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EVALUATING THE BIOGEOCHEMICAL FUNCTIONING OF A CONSTRUCTED FEN ON THE POST-MINING LANDSCAPE OF ATHABASCA OIL SANDS REGION, FORT MCMURRAY, ALBERTA, CANADA

Felix C. Nwaishi
Wilfrid Laurier University, nwai5240@mylaurier.ca

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EVALUATING THE BIOGEOCHEMICAL FUNCTIONING OF A CONSTRUCTED FEN ON THE POST-MINING LANDSCAPE OF ATHABASCA OIL SANDS REGION, FORT MCMURRAY, ALBERTA, CANADA

BY

Felix C. Nwaishi

B. Agric. Tech, Federal University of Technology Owerri, 2007

M.Sc., Manchester Metropolitan University, 2010

THESIS

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Abstract

Peatlands have a unique biogeochemical function, characterised by an imbalance between the rates of biomass accumulation and decomposition. These characteristics facilitate the ability of peatlands to support the sequestration of nutrients and carbon. In disturbed peatlands, these functions are compromised. Thus, reclamation targets amongst other key functions, the recovery of biogeochemical functioning. These functions could serve as a measure of recovery to conditions that are present in natural analogues. This thesis examines the recovery of microbially-mediated nutrient transformation processes in a fen peatland that was constructed on a post-mining landscape in the Athabasca oil sands region, Fort McMurray, Alberta. The major themes of this thesis examined (1) the concept of developing a functional-based approach for evaluating the functioning and trajectory of the constructed fen, (2) the impacts of donor-peat management practices on the resulting peat quality and the potential implications to the ecohydrological functioning of the constructed fen, (3) the evolution of above and below-ground nutrient transformation processes among different revegetation strategies in the constructed fen, and (4) the effect of revegetation and edaphic variables on the greenhouse gas (GHG) dynamics of the constructed fen.

The concept of developing a functional-based approach for evaluating the functioning of a constructed fen was examined by synthesizing the dominant processes of peatland development. The interactions and feedback processes that underlie various peatland ecosystem functions and their quantifiable variables were identified through this synthesis. This also highlighted the sensitivity of microbially-mediated biogeochemical processes to a range of variability in other ecosystem processes. As an alternative to the bio-indicator approach, microbially-mediated biogeochemical processes present potential functional indicators of ecosystem function.

The impact of donor-peat management practices on the ecohydrological functioning of the constructed fen was studied using cores extracted along transects in the donor fen before peat transfer, and after placement in the constructed fen. Relative to the properties of a reference fen, the donor-peat had a higher surface bulk density, and higher concentration of extractable nutrients. Transfer of peat to the constructed fen increased the near-surface bulk density, and decreased organic matter content and concentration of extractable nutrients.

Evolution of above and below-ground nutrient transformation processes were assessed among different revegetation strategies, over the first two growing seasons post-construction. Revegetation facilitated both above-ground productivity and the cycling of below-ground nutrients. Supply of labile substrates in the re-vegetated plots increased microbial potential activity, which was reflected in higher rates of respiration, nutrient acquisition and productivity. Nutrient dynamics within the constructed fen suggest that phosphorus limitation could hamper the establishment of a diverse plant community, whereas the build-up of microbial biomass appears to be NO_3^- limited. Ammonification, nitrogen mineralization and phosphorus availability were identified as potential functional indicators of the fen's recovery.

Finally, the effects of revegetation strategies and environmental characteristics of the constructed fen on GHG dynamics were examined. Relative to a natural fen, significantly lower ($p < 0.001$) fluxes of methane (CH_4) were observed in the constructed fen. This correlated with higher bulk density, lower organic matter content, and higher pH and SO_4^{2-} concentration. Revegetation did not stimulate CH_4 production, but increased carbon dioxide (CO_2) uptake and reduced the global warming potential (GWP) contribution of nitrous oxide (N_2O) by $63CO_2$-e m^{-2} yr^{-1} relative to the non-vegetated control. These studies provide a novel insight into the concept of assessment of a constructed fen ecosystem through the evolution of biogeochemical functioning.

Acknowledgements

I wish to acknowledge all those who have contributed to the success of this thesis. First, the inspiration from my creator, the love and support of my wife Cynthia, the motivating gaze of my daughter Kamsi, and the prayers and words of encouragement from my parents and siblings.

My heartfelt gratitude and appreciation goes to my supervisor, Dr. Rich Petrone for giving me the opportunity to study for a PhD under his edifying supervision, and for his unlimited moral, academic and financial support through all the stages of my research.

I would like to thank the members of my research committee: Dr. Jonathan Price for believing in me to work as a part of his innovative research, Dr. Robin Slawson for strengthening the diversity of my thesis committee, and the ever resourceful and accommodating Dr. Roxane Andersen for her support and guidance throughout the production of this thesis manuscript. I would also like to thank Dr. Merrin Macrae and Dr. Maria Strack for assistance with manuscript reviews and for allowing me to use their Labs for some of the biogeochemistry assays.

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For field and laboratory assistance, I wish to thank Adam Green, Scott Brown, George Sutherland, Vito Lam, Vinay Date and Kimberly Murray. Finally, I will remain eternally grateful to all those who encouraged and supported me through this four years journey, especially The Mezieobi's, Dr. Robin Sen and Dr. Ibrahim Rashid.

Declaration: Publications

This thesis is written in a manuscript format. Details of my contribution and that of others to each manuscript that form part of this thesis are indicated. Some minor editorial differences may exist between the published papers and the thesis chapters.

Chapter two is published as:

Nwaishi, F., Petrone, R. M., Price, J. S., & Andersen, R. (2015). Towards developing a functional-based approach for constructed peatlands evaluation in the Alberta Oil Sands Region, Canada. *Wetlands, 35*(2), 211-225.

This paper synthesized the dominant processes of peatland functioning in a continental boreal climate. The idea of this synthesis paper was conceptualized by F. Nwaishi, while the development of the manuscript proceeded with assistance from R. M. Petrone and R. Andersen; who suggested and provided the preliminary diagram that converted the synthesis of successional trajectory into a diagram, which was then enhanced and digitalized by F. Nwaishi (Figure. 2.2). The manuscript was written in its entirety by F. Nwaishi, while the final editing was provided by the co-authors and anonymous reviewers of the manuscript.

Chapter Three is published as:

Nwaishi, F., Petrone, R. M., Price, J. S., Ketcheson, S. J., Slawson, R., & Andersen, R. (2015). Impacts of donor-peat management practices on the functional characteristics of a constructed fen. *Ecological Engineering, 81*, 471-480.

This manuscript evaluated the impacts of donor-peat management practices on the ecohydrological functioning of the constructed fen. The manuscript focused mainly on biogeochemical functioning of the fen, however, a section on the hydrological functioning of the fen, which was added to widen the readership of the manuscript, was completed with contribution from S.J. Ketcheson and J.S. Price. All figures and tables were produced by F. Nwaishi, and final editing of the manuscript was provided by the co-authors and anonymous reviewers of the publication.

Chapter Four is published as:

Nwaishi, F., Petrone, R. M., Macrae, M. S., Price, J. S., Strack, M., Slawson, R., & Andersen, R. (2015). Above and below-ground nutrient cycling: criteria for assessing the biogeochemical functioning of a constructed fen. *Applied Soil Ecology*, doi:10.1016/j.apsoil.2015.10.015

This manuscript was solely prepared by F. Nwaishi; R. Andersen provided training and guidance on statistical analyses using the R software. Field measurements of CO_2 dynamics were collected by M. Strack's summer field assistants (Kimberly Murray and Mendel Perkins). The manuscript was reviewed by the co-authors, and the anonymous reviewers selected by the journal.

Chapter Five is undergoing review in the journal: Biogeochemistry and can be cited as:

Nwaishi, F., Petrone, R. M., Macrae, M. S., Price, J. S., Strack, M., Slawson, R., & Andersen, R. (*In review*). Preliminary assessment of greenhouse gas emissions from a fen peatland constructed on a post-oil sands mining landscape.

The entirety of this manuscript was also prepared by F. Nwaishi with final edits from the co-authors. R. Andersen also provided support during statistical analyses, by suggesting ways of running an error-free code for the Variation Partitioning analyses (Figure 5.3).

Statement of Originality

I hereby affirm that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Felix C. Nwaishi

September, 2015

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AOSR	Athabasca Oil Sands Region
AWCD	Average Well Colour Development
BD	Bulk Density
C/N	Carbon to Nitrogen Ratio
CLPP	Community Level Physiological Profile
CTRL	Control Treatment
EC	Electrical Conductivity
$ExNH_4$	Extractable Ammonium
$ExNO_3$	Extractable Nitrate
GEP	Gross Ecosystem Productivity
GHG	Greenhouse Gases
GWP	Global Warming Potential
K_V	Vertical hydraulic conductivity
K_H	Horizontal hydraulic conductivity
K_{SAT}	Saturated hydraulic conductivity
MC	Moisture Content
MLT	Moss Layer Transfer Treatment
MMO	Methane Monooxygenase
NAs	Naphthenic Acids
NEE	Net Ecosystem Exchange
NH_4SR	Ammonium Supply Rate

NO_3SR	Nitrate Supply Rate
NPP	Net Primary Productivity
OM	Organic Matter Content
OSPW	Oil Sands Process-affected Water
PAR	Photosynthetically Active Radiation
PFT	Plant Functional Type
PMIN	Phosphorous Mineralization
PMM	Peat-Mineral Mix
PRC	Principal Response Curve
PRS	Plant Root Simulator
PSR	Phosphorous Supply Rate
R	Respiration
SDL	Seedling Treatment
SMLT	Seedling and Moss Layer Transfer Treatment Combination
SRP	Soluble Reactive Phosphorous
TIN	Total Inorganic Nitrogen
TINSR	Total Inorganic Nitrogen Supply Rate
TLC	Tailing Line Corridor

Chapter One

General Introduction

1.1 Background and rationale of research

Peatlands are areas of a landscape where organic soils (peat) have accumulated to a minimum depth of 30 – 40 cm, and maintain a near-surface water table for most of the year (Clymo et al. 1998; Tarnocai 1998; Turunen et al. 2002). The global distribution of peatlands accounts for an estimated land area of 4.16 × 10^6 km^2, with most of the area (about 80 percent) situated in the northern hemisphere (Parish et al. 2008). In Canada, peatlands are the dominant wetland type, especially in the boreal region of northeastern Alberta, where peatlands comprise about 90% of the total wetlands area (Vitt et al. 1996). Fens, which are the minerotrophic variant of peatlands, are the most common land cover type in this region. Their contributions to regional and global biogeochemical cycles of carbon (C) and nutrients are well documented in the scientific literature (c.f. Gorham 1995; Blodau 2002; Lai 2009; Limpens et al. 2009). They also support vital ecosystem services such as the regulation of the regional water cycle (Ferone & Devito 2004; Devito et al. 2005), and the provision of unique ecological habitat for plants and animals (Chee & Vitt 1989). The significance of these peatland ecosystem services, especially with regards to carbon sequestration and regulation of regional climate, highlights the crucial need for proper management and conservation of the ecosystem. However, large areas of pristine peatlands are under continuous stress from anthropogenic disturbances, resulting in the degradation or complete loss of peatland ecosystems and services (Turetsky & Louis 2006; Connolly & Holden 2013).

For instance, boreal fens in the Athabasca region of Canada are being threatened by industrial developments for oil sands exploration and mining (Rooney et al. 2012). The method used in oil sands extraction is dependent on the depth of bitumen deposits within the area. Shallow deposits are recovered through open-pit mining (surface mining), while deeper deposits are removed *in situ* by steam assisted gravity drainage (Johnson & Miyanishi 2008). Although only 20 percent of bitumen can be accessed using open-pit mining, this method was used in most of the current mining-impacted areas in north-eastern Alberta. As of 2007, about 60 percent of bitumen crude was produced through open-pit mining (Energy Resources Conservation Board, 2008). However, current statistics show that open-pit mining now accounts for 48 percent of Alberta's total crude bitumen production as a result of advancement of *in-situ* recovery techniques (Lightbown 2015).

Open-pit mining has also been implicated for having the most significant and long-term impact on ecosystems as it involves the removal of functional peatland vegetation, and subsequent stripping of peat soil layers to access the bituminous oil sands deposit at depths below 70 meters (Johnson & Miyanishi 2008). Post-mining landscapes are fragmented and devoid of any remnants of the pre-disturbance landscape, except for the narrow strips of buffer zones located along the side of the Athabasca River. Thus, the implementation of ecosystem-scale reclamation is required to recreate a functional ecosystem with the ability to support the recovery of lost peatland functions. However, the physical and chemical constraints present in post-mining landscapes and the challenges associated with recreating the processes that supported peatland ecosystem services (*i.e.* under pristine conditions) present major limitations to the successful implementation of peatland reclamation (Audet et al. 2015). As a result of this challenge, other wetland types such as marshes and end-pit lakes were considered as alternative land-uses for offsetting the lost peatlands (Rooney et al. 2012). The novel work of Price et al. (2010) presented a conceptual model, which suggested that fens can be successfully recreated on post-mining landscapes if peat soils are salvaged before mining and placed in a hydrogeological setting that can sustain the requisite wetness conditions required for peatland vegetation establishment and biogeochemical functioning. This concept now makes it possible to attempt the recreation of peatlands following adoption and implemented by Suncor Energy Canada Inc.

1.2 Fen reclamation on post-mining landscapes and challenges

Construction of the experimental fen watershed commenced in the spring of 2010, within an area known as the tailings line corridor (TLC) on Suncor's Millennium mine lease. The post-mining landscape was reconfigured by filling the pit with composite tailing sand and petroleum coke before capping the fen area with a 2 m layer of peat from a donor fen. In the summer of 2013, peatland vegetation that are native to the region were reintroduced in the experimental fen using the revegetation design described by Borkenhagen & Cooper (2015) and presented in Figure 4.1. One of the major goals of fen reclamation is to recreate a biogeochemically-functional ecosystem that is able to maintain nutrient cycling for optimum productivity of a species-rich fen ecosystem, and also support long-term carbon sequestration functions.

Given that fen reclamation on the post-mining landscape is still at the experiment stage, the prospects of actualizing reclamation goals are associated with uncertainties resulting from knowledge gaps and the environmental constraints present in a constructed fen landscape. An example of a knowledge gap is evident in the lack of a standard framework that can guide the functional evaluation of the constructed fen. The unified analytical framework developed for the evaluation of reclaimed wetlands in the oil sands region (Rooney & Bayley 2011) is limited to structural evaluation, thus, unsuitable for functional evaluation of the constructed fen. Physicochemical properties of the donor-peat are modified by the management practices used in salvaging the peat substrate. The implication of such modification on the resulting biogeochemical functioning of the constructed fen is not known, and hence requires research attention. Effluents of salt and naphthenic acids from the tailing sand overburden used in constructing the fen and surrounding uplands are also expected to modify biogeochemical processes and water chemistry. The resulting water chemistry could affect the establishment of native fen vegetation species, and subsequent productivity of the fen. However, the effect of the interaction between water chemistry, vegetation and substrate quality on overall biogeochemical functioning (*e.g.* GHG dynamics and nutrient cycling) of the constructed fen is still unknown.

In view of these underlying uncertainties, management of the constructed fen might require a shift in paradigm towards emerging ideas in ecological restoration. The concept of novel and hybrid ecosystems have been introduced as an ideal approach for understanding “new ecosystems” (Hobbs et al. 2014; Perring et al. 2015). These concepts emphasize the need for reclamation to target the development of resilience in a recreated ecosystem, rather than trying to recreate exactly what was present in pre-disturbance landscapes (Hobbs et al. 2013). Hence, when setting reclamation targets, the successful recreation of a landscape that is not structurally similar but functionally equivalent to natural analogues can be accepted as desirable outcomes. Applying these concepts to the assessment of the constructed fen will allow a functional evaluation, which looks beyond the implications of the modified environmental characteristics of the constructed fen to focus on the functional potentials.

1.3 Objectives

The overall goal of this research is to advance our knowledge of biogeochemical processes in a constructed fen ecosystem and present a functional-based approach for evaluating the functioning and trajectory of a constructed fen. Specifically, the four main objectives are to:

1) initiate the development of a framework that could guide the evaluation of constructed fen ecosystems from a functional perspective;

2) examine the impacts of donor-peat management practices on the functional characteristics of a constructed fen;

3) evaluate the evolution of above and below-ground nutrient transformation processes among different revegetation strategies in the constructed fen; and

4) assess the effects of vegetation and physicochemical properties on the GHG dynamics of the constructed fen.

1.4 Structure of thesis

This thesis consists of six chapters that are structured to fit the multiple manuscript option. The first chapter presents a general introduction to the four major themes of this thesis, and also outlines the overall goal and specific objectives of the thesis, which ties back to the four major themes. Each of the subsequent four chapters presents a manuscript that addressed one of the four specific objectives of the thesis.

Chapter two is the literature review chapter, which addresses the first objective of this thesis. It provides a synthesis of dominant processes of peatland functional development and introduces the concept of a functional-based approach for evaluation of the constructed fen.

Chapter three addresses the second objective by examining the impacts of donor-peat management practices on the functioning of the constructed fen.

Chapter four addresses the third objective by evaluating above and below-ground nutrient transformation processes among different revegetation strategies in the constructed fen.

Chapter five addresses the last objective of the thesis by assessing the effects of vegetation and physicochemical properties of the constructed on GHG dynamics.

Chapter six presents a summary of the conclusions from the four manuscripts, highlights the major contributions to knowledge and recommends directions for future research towards the improvement of current reclamation practices.

Chapter Two

Towards Developing a Functional-Based Approach for Constructed Peatlands Evaluation in the Alberta Oil Sands Region, Canada

2.1 Introduction

Since the early 17th century, northern peatlands have become increasingly pressured from anthropogenic disturbances, as drainage for agricultural improvement became a common practice, and as the demand for peat as horticultural substrate and fuel grew (Martini et al., 2006). More recently, the discovery of oil sands deposits beneath some boreal forest peatlands in north-western North America have resulted in one of the world's largest industrial exploitation of pristine peatland ecosystems (Rooney et al. 2012). In this region, open-pit mining for oil sands involves the total stripping of peat layers, leaving landscapes with very large pits approximately 100 m in depth (Johnson & Miyanishi 2008). The outcome of this process is a complete loss of peatlands and their associated ecosystem services (ES) such as water storage and cycling, habitat support, and storage of carbon (C) and nutrients.

The environmental regulatory framework for Alberta oil sands development requires the energy industries to return post-mining landscapes to equivalent land capabilities, where "the ability of the land to support various land-uses after conservation and reclamation is similar to the ability that existed prior to industrial development on the land, but the individual land uses will not necessarily be identical to pre-disturbance conditions" (Alberta Environment, 2009). Because of the footprint of oil sands operations (*i.e.* a fragmented landscape without remnants of the pristine structure), this regulatory obligation can only be achieved through reclamation, which will involve the complete re-creation of landforms and ecosystems such as fen peatlands that dominated the pre-disturbance landscape in this region (Chee & Vitt 1989). However, considering the notion that several decades are required for the initiation of the peat accumulation function in a peatland (Clymo 1984), peatland reclamation presents a significant challenge. To begin to address this challenge, attempts are now being made to design fen ecosystems on the post-mining oil sands landscape. For example, Price et al. (2010) presented a pilot fen model, which suggested that the creation of fen peatlands on post-mining landscape may be possible if peat is salvaged before mining and placed in a hydrogeological setting that can sustain the requisite wetness conditions required for the establishment of peatland vegetation. The approach of peat transfer to a recreated site will fast-track the initiation of peat accumulation process in constructed peatlands, but it is not known if the transferred peat will support ecohydrological functions similar to those present in natural analogues in the long run. Hence, there is need to track the recovery of reclaimed oil sands peatlands, especially at the early stages of the pilot project.

Considering that fen reclamation is a new and untested concept in the Alberta oil sands region, there is a dearth of information on the appropriate ecological approach for monitoring the trajectory of reclaimed peatlands. At present, the framework available for the evaluation of reclaimed sites in this region was developed for other wetland forms such as open-water marshes, and is based on the concept of indicator species (Rooney & Bayley 2011). The appropriateness of such a framework for reclaimed peatland evaluation is contentious because relative to other wetland types, peatlands are functionally and structurally unique; they are products of the advanced stages of wetland succession (Bauer et al. 2003), support more vital functions (Vitt et al. 1996), and thus deliver additional ecosystem services that cannot be effectively assessed with the indicator species approach alone. Also, the presence of indicator species (Stapanian, Adams & Gara 2013) does not account for the functional state of a reclaimed ecosystem because an indicator species might be present in a reclaimed site, yet not functionally equivalent to natural analogues as a result of abiotic alterations (Dale & Beyeler 2001). That is, indicator species may be present although ecohydrological conditions (*i.e.* water use efficiency, nutrient cycling) are not suitable for the long-term sustainability of that species in the reclaimed system. Hence, if the framework used in evaluating wetland reclamation is applied to peatlands, such evaluations will be limited to assessing reclamation based on the vegetation community structure and biotic conditions without providing much insight on peatland ecosystem functioning: i.e. the interaction between biotic and abiotic ecosystem processes that supports the continuous flow of energy to sustain ecosystem services in the reclaimed peatland.

It can be argued that the indicator species approach has been used effectively to evaluate the attainment of restoration goals in restored vacuum-milled peatlands of eastern Canada (González et al. 2013), and perhaps peatland restoration concepts should be applicable to reclamation. However, the difference between the concept of peatland reclamation (*i.e*. recreating a peatland ecosystem where it has completely been removed from the landscape) and restoration (*i.e.* returning peatland functions on disturbed peatland remnants) presents the need for developing a rigorous framework that can capture the complexities associated with reclaimed peatlands. For instance, the planting design adopted in the on-going oil sands pilot fen reclamation project involves a combination of vegetation assemblage found in different fen types (saline, fresh water, rich and poor fen) within this region (Daly et al. 2012). The idea is to select for native species that can survive the altered abiotic conditions expected in this constructed ecosystem (Harris, 2007).

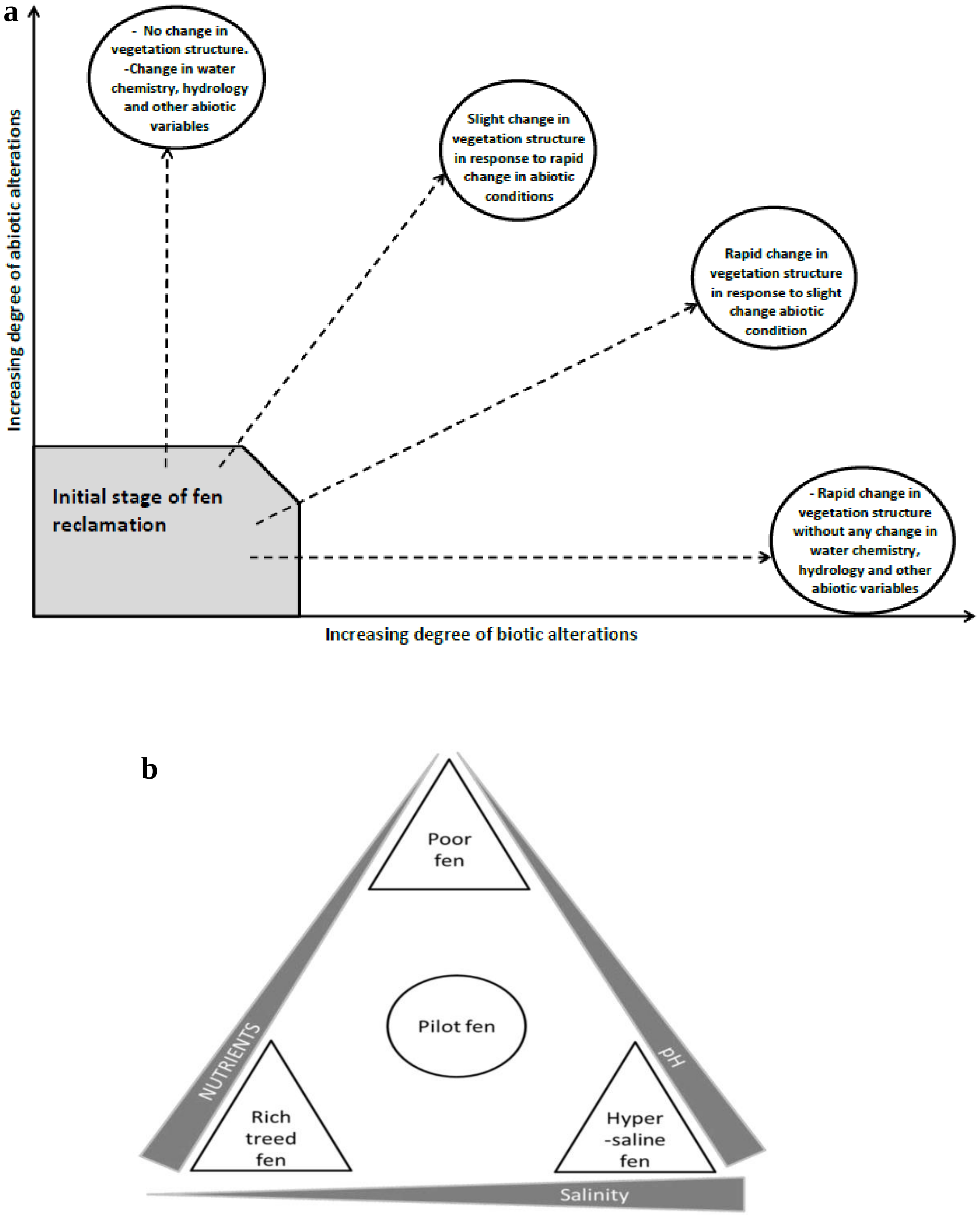


Figure 2.1: Schematic representation of : a) potential reclamation trajectories in response to a range of alterations in biotic and abiotic components of the constructed peatland ecosystem; and b) an example of possible endpoints that might result from the multiple trajectories, specifically in response to various chemical gradients.

This concept could result in multiple successional endpoints (Figure. 1a & b), making the indicator species approach inappropriate in the latter case because, unlike in restored bogs where the successional endpoint is known (*i.e.* the recovery of a typical sphagnum moss carpet), that of reclaimed oil sands fen is relatively unknown due to multiple possibilities (Figure. 1a & b). Interactions between the combined native vegetation species assemblages or invasion by non-native species and altered abiotic conditions (*e.g.* altered hydrology and water quality) in reclaimed sites could lead to the emergence of new ecosystems. This has been observed in recreated landscapes (Lindenmayer et al. 2008), which have been classified into hybrid and novel ecosystems based on the degree of transformation from natural analogues (Hobbs et al. 2006). A hybrid ecosystem is one that is functionally similar but structurally different (*e.g.* combination vegetation species that occur in different natural environments) from natural analogues, while novel ecosystems have functional and structural characteristics that are completely different from natural analogues (Hobbs et al. 2009). Based on the definition of "equivalent land capabilities" in the oil sands reclamation context, the land uses targeted by reclamation can be classified as hybrid ecosystems, which could evolve into novel ecosystems as a result of the altered biotic and abiotic conditions anticipated in fen ecosystems recreated on post-mining oil sands landscapes (Harris, 2007; Daly et al. 2012). The management of ecosystems with a combination of rare species and/or altered abiotic conditions will require adopting a novel approach that focus on understanding their functioning. Hence, there is a need to shift from the traditional indicator species approach to a comprehensive functional approach that can improve our understanding of the functional characteristics of these recreated ecosystems through a pilot study, which will inform future large scale reclamation projects.

Developing a functional-based reclamation evaluation framework requires an understanding of key functional processes in natural analogues, the identification of quantifiable measures of specific ecosystem functions targeted in reclamation, and a comprehensive monitoring program that can capture the evolutionary interactions between biotic and abiotic variables in the reclaimed ecosystem. This paper proposes a concept that will guide the evaluation of oil sands-reclaimed fen ecosystem from a functional perspective, which is more suitable for assessing what is essentially primary succession that has begun at some “non-initial” state, and could lead to novel ecosystems. The appropriateness of this concept was demonstrated by: 1) conducting a brief review to synthesize the processes that dominate peatland succession and how interactions between these processes supports specific ecosystem function; 2) identifying the key ecosystem variables that can be used as quantifiable measures of specific ecosystem functions; and 3) exploring the mechanisms of key ecosystem interactions to identify the most suitable integral indicator of ecosystem functioning. Hence, the aims of this paper are twofold: 1) to initiate the development of a framework for integrating the diverse research data generated from on-going oil sands reclamation pilot studies, towards understanding the functioning of constructed peatlands; 2) and to stimulate discussions on refining the current reclamation evaluation practices towards a functional-based approach that will be most appropriate for constructed peatland evaluation in the Alberta oil sands region.

