Aug-2010	Dark	Slurry	FW	CT	-0.0570
Aug-2010	Dark	Slurry	FW	CT	-0.1026
Aug-2010	Dark	Slurry	FW	SoCT	-0.0293
Aug-2010	Dark	Slurry	FW	SoCT	-0.0570
Aug-2010	Dark	Slurry	FW	SoCT	-0.0904
Aug-2010	Dark	Slurry	FW	SoCT	-0.0578
Aug-2010	Dark	Slurry	FW	SoCT	0.0098
Aug-2010	Dark	Slurry	FW	SoCT	-0.1059
Aug-2010	Dark	Slurry	FW	SoCT	0.0049
Aug-2010	Dark	Slurry	FW	SoCT	-0.1800
Aug-2010	Dark	Slurry	FW	SoCT	-0.0920

Aug-2010	Dark	Slurry	FW	SoSa	-0.0130
Aug-2010	Dark	Slurry	FW	SoSa	-0.1124
Aug-2010	Dark	Slurry	FW	SoSa	-0.0676
Aug-2010	Dark	Slurry	FW	SoSa	-0.0546
Aug-2010	Dark	Slurry	FW	SoSa	0.0033
Aug-2010	Dark	Slurry	FW	SoSa	-0.0204
Aug-2010	Dark	Slurry	FW	SoSa	-0.0171
Aug-2010	Dark	Slurry	FW	SoSa	-0.1913
Aug-2010	Dark	Slurry	FW	SoSa	-0.1669
Aug-2010	Dark	Slurry	FW	SoSo	-0.0440
Aug-2010	Dark	Slurry	FW	SoSo	-0.1067
Aug-2010	Dark	Slurry	FW	SoSo	-0.0977
Aug-2010	Dark	Slurry	FW	SoSo	-0.0432
Aug-2010	Dark	Slurry	FW	SoSo	-0.0252
Aug-2010	Dark	Slurry	FW	SoSo	-0.0879
Aug-2010	Dark	Slurry	FW	SoSo	-0.0024
Aug-2010	Dark	Slurry	FW	SoSo	-0.1783
Aug-2010	Dark	Slurry	FW	SoSo	-0.1002
Aug-2010	Dark	Slurry	OSPW	CT	-0.1840
Aug-2010	Dark	Slurry	OSPW	CT	-0.1213
Aug-2010	Dark	Slurry	OSPW	CT	-0.0741

Aug-2010	Dark	Slurry	OSPW	CT	-0.0627
Aug-2010	Dark	Slurry	OSPW	CT	-0.1050
Aug-2010	Dark	Slurry	OSPW	CT	0.0122
Aug-2010	Dark	Slurry	OSPW	CT	-0.0945
Aug-2010	Dark	Slurry	OSPW	CT	-0.0863
Aug-2010	Dark	Slurry	OSPW	CT	-0.0651
Aug-2010	Dark	Slurry	OSPW	SoCT	-0.2044
Aug-2010	Dark	Slurry	OSPW	SoCT	-0.1449
Aug-2010	Dark	Slurry	OSPW	SoCT	-0.1018
Aug-2010	Dark	Slurry	OSPW	SoCT	-0.0448
Aug-2010	Dark	Slurry	OSPW	SoCT	-0.1034
Aug-2010	Dark	Slurry	OSPW	SoCT	0.0252
Aug-2010	Dark	Slurry	OSPW	SoCT	-0.1181
Aug-2010	Dark	Slurry	OSPW	SoCT	0.1002
Aug-2010	Dark	Slurry	OSPW	SoCT	-0.0741
Aug-2010	Dark	Slurry	OSPW	SoSa	-0.0334
Aug-2010	Dark	Slurry	OSPW	SoSa	-0.1531
Aug-2010	Dark	Slurry	OSPW	SoSa	-0.1156
Aug-2010	Dark	Slurry	OSPW	SoSa	-0.1181
Aug-2010	Dark	Slurry	OSPW	SoSa	-0.1303
Aug-2010	Dark	Slurry	OSPW	SoSa	0.0326