2.2 Processes of peatland initiation and succession

Peatland development is initiated by physical processes, which are driven by environmental factors such as climate, relief and hydrogeology. The interactions between these external abiotic factors produce allogenic processes, which feedback on the internal ecosystem variables and autogenic processes such as plant and microbial community succession (Payette 1988). Primary peat formation, terrestrialization and paludification are the three main processes that have dominated the initiation of northern peatlands (Halsey et al. 1998; Ruppel et al. 2013). These three processes have been identified across North American boreal forest peatlands; with paludification being the dominant process over all northern peatlands (see Figure.2;Vitt 2006; Ruppel et al. 2013; Inisheva et al. 2013). Kuhry & Turunen (2006) described paludification as the inception of peat formation on formerly dry mineral soil substrate occupied by terrestrial vegetation, following such change in local hydrological conditions that result in the inundation or accumulation of runoff water in topographic lower points.

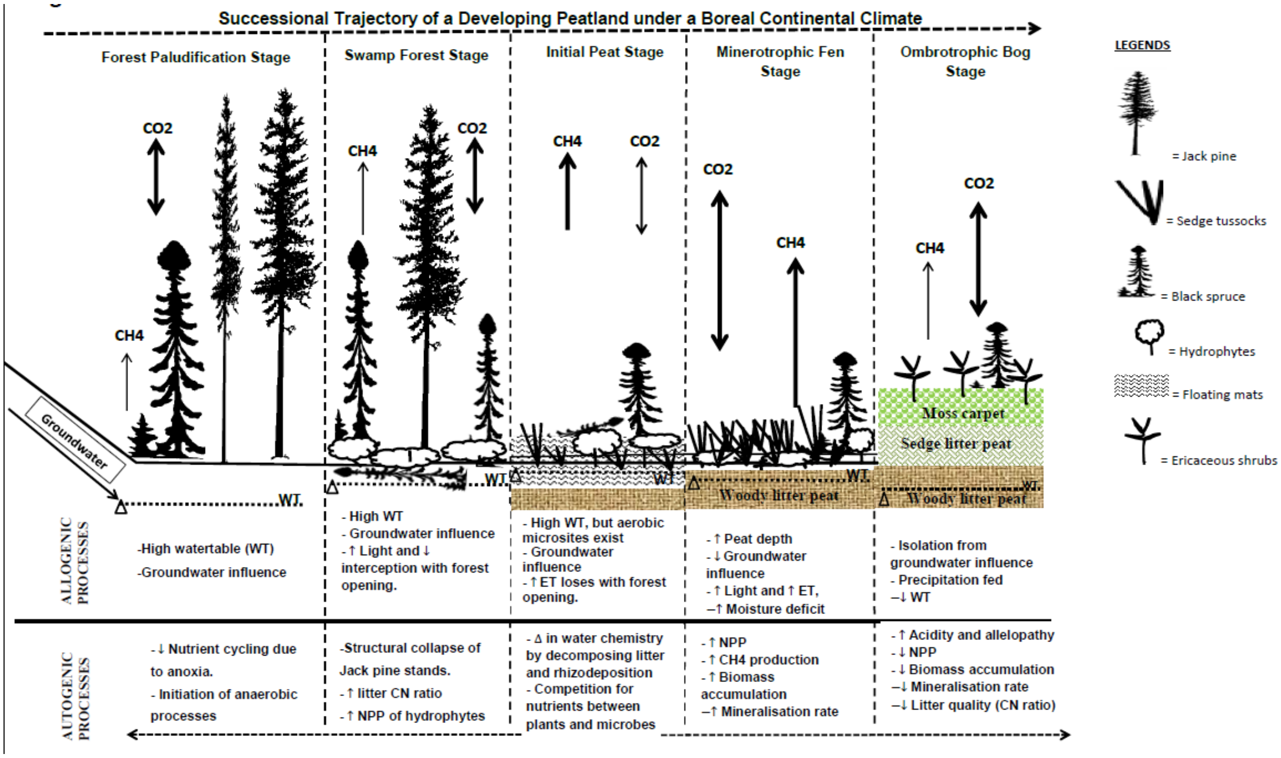


Figure 2.2: The successional stages of a natural peatland under an ideal continental boreal climate condition, highlighting dominant allogenic and autogenic processes at various stages of along the successional trajectory. The thicker arrows indicate the dominant flux at different stages. The horizontal lines extending from the middle to both ends of the trajectory line indicated that microbial mediated biogeochemical transformation are dominant in all successional stages due to microbial competition for nutrients.

The swamping of a formerly dry mineral soil substrate alters allogenic and autogenic processes such as depth of watertable and nutrient mineralization rates, respectively. Soil saturation leads to anaerobic soil conditions and slowed organic matter breakdown, which results in decreased nutrient cycling. The deposition of eroded nutrient-rich organic matter and dissolved sediments by runoff water into the paludified site increases anaerobic oxidation processes such as nitrous oxide (N_2O) and methane (CH_4) production (Smemo & Yavitt 2011). Thus, at the early stage of paludification, which is synonymous with the rewetting of dewatered peatlands (Zerbe et al. 2013), the presence of nutrient-rich substrates such as mineralized peat will make the paludified site a hot spot of greenhouse gas (GHG) production (McClain et al. 2003).

Persistence of inundated conditions in a typical boreal forest retards the development of *Pinus banksiana* (jack pine) roots due to oxygen deficiency and reduced nutrient cycling in the anoxic rooting zone (Tiner 1991). This leads to the gradual senescence of *Pinus banksiana* roots, then stands, creating more favorable conditions for hydrophytic plants and hydrophytic tolerant trees such as *Picea mariana* (black spruce). At this stage, the poor nutrient quality (high C/N ratio) of the decomposing *Pinus banksiana* litter further reduces nutrient cycling by altering the nutritional status of the decomposer communities (Thormann et al. 2001). Therefore, the litter quality (i.e. a function of C/N ratio) of the reclamation substrate will be one of the factors controlling the rate of nutrient cycling at the early stages of reclamation.

Following the structural collapse of *Pinus banksiana* stands, opening of the forest cover abets allogenic processes (e.g. reduced precipitation interception and low evapotranspiration losses) that creates conditions relevant to the invasion of hydrophilic plants, which marks the first stage of vegetation succession (Mitsch & Gosselink 2010; Tuittila et al. 2007). At this stage, the physiological structure of the site is similar to an open swamp, containing vascular plants that are adapted to the waterlogged conditions through the formation of tussocks, large intercellular spaces (aerenchyma) and floating mats (Rochefort et al., 2012). Part of the dead *Pinus banksiana* litter is deposited into the anoxic zone where decomposition will continue at a slower rate due to the metabolic energy constraints associated with phenolic inhibition under anoxic conditions (Shackle et al. 2000).

The aerated portion of the submerged *Pinus banksiana* trunks and the floating mats creates aerobic microsites that are colonized by aerobic microorganisms. Aerobic microsites are hot spots of litter breakdown where microbial secretion of extracellular enzymes like phenol oxidase causes efficient degradation of recalcitrant organic matter by releasing extracellular hydrolase enzymes from phenolic inhibition (Shackle et al. 2000; Freeman et al. 2004). Thus, aerobic microsites form the peat producing layer, or acrotelm in a developing peatland (Ingram, 1979). The partially decomposed plant materials (peat) produced in the acrotelm are submerged into the deeper anoxic zone, the catotelm, which forms the real peat accumulator in a peatland (Clymo et al. 1998).

The biochemical composition of decomposing litter combines with rhizodeposition to alter the water chemistry and biogeochemical processes of developing peatlands (Strack et al. 2006; Bradley et al. 2008). For instance, the persistence of mineroptrophy (nutrient-rich conditions) and high photorespiration increases the net primary production (NPP) of *Carex* sedges (Dise 2009). High productivity of Carex sedges have been associated with high CH_4 emission (positive feedback) as a result of their aerenchymatic tissues, which serves as conduits for transporting gases from the anoxic zone to the atmosphere (Yavitt et al. 2000; Lai 2009 and references therein). Hence, at the intermediate stage of peat development when minerotrophic sedges form the dominant plant functional types (PFT), the flux of CH_4 from the constructed peatland is expected to be at its peak in the absence of other external forcing factors such as sulfate (SO_4^{2-}) deposition (Dise & Verry 2001). Higher NPP also accelerates litter turnover (Laiho 2006), and the subsequent increase in sedge peat accumulation. Humification of accumulated peat leads to a catotelm that is characterized by higher bulk density, lower specific yield, pore size distribution and hydraulic conductivity relative to the acrotelm peat (Clymo 1992; Price 2003; Holden 2005; Petrone et al. 2008). These peat properties control the water regulation and storage functions in peatlands (Figure 2.3).

With increased humification of the catotelm peat, the upwelling of nutrient-rich pore water from the mineral substrate is retarded by three processes: 1) the production of organic acids during peat humification increases the competition between hydrogen ions and cations (Damman 1978); 2) the low hydraulic conductivity of the highly humified peat reduces the upwelling of cation-rich solutes through the peat matrix; and 3) diminishing head gradients as the elevation of the mound increases. Thus, a continuous increase in catotelm peat thickness gradually isolates the acrotelm from the minerotrophic groundwater, resulting to ombrotrophic (nutrient-poor) conditions (Vitt 2006). The appearance of *Sphagnum* mosses is a floristic indicator of ombrotrophy, a final stage in peatland succession (Mitsch & Gosselink 2010; Tuittila et al. 2012). Ombrotrophication is biogeochemically associated with reduced mineralization rates and NPP (Bayley et al. 2005); lower CH_4 and N_2O emission, with CO_2 being the major GHG (Regina et al. 1996); high acidity and production of allelochemical that slows the rate of nutrient cycling (Bradley et al., 2008).

2.3 Peatland ecological functions and related ecosystem variables

All the successional stages observed in natural peatlands may not occur in a constructed peatland, because reclamation attempts to skip initial successional stages by transferring peat from a donor peatland to a constructed site. Hence, from a functional perspective, it is expected that constructed peatlands may start from the intermediate stage of natural peatland succession. To be classified as an "equivalent land capability", ecohydrological conditions (especially biogeochemical functions) in the constructed peatland are also expected to align with those observed at the intermediate stage in natural analogues. Peatland reclamation targets the recovery of hydrologic regulation and water storage (Price 2003; Holden 2005), biogeochemical transformation (Limpens et al. 2008; Dise 2009), vegetation species succession (Bauer et al. 2003; Tuittila et al. 2007), primary production and decomposition rates (Clymo et al. 1998; Frolking et al. 2001). Since these ecosystem functions vary along the successional pathway of a developing peatland (Figure 2.2), our understanding of the functional state of a reclaimed peatland can be improved by aligning their functional characteristics with those observed in a natural analogue, to find a suitable reference along the successional pathway that can be used for evaluating a given site. However, assessing the functional state of an ecosystem requires identifying the quantifiable ecosystem variables that are associated with specific peatland ecosystem functions (Table 2.1).

2.3.1 Hydrologic regulation and water storage functions

Peat physical properties such as pore size distribution, specific yield, hydraulic conductivity and bulk density control the movement and storage of water in the peat (Boelter 1968). The hydrologic regulatory function of peatlands is a product of the range of variability between these peat properties within the acrotelm and catotelm (Figure 2.3; Holden, 2005).

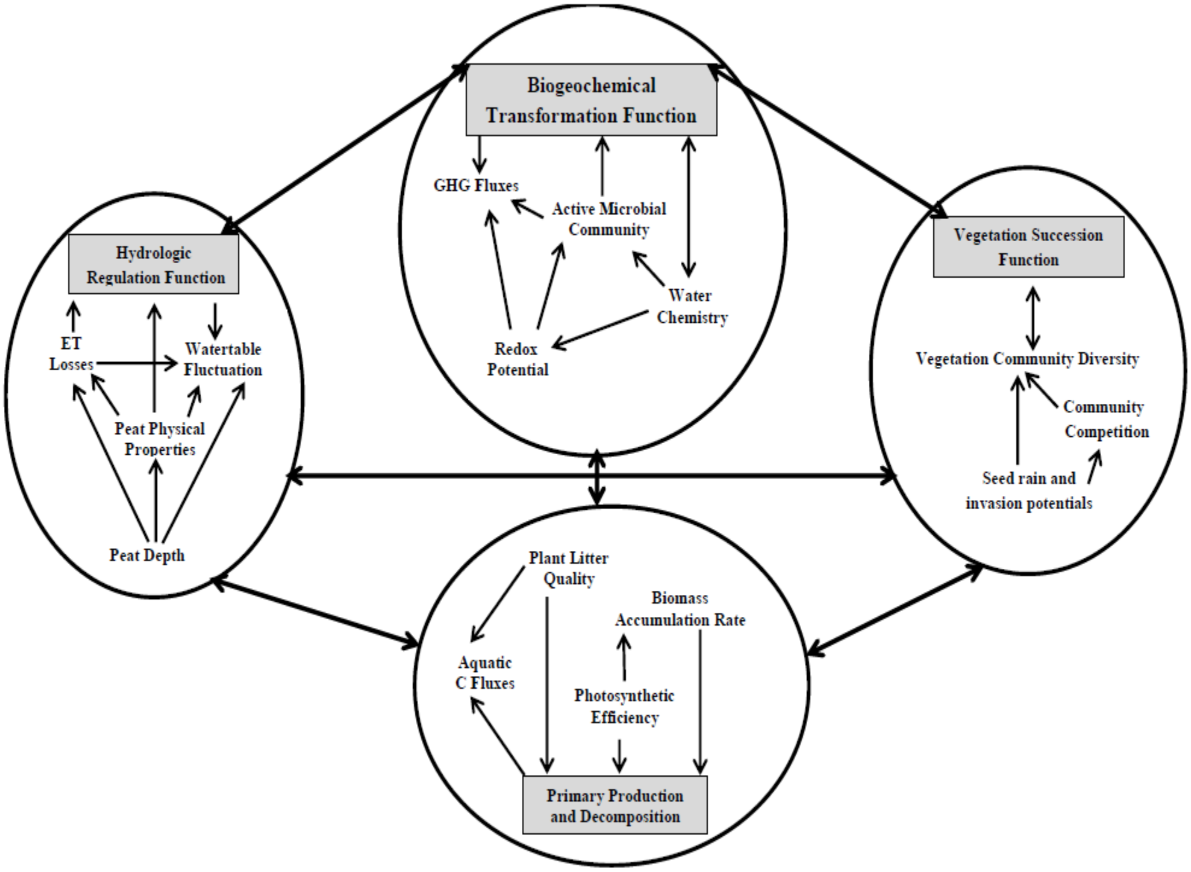
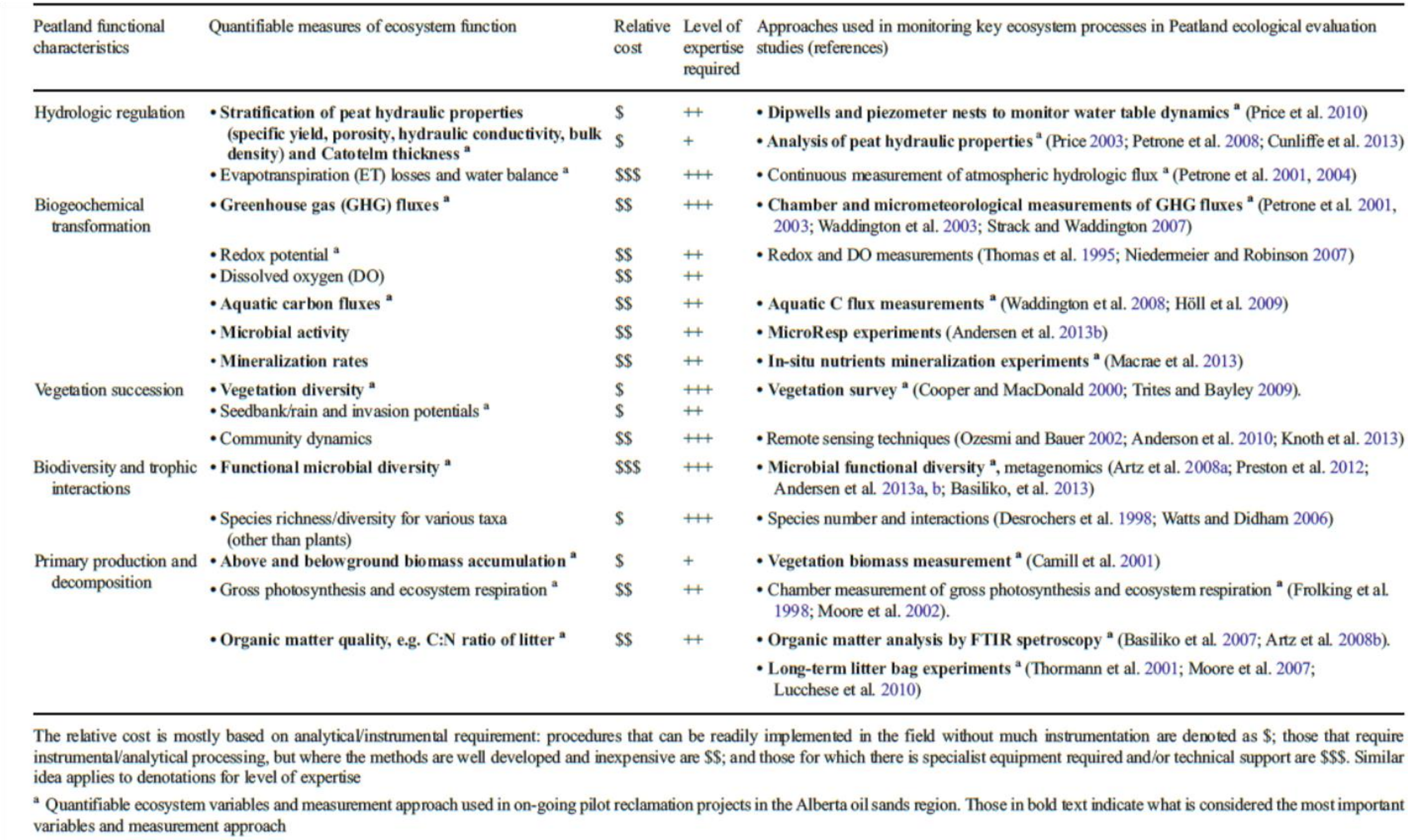


Figure 2.3: Conceptual diagram demonstrating the dominant interaction and feedbacks processes that support various ecosystem functions in peatlands. The *double-pointed lines* indicate a feedback interaction while *single-pointed ones* indicate a one-way interaction and points towards the dependant variable.

The partially decomposed property of plant litter in the acrotelm forms a porous medium through which water readily infiltrates into the peat layers. The ease of infiltration through the acrotelm is due to the presence of many large pores (high average pore size) in partially decomposed plant litter, which allow a greater proportion of the infiltrating water to be drained by gravity (Price et al., 2003). As water infiltrates into the peat matrix, the increase in watertable is modulated by the high specific yield, as is its decline as water is lost to drainage and evapotranspiration. During drier periods when the watertable is lower, the well-drained large-pore matrix in the upper acrotelm becomes a poor conductor of water and evapotranspiration losses are curtailed (Price & Whittington 2010). The rate of lateral seepage is defined by the hydraulic conductivity of the porous peat; a function of the degree of peat decomposition and compression. When the watertable is high, the acrotelm has high transmissivity and can readily shed water, whereas during dry periods when the watertable is low, the highly decomposed catotelm peat characterized by very small pores restricts lateral water loss to maintain water storage in peatlands. Consequently, peatlands have the ability to self-regulate their hydrology and keep water levels relatively stable (Rochefort, et al., 2012).

In an intact peat layer, hydraulic conductivity, average pore size and specific yield are expected to decrease down the diplotelmic profile (from acrotelm to catotelm), while bulk density increases (Clymo 1992). The gradual accumulation of decomposing litter during natural peatland development (Figure 2.2) creates the diplotelmic peat layers. This attribute is compromised in constructed peatlands because the process of site preparation and donor peat deposition results in a fragmented peat layer, which lacks the stratified properties that support the hydrologic regulatory functions of intact peat. The implications of this will be seen in the establishment limitations of native peatland vegetation species. For instance, regenerating mosses with large open pores are incapable of generating a strong capillary rise of water from the underlying fragmented peat because of the abrupt transition in peat hydraulic properties, notably water retention capacity (McCarter & Price 2013). Thus, the recovery of peat stratification, which can be assessed by monitoring the peat physical properties along the peat profile (Table 2.1), will be an effective proxy for evaluating the recovery of hydrologic regulatory functions in a reclaimed peatland. This evaluation is relatively easy to implement, and can be achieved within the frontier of funds available to small reclamation research groups.

Table 2.1: The major peatland ecosystem functional characteristics targeted in reclamation, the quantifiable variables associated with specific ecosystem functions, relative cost of evaluation, level of expertise required and the approach used in assessing key ecosystem processes in various peatland ecological evaluation studies.



Peatland functional characteristics	Quantifiable measures of ecosystem function	Relative cost	Level of expertise required	Approaches used in monitoring key ecosystem processes in Peatland ecological evaluation studies (references)
Hydrologic regulation	• **Stratification of peat hydraulic properties (specific yield, porosity, hydraulic conductivity, bulk density) and Catotelm thickness** [a]	$	++	• **Dipwells and piezometer nests to monitor water table dynamics** [a] (Price et al. 2010)
		$	+	• **Analysis of peat hydraulic properties** [a] (Price 2003; Petrone et al. 2008; Cunliffe et al. 2013)
	• Evapotranspiration (ET) losses and water balance [a]	$$$	+++	• Continuous measurement of atmospheric hydrologic flux [a] (Petrone et al. 2001, 2004)
Biogeochemical transformation	• **Greenhouse gas (GHG) fluxes** [a]	$$	+++	• **Chamber and micrometeorological measurements of GHG fluxes** [a] (Petrone et al. 2001, 2003; Waddington et al. 2003; Strack and Waddington 2007)
	• Redox potential [a]	$$	++	• Redox and DO measurements (Thomas et al. 1995; Niedermeier and Robinson 2007)
	• Dissolved oxygen (DO)	$$	++	
	• **Aquatic carbon fluxes** [a]	$$	++	• **Aquatic C flux measurements** [a] (Waddington et al. 2008; Höll et al. 2009)
	• **Microbial activity**	$$	++	• **MicroResp experiments** (Andersen et al. 2013b)
	• **Mineralization rates**	$$	++	• **In-situ nutrients mineralization experiments** [a] (Macrae et al. 2013)
Vegetation succession	• **Vegetation diversity** [a]	$	+++	• **Vegetation survey** [a] (Cooper and MacDonald 2000; Trites and Bayley 2009).
	• Seedbank/rain and invasion potentials [a]	$	++	
	• Community dynamics	$$	+++	• Remote sensing techniques (Ozesmi and Bauer 2002; Anderson et al. 2010; Knoth et al. 2013)
Biodiversity and trophic interactions	• **Functional microbial diversity** [a]	$$$	+++	• **Microbial functional diversity** [a], metagenomics (Artz et al. 2008a; Preston et al. 2012; Andersen et al. 2013a, b; Basiliko, et al. 2013)
	• Species richness/diversity for various taxa (other than plants)	$	+++	• Species number and interactions (Desrochers et al. 1998; Watts and Didham 2006)
Primary production and decomposition	• **Above and belowground biomass accumulation** [a]	$	+	• **Vegetation biomass measurement** [a] (Camill et al. 2001)
	• Gross photosynthesis and ecosystem respiration [a]	$$	++	• Chamber measurement of gross photosynthesis and ecosystem respiration [a] (Frolking et al. 1998; Moore et al. 2002).
	• **Organic matter quality, e.g. C:N ratio of litter** [a]	$$	++	• **Organic matter analysis by FTIR spectroscopy** [a] (Basiliko et al. 2007; Artz et al. 2008b).
				• **Long-term litter bag experiments** [a] (Thormann et al. 2001; Moore et al. 2007; Lucchese et al. 2010)

The relative cost is mostly based on analytical/instrumental requirement: procedures that can be readily implemented in the field without much instrumentation are denoted as $; those that require instrumental/analytical processing, but where the methods are well developed and inexpensive are $$; and those for which there is specialist equipment required and/or technical support are $$$. Similar idea applies to denotations for level of expertise

[a] Quantifiable ecosystem variables and measurement approach used in on-going pilot reclamation projects in the Alberta oil sands region. Those in bold text indicate what is considered the most important variables and measurement approach

The root architecture of reclamation pioneer vegetation (*Carex* species) presents some potential to facilitate the recovery of peat stratification during root growth and development in constructed peatlands. Mulching has been used to promote the recovery of micro hydrologic functions, such as soil moisture regulation in cutover peatland restoration (Price et al. 1998; Petrone et al. 2004).

2.3.2 Biogeochemical transformation functions

The hydrologic regulatory function in peatlands delineates the peat column into hydrological diplotelmic (oxic and anoxic) layers, which creates a redox gradient. Another important microbial ecological niche exists at the interface of the hydrological diplotelmic layers, a biogeochemical hotspot named the mesotelm (Clymo & Bryant 2008). This is the layer where the mean annual depth of watertable fluctuates within the acrotelm and can be quantitatively defined as plus or minus the standard deviation of the mean annual depth of watertable within the acrotelm. Hence, the peat column can therefore be described as a triplotelmic biogeochemical system.

Peatland biogeochemical transformations are products of the feedback interactions between of microbial activity and chemical dynamics (Figure 2.3; Hunter et al. 1998). The type, rate and pathways of biogeochemical processes are vertically stratified in response to the unique attributes of each of the peat layers. Microbial functional groups, which act as the biological engines of peatland biogeochemical transformation, show a vertical stratification along these triplotelmic peat layers (Artz 2009; Andersen et al. 2013a and references therein). In the oxic acrotelm, the availability of oxygen leads to an increase in the oxidation state of inorganic elements while oxygen limitations in the anoxic catotelm reduces the oxidation state of oxidized compounds. These changes in oxidation states involve redox reactions, which form the basis of microbially mediated biogeochemical processes in peatlands (Hunter et al. 1998; Falkowski et al. 2008).

Oxidized forms of inorganic compounds such as NO_3^- and Mn^{4+} are readily available to plant roots within the oxic peat layer since aerobic conditions efficiently sustain the energy demand of microbial activities. But in the anoxic peat layers, anaerobic microorganisms are more efficient at utilizing the oxidized forms of some plant essential nutrients (e.g. NO_3^-, Mn^{4+}, Fe^{3+} and SO_4^{2-}) as alternative electron acceptors. Consequently, this leads to the reduction in oxidation state of inorganic plant nutrients, and subsequent production of gaseous compounds like N_2O and CH_4 (Martikainen, 1996). As with the early stage of peatland development, reduction process may be a dominant function at the initial stage of constructed peatland development, when the acrotelm layer is very thin. Denitrification, the reduction process responsible for the removal of excess mineral nitrogen (N) compounds from the environment, is not a common process in natural peatlands (Dise & Verry 2001; Seitzinger et al. 2006). But in the case where peatlands receive elevated inputs of inorganic-N from wet and dry atmospheric deposition (e.g. ~25 kg N ha^{-1} yr^{-1} rate of deposition has been reported for sites adjacent to an active oil sands mine; Proemse et al. 2013), denitrification is dominant (Aerts 1997). Denitrification may also serve as a key mechanism for removing the excess N present in the mineralised donor peat used in constructed peatlands, which will be necessary for reducing N-toxicity to sensitive peatland plants (*e.g. Sphagnum moss*) and eutrophication of downstream ecosystems (improved water quality).

Methanogenesis and sulfur reduction (SR) are well-studied biogeochemical transformation processes in peatlands (Lai 2009 and references therein) and constructed wetlands

(Wu et al. 2013 and the references therein). Based on the electron tower theory (Laanbroek 1990), these transformations occur at very low redox potentials (about -150 mV) when the reduction process becomes strictly anaerobic, involving only obligate anaerobes like sulfur-reducing bacteria and methanogens. Within this negative redox gradient, SR outcompetes methanogenesis in the utilization of available substrate as a result of thermodynamic and kinetic advantages (Dise & Verry 2001; Vile et al. 2003; Gauci et al. 2005). Contrary to the previous thoughts that anaerobic CH_4 oxidation is solely dependent on SR, evidence from a recent study (Smemo & Yavitt 2007) suggests that other undiscovered anaerobic CH_4 oxidation pathways are present. This study further explained that the reduction in net CH_4 flux often observed after SO_4^{2-} addition to peatlands could be as a result of gross CH_4 production suppression and not the stimulation of anaerobic methane oxidation. This is based on the premise that in most cases, the SO_4^{2-} concentration of natural peatlands is below the kinetic energy threshold required to stimulate sulfate-dependent anaerobic methane oxidation (Schink 1997). Therefore, this lends more support to the circumstantial evidence that other unappreciated anaerobic pathways that involve methane oxidation or methanogenesis inhibition (e.g. reverse methanogenesis and NO_3^--dependent anaerobic methane oxidation) are present in peatlands, and could account for the imbalance between anaerobic methanogenesis and aerobic CH_4 consumption (Smemo & Yavitt 2011 and the references therein).

However, the specific electron acceptors involved in these anaerobic pathways were not determined by the current studies, which consistently demonstrated that in peat soils, the addition of common electron acceptors (i.e. NO_3^-, Fe^{3+} and SO_4^{2-}) do not stimulate anaerobic methane oxidation (Smemo & Yavitt 2007; Gupta et al. 2013). Perhaps these results can be fraught with the limitations associated with in-vitro studies, which fail to account for other controls that may complement these common electron acceptors under *in-situ* conditions (e.g. rhizosphere effect and re-oxidation associated with watertable fluctuations). In contrast to the electron acceptor/kinetic energy limitations present in natural peatlands, anaerobic CH_4 oxidation and/or suppression are expected to be dominant processes in constructed peatlands within the Alberta oil sands region. This follows the observation that ongoing industrial developments in this region are linked to elevated throughfall and bulk deposition of reactive N and S, with mean annual deposition rates of 25 kg N ha^{-1} $yr.^{-1}$ and 20 kg S ha^{-1} $yr.^{-1}$ measured on terrestrial sites near (~ 3 km) an active oil sands development (Fenn & Ross, 2010; Proemse et al. 2012; 2013). Although these deposition rates are low compared to those of other North American, European and Asian sites affected by elevated anthropogenic deposition (Dentener et al. 2006), the maximum rate of S deposition (39.2 kg SO_4-S ha^{-1} yr^{-1}) is higher than what was applied by Dise & Verry (2001) to stimulate thermodynamically favorable processes at the expense of methanogenesis.

Depending on the rate of methanogenesis and/or anaerobic CH_4 oxidation, part of the CH_4 produced in a peatland is liberated from the catotelm to the atmosphere by diffusion through the peat matrix, ebullition (release of bubbles from water-saturated peat) and plant transport (Strack et al. 2006; Lai 2009). In the mesotelm, part of the CH_4 diffusing through the peat pores undergoes aerobic oxidation by methane monooxygenase (MMO) enzyme activity of methanotrophic bacteria (Hanson & Hanson 1996). Considering the non-specific catalytic behaviour of monooxygenase enzyme and similarities between CH_4 and NH_4^+ (Holmes et al. 1995), ammonium monooxygenase activity can stimulate either CH_4 or NH_4^+ oxidation in the mesotelm. This presents a mechanism that can couple atmospheric N deposition and associated denitrification to CH_4 sink functions in reclaimed peatlands adjacent to active oil sands development. The mesotelm and other aerobic microsites within the anaerobic zones (e.g. the rhizosphere of sedges) are a very critical niche in the global C biogeochemical cycle because the oxidation of CH_4 can reduce the global warming potential of peatland CH_4 emission by 3.7 times per molecules per 100 years relative to CO_2 (Lashof & Ahuja 1990; Lelieveld et al. 1998; Shindell et al. 2005; Frolking & Roulet 2007). Hence, the development of a functional mesotelm in constructed peatlands is essential to mitigate the potential contribution of these peatlands to global warming. Since dominant hydrological regimes pose a major control on the periodically oxic/anoxic mesotelm (Clymo & Bryant 2008), the absence of a hydrologic regulatory function at the early stage of reclamation may result in limited aerobic CH_4 oxidation potential. This suggests that anaerobic CH_4 oxidation and/or methanogenesis inhibition could be the dominant pathways of CH_4 sink function until a functional mesotelm layer is formed in the constructed peatlands.

Ecosystem variables such as GHG fluxes are products of the biogeochemical transformation functions in peatlands, and can therefore be used as quantifiable proxies to assess the state of biogeochemical transformation functions in constructed peatlands. Also, since oxygen limitation in the saturated peat layer is a major factor that determines the rate of biogeochemical transformations (Armstrong 1967), the concentration of dissolved oxygen (DO) and redox potential can be used as a quantifiable ecosystem variables for assessing the potentially dominant redox process in constructed peatlands. Furthermore, measuring microbial activities, using recent advanced techniques in environmental genomics and stable isotope probing (Manefield et al. 2002; Whiteley et al. 2006) can be explored as a means to identify active taxa (through *in situ* extraction and analysis of rRNA) that can then be related to specific biogeochemical functions. Although this evaluation approach might seem unrealistic within the scope of small-scale research, these approaches are essential to identify the active portion of the microbial community and to associate specific micro-organisms with key processes under given environmental conditions (Basiliko et al. 2013). Alternatively, within the scope of limited resources, evaluation of temporal variability in watertable depth, microbial activity, nutrient mineralization rates and GHG fluxes can be explored to understand biogeochemical functioning of the ecosystem. Although it can be argued that these can only provide speculative information on the dominant microbially-mediated biogeochemical processes, the products of the processes (NO_3^-, Mn^{4+}, Fe^{3+} and SO_4^{2-}) can be easily analyzed to identify dominant functions.

2.3.3 Vegetation succession function

Peatland vegetation species succession is the function that enables peatlands to develop into a unique habitat that supports biodiversity, a vital ecosystem service in pristine peatlands. The water chemistry and dominant hydrologic conditions control the succession of vegetation species in peatlands (Figure 2.3; Tuittila et al. 2007). The recovery of vegetation succession functions in oil sands constructed peatlands is of utmost priority to reclamation stakeholders in the Alberta oil sands region. But concerns have been raised about the effect of industrial effluents of salinity and napthenates-affected water from substrate materials used in constructing surrounding hill slopes, on the recovery of this vital peatland function (Price et al. 2010a; Rooney & Bayley 2011). The response of reclamation vegetation assemblages to an altered abiotic environment is unknown. However, greenhouse studies (Rezanezhad et al. 2012; Pouliot et al. 2012) demonstrated that some vascular plants such as *Carex* species (e.g. *C. aquatilis*) as well as *Calamagrostis stricta*, can have stress-free growth in the current salinity and naphthenic acids (NAs) levels (~385 mg l^{-1} of Na salts and ~40 mg l^{-1} of NAs) present in oil sands process-affected water (OSPW). The same studies also showed that peat forming bryophyte species (e.g. *Bryum pseudotriquetrum*, *Dicranella cerviculata* and *Pohlia nutans*) could not tolerate these conditions. Considering that field conditions are more extreme relative to mesocosm conditions, the anticipated poor water quality presents a major limitation to the field establishment of diverse, native peat-forming vegetation species in oil sands constructed peatlands. Field observations have also shown that in wetlands where salinity tolerant vascular plants are dominant, biodiversity is very low, leading to a “green desert” of vigorous plant stands with low diversity of insects and vertebrates (Trites & Bayley 2009; Foote et al., 2013).

The findings from these studies suggest that establishing a diverse and analogous peatland vegetation community may not be feasible at the early stages of constructed peatland development in the Athabasca oil sands region. Since some minerotrophic vascular plants have shown a potential to become a tolerant pioneer species, constructed peatlands in this region may follow a PFTs succession similar to that observed in natural peatlands. However, the compromised chemical and hydrologic gradients anticipated in these sites might combine with invasive species competition to derange the recovery of native peatland vegetation succession function. This lends more supports to the inappropriateness of using only an indicator species approach for the evaluation of constructed peatlands in the oil sands region. Continuous (annual growing season) vegetation surveys are being used to keep track of community competition, invasion potentials and vegetation succession in constructed peatlands. This measurement is very important, but relatively labour-intensive in a large-scale study. Hence, there is a need for further research on the enhancement of vegetation surveying through remote sensing techniques.

2.3.4 Primary production and decomposition functions

The capacity of peatlands to store carbon is due to an imbalance between the rates of NPP and decomposition, driven by a combination of hydrologic gradients, litter quality and water chemistry (Thormann et al. 2001; Turetsky & Ripley 2005; Laiho 2006). NPP is a function of the photosynthetic efficiency of plants (Figure 2.3), and therefore varies among different peatland PFTs (Laine et al. 2012; Tuittila et al. 2012). A study of peatland NPP suggests that among the PFTs present during northern peatland succession, minerotrophic sedges have the highest NPP, while ombrotrophic forbs have the lowest (Frolking et al. 2010). Considering the open structure of constructed peatlands, the combination of high light saturation potential in sedges (Busch & Lösch 1998), adequate photosynthetic active radiation (PAR) in the continental boreal climate (Frolking et al. 1998), and atmospheric N input from oil sands activities (Proemse et al. 2013) may result in very high NPP for pioneer salinity tolerant *Carex* species. In addition, high levels of nutrient deposition will also alter the litter quality of pioneer vegetation by reducing the C: N ratio, which will accelerate litter decomposability (Figure 2.3; Aerts et al. 1995), and consequently affect the rates of carbon accumulation as peat (Bragazza et al. 2006). Hence, maintaining a near surface water table is essential to peat accumulation in reclaimed peatlands receiving high nutrient inputs and producing refractory litter. It is uncertain, however, whether the fragmented peat substrate used in reclamation can maintain a stable hydrologic regime and support near surface anoxia for most of the growing season. Over time, if the hydrologic regulatory function is not recovered, total decompsition may exceed NPP leading to net carbon loss.

2.4 Interactions between biotic and abiotic components of peatland functions

The goal of peatland reclamation is focused on creating a self-sustaining ecosystem that is carbon-accumulating, capable of supporting a representative assemblage of species, and resilient to normal periodic stresses (Daly et al. 2012). For an ecosystem to be self-sustaining, the key ecosystem processes that support various ecosystem functions need to be tightly coupled, in order to maintain the continuous flow of energy required for sustained delivery of ecosystem services. Evaluating the actualization of reclamation goals will require an integrated hydrological, biogeochemical and ecological research monitoring program that can capture the complex interactions between interrelated components of various ecosystem functions.

Exploring the interactions and feedback mechanisms that underline the tight coupling between ecosystem processes and functions will guide the integration of reclamation monitoring data towards evaluating the functional state of a reclaimed ecosystem. The conceptual model, (Figure 2.3) illustrates the mechanisms that sustain the ecosystem processes of peatland development and succession (Figure 2.2). Considering the multiple feedbacks that may result from simultaneous ecosystem processes, it is worthy to note that these interactions are non-unidirectional in nature. Interactions between components of different ecosystem functions often result in inter-functional dependency, a control feedback mechanism. For instance, with regards to the interaction between hydrologic regulation and vegetation succession functions, the phenological characteristics (e.g. stomatal conductance and root architecture) of peatland vegetation regulated ET losses, water use efficiency and consequently, hydrologic fluxes (Petrone et al. 2007b; Brown et al. 2010). As a feedback mechanism, vegetation communities will also shift in response to changes in hydrologic conditions (Laiho 2006). The dependency of NPP and decomposition on photosynthetic efficiency and litter quality respectively, creates a similar inter-functional link between vegetation succession and peatland carbon accumulation functions (Bauer et al. 2003).

A strong feedback interaction between biogeochemical transformation and vegetation succession functions is evident in the interdependency between vegetation communities, water chemistry, microbial communities and nutrient cycling (e.g. mineralization and GHG fluxes). Similarly, the redox-sensitivity of biogeochemical processes leads to a tight coupling between hydrologic regulation and biogeochemical transformation functions (Niedermeier & Robinson 2007). The response sensitivity of these feedback mechanisms varies among the levels of interaction, and can be explored as an indicator of the functional characteristics of reclaimed peatland ecosystems. Microbially mediated biogeochemical processes are very sensitive, respond rapidly to changes in conditions and are quantifiable. They also depend and feedback on all the other ecosystem functional components such as water table fluctuations and redox gradients, plant litter quality, and vegetation community diversity. Hence, microbially mediated biogeochemical processes will be a suitable indicator of ecosystem functioning.

Such functional evaluation can be achieved by quantifying measures of ecosystem processes that interact with biogeochemical transformation functions (Figure 2.3), using the most important variables highlighted in Table 2.1. For example, a practicable approach to undertake this functional evaluation will involve monitoring the growing season’s hydrologic variability (water table fluctuations), a function of peat stratification. In a functional ecosystem, microbial activities such as decomposition and mineralization will be responsive to seasonal variability in hydrologic conditions due to redox gradients (Figure 2.3). As a result, the rate of nutrient transformation and supply rates will determine vegetation productivity and community diversity in the short-term and long-term respectively. Vegetation productivity and community diversity will feedback on litter quality, which interacts with microbial activity and hydrologic conditions to determine the degree of organic matter sequestration, a targeted function in peatland reclamation. The functional state and trajectory of the constructed peatland can then be delineated by relating their functional characteristics (e.g. microbial carbon utilization profile) to those of different possible natural analogues (e.g. Figure 2.1b; saline fen and rich fen).

2.5 Conclusion and recommendation for future fen reclamation projects

Based on the limitations associated with the contemporary bio-indicator “tick box” approach of wetland evaluation, this study presents the concept of a functional-based approach that will be more appropriate for the evaluation of constructed peatlands in the Alberta oil sands region. The appropriateness of this concept is grounded on the potentials to define the functional characteristics that might evolve in an ecosystem where the range of variability in biotic and abiotic conditions can result to multiple trajectories and endpoints. Hence, this concept addresses the need to develop an integrated functional-based approach for the management of novel ecosystems that could evolve in constructed peatlands.

Adopting this concept in fen reclamation projects is feasible since it is based on the integration of quantifiable ecosystem processes that have been extensively studied in natural and restored peatlands (Table 2.1). But since it can be argued that some of these measurements are expensive, highly labour and time-intensive, or require advanced scientific expertise; the current study highlighted the most important variables that can be used to achieve meaningful results, especially within the frontiers of a small research group with limited funds. However, considering that this concept of fen reclamation is still at the pilot stage, it is premature to determine if the functional evaluation of constructed peatland can be significantly simplified to a less cost and labour-intensive venture, considering the targeted functions that need to be assessed in this peatlands. This approach presents the first attempt to develop a cost-efficient functional based approach to evaluate constructed oil sand peatlands, and opens a horizon for future research on the subject matter.

Considering that energy industries are obliged to ensure that what they reclaim is functioning as natural analogues, it is certain that a functional approach will ensure this, whereas the indicator species approach might lead to wrong conclusions about ecosystem processes (e.g. GHG emissions) due to altered abiotic conditions. It is assumed that the industry and environmental regulators in this region appreciate the need to develop a process-based evaluation approach, as they are already investing in pilot fen projects where this concept will be tested. Although these pilot studies are cost-intensive, the cost associated with these cannot be matched with the environmental cost of losing peatland ecosystem services in the first place. Once sufficient insight about the functional characteristics of the constructed peatlands have been established from these pilot studies, reclamation manager can then make informed decision about the key variables and processes that are most relevant in the context of cost-efficient, future large scale peatland reclamation projects in the Alberta oil sands region.

Chapter Three

Impacts of Donor-Peat Management Practices on the Functional Characteristics of a Constructed Fen

3.1 Introduction

The partially decomposed organic materials that form the peat layers in boreal forest peatlands are some of the vital resources impacted by oil sands development in Northeastern Alberta, Canada. In this region, the recovery of bituminous oil sands through open-pit mining presents a major threat to peatlands, as it involves the total stripping and stockpiling of peat layers that have accumulated over thousands of years (Yeh et al. 2010; Rooney & Bayley 2011). In an effort to foster environmental stewardship, oil sands operators are attempting the reclamation of functional peatland ecosystems on post-mining landscapes (Daly et al. 2012). One of the approaches currently under scientific investigation involves the transfer of salvaged peat from a donor peatland to a constructed landscape, where the peat serves as a vegetation establishment substrate, and also attenuates the upwelling of dissolved solutes from the underlying tailing sediments to the rooting zone (Price et al. 2010). The operational practice used in salvaging donor peat, which involves clearing of natural peatland vegetation, dewatering (i.e. the complete draining of a wetland) and transfer of fragmented peat with earth-moving equipment, may impact the residual peat quality relative to that of intact peat in natural analogues (Macyk & Drozdowski 2008; Makenzie 2012). It is well established that peat quality (i.e. the distinctive physicochemical properties of peat) controls major peatland functional characteristics such as; hydrologic regulation and water storage (Price & Schlotzhauer 1999), biogeochemical transformations (Updegraff et al. 1995), vegetation establishment (Salonen 1994) and organic matter turnover (Szumigalski & Bayley 1996). Therefore, donor peat quality has the potential to influence the functional characteristic and development of constructed peatlands (Nwaishi et al. 2015a), especially in the early stages of vegetation establishment.

Within the oils sands operation, conventional peat salvage practice involves the windrowing of peat as “peat-mineral mix”, which is then stockpiled until it can be used as a reclamation cover-soil for upland and wetland sites (Kong et al. 1980; McMillan et al. 2007). Where practicable, peat is directly placed on a reclamation landscape rather than stockpiled. However, timing does not always allow for this, as the current rate of resource extraction far exceeds that of reclamation (Woynillowicz et al. 2005). Although best management practices (BMPs) have been developed for the conservation of stockpiled peat (peat-mineral mix) used as cover-soil in upland reclamation (Makenzie 2012), these standards need to be updated to facilitate applicability to the conservation of donor peat slated for fen reclamation. For instance, the ongoing Suncor fen reclamation project eliminated the practice of windrowing, and adopted the in-situ loading of donor peat and direct transfer to the constructed site. Verifying the appropriateness of this method raises the need to evaluate the potential effect on peat quality.

Before salvaging the donor peat used in the pilot fen, the peatland was also dewatered as part of the mine plan. The dewatered peat was transferred to the constructed site after about 2 years, which allowed enough time for accelerated peat decomposition. Previous studies have shown that dewatering of peatland leads to the aeration of formerly anoxic peat layer (Laiho et al. 1998; Holden et al. 2004), which reduces the microbial metabolic energy constraint that was dominant under anoxia (Freeman et al. 1996; Freeman et al. 2004). With increased metabolic energy, heterotrophic microorganisms become more efficient in decomposing the labile organic matter within the peat layers (Turetsky 2004; Laiho 2006), which leads to structural collapse of large pores within the acrotelm, surface subsidence, compaction and shrinking of peat (Whittington & Price 2006). The latter translates to increased bulk density, and subsequent decline in specific yield and hydraulic conductivity in the decomposed peat layers (Price et al. 2003; Petrone et al. 2008). Higher peat bulk density also increases the surface area of decomposed peat, enhancing the tendency for more nutrient adsorption (Hargrave 1972; Laiho et al. 1998; Sundström et al. 2000). Nutrient availability in drained peat is also altered by the re-oxidation of reduced inorganic compounds (*e.g.* NH_4^+ to NO_3^-) in the former anoxic layers.

The disturbance associated with earth-moving equipment used in in-situ loading, transfer and placement of donor peat modifies the physical structure of peat. It also leads to the fragmentation of the naturally stratified peat layers (acrotelm, mesotelm and catotelm), which control vital ecohydrological functions such as water table regulation (Clymo et al. 1998; Graf, 2009) and vertical stratification of microbially mediated biogeochemical processes in natural analogues (Andersen et al. 2013 and references therein). The implication of this physical and biogeochemical disruption to the peat profile of a constructed fen has never been studied. Addressing this knowledge gap will require the evaluation of quantifiable measures of biogeochemical and hydrologic functioning in the constructed fen, following donor peat placement and vegetation establishment.

Using the first pilot project that used fragmented in-situ-loaded peat to attempt fen reclamation on post-mining landscapes, a field study was conducted with the following objectives: 1) to examine the impacts of dewatering on the physicochemical properties of the donor peat by comparing the dewatered peat and intact peat from a natural reference site; 2) to evaluate the impact of in-situ loading and fragmentation on peat quality by comparing the physicochemical and hydrophysical properties of the donor peat, before and after transfer to the constructed fen and; 3) to discuss the potential effects of the residual peat quality on hydrologic and biogeochemical functioning of the constructed fen.

The study hypothesizes that; 1) the concentrations of bioavailable nutrients will be higher in the donor peat relative to natural analogues; 2) the disturbance associated with earth-moving equipment will affect the physical properties of the relocated donor peat, while the chemical properties will be influenced by rewetting with run-off water from surrounding slopes, which will likely lead to the leaching of essentials nutrients like NO_3^- and PO_4^{3-} due to increased mobility (Damman 1978) and; 3) synthesizing the response of ecosystem processes to the modification of physicochemical properties will reveal the potential implication of altered peat quality on the ecohydrological functioning of constructed fen.

3.2 Materials and methods

3.2.1 Study sites description

The study was conducted at three different sites located within the oil sands development area, about 50 km north of Fort McMurray, Alberta. The area is characterized by a continental boreal climate with short summers and long cold winters. The mean annual temperature and total precipitation for the region was reported as 1 °C and 418.6 mm, respectively, based on 30-year monthly mean values (1981 – 2010) (Environment Canada, 2014). About 76 % of the annual mean precipitation occurs as rainfall, which peaks during the growing season (May to August), and coincides with the warmest months of the year when evaporative demands are very high (Petrone et al. 2007). Based on comprehensive regional vegetation, soil, and tree production inventories, Beckingham & Archibald, (1996) classified this sub-region of the Boreal Forest as a Boreal Mixed-wood Ecological Area.

The first study site is a natural peatland (reference fen); located northwest of Fort McMurray Alberta (56°56.298'N 111°32.898'W). The reference fen is surrounded by a typical upland coniferous forest of poor to medium nutrient regime. A comprehensive vegetation survey characterized the site, which contains both treed poor fen and treed rich fen ecosite phases (A. Borkenhagen, unpublished, Colorado State University**)**. Sampling for this study was conducted in the treed rich fen ecosites phase, with a tree cover of about 25% to 50%. The survey shows that this ecosite is dominated by the following shrubs, sedges, and moss species: *Larix laricina, Betula glandulosa, Equisetum fluviatile, Maianthemum trifolium, Carex prairea, Carex diandra, Stellaria longipe*, and numerous moss species (Crum & Anderson 1981; Moss & Packer 1983; Brodo et al. 2001). The peat depths at this site range between 0.6 to 1.6 m, and the geologic material underneath the peat is a thin layer (~ 5 m) of fluvial sand that overlay the McMurray lowland formation. The second study site is a dewatered peatland (donor fen), located in the southern end of Suncor’s Millennium lease (56°54.258'N 111°19.610'W). The site was dewatered and cleared in early 2010 (January to March). Before clearing, the vegetation structure of the site was similar to that of the reference fen, but included more treed rich fen ecosites. Peat within the donor site ranged from a maximum thickness of 255 cm to a minimum of <10 cm at the periphery of the peatland (average peat thickness = 145 cm, n = 14 locations).

The third site is a 3 ha constructed fen located in an area known as the tailings line corridor (TLC) on Suncor’s Millennium lease (56°55.944'N 111°25.035'W). The site is bounded by reclaimed slopes in the west and east corners, a patch of natural forest in the south, and an active mine haul-road in the northeast corner. The fen watershed was constructed between the spring of 2010 and winter of 2013. Details of the design, construction and revegetation approach were described earlier in Price et al. (2010) and Daly et al. (2012). To form the fen, peat from the donor fen was placed 2 m deep over a thin (0.5 m) petroleum coke layer designed to enhance the hydrological connection between the constructed upland aquifer and the fen.

3.2.2 Methods

The field study for this research was initiated in the fall of 2012 in the reference and donor sites. Two sets (side by side) of 100 cm deep triplicate peat cores (c. 10 m apart) were collected along transects in the reference and donor sites using a Russian peat corer (Eijkelkamp Agrisearch Equipment, Netherlands). Coring for the deeper peat layers (i.e. the first 50 to 100 cm depths) was executed following De Vleeschouwer et al. (2010) to avoid compression of the peat core. In the reference site, peat cores were collected at the interface of microforms (hummocks and hollows) to eliminate any micro-topographic influence and allow for relative comparison with fragmented donor peat. Selection of coring spots was targeted at capturing the vegetation assemblage representative of the ecosites. The intact cores collected were transferred to a semi-cylindrical PVC pipe (split lengthwise), and tightly sealed with plastic wrap before transportation to the Biogeochemistry Lab at the University of Waterloo for analysis.

In the laboratory, peat cores from the reference and donor fens were divided into the following depth categories; 0-5, 6-15, 16-45, 46-75 and 76-100 cm representing the upper acrotelm, lower acrotelm (or mesotelm), upper catotelm, middle catotelm and lower catotelm respectively. The subsamples were analyzed for selected soil quality indices, which consists of bulk density, organic matter content, C/N ratio, gravimetric moisture content, soil pH, electrical conductivity (EC) and extractable nutrients. These soil properties were selected as candidate indices for peat quality assessment because of their key role in supporting biogeochemical functioning in peatlands. For the analysis, subsamples from the first sets of triplicate peat cores were oven-dried at 80 ^oC for 24 hours to determine gravimetric moisture content and bulk density (g/cm^3). A known portion of the oven-dried peat samples were further analyzed for organic matter (OM) content, calculated by loss on ignition (LOI) following Rowell (1995).

Subsamples from the second set of the triplicate peat cores were homogenized individually in a Ziploc bag before removing two aliquots (~ 5g in dry weight). The first aliquot was leached in 50 ml of 2M KCl to extract NO_3^- and NH_4^+, while the second aliquot was extracted in 50 ml distilled-deionized water for the determination of soluble reactive phosphorous (SRP). These extracts were analyzed using a colorimetric technique (Bran Luebbe AA3 autoanalyzer, Seal Analytical, USA). Electrical conductivity (EC) and pH were also measured in peat-deionized water slurry (1:5 ratios) with an Accumet™ AP85 pH/Conductivity meter (Thermo Fisher Scientific Inc., Canada).

The constructed fen was sampled in the summer of 2013 after the re-deposited donor peat (which was transferred to the constructed site between December 2012 and January 2013) has undergone consolidation and rewetting through winter freezing and spring thaw cycles, respectively. Prior to in-situ loading of the donor peat, the upper 30 cm was removed to avoid weed seedbank transfer from the donor to the constructed fen, which would complicate vegetation establishment experiments. Accordingly, for consistent comparison and illustrative purposes, the new "0 cm depth" of the donor peat presented in this paper was actually peat located at 30 cm depth in the donor site. Two sets of shallow (15 cm deep) cores were collected side by side from 4 points located along each of the 3 east-west running transects (c. 5 m apart) in the constructed fen (n = 12). The first set of these samples was processed for nutrient extraction within 12 hours of collection in the Chemistry Lab at Keyano College, Fort McMurray

Alberta, and later the nutrient extracts were sent to the Biogeochemistry Lab at University of Waterloo for analysis. The second set was analysed for bulk density, OM, C/N ratio, gravimetric moisture content, soil pH, electrical conductivity (EC) and extractable nutrients using the methods described earlier.

The carbon to nitrogen (C/N) ratio of peat was quantified as the ratio of total mass of C and N derived from ^{13}C and ^{15}N in different sets of shallow peat cores (15 cm deep) collected from the three sites. Peat samples for C/N ratio analysis were processed in the Environmental Isotope Lab (EIL) at the University of Waterloo, following the approach described in Krüger et al. (2014). Stable C and N isotope analyses were measured in processed peat samples using the Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen-Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108 - Italy). C and N Isotope signatures are expressed in the common δ-notation $\delta\text{-‰} = ((R_{sample}/R_{standard}) - 1) \times 1000$, relative to the Vienna PeeDee Belemnite (V-PDB) standard or N_2 in ambient air for ^{13}C and ^{15}N data respectively. The analytical precision for ^{13}C and ^{15}N were below ±0.5‰; deviation between duplicate samples was <±0.1‰. A standard was analyzed for $\delta^{13}C$ and $\delta^{15}N$ following every 5 peat samples (n =15).

For the hydrophysical analyses, four additional cores (~90 cm deep) were extracted from separate locations within the donor site in the summer of 2012 using a Wardenaar box corer. Four cores were also extracted from the constructed fen for comparison; however, issues with the Wardenaar corer in the fragmented peat limited extraction to a depth of 60 cm. All cores were carefully transported back to the Wetlands Hydrology Laboratory at University of Waterloo. In the laboratory, the upper 30 cm of each peat cores extracted from the donor site was discarded for accurate comparison with the donor peat layers, as described previously. The cores were subsampled at 10 cm intervals below the “new 0 cm depth”, and each subsample was analyzed for specific yield and porosity following standard methods (Price 1996) and both vertical (K_V) and horizontal (K_H) saturated hydraulic conductivity (K_{SAT}) using the constant head method described by Freeze & Cherry (1977).

3.2.3 Statistical analyses

The data sets obtained from laboratory studies were subjected to a normality test prior to statistical analyses, and appropriate transformations were applied when necessary. Statistical analyses were performed using SPSS Software Version 22 (IBM Corporation, USA). Since peat decomposition increases with depth, the effect of donor peat dewatering was expected to vary with depth. A two-way factorial analysis of variance (ANOVAs) was used to test the effect dewatering on peat quality indices, while controlling for site (reference and donor) and depth categories in each of the sites, and also the interaction between both. ANOVA was also used to test the effect of peat salvage and placement practices on residual peat quality and potentials to support fen vegetation establishment, by comparing rhizosphere peat (0-15cm depth) from the three study sites. Post hoc analyses (Tukey) were performed using SPSS. Correlation analyses were also performed to evaluate the interrelations among peat properties and nutrient transformation processes in the constructed fen. The significant level for all statistical tests was $p \leq 0.05$.

3.3 Results

3.3.1 Peat physical properties

Generally, results showed that the physical properties of the peat varied among the three sites (Figure 3.1a to 1c). Since peat for physiochemical analysis of the constructed fen was collected from the rhizosphere depth (0-15cm), the data reported as "near-surface value" for the donor and reference sites are averages for 0-5cm and 6-15 cm from these sites. In the intact cores from the reference and donor fens, bulk density increased with depth (Figure 3.1a), and the near-surface value was similar ($p > 0.05$) between the reference (0.149 g/cm^3) and donor (0.157 g/cm^3) peat.