Aug-2010	Dark	Slurry	OSPW	SoSa	-0.1447
Aug-2010	Dark	Slurry	OSPW	SoSa	-0.1018
Aug-2010	Dark	Slurry	OSPW	SoSa	-0.0204
Aug-2010	Dark	Slurry	OSPW	SoSo	-0.0953
Aug-2010	Dark	Slurry	OSPW	SoSo	-0.0888
Aug-2010	Dark	Slurry	OSPW	SoSo	-0.0977
Aug-2010	Dark	Slurry	OSPW	SoSo	-0.0432
Aug-2010	Dark	Slurry	OSPW	SoSo	-0.1099
Aug-2010	Dark	Slurry	OSPW	SoSo	0.0057
Aug-2010	Dark	Slurry	OSPW	SoSo	-0.1433
Aug-2010	Dark	Slurry	OSPW	SoSo	-0.0936
Aug-2010	Dark	Slurry	OSPW	SoSo	-0.0383
Aug-2010	Light	Control	FW	CT	0.0064
Aug-2010	Light	Control	FW	CT	0.0127
Aug-2010	Light	Control	FW	CT	0.0273
Aug-2010	Light	Control	FW	SoCT	0.0636
Aug-2010	Light	Control	FW	SoCT	0.0227
Aug-2010	Light	Control	FW	SoCT	0.0482
Aug-2010	Light	Control	FW	SoSa	0.0045
Aug-2010	Light	Control	FW	SoSa	-0.0245
Aug-2010	Light	Control	FW	SoSa	0.0318

Aug-2010	Light	Control	FW	SoSo	-0.0254
Aug-2010	Light	Control	FW	SoSo	0.0354
Aug-2010	Light	Control	FW	SoSo	0.0000
Aug-2010	Light	Control	OSPW	CT	-0.0491
Aug-2010	Light	Control	OSPW	CT	0.0572
Aug-2010	Light	Control	OSPW	CT	0.0618
Aug-2010	Light	Control	OSPW	SoCT	-0.0472
Aug-2010	Light	Control	OSPW	SoCT	0.0972
Aug-2010	Light	Control	OSPW	SoCT	0.0836
Aug-2010	Light	Control	OSPW	SoSa	-0.0518
Aug-2010	Light	Control	OSPW	SoSa	0.0500
Aug-2010	Light	Control	OSPW	SoSa	0.0191
Aug-2010	Light	Control	OSPW	SoSo	-0.0391
Aug-2010	Light	Control	OSPW	SoSo	0.0273
Aug-2010	Light	Control	OSPW	SoSo	0.0700
Aug-2010	Light	Mat	FW	CT	-0.0136
Aug-2010	Light	Mat	FW	CT	-0.0045
Aug-2010	Light	Mat	FW	CT	0.0772
Aug-2010	Light	Mat	FW	SoCT	0.0372
Aug-2010	Light	Mat	FW	SoCT	-0.0354
Aug-2010	Light	Mat	FW	SoCT	0.0636

Aug-2010	Light	Mat	FW	SoSa	0.0827
Aug-2010	Light	Mat	FW	SoSa	-0.0036
Aug-2010	Light	Mat	FW	SoSa	-0.0008
Aug-2010	Light	Mat	FW	SoSo	0.1599
Aug-2010	Light	Mat	FW	SoSo	-0.0463
Aug-2010	Light	Mat	FW	SoSo	0.0463
Aug-2010	Light	Mat	OSPW	CT	0.0454
Aug-2010	Light	Mat	OSPW	CT	-0.0581
Aug-2010	Light	Mat	OSPW	CT	0.0990
Aug-2010	Light	Mat	OSPW	SoCT	0.0136
Aug-2010	Light	Mat	OSPW	SoCT	-0.1354
Aug-2010	Light	Mat	OSPW	SoCT	0.0718
Aug-2010	Light	Mat	OSPW	SoSa	0.0663
Aug-2010	Light	Mat	OSPW	SoSa	0.0618
Aug-2010	Light	Mat	OSPW	SoSa	0.1763
Aug-2010	Light	Mat	OSPW	SoSo	0.0100
Aug-2010	Light	Mat	OSPW	SoSo	-0.1681
Aug-2010	Light	Mat	OSPW	SoSo	-0.0118
Aug-2010	Light	Slurry	FW	CT	0.0300
Aug-2010	Light	Slurry	FW	CT	0.0954
Aug-2010	Light	Slurry	FW	CT	-0.0118