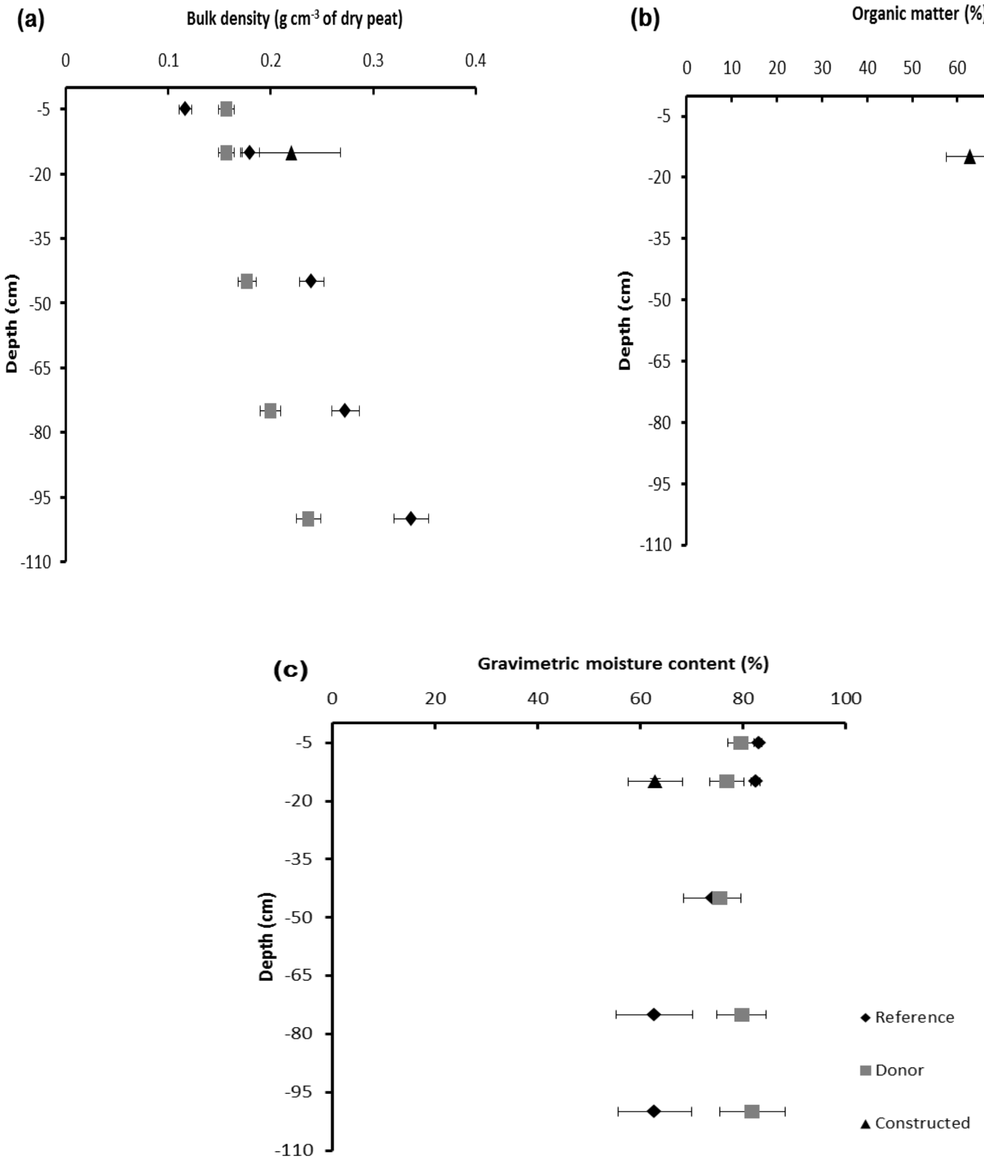


Figure 3.1: Mean and standard deviations (error bars) of peat bulk density (a), organic matter (b) and gravimetric moisture content (c). The mean values for reference (dark diamond shape) and Donor sites (grey square shape) were measured along the peat profile (0 to 100 cm depth, n = 3), while that of the Constructed fen (dark triangle shape) was measured in the near-surface depth (0-15 cm depth n = 12).

Bulk density varied significantly with depth ($p < 0.001$), and between the reference and donor fens ($p < 0.05$). In the constructed fen, the near-surface bulk density (0.193 g/cm^3 to 0.359 g/cm^3) was within the range observed in subsurface layers of the intact reference and donor peat (Figure 3.1a). Hence, it was significantly higher ($p < 0.05$) than that of the reference and donor fens. Correlation analysis indicated that bulk density was negatively correlated ($p < 0.05$, Table 3.1) with organic matter (OM) and moisture content.

Table 3.1: Correlation coefficients matrix (Spearman's ρ) between the selected peat quality indices along the peat profile of the reference fen. Significant correlations are shown in bold.

Characteristics	BD	OM	MC	pH	EC	NO_3-N	NH_4-N	TIN
OM (%)	-0.929**							
MC (%)	-0.786*	0.714*						
pH	0.946**	-0.946**	-0.778*					
EC (µS cm^{-1})	0.833*	-0.905*	-0.810*	0.826*				
NO_3-N (µg g^{-1})	-802**	0.492	0.806**	0.382	0.236			
NH_4-N (µg g^{-1})	-0.395	-0.073	0.830**	0.830**	-0.152	0.588		
TIN (µg g^{-1})	-0.480	0.018	0.879**	0.794**	-0.115	0.721*	0.964**	
SRP(µg g^{-1})	-0.675*	0.529	0.479	0.321	-0.503	0.467	0.527	0.576

N = 10; BD = Bulk density; OM = Organic matter; MC = Moisture content; EC = Electrical Conductivity

** = P ≤ 0.01; * = P ≤ 0.05

Near-surface OM content was significantly lower ($p < 0.05$) in the donor (82 % ± 4.46) than in the reference fen (90 % ± 1.05). In the subsurface layers of both sites, OM did not vary with depth ($p > 0.05$, Figure 3.1b). Following donor peat transfer and placement in the constructed fen, there was a significant reduction in OM ($p < 0.05$) to about 63% ± 5.36. Moisture content in the near-surface layer was higher ($p < 0.05$) in the reference fen than in the donor and constructed fen (Figure 3.1c). Moisture content correlated positively with organic matter and water extractable nutrients, but was negatively correlated with EC and pH (Table 3.1).

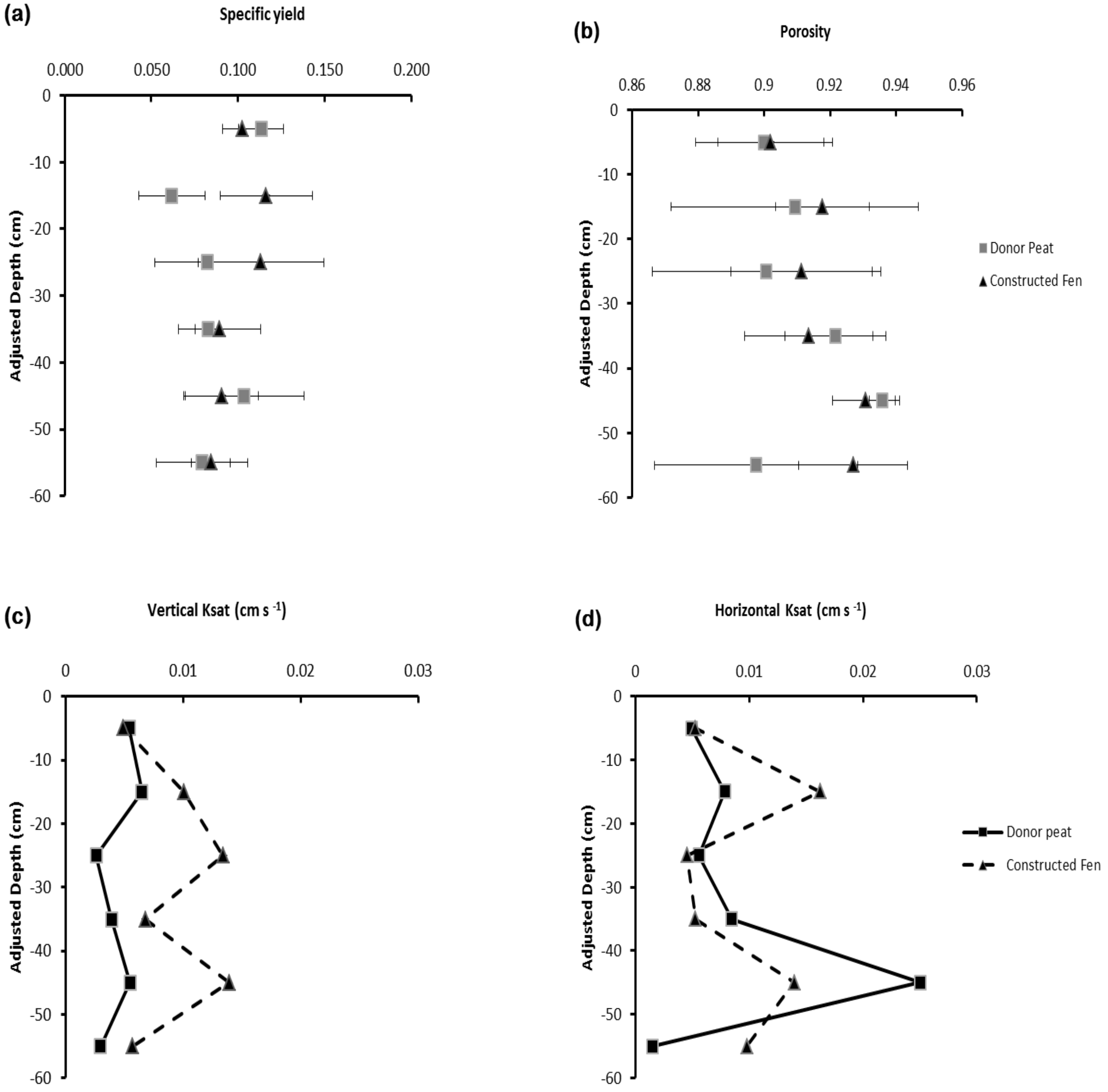


Figure 3.2: Hydrophysical properties of Donor and Constructed fens' peat: (a) specific yield, (b) porosity, (c) vertical saturated hydraulic conductivity and (d) horizontal saturated hydraulic conductivity.

For peat hydrophysical properties, porosity showed no discernable difference between the donor and constructed fen peats; however, there was a slight increase in porosity with depth in both sites (Figure 3.2). Likewise, K_H of the donor and constructed fen peats were similar, ranging from 3 x 10^{-3} to 3 x 10^{-2} cm s^{-1}. Interestingly, K_V of the constructed fen peat was consistently higher than that of the donor peat with depth (Figure 3.2c), which is a consequence of the large changes to the peat structure during the extraction-transportation-placement process. Consequently, the horizontal/vertical anisotropy ratio was reduced from 1.5 (donor peat) to 1 (constructed fen peat).

3.3.2 Peat chemical properties

The pH of the donor peat (7.2 to 7.8) was significantly higher than that of the reference peat (6.5 to 6.8) ($p < 0.05$, Figure 3.3a), but did not vary with depth at either site. Fragmentation of donor peat did not have any significant effect ($p > 0.05$) on the near-surface pH of the constructed fen peat (7.7 ± 0.1). pH correlated positively with EC, NH_4-N and TIN (Table 3.1). EC was significantly lower ($p < 0.001$) in the reference fen (37 to 129µS/cm) compared with the donor fen (145 to 184µS/cm) peat. An abrupt increase in EC (from 169 µS/cm ±15 to 556 µS/cm ± 88) was observed after donor peat transfer to the constructed fen, which was thus significantly higher than in the two other sites ($p < 0.001$, Figure 3.3b).

The concentrations of NO_3^- and NH_4^+ decreased with depth in both reference and donor peat profiles (Figure 3.3c). In the reference peat, total inorganic nitrogen (TIN, i.e. the sum of NO_3^- and NH_4^+) was dominated by NH_4^+ (~ 76% of TIN). Conversely, in the dewatered donor peat, the contribution of NH_4^+ to TIN dropped to ~39%, making NO_3^- the dominant form of TIN

(~ 61%). The major differences in the N concentration of both sites were observed in the near-surface layers (p < 0.001, Figure 3.3c, d and e). Relative to the donor peat, NO_3^- concentration decreased significantly (p < 0.001) in the near-surface layers of the constructed fen (8.23 µg g^{-1} ± 3.8); reinstating NH_4^+ as the dominant source to TIN (~ 70%). SRP was low within the reference peat layers and decreased with depth (0.78 to 0.09 µg g^{-1}). Higher concentrations of SRP (7.77 to 2.36 µg g^{-1}) were observed in the near-surface layers of the donor peat, but concentrations decreased (0.11 µg g^{-1} ± 0.04, p < 0.001) in the constructed fen (Figure 3.3f).

Carbon isotopic signature ($\delta^{13}C$) in the near-surface layers ranged between -28.95‰ and -25.56‰ across the three sites. The constructed fen peat was more $\delta^{13}C$ enriched than the other two sites, with the lowest enrichment observed in the reference fen (Table 3.2). Post-hoc analyses (Tukey) showed that there was no significant difference between the mean $\delta^{13}C$ enrichment of the reference and donor peat, but the higher mean $\delta^{13}C$ (-26.53±0.20) observed in the constructed fen was significantly different from the other sites (p < 0.05). A significant negative correlation (ρ = -0.794; p = 0.006) was found between $\delta^{13}C$ and % C content of peat using Spearman ranked correlation. The $\delta^{15}N$ signatures in the peat samples ranged between -3.66‰ and 0.68‰ across all the sites. Relative to $\delta^{15}N$ signatures in the reference fen peat (Table 3.2), lower values were observed in the donor fen peat (~ -1.34‰ ±1.63). There was no significant difference (p > 0.05) in $\delta^{15}N$ signatures across the three sites, but a post hoc test showed that it differed significantly (p < 0.05) between the reference and donor fen peat. C/N ratio ranges between 18.9 to 31, 26.4 to 44 and 20.9 to 23 in the reference, donor and constructed fen respectively. The mean C/N ratio was significantly different (p < 0.05) across the three sites. Strong negative correlation was observed between $\delta^{15}N$ signatures and C/N ratio across the sites (ρ = -0.706; P = 0.003).

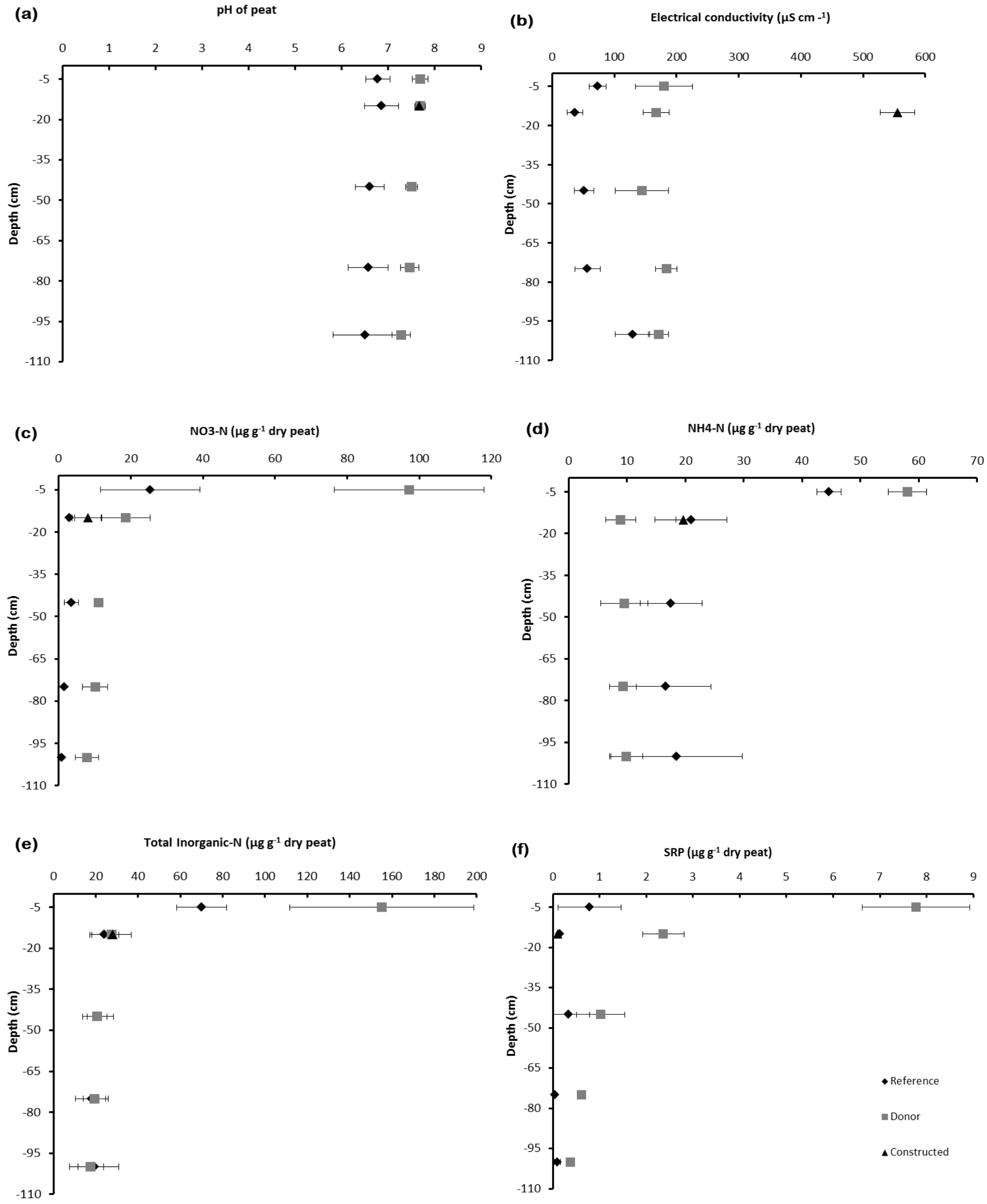


Figure 3.3: Mean and standard deviations (error bars) of peat pH (a), electrical conductivity (b), NO_3-N (c), NH_4-N (d), TIN (e) and SRP (f). The mean values for Reference (dark diamond shape) and Donor sites (grey square shape) were measured along the peat profile (0 to 100 cm depth, n = 3), while that of Constructed fen (dark triangle shape) was measured in the near-surface depth (0-15 cm depth n = 12).

Table 3.2 Stable isotope compositions and elemental C/N ratios of surface (0-15 cm) peat samples from the three study sites, mean values (n = 5) of peat sub-samples (± standard deviation).

Sites	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	% weight Carbon	% weight Nitrogen	C/N Ratio
Reference	-28.38(0.40)	0.31(0.37)	33.63(16.3)	1.25(0.64)	26.00(5.24)
Donor	-27.43(1.51)	-1.34(1.63)	37.44(2.64)	1.13(0.18)	34.02(7.04)
Constructed	-26.53(0.20)	-0.06(0.41)	29.33(3.48)	1.30(0.16)	22.54(0.98)

3.4 Discussion

3.4.1 Donor fen dewatering and potential impacts on peat properties

Given that there was only one donor and one reference site in this study, it is impossible to differentiate between the effect of dewatering and the inherent differences between two sites. However, the donor site, prior to drainage, supported the same vegetation assemblages as the reference fen site and this gives more confidence in attributing the observed differences to dewatering. Dewatering of peatlands generally alters peat physical characteristics, which feedback on biogeochemical processes such as organic matter decomposition and nutrient cycling (Silins & Rothwell 1998; Prévost et al. 1999; Holden et al. 2004). Results suggest that the effects of dewatering on peat properties varies with depth. Kong et al. (1980) observed a similar pattern of variation in the physical properties of stockpiled peat in the Alberta Oil Sands Region (AOSR). Studies have shown that well-decomposed peat generally displays high bulk densities (Boelter 1968; Clymo 1984). Thus, given the increased aeration (decomposition) associated with donor fen drainage, higher mean bulk density was expected in the donor fen relative to the reference fen, but this was not the case. However, relative to the reference fen, a significantly lower bulk density was observed in the subsurface layers of the donor peat. One possibility is that the donor site initially had a lower bulk density than the reference site, which has now increased in the near-surface layers following dewatering of the donor peat as a consequence of surface subsidence (Price & Schlotzhauer 1999).

Accelerated decomposition in the dewatered peat could lead to lower organic matter content, especially in the near-surface layers, which was observed here and supported by a strong negative correlation between bulk density and organic matter content (Table 3.1). Kong et al. (1980) reported similar results for stockpiled peat, as organic matter content decreased following peat decomposition and increased bulk density. High bulk density is generally associated with smaller pore size distribution (Price 1997; Silins & Rothwell 1998; Petrone et al. 2008), which explains the observed significant negative correlation between moisture content and bulk density. Similar to findings reported by Stapanian et al. (2013b), these results confirmed the positive correlation between moisture content and organic matter, which is critical to peat water-holding capacity in the constructed fen. Overall, the effect of donor peat dewatering on peat physical characteristics seems to be tightly coupled with the alteration of peat physical structure.

Studies looking at the impact of drainage on peat chemistry have reported an increase in the concentration of some essential elements with increasing bulk density (Heathwaite 1990; Laiho et al. 1998; Sundström et al. 2000). This relationship could be due to an increase in the surface area of decomposed organic materials (humus) associated with high peat bulk density (Hargrave 1972). Hence, the saturation of base cations (e.g. Ca^{2+} and Na^{+}) in pore water translates to increased alkalinity and EC (Bragazza & Gerdol 2002). The significant positive correlation between pH and EC ($p < 0.05$, Table 3.1) could be explained by the tight coupling between alkalinity and the accumulation of salts forming cations.

Donor fen dewatering could feedback on the interactions between peat chemical and physical properties. For instance, the surface area available for ion adsorption is known to increase in well-degraded organic matter (Hargrave 1972; Crist et al. 1996). This implies that the further degradation of donor peat could exacerbate the displacement of nutrient anions (e.g. NO_3^- and PO_4^{3-}) due to higher affinity for base cations (e.g. Mg^{2+}, Ca^{2+} and Na^+) on the ion exchange site, therefore creating a greater tendency for base saturation and salinity in the constructed fen, which is supported by the significant negative correlation between bulk density and nutrient anions (Table 3.2).

The higher concentrations of extractable NO_3^- and SRP in the near-surface layers of the donor peat relative to the reference peat may also reflect site-specific differences associated with groundwater influence or could be an effect of dewatering, since previous studies have shown that increased areation in dewatered peatland accelerates nutrient mineralization and subsequent release to porewater (Laiho et al. 1998; Macrae et al. 2013). A shift in the proportional contributions of NO_3^- and NH_4^+ towards TIN can be used to assess the effect of dewatering on net N-mineralization. Based on this concept, studies looking at N-mineralization in northern peatlands have shown that most of the TIN in undrained peat are present as NH_4^+ (Verhoeven et al. 1990; Westbrook & Devito 2004; Bayley et al. 2005), whereas in drained peatlands, NO_3^- comprises a larger proportion of TIN (Macrae et al. 2013). These results agree with these studies: a larger proportion of TIN in the donor fen was present as NO_3^- while the opposite was true for the reference fen. This explains the observed difference in the dominant form of TIN between the reference and donor peat and supports the argument that N-mineralization in the donor fen was altered by peat dewatering effects. Considering the decreasing tendency for anion adsorption with increasing surface area in decomposed organic materials, the larger contribution of NO_3^- to

TIN may decline with further degradation of peat, making NH_4^+ the dominant form of TIN, especially under anoxic alkaline conditions (Bragazza & Gerdol 2002).

As observed in previous studies, SRP is usually a limiting nutrient in peatlands, especially in the subsurface layers of peat (Boeye et al. 1999; Andersen et al. 2013b). Due to the redox sensitivity of SRP, changes in the hydrologic regimes of wetlands often affects the mobilization of SRP through chemical precipitation (Shenker et al. 2005). This suggests that the significantly higher concentration of SRP ($P < 0.05$) observed in the donor peat, especially in the near-surface layers, maybe a consequence of dewatering effects such as oxygen-induced iron (Fe) precipitation (Zak et al. 2010).

The use of ^{13}C and ^{15}N isotope signatures as a surrogate for assessing organic matter quality in the near-surface layers of the study sites is explored here. Based on these analysis, the observed δ^{13}C enrichment in the donor peat, suggests that the organic matter quality was reduced as a result of changes in microbial metabolic pathways following donor peat aeration (Alewell et al. 2011). This coincides with a significant decrease in δ^{15}N and subsequently, a higher C/N ratio in the donor peat. The significant negative correlation between δ^{13}C and % C supports the notion that the total C component of the donor peat's C/N ratio may be dominated by lignin-derived C compounds (Benner et al. 1987; Esmeijer-Liu et al. 2012). Lignin-derived C compounds are less easily metabolised by microbial communities due to the energy constraints associated with the metabolism of these compounds (Andersen et al. 2010). Hence the dominance of lignin-derived C compounds associated with donor peat degradation could have a negative impact on microbially mediated biogeochemical processes in the constructed fen.

3.4.2 Impact of donor peats' post-tranfer modifications on the functional characteristics of the constructed fen

Discernible differences in near-surface peat properties were observed following the transfer of peat to the constructed fen. Data analysis suggests that the modifications resulting from peat transfer and placement had an additional effect on peat properties. However, considering that the constructed fen is prone to sediment and solute loading from surrounding slopes, these effects cannot be solely attributed to the operational practices associated with donor peat transfer and placement. For instance, a closer look at bulk density results (data not shown) reveals that plots nearest to the slopes had the highest bulk density values. Although high surface bulk density was expected due to the removal of the upper 30 cm layer prior to donor peat transfer, the relationship between high bulk density and proximity to sediment-rich slopes attributes highest surface bulk density values to sediment loading (edge effect). Consequently, the mean surface bulk density of the constructed fen was similar to those measured in the subsurface layers (75-100 cm deep) of stratified reference peat (Figure 3.1a).

The observed degradation in peat physical properties along the periphery of the constructed fen is important as it may compromise the ecohydrological functioning of the constructed fen. For instance, the increased surface bulk density will result in a subsequent decline in specific yield and hydraulic conductivity of the upper peat layer (Price et al. 2003; Whittington & Price 2006). The outcome is poor infiltration capacity, and subsequent enhancement of overland flow along the margins. Recent site observations identified the formation of a sediment crust lens beneath ponds along the fens's margins, which exacerbates the competitive advantage of invasive vascular plants such as *Typha spp* over peat-forming mosses, as the latter are incapable of sustaining efficient water retention capacity under degraded peat substrates (McCarter & Price 2013). The invasion niche created at the margins of the constructed fen could extend into other parts of the fen where conditions are similar, undermining the establishment of fen vegetation.

The variability in hydrophysical properties with depth also suggests that random placement and fragmentation of the donor peat is partly responsible for inconsistencies between the surface and subsurface properties of the placed peat. For instance, higher degree of variability in K_V of the constructed fen (Figure 3.2c) could cause percolating water to follow preferential flow paths in the unsaturated zone during precipitation events. Over time, there is potential for more permanent preferential flow paths to be sustained within the fragmented peat column. These would extend either vertically or horizontally with the development and extention of vascular plants' root architecture, which tends to shift towards the direction of water and nutrient sources (Rydin & Jeglum 2006). Such preferential flow paths could represent a substantial mechanism of water transport within the constructed fen if they become incorporated into the saturated zone with a rise in water table.

Although in-situ loading, transfer and placement processes did not have an appreciable impact on the porosity or K_H of the peat (Figure 3.2d), changes to the peat structure resulted in substantial differences to the K_V of the placed peat within the constructed fen. During the accumulation of peat in natural fen peatlands, dead vegetation materials (especially forbs and sedges) are typically oriented in a horizontal direction within the peat profile, as these plant materials die and bend over 90 degrees to the vertical direction of growth (Dai & Sparling 1973). Although often complicated relationships, peatlands typically have horizontal/vertical anisotropy ratios greater than 1 (Beckwith et al. 2003) (indicates that $K_H > K_V$) and ratios as high as 4 have been documented (Schlotzhauer & Price 1999). The reduction in anisotropy in the placed peat (from 1.5 to 1) indicates that the layered structure of natural sedge peat deposits was largely disrupted during the transfer and placement process. This will result in greater vertical groundwater fluxes than would be observed in a more anisotropic system with lower K_V, which will inevitably influence (increase) the vertical fluxes of solutes through the placed peat deposit as well. Nonetheless, the influence of this change to the peat structure, albeit important, will likely have less of an impact on water stores and fluxes than the level of heterogeneity encountered within the placed peat deposit.