Aug-2010	Light	Slurry	FW	CT	0.0227
Aug-2010	Light	Slurry	FW	CT	0.0681
Aug-2010	Light	Slurry	FW	CT	-0.0245
Aug-2010	Light	Slurry	FW	CT	0.0009
Aug-2010	Light	Slurry	FW	CT	0.0173
Aug-2010	Light	Slurry	FW	CT	-0.0482
Aug-2010	Light	Slurry	FW	SoCT	0.0718
Aug-2010	Light	Slurry	FW	SoCT	0.0436
Aug-2010	Light	Slurry	FW	SoCT	-0.0609
Aug-2010	Light	Slurry	FW	SoCT	-0.0372
Aug-2010	Light	Slurry	FW	SoCT	-0.0654
Aug-2010	Light	Slurry	FW	SoCT	-0.0318
Aug-2010	Light	Slurry	FW	SoCT	-0.0064
Aug-2010	Light	Slurry	FW	SoCT	-0.0318
Aug-2010	Light	Slurry	FW	SoCT	-0.1326
Aug-2010	Light	Slurry	FW	SoSa	0.1036
Aug-2010	Light	Slurry	FW	SoSa	-0.0273
Aug-2010	Light	Slurry	FW	SoSa	-0.0781
Aug-2010	Light	Slurry	FW	SoSa	-0.0500
Aug-2010	Light	Slurry	FW	SoSa	-0.0627
Aug-2010	Light	Slurry	FW	SoSa	-0.0382

Aug-2010	Light	Slurry	FW	SoSa	-0.0018
Aug-2010	Light	Slurry	FW	SoSa	-0.0064
Aug-2010	Light	Slurry	FW	SoSa	-0.0845
Aug-2010	Light	Slurry	FW	SoSo	0.0945
Aug-2010	Light	Slurry	FW	SoSo	-0.0391
Aug-2010	Light	Slurry	FW	SoSo	-0.0536
Aug-2010	Light	Slurry	FW	SoSo	-0.0182
Aug-2010	Light	Slurry	FW	SoSo	-0.0827
Aug-2010	Light	Slurry	FW	SoSo	-0.0300
Aug-2010	Light	Slurry	FW	SoSo	0.0064
Aug-2010	Light	Slurry	FW	SoSo	-0.0173
Aug-2010	Light	Slurry	FW	SoSo	-0.1563
Aug-2010	Light	Slurry	OSPW	CT	0.0781
Aug-2010	Light	Slurry	OSPW	CT	-0.0245
Aug-2010	Light	Slurry	OSPW	CT	0.1154
Aug-2010	Light	Slurry	OSPW	CT	0.1772
Aug-2010	Light	Slurry	OSPW	CT	0.0709
Aug-2010	Light	Slurry	OSPW	CT	-0.0003
Aug-2010	Light	Slurry	OSPW	CT	0.1226
Aug-2010	Light	Slurry	OSPW	CT	0.1726
Aug-2010	Light	Slurry	OSPW	CT	0.0927

Aug-2010	Light	Slurry	OSPW	SoCT	-0.2244
Aug-2010	Light	Slurry	OSPW	SoCT	-0.0899
Aug-2010	Light	Slurry	OSPW	SoCT	0.0881
Aug-2010	Light	Slurry	OSPW	SoCT	0.0191
Aug-2010	Light	Slurry	OSPW	SoCT	0.0300
Aug-2010	Light	Slurry	OSPW	SoCT	-0.0027
Aug-2010	Light	Slurry	OSPW	SoCT	-0.1226
Aug-2010	Light	Slurry	OSPW	SoCT	0.1481
Aug-2010	Light	Slurry	OSPW	SoCT	0.1099
Aug-2010	Light	Slurry	OSPW	SoSa	-0.0809
Aug-2010	Light	Slurry	OSPW	SoSa	0.0827
Aug-2010	Light	Slurry	OSPW	SoSa	0.1372
Aug-2010	Light	Slurry	OSPW	SoSa	0.0536
Aug-2010	Light	Slurry	OSPW	SoSa	0.0100
Aug-2010	Light	Slurry	OSPW	SoSa	0.0127
Aug-2010	Light	Slurry	OSPW	SoSa	-0.1163
Aug-2010	Light	Slurry	OSPW	SoSa	0.0572
Aug-2010	Light	Slurry	OSPW	SoSa	0.0600
Aug-2010	Light	Slurry	OSPW	SoSo	-0.1272
Aug-2010	Light	Slurry	OSPW	SoSo	0.0645
Aug-2010	Light	Slurry	OSPW	SoSo	0.0954

Aug-2010	Light	Slurry	OSPW	SoSo	0.0109
Aug-2010	Light	Slurry	OSPW	SoSo	-0.0164
Aug-2010	Light	Slurry	OSPW	SoSo	-0.0236
Aug-2010	Light	Slurry	OSPW	SoSo	-0.0454
Aug-2010	Light	Slurry	OSPW	SoSo	0.1236
Aug-2010	Light	Slurry	OSPW	SoSo	0.0536

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