As in cutover (harvested) peatlands in other parts of Canada (Price & Ketcheson 2009), the hydrologic self-regulatory function intrinsic in intact peatlands is compromised in a peatland constructed with fragmented donor peat. The consequential persistence of anoxic conditions will affect biogeochemical transformation functions such as the loss of NO_3^- and mobilization of P through redox processes and leaching (Shenker et al. 2005; Seitzinger et al. 2006; Niedermeier & Robinson 2007). Although this could explain in part, the lower concentration of NO_3^- and SRP observed in the surface layers of the constructed fen, it is more likely a consequence of the removal of the upper peat layers where most of these nutrients accumulate (Damman 1978). In any case, the loss of nutrients through removal of upper layer and/or rewetting of the donor peat resulted in nutrient concentrations similar to those of the reference site, which was necessary to reinstate the “optimum nutrient concentration” present in natural analogues (Kleimeier et al. 2014).

Consequentially, the removal of upper peat would result in the decline of near-surface microbial biomass, similar to conditions observed in bogs exploited for horticultural peat (Andersen et al. 2006). Reduced microbial biomass would be exacerbated by the high surface bulk density of humified donor peat, and could lead to microbial energy conservation (starvationsurvival state) and low nutrient transformation rate in the developing acrotelm (Fisk et al. 2003). Since the addition of fresh substrates through plant litter and root exudates can promote microbial activity (Zak et al. 2003), practices such as moss-transfer for vegetation establishment maybe important for restoring the lost active microbial biomass in the upper peat layer of a constructed fen. Following reclamation, active microbial groups could facilitate microbial biomass build-up by retaining mineralized nutrients in the microbial loop (Andersen et al. 2013b), leading to nutrient immobilization. Hence monitoring the evolution of microbial activies in relation to nutrients transformation and recovery of nutrient pools will be essential to understanding the constructed fens' trajectory.

The limited infiltration capacity of fragmented donor peat will abet the accumulation of solutes on the peat surface. Consequently, the high EC observed in the surface layers of the constructed fen could be associated with the accumulation of sodic solutes from advective transport and flushing of tailing-enriched sediments used in the reclamation of adjacent slopes. The significant negative correlation between moisture content and EC (Table 3.1) suggests a dilution effect of water on sodic solutes. This also indicated that practices like mulching, which supports moisture conservation will also reduce salinity stress in the constructed fen. Although the C/N ratio of the constructed fen peat was expected to be higher because of the high bulk density and low organic matter, results showed that it was lower than both the reference and donor fen peat C/N ratios (Table 3.2). N addition from atmospheric deposition at the site could have led to higher $\delta^{15}N$ and percentage total N and a subsequent decrease in C/N ratio (Esmeijer-Liu et al. 2012). This is possible since the constructed fen is sited in the hub of industrial activities, where atmospheric N deposition has been recently reported (Proemse et al. 2013).

3.5 Conclusion and recomendations for future fen reclamation projects in the Athabasca Oil Sands Region

The comparison in this study was limited by site-specific differences such as peat depth and the influence of underlying geologic materials. To eliminate the effect of such differences in the interpretation of results, future peatland reclamation projects should conduct a comprehensive analysis of the donor peat before the commencement of dewatering and, immediately before transfer to the constructed landscape. This is crucial to improve our understanding of the functional characteristics and anticipated trajectory of a constructed fen in the AOSR. Hence, at the pilot stage of fen creation in the AOSR, analysis of key indicators of donor peat quality should be incorporated and adopted as part of the mine commissioning procedures for mine sites located on prospective donor peatlands.

The ecohydrological functions supported by peatlands are controlled by the natural stratification observed in intact peat layers (Graft et al. 2009). Results showed that current peat placement practices, which result in fragmentation and inversion of the peat layers, will modify the trajectory to recovery of ecohydrological conditions required to support biogeochemcial and hydrological functions in the constructed fen. Based on the findings of this study, future reclamation projects can work towards improving the quality of reclamation substrate by reducing the timeframe between donor peat dewatering and transfer to the constructed landscape. Also, a timeline that eliminates accelerated growing season decomposition should be considered when selecting the timing for donor peat dewatering. For instance, dewatering can be commenced towards the end of fall season, then followed by donor peat transfer by mid-winter, when trafficability over the dewatered site is more feasible.

Since results also shows that major alterations to peat physical structure occurred after peat transfer to the constructed fen, reclamation stakeholders should explore efficient means of improving the resultant poor surface properties. This can be achieved by ensuring the strict adherence to environmental regulatory guidelines when selecting cover materials for construction of surrounding upland and slopes. The installation of fine wire mesh at the margins of the constructed fen, would check the loading of mineral sediments from surrounding slopes. Heterogeneity in surface structure of the placed peat can be minimized by implementing appropriate surface reconfiguration techniques such as ploughing and harrowing. Surface reconfiguration could improve physical structure of peat, hydrologic conditions and also enhance the successful establishment of peat-forming vegetation, which is essential for reclaiming the carbon sequestration potential of a constructed fen.

Chapter Four

Above and Below-ground Nutrient Cycling: Criteria for Assessing the Biogeochemical Functioning of a Constructed Fen

4.1 Introduction

The often observed overlap between deposits of natural resources and pristine ecosystems (Durán et al. 2013; Kobayashi et al. 2014) suggests that meeting the increasing global resource demand through mining will inevitably be associated with the loss of vital ecosystems and supported services (Sims et al. 2013; Soni et al. 2014). Resource exploration sites are known hotspots of environmental change, where the need to ensure sustainability remains a critical challenge to environmental stakeholders (United Nations 2012; Giurco & Cooper 2012; Audet et al. 2015). As with any responsible mining operation around the world, implementation of ecosystem-scale ecological reclamation is a regulatory requirement and a major aspect of mine-closure procedures in the Athabasca oil sands region (AOSR), Canada. In this region, minerotrophic peatlands (fens) are the dominant wetland type in pre-mining landscapes (Vitt et al. 1996). Fens such as those found in the region, are known to support vital ecosystem services such as the regulation of nutrient cycling and carbon sequestration, and hence are of major importance to regional and global biogeochemical cycles (Vitt et al. 2000; Blodau 2002). These peatlands are lost through oil sands exploration (Rooney et al. 2012); however, reclamations efforts are testing the feasibility of re-creating functional and self-sustaining fen ecosystems on post-mining landscapes (Daly et al. 2012).

Currently, there are two pilot fen projects in the Athabasca oil sands region, which were built by reconfiguration of the post-mined landscape with overburden materials (Ketcheson et al. 2015). To form the fen watershed, a shallow basin created within the constructed landscape was capped with a layer of peat (0.5 to 2 m) from a donor site (Price et al. 2010; Wytrykush et al. 2012). In the fen created on the Suncor Energy Inc. site, vegetation was introduced using four revegetation strategies: a seedling treatment; a moss layer transfer treatment; a combination of seedling with moss transfer; and a control (no vegetation) (Daly et al. 2012). Following implementation of these revegetation strategies, a comprehensive ecohydrological monitoring program was initiated to assess the development of ecological and hydrological processes in the constructed fen. One major objective of this monitoring program is to identify the revegetation strategy that has the highest potential to facilitate the recovery of ecosystem biogeochemical functioning in the constructed fen, and ensure a stable successional trajectory.

Evaluating the functional state of the constructed fen through a comprehensive ecohydrological monitoring program is imperative to understanding the reclamation trajectory. However, an evaluation based on microbially-mediated biogeochemical processes has been proposed as essential for assessing the functional state of the fen (Nwaishi et al. 2015a). The central aspect of this evaluation involves exploring the recovery of biogeochemical transformation functioning, an integral mechanism of peatland ecosystem functioning. The appropriateness of this concept is grounded on the potential of exploring the sensitivity of microbial-mediated processes to a range of variability in biotic and abiotic conditions (Keller et al. 2006; Jaatinen et al. 2008; Artz et al. 2008; Lin et al. 2012), which can then be used as a criterion to define the functional characteristics that might evolve in a constructed ecosystem

(Harris 2003; Ruiz-Jaen & Aide 2005), where multiple successional pathways are possible and an endpoint is unknown.

Under pristine conditions, biogeochemical transformations support the cycling of various forms of nutrients, and maintain the continuous flow of energy and matter between ecosystem trophic levels. The overall ecosystem biogeochemical cycling is a combination of above-ground (*e.g.* net ecosystem exchange of CO_2 and gross productivity) and below-ground (*e.g.* nutrient cycling processes and microbial activity) components. Soil microbes are the biological engine of the below-ground component of biogeochemical processes, and soil edaphic variables such as substrate quality, water chemistry, moisture conditions and peat temperature control microbial activities, and thus the mineralization rate (Updegraff et al. 1995; Westbrook et al. 2006). At the same time, mineralization is often a major control on nutrient turnover, net ecosystem exchange of CO_2 and subsequent productivity of surface vegetation in peatlands (Keller et al. 2006). This feedback loop creates a tight coupling between above and below-ground biogeochemical processes in natural peatlands. Plants serve as the biological link connecting above and below-ground biogeochemical processes by supporting microbial activities through rhizodeposition, and depending on microbially-mediated nutrient supply for above and below-ground biomass productivity (Wardle et al. 2004; Van Der Heijden et al. 2008; Bragazza et al. 2015). Since the biogeochemical functioning of an ecosystem is sustained by the feedback mechanism between above and below-ground processes, assessing the recovery of this mechanism may be a useful criterion for defining the functional state of a constructed fen ecosystem (Wardle & Peltzer 2007; Nwaishi et al. 2015a).

Because fen construction is still at an experimental stage, there is a dearth of information on the state of biogeochemical functionality of these new ecosystems. Previous studies have focused on natural peatlands and provided some indications of key controls on processes such as nutrient mineralization (Bridgham et al. 1998; Aerts et al. 1999), organic matter accumulation (Belyea & Clymo 2001) and carbon cycling (Bellisario et al. 1999; Blodau 2002; Keller et al. 2006; Strack et al. 2009). However, it is not yet known if the mechanisms that sustain the feedback loop between above and below-ground processes can be established in constructed fens. Indeed, since the biotic and abiotic controls of biogeochemical processes are compromised in post-mining landscapes (Johnson & Miyanishi 2008; Rooney & Bayley 2011), there is a possibility that nutrient cycling processes will also be modified. For instance, Nwaishi et al. (2015b) showed that the peat soil used as vegetation establishment substrate in Suncor's constructed fen has a higher bulk density, and lower organic matter and moisture content than undisturbed sites, due to drainage and subsequent decomposition of peat before transfer to the constructed fen. Studies examining the effect of peat degradation on nutrient dynamics suggest that the quality of peat substrate is a major control on biogeochemical processes (Updegraff et al. 1995), which could be attributed to the substrate dependency of below-ground microbial communities (Fisk et al. 2003; Andersen et al. 2010a). Hence, the introduction of labile substrates to the constructed fen is crucial to the recovery of microbially-mediated processes. Although revegetation introduces new plant materials into the constructed ecosystem, the different revegetation strategies under trial in the constructed fen will likely have different effects on below-ground microbial community and function (Kowalchuk et al. 2002; Fisk et al. 2003; Zak et al. 2003), and also on available nutrient pools due to differences in vegetation nutrient demands (Aerts et al. 1999).

This study explores the temporal trends in microbially-mediated processes of carbon (C), nitrogen (N) and phosphorus (P) cycling, as quantifiable proxies of biogeochemical transformation function, by building on established knowledge from previous peatland studies (Strack et al. 2009; Andersen et al. 2013b; Macrae et al. 2013; Wood et al. 2015). This study reports on nutrient dynamics and edaphic conditions in the constructed fen over the first and second growing seasons post-construction. The specific objectives of the study are: 1) to evaluate the effect of different revegetation treatments on temporal variability in above and below-ground processes, relative to a natural analogue; 2) to determine the effect of revegetation treatments on the strength of the association between above and below-ground processes, and the relationship with environmental variables; and 3) to identify ecosystem processes that can be explored as potential key indicators of biogeochemical functions in the constructed fen.

The study hypothesizes that nutrient dynamics in the moss transfer plots will mirror the processes dominant in natural analogues, while soil nutrient pools will be reduced in seedling plots in response to high nutrient demands by vegetation. Given the recalcitrant nature of the peat substrate, it also hypothesized that following revegetation; microbial nutrient immobilization will be the dominant process, especially in the seedling plots, due to higher microbial biomass nutrient demands in the rhizosphere of vascular plants.

4.2 Materials and methods

4.2.1 Site description and preparation

The field research for this study took place at a natural peatland (Poplar fen) and a constructed fen on the Suncor Energy Inc. Millennium lease (Suncor pilot fen). Both are located within the oil sands development area, about 50 km north of Fort McMurray, Alberta. A general description of the study area, and specific information about the two study sites were presented in Nwaishi et al. (2015b). Six monitoring plots (measuring 2 m^2 and 10 m apart) that represent typical site conditions were selected in the natural peatland to serve as a reference for the study (REF). In each reference plot, monitoring spots were located at the interface of micro-topographic features (hummocks and hollows) for relative comparison with the constructed fen, which lacks microforms. The 3 ha Suncor pilot fen was constructed with 2 m of peat, whose source was from a treed fen similar to the reference fen.

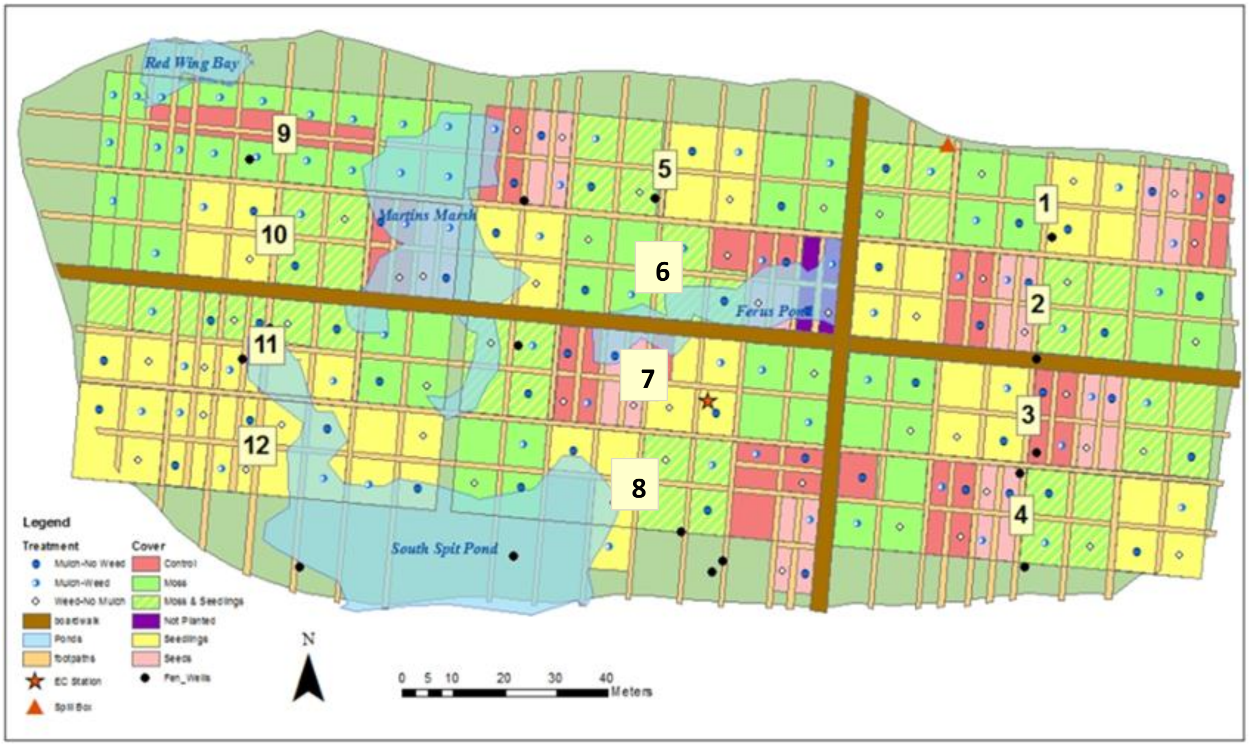


Figure 4.1: An overview of the constructed fen's experimental split-plot design. The design is replicated in 12 blocks and consists of 5 planting treatment plots of control (red), seeds (pink), seedlings (yellow), moss transfer (green), and seedlings + moss transfer (yellow and green). The plots are split into mulch + no weed (blue circle), mulch + weed (white and blue circle), no mulch + weed (diamond shape), and no weed + no mulch (no circle). Image created and supplied by Andrea Borkenhagen (2013)

The experimental design was laid out as series of blocks and planted in the summer of 2013, using a randomized split-block design with 12 replicates (Figure 4.1). Each block had four main vegetation plots that were approximately 16 x 17 m and included: 1) seedlings only (SDL); 2) moss layer transfers (MLT); 3) seedlings with moss transfer (SMLT); 4) a control treatment (CTRL). It is worthy to note that the CTRL plots were split into two, to accommodate a seed sowing treatment. However, only the untreated CTRL (unseeded) plot was used as the CTRL for this study. Each vegetation plot was split into four 8 x 8.5 m sub-plots to test the effects of wood-strand mulch/weeding treatment, mulch/no-weeding treatment, no-mulched/weeding, and no-mulched/no-weeding. For the purpose of this study, monitoring was only conducted on the mulch/weeding and no-mulch/weeding sub-plots. The study chose to focus on weeded treatments to allow for a relative comparison of the results with natural analogues with similar peatland plant communities. Thus, in all the plots, unwanted species were clipped at the base throughout the seasons and disposed of off-site. Following Poulin et al. (2013), the species targeted in the weeding campaigns included *Ranunculus sceleratus*, *Sonchus arvensis*, *Typha latifolia*, *Brassicaceae* sp., *Chenopodium album*, *Urtica dioica*, *Matricaria matricarioides,* and *Senecio congestus*.

The harvested moss layer used in the MLT consisted of plant propagules and target moss species that are regionally abundant (Chee & Vitt 1989), and have exhibited suitable tolerance to oil sands process water (Pouliot et al. 2012). The dominant moss species that were introduced through the moss layer transfer included; *Campylium stellatum* and *Tomenthypnum spp*. Vascular plants were also transferred with the moss layer as rhizomes and seeds present in the transferred materials. The SDL consist of saline (*Juncus balticus* seedlings, *Triglochin martima*) and freshwater (*Carex aquatilis*, *Betula glandulosa*, *Oxycoccus microcarpus*, *Calamagrostis inexpansa* seedling, *Sarricena purpurens*) peatland plant species. Some of these seedlings did not establish successfully. Consequently, in the second growing season (May to August, 2014), the SDL and SMLT plots were dominated by *Carex aquatilis* and *Juncus balticus*. However, relative to the SDL plots, the SMLT plots hosted a more diverse plant community. *Carex aquatilis* and the weeds targeted in the weeding campaigns spontaneously established on some of the CTRL plots in the second growing season. Due to the high degree of heterogeneity within the site, 24 monitoring sub-plots, consisting of four treatments and six replicates (n = 6 per plot for each of the 4 treatments) were selected to represent typical site conditions.

4.2.2 Peat physicochemical properties

Comprehensive ecological monitoring of the constructed fen commenced in July 2013, following completion of vegetation introduction. However, before vegetation was planted on the proposed monitoring plots, a set of two shallow peat cores (15 cm deep) were collected side by side in each plot for peat physical and chemical analysis. This sampling campaign was repeated again at the end of the first growing season, and beginning and end of the second growing season. In all cases, samples were processed for bulk density, organic matter content, gravimetric moisture content, soil pH, electrical conductivity and extractable nutrients (KCl-extractable N and water-extractable P) using the standard techniques as described in detail in Nwaishi et al. (2015b).

4.2.3 Nutrient mineralization and supply rates

The rates of net nutrient mineralization and supply to plant roots were measured simultaneously in all selected monitoring plots. The measurements were conducted twice in the first growing season, and three times in the second growing season to capture early (May to June), middle (June to July) and late (July to August) season nutrient dynamics. In the second growing season, monitoring was extended to the natural site (REF) for comparison with the constructed fen. Each measurement cycle in both growing seasons lasted approximately 30 days. For the mineralization experiment, two adjacent "paired" peat cores (15 cm deep) were extracted from all the monitoring plots using a PVC pipe that was cut to the specified depth. One of the extracted cores, referred to as the 'pre-incubation' core was processed and analyzed for pre-incubation concentrations of extractable ammonium (NH_4^+), nitrate (NO_3^-) and soluble reactive phosphorus (SRP) as described by Hart et al. (1994). The second core was used as the incubation core for *in-situ* net mineralization, following the buried-bag method described by Eno (1960). After the incubation period, cores in the buried bags were recovered and returned to the lab in a cooler filled with ice-packs, and processed for extractable NO_3^-, NH_4^+ and SRP (Hart et al. 1994).

Processing of peat samples for extractable nutrients involved the removal of two sub-samples (~ 10 g wet weight) from each sample and placement in a specimen cup. One sub-sample was extracted with 50 ml of 2M KCl (for determination of NH_4^+ and NO_3^-), while the second sub-sample was extracted with double de-ionized water (for determination of SRP). The solutions were shaken for 1 hr to dissociate the peat, and the extractants were gravity filtered into another specimen cup using a 1 µm porosity filter paper (Whatman no. 42). Filtered extractants from the processed pre-incubation and incubation cores were frozen until analysis at the Biogeochemistry Lab at University of Waterloo (Bran Luebbe AA3, Seal Analytical, Seattle, U.S.A., Methods G-102-93 (NH_4^+), G-109-94 (NO_3^- + NO_2^-), and G-103-93 (SRP)). Since the drying of samples can enhance extractable nutrient pools (*e.g.* Pote et al. 1996), field moist samples were used for nutrient extractions. However, to permit the expression of extractable nutrient pools and net mineralization rates in per unit dry weight of peat (Macrae et al. 2013), the moisture content of the each peat sample was determined. For every incubation period, net nitrification, net ammonification and net P mineralization rates (µg g^{-1} dry peat day^{-1}) were estimated as the average difference between the post-incubation concentrations of NO_3^-, NH_4^+ and SRP in the incubated core, and the pre-incubation concentrations of NO_3^-, NH_4^+ and SRP in the pre-incubated cores. Hence, negative values were used to denote nutrient immobilization, while positive values denote net nutrient release to pore water. Net N mineralization rates were estimated as the sum of net nitrification rate and net ammonification rates.

Nutrient supply rates were measured using plant root simulator (PRS) ^{TM} probes (Western Ag Innovations Inc., Saskatoon, SK). The PRS^{TM} probe is a 10 cm^2 resin membrane held in a plastic applicator handle that can be easily inserted into the soil for *in-situ* measurement of nutrient anions and cations supply to soil solution, when left to incubate for a set period (MacKenzie & Quideau 2010). Nutrient supply estimates (µg 10 cm^{-2} incubation $period^{-1}$) obtained with the probes integrate the effect of temperature and moisture on nutrient fluxes during the incubation period (Qian & Schoenau 1995; 2002). The resin membrane in the anion probes (orange colour) are positively-charged to simultaneously attract and adsorb all negatively-charged anions, such as NO_3^-, phosphate ($H_2PO_4^-$, HPO_4^{2-}), and sulphate (SO_4^{2-}).

Conversely, the membrane in the cation probes (purple colour) is negatively-charged, to simultaneously attract and adsorb all positively-charged cations, such as ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}).

Prior to use in the field, the anion and cation membranes were recharged by saturation with HCO_3^- and Na^+ ions, respectively. Thus, when buried, soil ions displace the counter-ions at a rate that depends on their activity and diffusion rate in soil solution (Western Ag Innovations Inc., Saskatoon, SK). The probes were buried vertically to a depth of 15 cm, at four randomly selected spots in each monitoring plot, and left to incubate (concurrent with nutrient mineralization incubations). At the end of each incubation cycle, the probes were recovered from the plots, washed with deionized water, and stored in a cool box, which was returned to Western Ag Innovations Inc. at the end of each growing season for elution with 0.5 M HCl and subsequent nutrient analysis. Western Ag Innovations determines NO_3^-, NH_4^+, and PO_4^{3-} with the same standard colorimetric technique used in analyzing the mineralization incubation nutrient concentration.

4.2.4 Analysis of microbial potential activity and functional diversity

The community level physiological profiles (CLPP) of belowground microbes was explored as a measure of microbial potential activity and functional diversity, using the MicroResp^{TM} system (Campbell et al. 2003), following a protocol modified for peat as in Andersen et al. (2013b). Samples for the analysis were collected from all the monitoring plots, within 2 weeks after the commencement of mineralization and ionic resin incubation experiments. Due to logistical challenges, the analysis was done once in the first growing season (during the late season incubation), but was completed three times (early, middle and late) in the second growing season. For each of these sampling campaigns, peat samples were collected at a depth of 0-15 cm at 5 spots within four randomly selected 2 m^2 quadrats (adjacent to the buried bags and probes) in each monitoring plot. The samples were homogenised in a Ziploc bag, and kept at 4 ^{o}C during transfer from the field to the lab, where analysis commenced within a week of sample collection.

In the lab, a portion of each peat sample was used to determine the moisture content gravimetrically. For the analysis, each deep well plate (96 wells) contained samples from three monitoring plots (32 each). About 0.30 g of peat was weighed into each well, and incubated for 72 h at 25 ^{o} C, in the absence of light. After the incubation, 15 carbon sources and water (as a negative control) were added to the peat in each deep well, and replicated twice for each sample. Fourteen (14) out of the 15 carbon sources used in this study included a selection of carbohydrates (fructose, arabinose, glucose, trehalose, N-acetylglucosamine), amino acids (arginine, lysine, alanine) and carboxylic acid (oxalic acid, citric acid, malic acid, γ-aminobutyric acid, α-ketoglutarate, cysteine) commonly found in root exudates. Oil sands process water (OSPW) was also used as a carbon source, since it is anticipated to be the main water source from the constituent materials used in constructing the slopes surrounding the fen (Nwaishi et al. 2015a). It is worthy to note that cysteine, oxalic acid and OSPW were not available during the first growing season's analysis.

Detection plates, containing indicator gel made from selected compounds were read in a microplate reader at an absorbance wavelength of 570 nm before and after 6 h of incubation at 25 ^{o}C. The difference between the absorbance for each deep well after 6 h and at time zero (0) was used to estimate the rates of CO_2 production from individual wells. Microbial potential activity for each sample was estimated as the average well colour development (AWCD) = $\Sigma pi \,/ n\ (p)$, where Σpi is the sum of respiration activities induced by response of soil microbes to all substrates and n (p) is the number of carbon substrates used. Microbial functional diversity consists of both catabolic richness and evenness (Zak et al. 1994; Degens et al. 2001). Since all the substrates were utilized by soil microbes, this study focused on the comparison of catabolic evenness as a measure of functional diversity between plots. Catabolic evenness, the variability in substrate use across the range of substrates tested, was estimated from the respiration response profiles as $E = 1/\Sigma pi^2$ (Magurran 1988; Degens et al. 2001).

4.2.5 Field measurement of net exchange of CO_2

Fluxes of CO_2 were measured from all the monitoring plots in the natural and constructed fen. Measurements were made simultaneously with all the nutrient incubation experiment cycles, on a biweekly basis. An average of all the flux measurements obtained for individual plots, during the growing season was used to estimate the average daily plot-scale fluxes for the same periods as the other experiments in both growing seasons. In the SDL and SMLT plots, flux measurements were duplicated to capture any difference between the saline (*Juncus balticus*) and fresh water (*Carex aquatilis*) vegetation communities.

The procedure and instruments used in measuring CO_2 fluxes followed the description of Munir et al. (2014). Briefly, a chamber made of clear acrylic, with a dimension of 60 × 60 × 30 cm (L×W ×H), was placed over a 60 × 60 cm steel collar, and permanently inserted ~ 20 cm into the ground for the measurement. Instantaneous CO_2 concentration in the chamber was monitored with a portable infrared gas analyser (PP Systems, USA, EGM - 4). To achieve equilibrium in CO_2 concentration between measurements, the chamber was lifted from the collar until temperature and CO_2 concentrations returned to ambient levels. Two small battery-operated fans were attached to the chamber to continuously circulate the air inside during the measurement. Other measurements taken simultaneously with the flux measurement included the temperature inside the chamber, measured with a thermocouple thermometer (VWR Int., USA); and photosynthetically active radiation (PAR), measured with a quantum sensor (PP Systems, USA) placed at the top of the chamber. All measurements were taken at 15 s intervals for up to 1.75 min. Soil temperature was also measured in all the plots, at the depths of 2, 5, 10, 15, and 20 cm using a thermocouple.

Plot-scale net exchange of CO_2 (NEE_{ps}) was calculated using linear change in CO_2 concentration inside the chamber headspace with time, as a function of volume, air temperature and pressure inside the chamber, according to the ideal gas law. To obtain an estimate of plot scale respiration (R_{ps}), CO_2 flux measurements were also taken in the dark by covering the clear chamber with an opaque shroud. Plot scale productivity (GEP_{ps}) was estimated as the difference between NEE_{ps} and R_{ps}.

4.2.6 Statistical analyses

R (R Development Core Team, 2013) was used to perform all the statistical analyses. Prior to analyses, all the response variables were tested for normality of distribution, and when required, appropriate transformations were implemented to meet the assumptions of parametric statistics. The effect of the mulch treatment on all the measured variables was tested, and only found significant effects on EC. Hence, for data analysis, the mulch and no-mulch plots were treated as replicates of the revegetation treatments. One-way analysis of variance (function “aov”, package “stats” (Chambers et al. 1992) was used to test the difference in soil physicochemical properties and environmental variables between the study plots. Linear mixed effect model (function “lme”, package “nlme” (Pinheiro et al. 2011) was used to test the effect of revegetation treatments (fixed factor) on above and below-ground processes over the three growing season monitoring cycles (random factor). A multiple comparison test for significant difference between the revegetation treatments was conducted on the model output using post hoc analyses (Tukey) built into a general linear hypothesis function (“glht”, package “multcomp” (Bretz et al. 2015)).

The temporal variability in above and below-ground processes was also tested with a one-way permutational ANOVAs (function “lm”, package “stats”). Pearson’s correlation analysis was used to analyse the strength of associations (potential biological link) between above and below-ground processes in each revegetation treatment. To identify ecosystem processes that are potential indicators of ecosystem function, principal response curve (function “prc”, package “vegan”) was used. PRC was suggested as an efficient approach to identify drivers of change in the context of ecological restoration (Poulin et al. 2013). Growing season nutrient cycling data, collected in the first 14 months after revegetation of the constructed fen was used to construct the PRC graphs (Figure 5a and 6b). Data collection within the 14 months period focused on the frost-free months; as below-ground processes are known to be slow during the winter months as a result of changes in edaphic conditions such as temperature (Devito et al. 1999). The significant level for all statistical tests was $p \leq 0.05$.

4.3 Results

4.3.1 Physicochemical properties and environmental variables

Relative to the natural fen (REF), peat in the constructed fen plots had significantly lower organic matter ($F_{4, 90} = 27.9$, $p < 0.001$), and peat moisture content ($F_{4, 90} = 16.37$, $p < 0.001$) but higher bulk density ($F_{4, 90} = 19.79$, $p < 0.001$) and EC ($F_{4, 91} = 10.77$, $p < 0.001$; Table 4.1). Post hoc tests indicate that in the constructed fen, re-introduction of vegetation through the moss transfer treatment (i.e. MLT and SMLT) significantly increased the organic matter content ($p =$

0.008) and reduced bulk density ($p = 0.005$) over time, especially when combined with the seedling treatment (SMLT). Peat with high organic matter content also had higher moisture content ($R = 0.74$, $p < 0.001$), lower EC ($R = - 0.54$, $p < 0.001$) and lower bulk densities ($R = - 0.89$, $p < 0.001$). Bulk density correlated negatively with moisture content ($R = - 0.83$, $p < 0.001$) and positively with EC ($R = 0.45$, $p < 0.001$).

Environmental control variables such as water table depth, peat temperature and PAR showed a similar seasonal trend in all the sites, but also varied between the natural and constructed fen plots (Table 4.2). Water table depths decreased throughout the growing season, whereas peat temperature increased; however, water table position and temperature were not correlated ($p > 0.05$). Relative to the natural fen, the constructed fen had a deeper water table ($F_{4, 133} = 5.18$ $p < 0.001$), higher temperature ($F_{4, 133} = 11.3$, $p < 0.001$) and PAR ($F_{4, 109} = 6.38$, $p < 0.001$; Table 4.2). The lower water table in the constructed fen was consistent for all plots except the SMLT plots that maintained a shallow water table that was not significantly deeper than that of the natural fen ($p > 0.05$; Table 4.2).

4.3.2 Nutrient dynamics

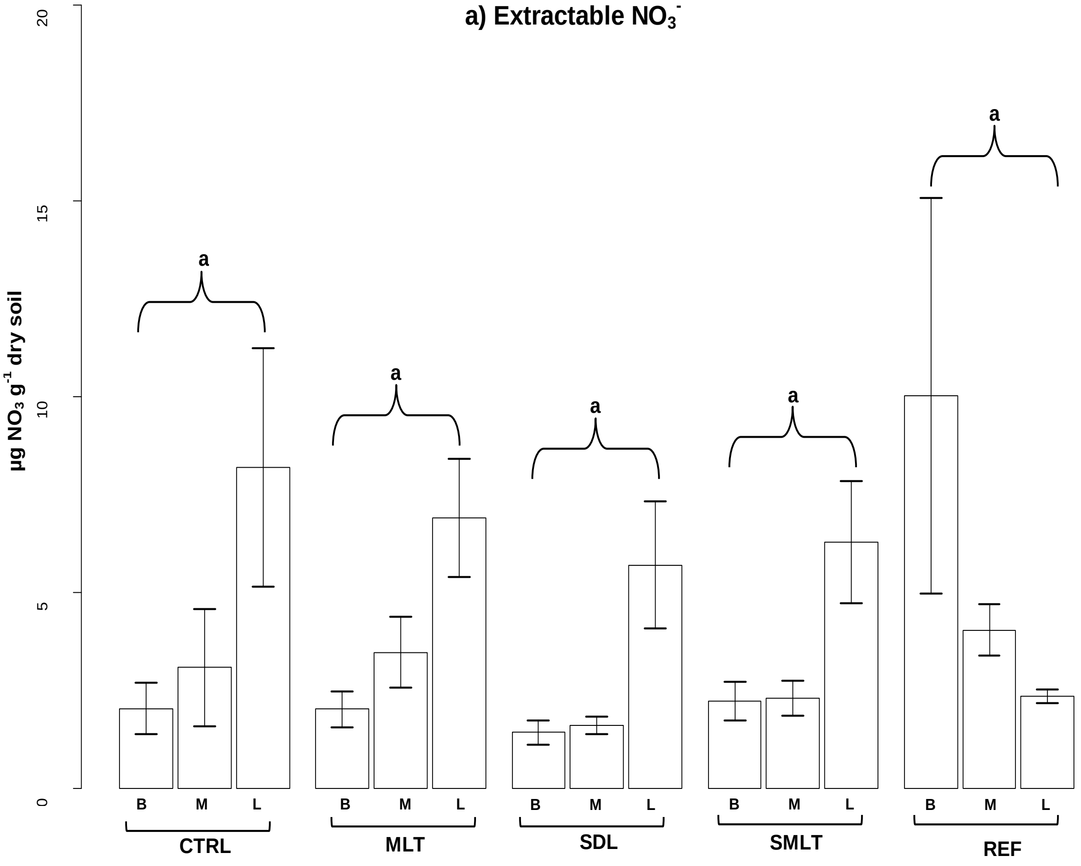
Seasonal patterns in NO_3-N dynamics were observed in all the sites. In the constructed fen, concentration of extractable NO_3^- pools increased through the growing season but decreased in the REF (Figure 4.2a). However, when the three incubations were pooled, extractable NO_3^- pools did not differ among the REF and constructed fen treatments.

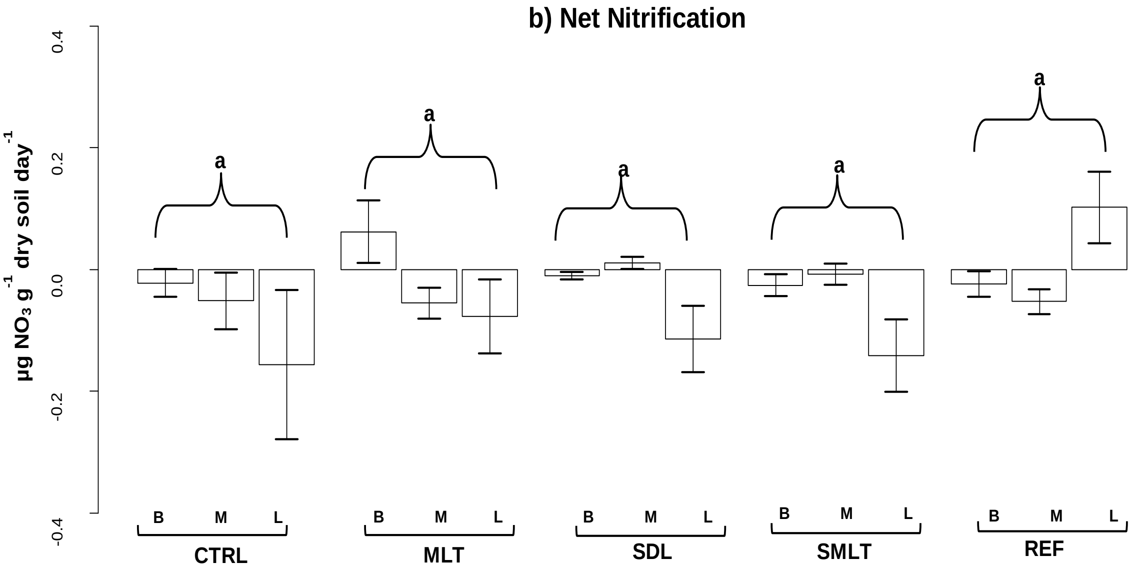
Table 4.1: Physicochemical properties (mean ± std. dev.) of the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) and the Natural reference site (REF) showing organic matter, bulk density, pH and electrical conductivity (EC). The characters a, b, and c are used to indicate significant differences (Tukey HSD test) between treatments.

Sites	Organic Matter (%)	Bulk density (g cm^{-3})	pH	EC (µs cm^{-1})
REF	85 ± 3^{c}	0.09 ±0.01^{a}	6.9 ± 0.4^{a}	226 ± 68^{a}
MLT	66 ± 4^{b}	0.24 ±0.05^{bc}	7.8 ± 0.1^{b}	1022 ± 426^{b}
SDL	61 ± 8^{ab}	0.25 ±0.06^{bc}	7.8 ± 0.2^{b}	1211 ± 566^{b}
SMLT	65 ± 6^{b}	0.22 ±0.03^{b}	7.8 ± 0.2^{b}	1043 ± 527^{b}
CTRL	57 ±12^{a}	0.29 ±0.11^{c}	7.8 ± 0.2^{b}	1226 ± 656^{b}

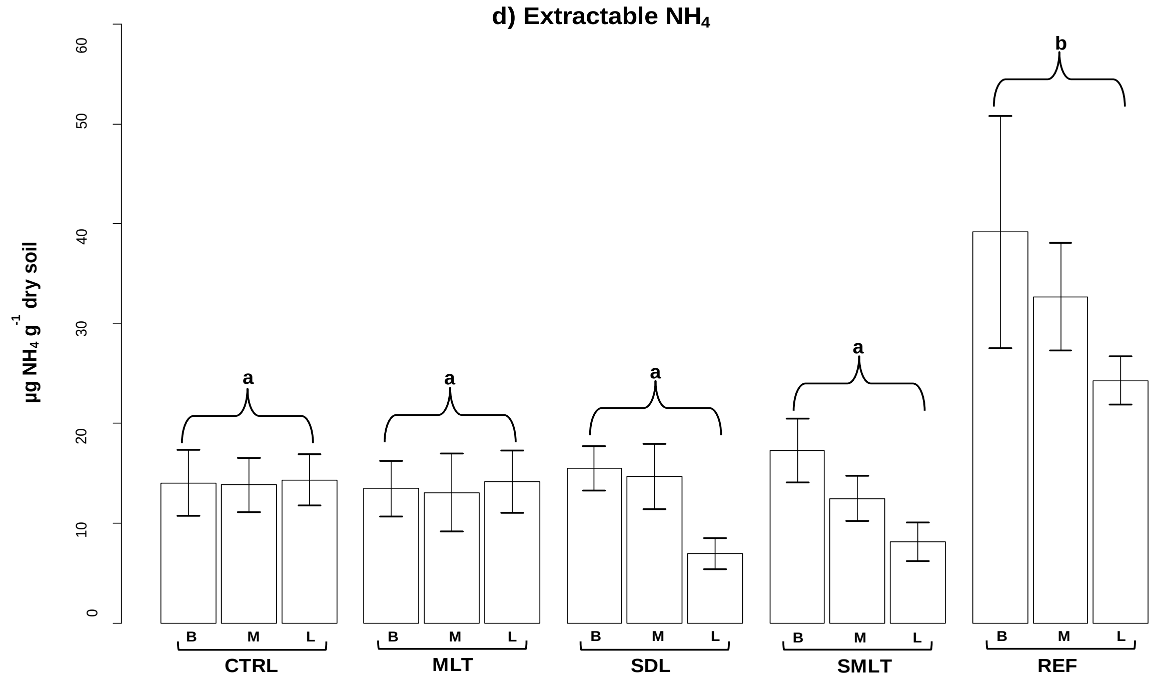
Table 4.2: Growing season profiles of environmental variables showing means (± std. dev.) of water table, moisture content, average peat temperature and photosynthetically active radiation (PAR) at the Beginning (B), Middle (M) and Late (L) periods of the growth seasons across the study sites.

Sites	Watertable Depth (cm)			Moisture Content (% Grav.)			Average Peat Temp. 0 -15 cm depth (^{o}C)			PAR (µmol m^{-2} s^{-1})		
	Begin	Mid	Late	Begin	Mid	Late	Begin	Mid	Late	Begin	Mid	Late
REF	0.8 ± 0.2	- 0.2 ±0.4	- 5.3 ± 0.5	90 ± 2	90 ± 1	89 ± 1	11.4 ± 1.7	14.7 ± 1.2	17.3 ± 0.7	359 ± 289	423 ± 415	267 ± 126
MLT	-11.5 ±14	-11.5 ±13	- 9.4 ±13	78 ± 2	78 ± 4	79 ± 2	15.8 ± 2.3	17.6 ± 1.2	18.2 ± 1.8	880 ± 156	1039 ± 450	882 ± 352
SDL	- 3.7 ± 5	- 6.6 ± 5	- 7.1 ± 5	78 ± 2	77 ± 6	76 ± 7	17.4 ± 2.6	17.7 ± 1.3	18.3 ± 1.9	578 ± 333	1039 ± 438	821 ± 330
SMLT	- 1.0 ± 2	- 3.9 ± 3	- 4.4 ± 4	82 ± 2	81 ± 3	77 ± 7	16.2 ± 0.6	17.4 ± 0.5	17.9 ± 1.4	534 ± 106	929 ± 439	787 ± 386
CTRL	- 3.7 ± 5	- 7.2 ± 8	- 6.5 ± 8	76 ± 7	73 ±10	71 ±11	15.5 ± 1.5	17.0 ± 1.1	18.3 ± 1.7	465 ± 309	1057 ± 307	772 ± 277


a) Extractable NO_3^-


b) Net Nitrification


c) NO$_3$ Supply Rate
µg NO$_3$ 10 cm^{-2} day^{-1}
0.0
0.1
0.2
0.3
0.4
0.5
a
a
a
a
a
B
M
L
B
M
L
B
M
L
B
M
L
B
M
L
CTRL
MLT
SDL
SMLT
REF


d) Extractable NH$_4$
µg NH$_4$ g^{-1} dry soil
0
10
20
30
40
50
60
b
a
a
a
a
B
M
L
B
M
L
B
M
L
B
M
L
B
M
L
CTRL
MLT
SDL
SMLT
REF

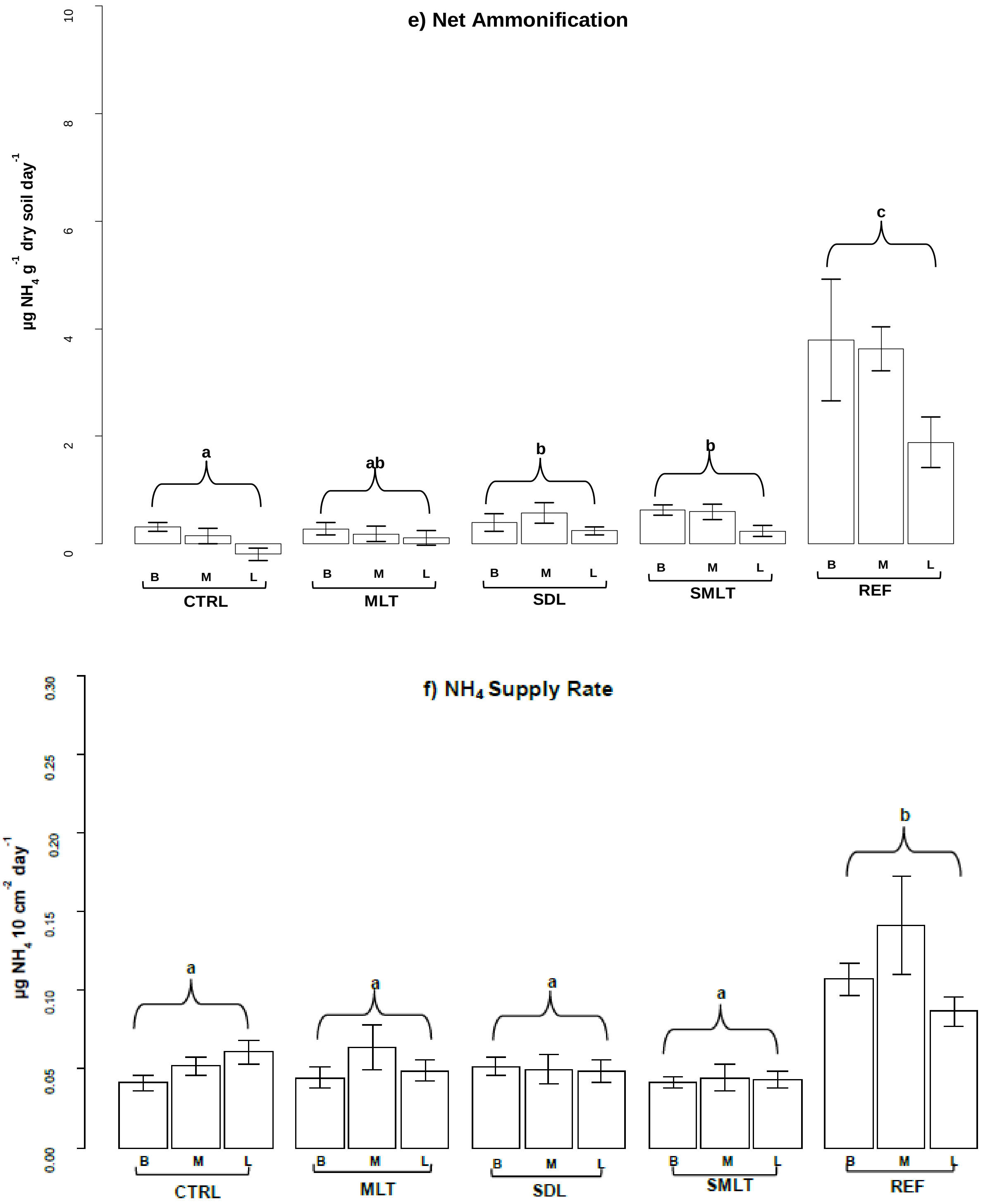
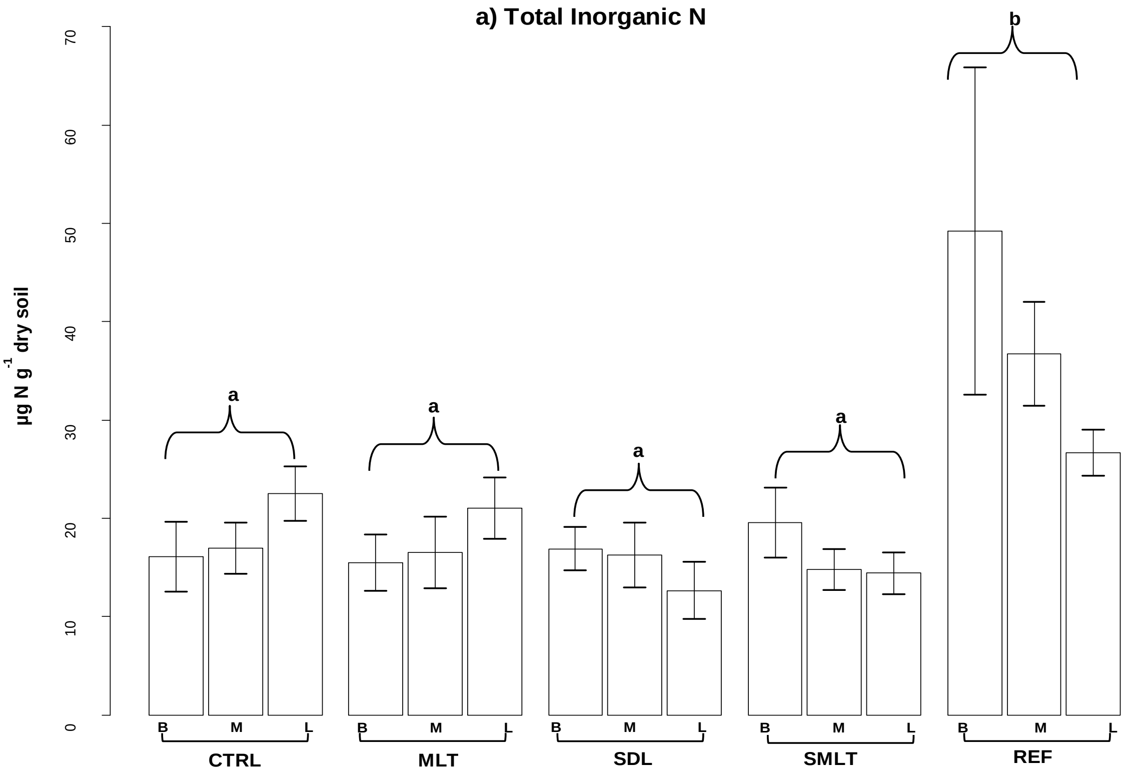
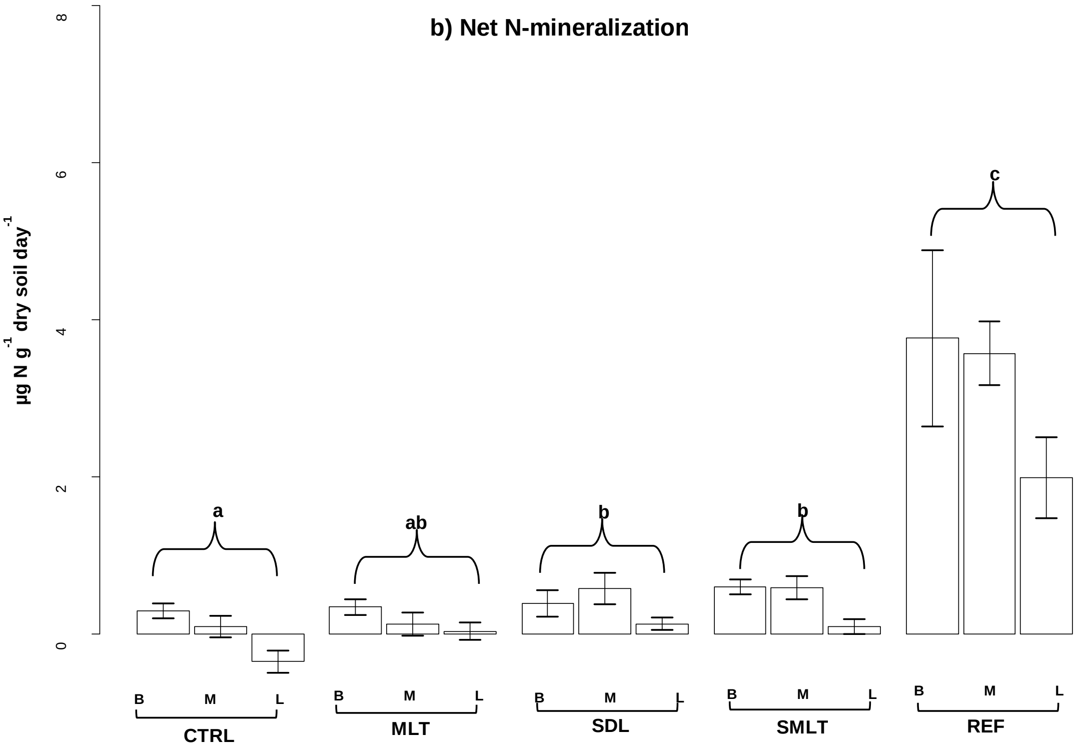


Figure 4.2: Growing season dynamics of extractable NO_3^- (a) and NH_4^+ (d) pools (mean ± std. err.), daily net nitrification rate (b) and ammonification rates (e; mean ± std. err.), and daily NO_3^- (c) and NH_4^+ (f) supply rates (mean ± std. err.) across the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) and the Natural reference site (REF) in the Beginning (B), Middle (M) and Late (L) periods of the growth seasons. The characters a, b, and c are used to indicate significant differences (Tukey HSD test) between treatments.

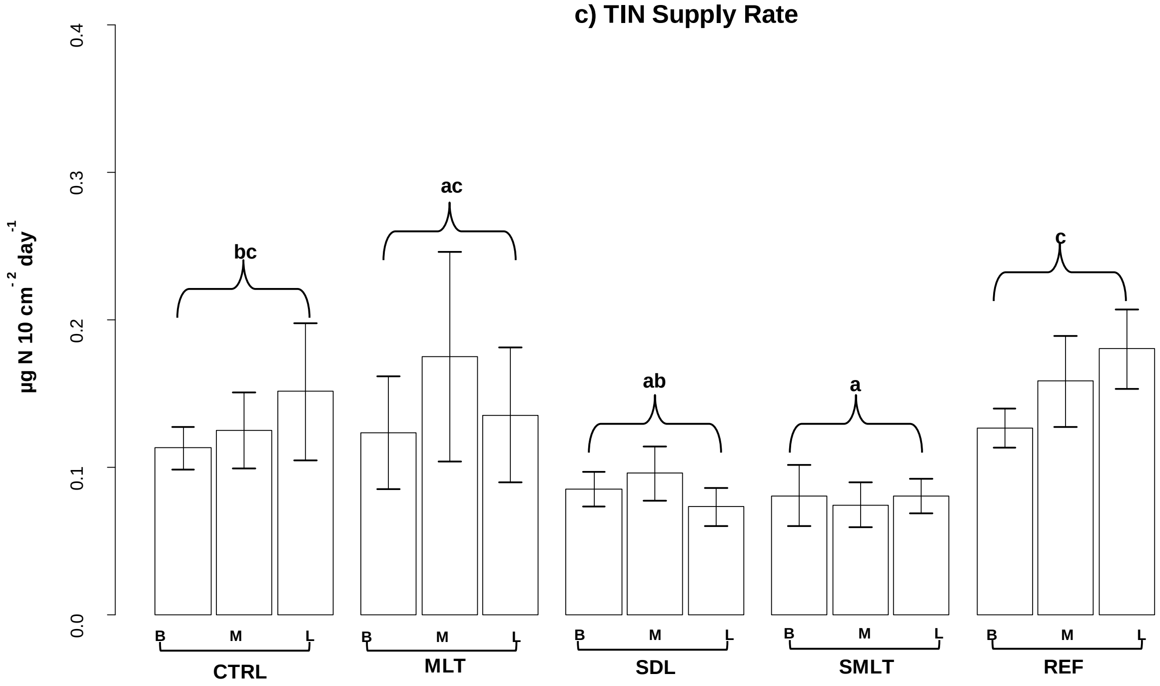
Net nitrification was dominated by negative rates (i.e. immobilization or denitrification) at all the sites, except in the REF where daily net nitrification rates increased later in growing season (Figure 4.2b). This late season increase in net nitrification was reflected in an increase of NO_3^- supply in the natural fen (Figure 4.2c). In contrast to what was observed for extractable NO_3^- pools (Figure 4.2a), no distinct seasonal pattern was observed for NO_3^- supply rates in the constructed fen (Figure 4.2c). NO_3^- supply rates were greater in the CTRL and MLT plots relative to the REF and plots with vascular plants (SDL and SMLT). A strong correlation ($R = 0.82$, $p < 0.001$) was observed between NO_3-N cycling processes in the natural fen whereas in the constructed fen, a stronger correlation was observed between extractable NO_3^- pools and net nitrification rates in the SDL ($R = - 0.95$, $p < 0.001$) and SMLT ($R = - 0.97$, $p < 0.001$) plots. NO_3^-–N cycling processes were comparable across the study sites, but proceeded at significantly different rates at different times over the growing season ($F_{1,\ 136} = 3.33$, $p = 0.03$). Concentration of extractable NH_4^+-N pool was lower in the constructed fen (Figure 4.2d; $F_{4,\ 131} = 8.77$, $p < 0.001$), and did not vary over the growing season except in the SMLT plot, which followed a decreasing seasonal pattern similar to that observed in REF (Figure 4.2d).

Net ammonification rates showed a decreasing seasonal pattern in the REF, but remained positive for all sites through the growing season. Net ammonification rates were significantly lower in the constructed fen ($F_{4,\ 131} = 28.94$, $p < 0.001$) but did not differ among treatments within the constructed fen. Given that NH_4^+ dominated total inorganic N (TIN) cycling processes across sites, net N-mineralization (Figure 4.2b; $F_{4,\ 131} = 48.22$, $p < 0.001$) and concentrations of TIN pools (Figure 4.3a; $F_{4,\ 131} = 5.51$, $p < 0.001$) were higher in the REF and also decreased over the growing season. Among the constructed fen plots, similar seasonal patterns and higher rates of net N-mineralization were observed in the SDL and SMLT plot (Figure 4.3b).

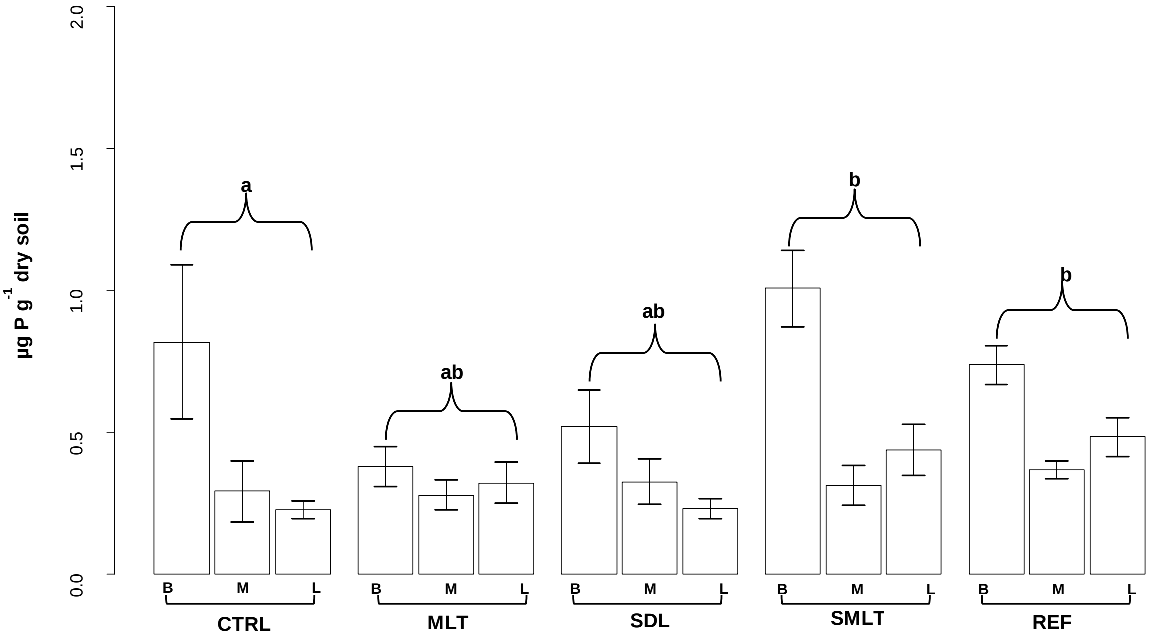

a) Total Inorganic N

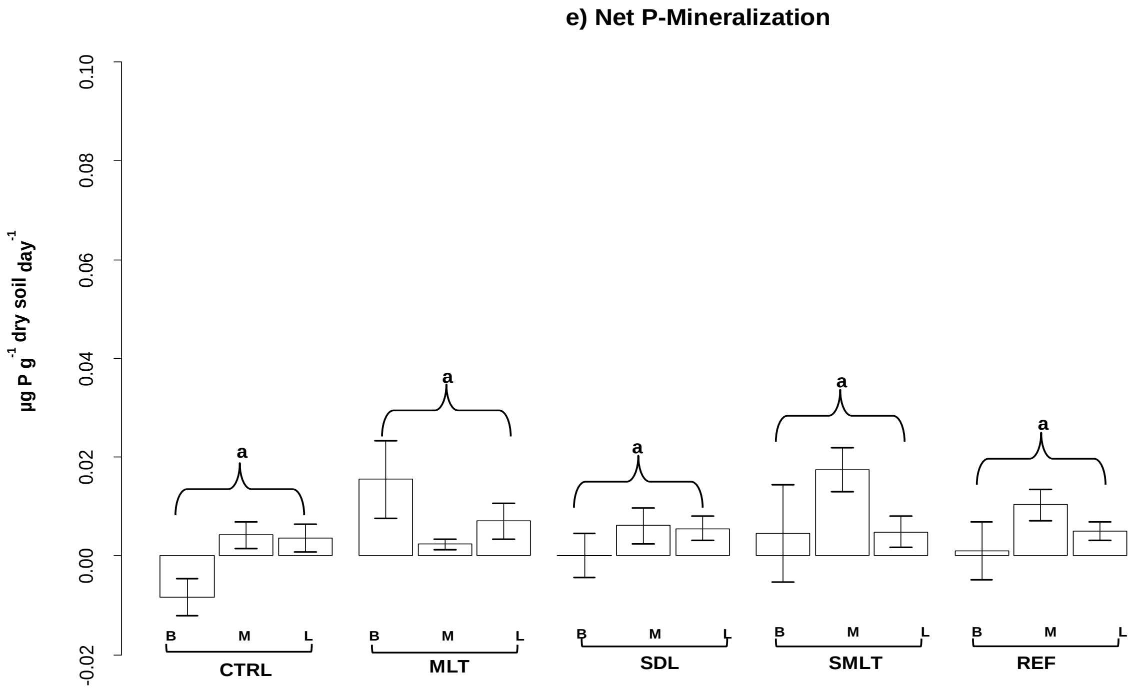

b) Net N-mineralization

c) TIN Supply Rate

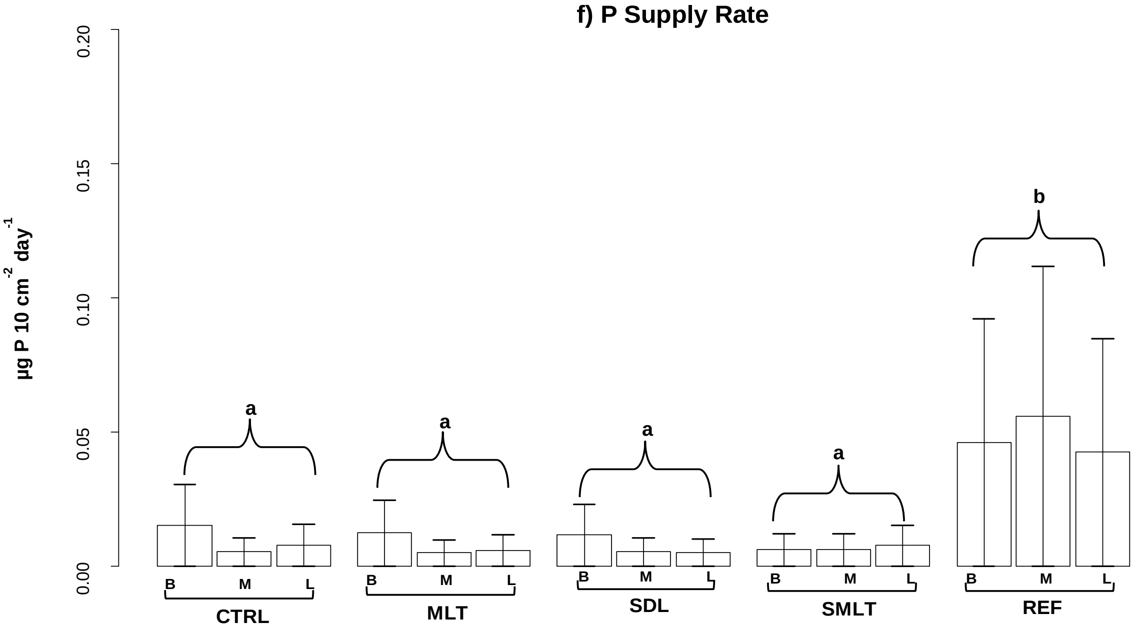


d) SRP





e) Net P-Mineralization



f) P Supply Rate

Figure 4.3: Growing season's dynamics of total inorganic N (a) and SRP (d) pools (mean ± std. err.), daily net N (b) and P (e) mineralization rates (mean ± std. err.), and daily N (c) and P (f) supply rates (mean ± std. err.) across the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) and the Natural reference site (REF) in the Beginning (B), Middle (M) and Late (L) periods of the growth seasons. The characters a, b, and c are used to indicate significant differences (Tukey HSD test) between treatments.

Net P mineralization rates (Figure 4.3e) did not vary significantly ($F_{4, 131} = 1.83$, $p > 0.05$) among the study sites. Seasonal patterns in the concentration of SRP pools (Figure 4.3d) were similar between the natural fen and the plots that received the moss treatment, but different from the CTRL and SDL plots. Concentrations of SRP were significantly higher ($F_{4, 131} = 4.03$, $p < 0.001$) in the REF and SMLT than all the other study plots. The daily supply rate of SRP (Figure 4.3f) was negligible in the constructed fen plots, and was significantly higher ($F_{4, 131} = 24.67$, $p < 0.001$) in the natural fen. There was no significant association ($R = 0.02$, $p > 0.05$) between P cycling processes in all the sites.

4.3.3 CO_2 exchange and plot scale productivity

Average seasonal NEE_{ps} rates differed significantly among the study sites (Figure 4.4a; $F_{4, 107} = 2.71$, $p = 0.03$). However, post hoc tests showed that the difference between NEE_{ps} of CTRL and SMLT ($p = 0.009$, Figure 4.4a) was a major source of variation within the constructed fen. Relative to the CTRL plots, re-introduction of vegetation in the constructed fen significantly increased the NEE_{ps} sink potential and consequently, higher average seasonal rates of R_{ps} and GEP_{ps}, especially in the SMLT plots (Figure 4.4b and c; $p < 0.001$). Generally, among the constructed fen plots, average seasonal rates of NEE_{ps}, R_{ps} and GEP_{ps} increased with an increase in vegetation density and diversity as follows: SMLT > SDL > MLT > CTRL.

4.3.4 Microbial potential activity and functional diversity

The microbial potential activity was not significantly different between the study sites (Figure 4.5a; $F_{4, 107} = 1.33$, $p > 0.05$), but it varied significantly over the growing season ($F_{1, 112} = 10.15$, $p = 0.002$). Apart from the MLT plot, microbial potential activity in all of the other monitoring sites decreased linearly over the growing season (Figure 4.5a).

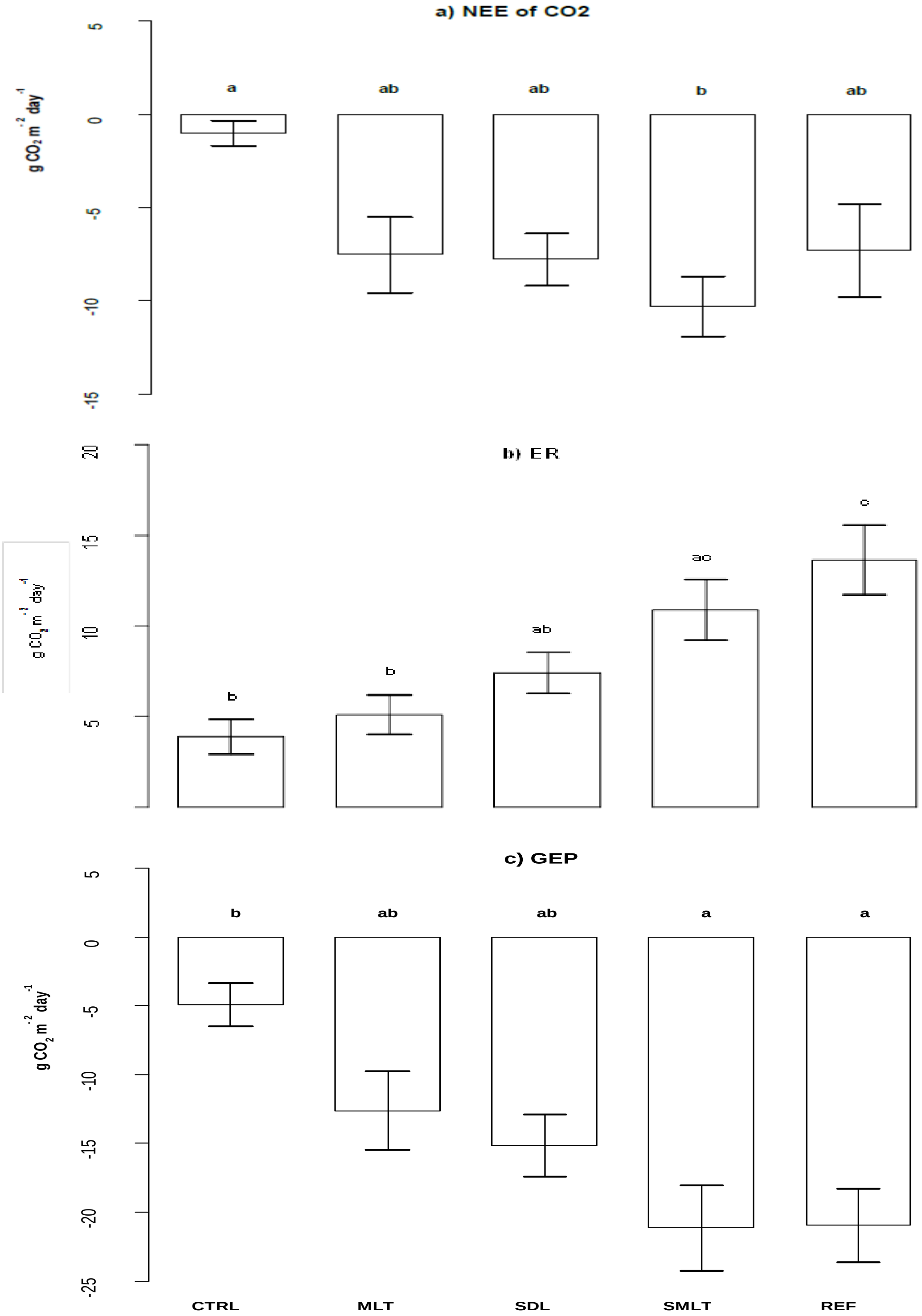


Figure 4.4: Average (± std. err) growing season's measurement of a) Net ecosystem exchange of CO_2 (NEE), b) Ecosystem respiration (ER) and c) Gross ecosystem productivity (GEP) across the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) and the Natural reference site (REF). Negative values indicate CO_2 removal from the atmosphere, while positive means release to the atmosphere. The characters a, b, and c are used to indicate significant differences (Tukey HSD test) between treatments.

However, among the sites that followed a similar linear decrease in microbial potential activity, the CTRL plots had the lowest activity rate at the early season peak (Figure 4.5a). Microbial potential activity was positively correlated with concentrations of extractable N ($R = 0.43$, $p < 0.036$) and SRP ($R = 0.65$, $p < 0.001$) in the constructed fen, but with only SRP in the natural fen ($R = 0.52$, $p = 0.026$). Microbial functional diversity (catabolic evenness), decreased significantly over time in the constructed fen plots ($F_{1,\ 112} = 11.3$, $p < 0.001$), but remained stable throughout the growing season in the natural fen (Figure 4.5b).

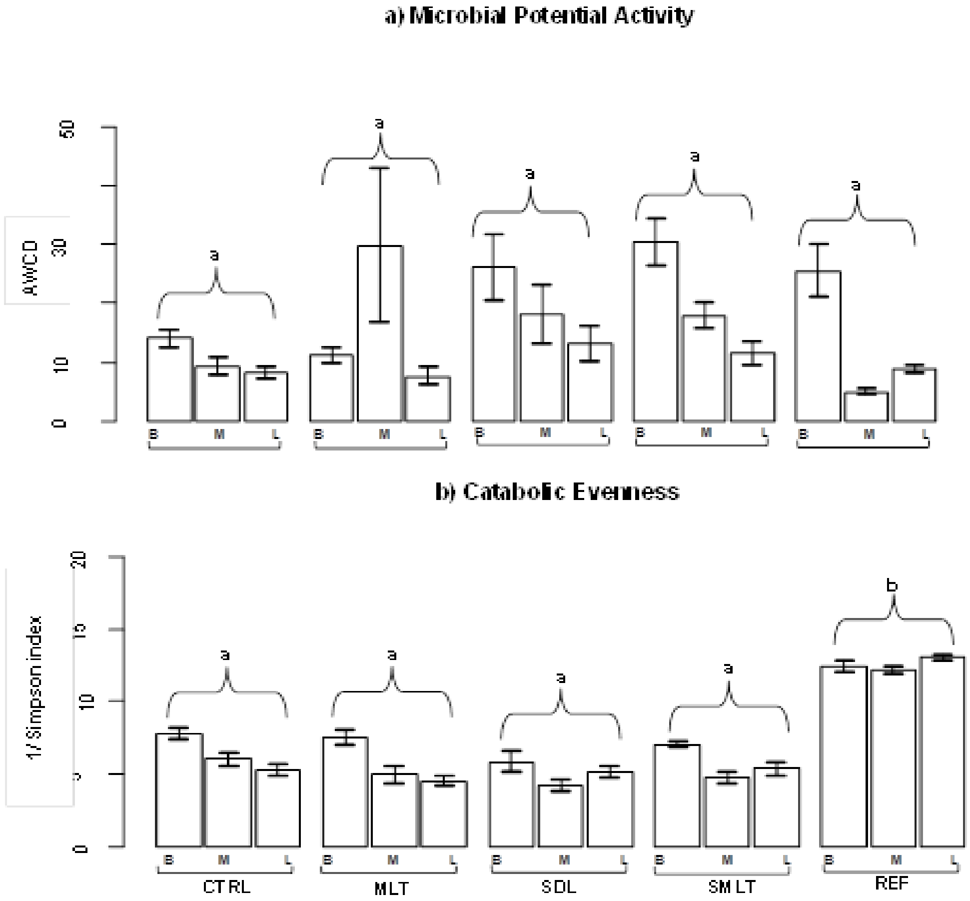


Figure 4.5: Patterns of a) Microbial potential activity (mean ± std. err) and b) Catabolic evenness (mean ± std. err) during the growing seasons across the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) and the Natural reference site (REF) in the Beginning (B), Middle (M) and Late (L) periods of the growth seasons. The characters a, b, and c are used to indicate significant differences (Tukey HSD test) between treatments.

4.4 Discussion

4.4.1 Impact of revegetation strategies on the recovery edaphic variables

A major challenge in reclamation is recreating suitable edaphic conditions such as the peat physical properties that support ecosystem processes of natural analogues (Audet et al. 2015). Consistent with the findings of a previous study (Nwaishi et al. 2015b), results this study confirm that two growing seasons after revegetation of the constructed fen, characteristics of the peat (*e.g.* moisture content, organic matter and bulk density) still vary significantly from that of the natural fen. The key role of these peat characteristics in nutrient transformation processes (Updegraff et al. 1995) implies that poor peat physical quality would likely influence the biogeochemical functioning of the constructed fen. However, the finding that organic matter content and bulk density in MLT and SMLT treatments evolved towards conditions at REF is in line with the idea that revegetation should facilitate the recovery of natural-like peat characteristics (Nwaishi et al. 2015b).

Thus, introducing fragments from a natural peatlands in reclamation such as in the moss transfer plots, appears to be an efficient means of facilitating the recovery of a functional soil (Bradshaw 1997; Mackenzie & Naeth 2010). Similar to other studies (Bradshaw 2000; Tordoff et al. 2000), the improved soil characteristics observed in the SMLT plots supports the idea that artificially introducing plants can complement the re-establishment and influence of transferred live vegetation (moss) by providing structural protection and suitable microclimate, especially under extreme edaphic conditions such as those present in the constructed fen (Nwaishi et al. 2015b). Result shows that the mosses in the MLT plots had the deepest water table, while in the SMLT plots where MLT was combined with SDL, a modulation of near surface water table was observed (Table 4.2). Although this could be attributed to the inability of regenerating mosses to sustain a strong capillary rise of water and efficient water retention capacity under degraded peat substrate (McCarter & Price 2013), a recent study did not report any significant control of vegetation type on soil water dynamics in the constructed fen (Scarlett, 2015).

4.4.2 Belowground processes in the natural and constructed fens

Revegetation strategies implemented in the constructed fen influenced belowground processes in different ways, and these processes also varied from the natural fen. This study suggest that the revegetation of degraded peat facilitates the supply of photosynthetically-derived carbon sources (through rhizodeposition), which stimulates belowground microbial activity, and subsequent increase in nutrient uptake (Andersen et al. 2013a; Elliott et al. 2015). For instance, relative to the natural fen, net ammonification (the process of transforming organic-bound N to NH_4^+) was likely limited by substrate constraints (recalcitrant peat) in the constructed fen. However, the input of fresh carbon by the rhizosphere in plots re-vegetated with seedling treatments (SDL and SMLT) could over-ride the substrate limitation, resulting in net ammonification rates that are significantly higher ($p = 0.003$) than rates observed in the un-vegetated CTRL. This is similar to what was reported in studies looking at microbial processes in oil sand sites reclaimed using a mixture of peat and mineral soil as a soil capping layer (peat-mineral mix, PMM), where nitrogen in the pore water and roots derived from the PMM were the most important stimulants for microbial growth (Dimitriu et al. 2010; Noah et al. 2014)

In the natural fen, seasonal variations in the rate and direction of N-cycling processes (Figure 4.3) suggests that belowground processes change from anaerobic dominance (*e.g.* ammonification) to become aerobically favorable later in the season, in response to redox conditions driven by water table fluctuation (Table 4.2). Lower rates of microbial potential activity following seasonal water table drawdown adds support to a dominance of anaerobic microbial nutrient transformation processes in natural peatlands (Croft et al. 2001; Andersen et al. 2006). Although anaerobic N-mineralization also dominated N-cycling processes in the constructed fen, NO_3^- mineralization (nitrification) rates did not increase (Figure 4.2b) as redox conditions became aerobically favorable with water table drawdown later in the growing season (Table 4.2). This could be attributed to a dominance of ammonifying bacteria biomass over nitrifying bacteria in the exposed catotelm peat, which is anticipated due to absence of the original acrotelm peat (a niche for nitrifying bacteria) in the constructed fen (Nwaishi et al. 2015b). Given that microbial catabolic evenness in the constructed fen (Figure 4.5b) correlated positively with water table drawdown (Table 4.2), it can be argued that the catabolic potential may be adversely affected by temporal variation in edaphic conditions due to low functional diversity (Allison & Martiny 2008) in the reclamation plots. Further evaluation of the microbial community through molecular approaches will help connect belowground processes with changes in key groups of organisms that may be associated with disturbances typically found in post oil-sand landscapes, such as potential contaminants (*e.g.* poly-aromatic hydrocarbons) (Noah et al. 2014).

The difference in NO_3^- supply rates between the CTRL and seedling treatment plots (SDL and SMLT; Figure 4.2c) suggests a higher competitive demand between vegetation and microbial communities in the rhizosphere of these plots. Despite the seasonal decline in nitrification rate (Figure 4.2b), there was an increasing concentration of extractable NO_3^- in the constructed fen later in the growing season (Figure 4.2a), which is not reflected in the supply rate (Figure 4.2c). Indeed, the strong positive correlation between late season spikes in extractable NO_3^- pools and immobilization of NO_3^- in the constructed fen concurs with the hypothesis that following revegetation; microbial nutrient immobilization will be a dominant process (Weintraub

& Schimel 2003; Westbrook & Devito 2004). This is especially true in the seedling plots, as microbes will favour biomass build-up. Similarly, studies looking at microbial communities in degraded peat have shown that the presence of biological drivers such as rhizosphere effect reactivates microbial activity, which leads to subsequent immobilization of available N pools and development of an active microbial biomass (Fisk et al. 2003; Andersen et al. 2006; Artz 2009; Urbanová et al. 2011). Consistent with these studies, results suggest that interactions between the chemical and biological component of belowground processes are central to the development of a functional microbial community, without which the recovery of biogeochemical functioning in the constructed fen will not be possible. This functional recovery can be delineated as the time in subsequent growing seasons, from which microbial immobilization of NO_3^- is compensated by increased mineralization rate, leading to the release of NO_3^- for vegetative uptake.

The ratio of NH_4^+/ NO_3^- has been shown to be higher in natural peatlands, while lower ratios have been reported as a consequence of accelerated nitrification associated with peatland disturbances (Macrae et al. 2013; Nwaishi et al. 2015b; Elliott et al. 2015). Therefore, the balance between NH_4^+/ NO_3^- concentrations can be explored as a functional indicator of the status of N-cycling in the constructed fen. Unlike the low ratio previously reported by Nwaishi et al (2015b), results indicate that the constructed fen now has a higher NH_4^+/ NO_3^- ratio, which is not significantly different from that of the natural fen ($F_{4,\,131} = 1.20$, $p > 0.05$). This balance of inorganic-N ratio may be explained by either the reduction of NO_3^- following rewetting of the constructed fen or the high assimilation of NO_3^- in the rhizosphere of vascular plants (Limpens et al. 2004; Wiedermann et al. 2009), which are the dominant plant species in the constructed fen. Although N-mineralization rates were very low in the constructed fen plots (Figure 4.3b), the concentration of N available for plant assimilation was similar to that of the natural fen, where higher mineralization was observed (Figure 4.3c). The latter suggest the possibility of an exogenous N source overriding microbial N immobilization in the constructed fen (Gerdol et al. 2006). A study looking at exogenous N deposition in the Athabasca Oil Sands Region has shown that terrestrial sites within ~30 km radius from the main oil sands developments are significantly affected by industrial contributions to atmospheric NO_3^- and NH_4^+ deposition (Proemse et al. 2013). The location of the constructed fen makes it susceptible to elevated deposition of inorganic N from adjacent industrial sites, which will consequently affect the inorganic N ratio and the balance of N cycling processes.

The reclamation approach used in the constructed fen involved the removal of the upper (30 cm) peat horizon to avoid complicating the vegetation re-introduction experiment with seedbanks transferred from the donor to the constructed fen. Considering that higher P concentrations are usually found in the lost upper peat horizon (Litaor et al. 2005; Andersen et al. 2006; Damman 1978), a lower concentration of P was anticipated in the constructed fen. Conversely, results shows that re-introduction of live moss and vascular plant rhizomes as part of MLT increased the average seasonal concentration of SRP, especially in the SMLT plot, which did not vary significantly from the natural fen. Consistent with previous studies (Chapin et al. 2003; Andersen et al. 2013b), P mineralization was negligible in both the natural and constructed fen over the two study periods. However, the concentration of SRP available for plant root assimilation was significantly higher in the natural fen (Figure 4.3f), where the upper peat layer is still intact, and the chance of leaching is reduced by a stable water table.

4.4.3 Effect of revegetation strategies on above and below-ground linkages

The role of plants as the biological link between above and below-ground processes have been hypothesized and tested in ecological studies (Kowalchuk et al. 2002; Wardle et al. 2004; Wardle

& Peltzer 2007). In the species-rich natural fen, a strong positive association between above-ground plant productivity and below-ground microbial activities were observed (Table 4.3). Nitrification and P availability are also paramount to the significant correlation between above and below-ground processes in the natural fen.

Table 4.3 Pearson's correlation coefficients for significantly correlated interactions between above and below-ground processes in the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) and the Natural reference site (REF).

Vegetation Treatments	Aboveground	Belowground							
		SRP	P Supply Rate	Microbial Activity	Nitrification Rate	Extractable NO_3	NO_3 Supply Rate	NH_4 Supply Rate	TIN Supply Rate
REF	NEE	0.53[a]		0.61[b]	0.65[b]		0.55[b]		
	GEP	0.74[b]		0.75[c]					
	ER	-0.46[a]	0.47[a]						
SMLT	NEE		0.41[a]		-0.52[b]	0.56[b]	0.61[b]	0.68[c]	0.73[c]
	GEP				-0.59[c]	0.61[b]	0.62[c]	0.70[c]	0.74[c]
	ER				0.59[c]	-0.58[c]	-0.56[b]	-0.64[c]	-0.68[c]
SDL	NEE								
	GEP						0.55[b]	0.64[c]	0.66[c]
	ER						-0.58[b]	-0.74[c]	-0.73[c]
MLT	NEE	-0.49[a]				0.52[b]			
	GEP	-0.55[b]				0.55[b]			
	ER	0.55[b]				-0.47[a]			
CTRL	NEE				-0.51[a]				
	GEP								
	ER								

[a] **Significant at the 0.05 probability level;**

[b] **Significant at the 0.01 probability level;**

[c] **Significant at the 0.001 probability level.**

Interestingly, these below-ground processes are both known to be limited in natural peatlands (Devito et al. 1999; Chapin et al. 2003). Thus, peatlands found in the vicinity of industrial setting might experience an increase in productivity linked with greater availability of nutrients that are usually found to be limited in natural peatlands.

Relative to CTRL, the re-vegetated plots in the constructed fen showed stronger correlations between above-ground productivity and below-ground nutrient cycling processes. The significant positive correlation between above-ground productivity and N cycling processes in the re-vegetated plots (Table 4.3) suggest that higher productivity, especially in the seedling plots, is associated with N availability. However, P availability significantly correlated with above-ground processes in plots where mosses were established. Similar patterns were reported by studies looking at N and P availability in species-rich ecosystems, which suggested that the P requirements of mosses are larger than those of vascular plants; hence P limitation and N availability decreases above-ground productivity of mosses and may alter the plant species composition to the benefit of vascular plants (Aerts et al. 1992; Niinemets & Kull 2005; Andersen et al. 2006; Limpens et al. 2011). The highest productivity observed in the vascular plant plots (Figure 4.4c), with N/P ratios greater than 20 (data not shown) indicates that the nutrient status in the constructed fen is more favourable to vascular plants establishment. In agreement with findings from restoration studies on post-harvested peatlands in Eastern Canada (Andersen et al. 2010b), results also suggest that the availability of P will be a good indicator of the successful re-establishment of a species-rich plant community in the constructed fen, but P fertilization might be required to enhance above-ground productivity of mosses at the initial stage of revegetation.

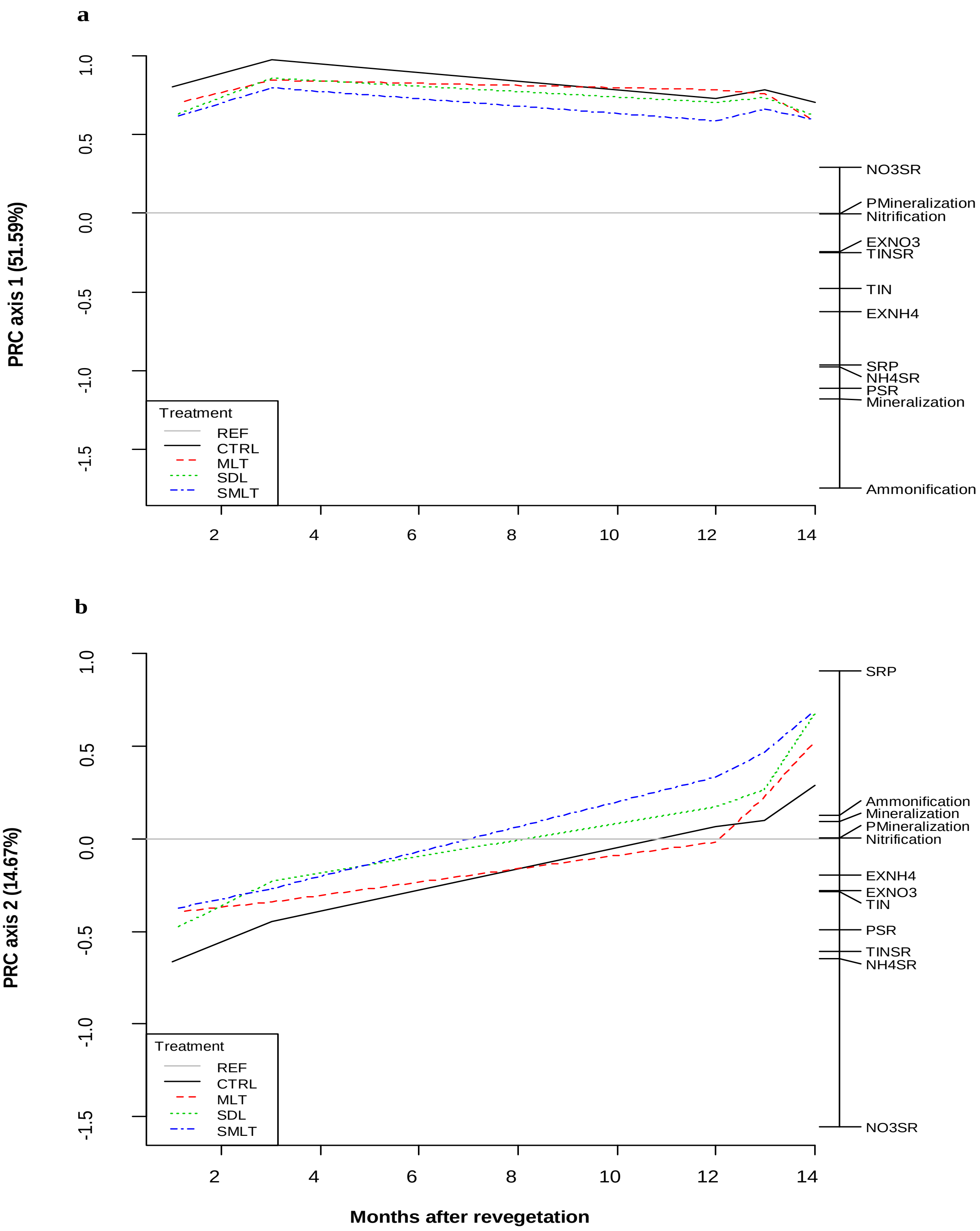


Figure 4.6: PRC for the first axis of RDA testing the effect of site × time on below-ground processes and showing a) the dominant temporal trajectory in nutrient cycling processes and b) representing a secondary temporal trajectory in nutrient cycling processes across the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) relative to the zero line, which represents the Natural reference site (REF).

4.4.4 Temporal changes in below-ground processes and functional trajectory of the constructed fen

Principal Response Curves (PRC) have been suggested as an effective approach for tracking the trajectory of reclaimed sites relative to a natural reference in ecological monitoring (Poulin et al. 2013; Andersen et al. 2010b). A PRC analysis was used to summarize the dominant temporal patterns in below-ground processes, and also to identify the key processes that can be used as functional indicator of ecosystem trajectory. From the first axis of the RDA (Figure 4.6a), it appears that ammonification (Figure 4.2e) followed by N-mineralization (Figure 4.3b) and P supply (Figure 4.3f) were the dominant processes driving the temporal differences between the natural and constructed fen plots over the period studied. The observed differences increased at the middle of the growing seasons, which corresponds with the summer peak, when below-ground processes have been reported to be most active (Andersen et al. 2010a; 2013; Macrae et al. 2013). The results also show that the vegetated plots, especially the SMLT were on a closer trajectory to the natural fen than the CTRL, and seemingly became more comparable to each other towards the end of the second growing season (converging lines). Given that the peak of the difference between the constructed fen and the natural fen is higher in the first than the second growing season, in the future, it will be interesting to see if the trajectory of these dominant processes in the vegetated plots continues to follow a downward trend towards the natural reference, or if it will remain different than the reference over time, as was observed in other ecological restoration studies (Mastrogianni et al. 2014; Elliott et al. 2015).

The second axis of the RDA (Figure 4.6b) highlights the secondary temporal trajectory of below-ground processes in the constructed fen relative to the natural fen. The PRC shows that relative to the REF, the concentration of SRP in the nutrient pools is increasing over time in the constructed fen plots, while N supply processes (NO_3, NH_4, and TIN) are decreasing. Considering the variability in timing of the treatment lines crossing through the reference point (7 months for SMLT, 8 for SDL, 10 for CTRL and 12 for MLT), it can be hypothesized that the higher demand for N in vascular plant-dominated plots could be driving a reduction in the N/P ratio over time, leading to the increasing SRP concentration in the nutrient pools. Lowering of the water table and subsequent redox conditions could also be contributing to the late season increase in SRP concentration (Shenker et al. 2005; Zak et al. 2010). With the exception of the CTRL, convergence of the trajectory lines for all the re-vegetated plots at the beginning and end of this preliminary monitoring highlight the influence of vegetation on the recovery of below-ground process. Given that PRC results are greatly influenced by the choice of reference site, in the future, the identification of potential functional indicators will be most efficient if data from more than one reference site within the study region is used in constructing the PRC plots (Suganuma & Durigan 2015)

4.5 Conclusion and recommendations for future monitoring of the constructed fen

By exploring the temporal patterns in above and below-ground processes, this study highlights the importance of integrating these processes in reclamation monitoring. Re-introduction of vegetation through the combination of moss layer transfer and seedling establishment improved the peat quality (*e.g.* higher organic matter) relative to the non-vegetated CTRL plots. The improved peat quality and associated supply of labile substrates reactivated microbial communities driving below-ground processes, which was reflected in higher rates of above and below-ground respiration in plots with most diverse plant species, specifically the SMLT plots.

Higher rates of plant productivity, nutrient cycling and acquisition observed in the SMLT plots could be explained as the key responses commonly associated with species-rich plant communities in restoration experiments. Thus, the biological link between above and below-ground processes are most likely to be established in plots where fresh substrate supply through rhizodeposition and/or application of a live moss layer is capable of reactivating dominant microbial-mediated processes.

From a management perspective, it is recommended that future ecological monitoring aimed at tracking the recovery of biogeochemical functions in constructed fens should initially focus on the state of microbial-mediated nutrient cycling processes before progressing to their output (*e.g.* nutrient pools) and subsequent above-ground productivity. Based on the findings of this study, ammonification and N-mineralization present potential functional indicators of a fen's trajectory towards conditions present in the natural reference. Finally, timing of monitoring within the growing season is also important to capture the range of seasonal variabilities, while a continuous multi-year monitoring will be useful to identifying inter-annual variabilities for predicting long-term trajectory of biogeochemical functions in the constructed fen.

Chapter Five

Preliminary Assessment of Greenhouse Gas Emissions from a Fen Peatland Constructed on a Post-Oil Sands Mining Landscape

5.1 Introduction

Industrial development processes such as the mining of natural resources are often associated with land-use change. On a global scale, changing land-use patterns have been identified as one of the major factors that affect the exchange of greenhouse gases (GHG) such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) between terrestrial ecosystems and the atmosphere (IPCC 2000; Greenhalgh & Daviet 2006). The effect of land-use change on GHG emissions is heightened in cases where the end-product of the changed land-use upsets the natural source – sink balance of an ecosystem, contributing to enhanced GHG emissions. In Canada for instance, the expansion of oil sands development has been identified as a potential major contributing component of the national GHG emission budget (Grant et al. 2013; Environment Canada 2014). Although the process of upgrading the bitumen to synthetic crude oil is the primary process leading to intensive GHG emission, the land-use changes associated with bitumen extraction from large areas of uplands and wetlands may also be significant sources of GHG emissions (Charpentier et al. 2009; Yeh et al. 2010). Over the years, the oil sands industries have worked towards the development of efficient technologies to improve GHG efficiency and reduce the footprint of the industrial development processes (Flint 2004; Wang & Naterer 2010). The reclamation of disturbed land may represent an ecological means of reducing the GHG footprint of the industry; however, this has not yet been explored.

Research is ongoing to evaluate the possible reduction of environmental impacts of land-use change through forest (upland) and wetland reclamation (Fung & Macyk 2000; Hyndman & Sobkowicz 2010; Foote 2012; Audet et al. 2015). Although the regrowth of forests leads to the accumulation of carbon (C) in above and belowground biomass, the choice of constructing wetlands on post-mining landscapes to sequester C could either have a negative or positive feedback on the overall GHG footprint of the industry. When used for mining waste water treatment, constructed wetlands (*e.g.* ponds and marshes) can act as sources of GHGs (Mander et al. 2005; Liikanen et al. 2006) as these wetland types are nutrient-rich and lack the hydrologic regulatory function that sustains the anoxic conditions required for long-term nutrient sequestration (Holden 2005). In contrast, peatlands sequester more carbon and nutrients than wetlands with mineral soil (*e.g.* marshes), and therefore have greater potential to act as a net sink of GHGs (Roulet 2000) relative to other wetland types. However, the C sequestration potential of peatland depends on hydrology, vegetation type, litter substrate quality, water chemistry and other edaphic variables such as soil temperature. Thus, a constructed fen could present an ecological option of GHG emission reduction technology, with the dual benefit of reclaiming lost landscape functions and contributing towards a reduction of the industry's GHG emission footprint.

In the Athabasca oil sands region, constructed fens are one of the land-use types targeted in ongoing landscape reclamation projects. Relative to the characteristics of natural fens, the modification of physicochemical properties in the constructed fen has resulted in an array of uncertainties regarding its functional potentials. For instance, the fragmentation of donor-peat during transfer and placement in constructed fen alters the vertical stratification of peat layers (Nwaishi, et al. 2015b) that have been observed to control self-regulated hydrology and redox processes in natural analogues (Strack & Waddington 2007; Nwaishi, et al. 2015a). The stratified peat layers also support different microbial groups that mediate biogeochemical transformation (Krab et al. 2010; Andersen et al. 2013a; Elliott et al. 2015). Recreating hydrologic conditions that are self-regulated within the constructed fen may require several years, and the effects this will have on the redox processes that produce GHG fluxes is not known. Similarly, the displacement of microbial niches (*e.g.* exposure of catotelm microbial communities such as methanogens and sulfur reducers to the upper oxic layers) in the peat column of the constructed fen will likely compromise biogeochemical transformation processes and hence, GHG fluxes (Schimel & Gulledge 1998).

The management practices used in salvaging the donor-peat were reported to have potentially degraded the substrate quality and modified the nutrient balance, as they involved draining and aeration of the donor-peat for approximately two years before transfer to the constructed fen (Nwaishi, et al. 2015b). It is anticipated that this may result in GHG emissions in the short term. Drained peatlands can easily change from a CO_2 sink to a source and potentially emit N_2O as the increased humification of the peat accelerates nitrogen (N) cycling (Freeman et al. 1992; Martikainen et al. 1993; Moore & Roulet 1993). Rewetting of drained peat during reclamation could also shift the C balance towards a CH_4 source (IPCC 2013; Beyer & Höper 2014; Vanselow-Algan et al. 2015). However, this will depend on the stability of the recreated hydrology and substrate quality (Wilson et al. 2009; Waddington & Day 2007).

Vegetation species can also affect GHG fluxes in peatlands. For example, the presence of vascular plants have been reported to enhance CH_4 production in peatlands (Joabsson et al. 1999; Trinder et al. 2009; Mahmood & Strack 2011). Thus, it is essential to understand how different revegetation strategies used in constructed fens affect the dynamics of GHG fluxes.

Notwithstanding the enhancement of CH_4 production by vascular plants, the presence of elevated concentrations of inorganic compounds such as sulfate (SO_4^{2-}) and iron III oxide (Fe^{3+}) have been reported to supress methanogenesis in vascular plant-dominated sites due to different levels of efficiency in microbially mediated reduction processes (Roden & Wetzel 1996; Dise & Verry 2001; Gauci et al. 2004). Location of the constructed fen at the hub of industrial activities that release elevated concentrations of reactive S (~ 20 kg S ha^{-1} yr^{-1} (Proemse et al. 2012; Proemse et al. 2013) will favour sulfate-dependent anaerobic methane oxidation (Schink 1997). Moreover, the tailing sand used in constructing the surrounding upland slopes contain elevated concentrations of Mn^{4+}, Fe^{3+} and SO_4^{2-} (Rowland et al. 2009), which can serve as alternative electron acceptors for reduction processes and subsequently affect GHG emissions from the constructed fen (Fedorak et al. 2002).

In view of the underlying uncertainties surrounding the biogeochemical functioning of constructed fens, especially in relation to GHG emissions, a study was conducted to quantify the emissions of CO_2, CH_4 and N_2O over the first two growing seasons following construction of a fen system near Fort McMurray, Alberta. Here, the fen reclamation concept involves recreating hydrologic conditions that will support the re-establishment of fen vegetation communities on a peat substrate that was transferred from a lease site (an area slated for mining) to the constructed landscape (Price et al. 2010a; Pollard et al. 2012). The specific objectives of the study are: 1) to evaluate the effect of different vegetation re-introduction strategies on average seasonal GHG emissions, relative to a natural reference system; and 2) to relate the quantified emissions to edaphic factors (e.g. substrate quality) and environmental variables (soil temperature, moisture content, water table, and water chemistry) that control GHG exchange between terrestrial ecosystems and the atmosphere.

5.2 Materials and methods

5.2.1 Study sites

The constructed fen is located on one of the oil sands lease areas (56^{o}55.944$^{'}$ N 111^{o}25.035$^{'}$ W), about 50 km north of Fort McMurray, Alberta. A natural fen that is further away from active industrial development, but within the oil sands region (56^{o}56.298$^{'}$ N 111^{o}32.898$^{'}$ W) was used as a reference site (REF) for this study. Detailed descriptions of both sites have been previously reported (Nwaishi et al. 2015b). Briefly, the natural fen is a treed rich fen, dominated by shrubs, mosses and sedges, while the constructed fen is a 3 ha fen watershed built between the spring of 2010 and winter of 2013. Revegetation strategies used in the constructed fen included: 1) seedlings only treatment (SDL); 2) moss layer transfer (MLT); 3) seedlings with moss layer combination (SMLT); and 4) a control treatment (CTRL). Among the vascular plants re-introduced in the constructed fen, *Carex aquatilis* and *Juncus balticus* dominated the SDL and SMLT plots. However, the SMLT plots comprise a more diverse plant community than the SDL plots. In the natural fen, monitoring was conducted on six plots (2 x 2 m), and in the constructed fen, study plots were replicated six times for each of the four treatments (i.e. 24 study plots). It is worthy to note that field measurements for this study were only extended to the natural fen in the second growing season (May to August, 2014).

5.2.2 Monitoring edaphic conditions in the study sites

Study site instrumentation and monitoring commenced in July 2013, immediately following vegetation re-introduction in the constructed fen. The study plots were instrumented with wells to monitor growing season changes in water table depth. Shallow peat cores (15 cm deep) were extracted at the beginning and end of each growing season, and processed for organic matter, bulk density, gravimetric moisture content and soil pH. These analyses followed the standard procedures and some modifications described in details in Nwaishi et al. (2015b). The average of beginning and end of season measurements were used to derive a seasonal value for each edaphic variable.

5.2.3 Determination of pore-water ionic concentrations

Plant root simulator (PRS) ^{TM} probes (Western Ag Innovations Inc., Saskatoon, SK) were used to monitor the seasonal dynamics of nutrient cations and anions in the study sites. The principles underlying the application of these ion exchange resin probes in environmental research were described earlier (Qian & Schoenau 2002), and has been used successfully in reclaimed oil sand landscapes (Rowland et al. 2009; MacKenzie & Quideau 2010; Percy et al. 2012). For this study, four pairs of the anion and cation probes were buried vertically in each study plot, and allowed to incubate for 30 days. Burial depth was maintained within the uppermost soil layer (15 cm depth) where most soil nutrients are released and roots are most active (the rhizosphere).

In the first growing season, two incubation cycles were completed between July and August, 2013, while three incubation cycles were completed in the second growing season between May and August, 2014. Following each incubation cycle, the buried probes were recovered from the peat, washed with deionized water, and shipped in a cool box to Western Ag Innovations Inc. for analyses. Analyses of the probes involved elution of the ionic resin membrane with 0.5 M HCl before determination of captured anions and cations concentration in the eluate. Standard colorimetric technique on an automated flow injection analysis system (Bran and Lubbe, Inc., Buffalo, NY) was used to determine the concentrations of NO_3^- and NH_4^+, while inductively-coupled plasma spectrometry (PerkinElmer Optima 3000-DV, PerkinElmer Inc., Shelton, CT) was used to measure the concentrations of SO_4^{2-}, $Ca^{2+,}$ K^+, Mg^{2+}, Mn^{2+}, and Fe^{3+} in the eluate.

5.2.4 Measurement of greenhouse gas fluxes

In-situ fluxes of CO_2, CH_4 and N_2O were determined by dynamic closed-chamber technique in all the study plots (Solondz et al. 2008; Petrone et al. 2011). Flux measurements were completed up to 6 times during the course of each growing season. In the constructed fen, a total of 36 steel collars (CTRL and MLT replicated 6 times, while SDL and SMLT had 12 replicates each) covering an area of 0.36 m^2 (60 × 60 cm) each were installed to represent all the re-vegetation strategies within the range of typical site conditions. Six of the same collars that were already installed in the natural fen from previous year's flux measurements were used as a reference in this study. To determine the net ecosystem exchange of CO_2 (NEE*ps*) at plot-scale, a closed chamber made of a transparent acrylic, with a dimensions of 60 × 60 × 30 cm (L×W×H), was placed over the steel collar, fitting into a groove to achieve a tight seal. Measurements for NEE*ps* were repeated under different light levels created using shades, while a dark measurement was obtained to represent the total CO_2 efflux from ecosystem respiration (ER) by soil organisms and plants (Solondz et al. 2008; Petrone et al. 2011). Gross ecosystem photosynthesis was estimated as the difference between NEE*ps* and ER. CO_2 flux measurements were not taken simultaneously with CH_4 and N_2O due to the longer incubation period required to get a CH_4 flux.

Concentration of CO_2 in the chamber was determined with a portable infrared gas analyser (PP Systems, USA, EGM - 4). Measurements were taken at 15 s intervals for up to 1.75 min (Solondz et al., 2008; Petrone et al., 2011; Munir et al. 2014). To achieve equilibrium in CO_2 concentration between measurements, the chamber was lifted from the collar until temperature and CO_2 concentrations returned to ambient levels. Two small battery-operated fans were attached to the chamber to continuously circulate the air inside during the measurement. The incubation for CH_4 and N_2O fluxes lasted for 30 min, and measurements were taken at time zero and at every 15 min intervals. Concentrations of CH_4 and N_2O in the chamber were determined using a Gasmet DX-4015 Fourier-Transform Infrared Gas Analyzer (FTIR-GA) (Gasmet Technologies Oy, Helsinki, Finland), following the procedure described by Brummell et al. (2012). Briefly, the gas analyzer has an in-built pump connected to a high sensitivity sample cell, which collects a complete infrared spectrum at 100 ms intervals, with a multiple spectra averaged over a pre-selected measurement time (80 s). The resulting spectrum gas concentrations are then calculated using a laptop operated software (Calcmet^{TM} ver. 2005.1), which employs a modified classical least squares analysis algorithm to simultaneously detect and quantify ambient gases in near-real-time.

CO_2 (g m$^{2-1}$ d^{-1}), CH_4 and N_2O fluxes (mg m^{-2} d^{-1}) were calculated from the linear change in concentration of these gases inside the chamber headspace with incubation time, as a function of volume, air temperature and pressure inside the chamber, according to the ideal gas law. It is worthy to note that the fluxes reported for REF are below canopy fluxes, which does not account for tree roots respiration as these were removed from collar sites. Edaphic variables such as peat temperature, moisture content and water table were also determined simultaneous with the gas flux measurements.

5.2.5 Statistical analyses

All the statistical analyses were performed with R (R Development Core Team, 2013). The data sets obtained from field measurements were subjected to normality testing, and when required, appropriate transformations were implemented to meet the assumptions of parametric statistics. Linear mixed effect model (function “lme”, package “nlme” (Pinheiro et al. 2011) was used to test the effect of different vegetation re-introduction strategies on average seasonal GHG emissions, relative to a natural reference system. To test for significant differences between revegetation strategies, a multiple comparison test was conducted on the model output using post hoc analyses (Tukey) built into a general linear hypothesis function ("glht", package "multcomp" (Bretz et al. 2015)). Pearson correlation coefficients (two-tailed significance) were determined across all sites for edaphic factors (e.g. substrate quality), environmental variables (soil temperature, moisture content, water table, and water chemistry) and gas fluxes. After identifying the edaphic factors and environmental variables that correlated with GHGs, variation partitioning (function "varpart", package "vegan" (Peres-Neto et al. 2006)) was then used to test the proportion of variation in the patterns of GHG fluxes explained by these environmental characteristics. The accepted significance level for all statistical tests was $p \leq 0.05$.

5.3. Results

5.3.1 Edaphic factors and environmental variables

Organic matter, bulk density and moisture content showed similar patterns of variation among the study sites (Table 5.1). The constructed fen had higher bulk density ($F_{4,\ 90} = 19.79$, $p < 0.001$), lower organic matter ($F_{4,\ 90} = 27.9$, $p < 0.001$) and moisture content ($F_{4,\ 90} = 16.37$, $p < 0.001$) relative to the REF. Within the constructed fen, re-vegetation through moss layer transfer increased organic matter and moisture content, but reduced bulk density in the MLT and SMLT plots. Consequently, plots with low bulk density also had higher organic matter ($R = –0.89$, $p < 0.001$) and moisture content ($R = - 0.83$, $p < 0.001$). Average peat temperature in the upper 0 to 15 cm depth was similar among the constructed fen plots and warmer by approximately 3 ^{o} C relative to the REF ($F_{4,\ 133} = 11.3$, $p < 0.001$). Peat water table depths were within 11 cm of the surface across all sites, with deeper water tables observed in the constructed fen relative to the

REF ($F_{4, 133} = 5.18$ $p < 0.001$). However, post hoc tests indicated that the water table depth in the SMLT plots did not differ significantly from the shallow water table observed in the REF ($p > 0.05$, Table 5.1).

Table 5.1: Edaphic properties (mean ± std. dev.) of the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) and the Natural reference site (REF) showing organic matter, bulk density, water table, gravimetric moisture content, and peat temperature. The characters a, b, and c are used to indicate significant differences (Tukey HSD test) among treatments.

Study Sites	Organic Matter (%)	Bulk Density (g /cm^3)	Water table Depth (cm)	Moisture Content (% Grav.)	Peat Temp. 0 -15 cm depth (^{o}C)
REF	85 ± 3^{a}	0.09 ± 0.01^{a}	-1.6 ± 0.5^{a}	89 ± 1^{a}	14.4 ± 2.7^{a}
MLT	66 ± 4^{b}	0.24 ± 0.05^{b}	-10.7 ± 12.8^{b}	79 ± 3^{b}	17.5 ± 1.8^{b}
SDL	61 ± 8^{bc}	0.25 ± 0.06^{bc}	-6.2 ± 7.3^{ab}	77 ± 6^{bc}	17.9 ± 1.8^{b}
SMLT	65 ± 6^{b}	0.22 ± 0.03^{b}	-3.5 ± 3.4^{a}	79 ± 5^{b}	17.4 ± 1.1^{b}
CTRL	57 ± 12^{c}	0.29 ± 0.11^{c}	-6.2 ± 0.1^{ab}	73 ± 10^{c}	17.2 ± 1.8^{b}

5.3.2 Peat pore-water chemistry

The pH of the study sites ranged between slightly alkaline in the REF to strongly alkaline among the constructed fen plots (Table 5.2, $F_{4, 103} = 41.82$, $p < 0.001$). Similarly, electrical conductivity (EC) was significantly higher ($F_{4, 103} = 14.29$, $p < 0.001$) among the constructed fen plots than the REF. In all the study sites, pH correlated positively but weakly with EC ($R = 0.24$, $p = 0.01$). Pore-water supply rates of NO_3^-, Ca^{2+}, and Mg^{2+} did not vary across the study sites.

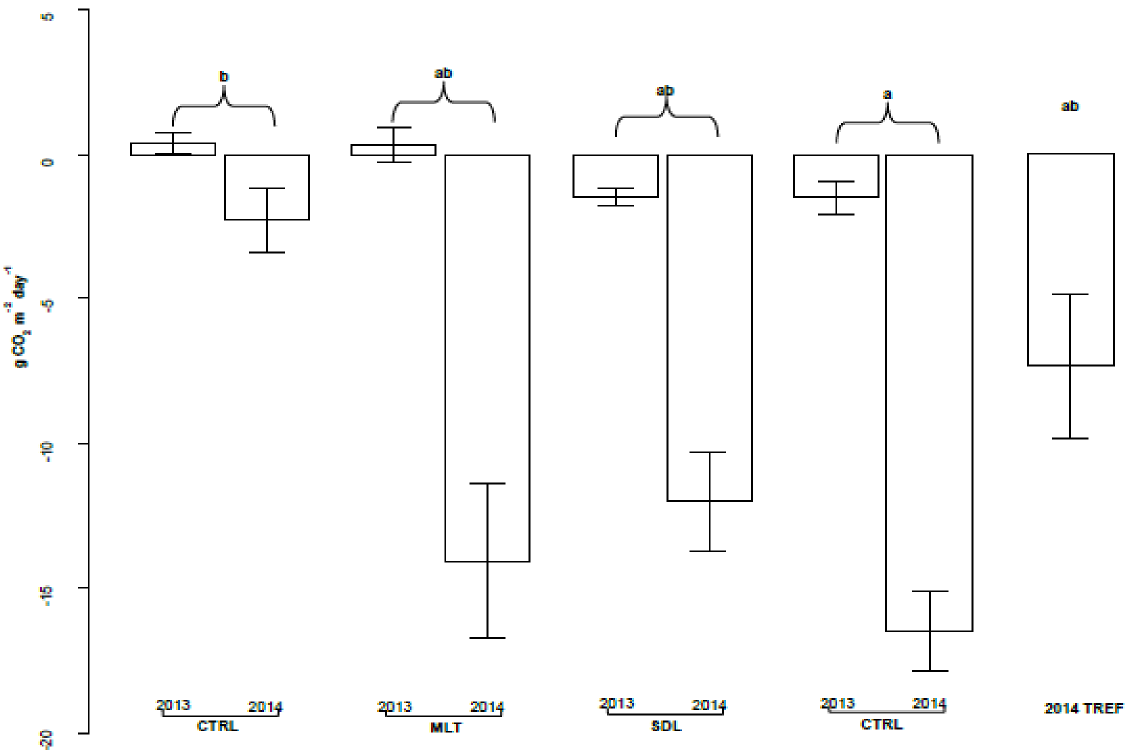
Table 5.2: Pore-water chemistry of the study sites showing means (± std. dev.) of pH, electrical conductivity (EC), and average daily rates of major nutrient elements supply over the two growing seasons. The characters a, b, and c are used to indicate significant differences (Tukey HSD test) among treatments.

Study Sites	pH	Electrical Conductivity (µs cm^{-1})	NO_3	SO_4	Ca	Mg	K	Fe	Mn
			$\leftarrow$ µg 10cm^{-2} day^{-1} $\rightarrow$						
REF	6.9 (0.4)^{a}	226 (68)^{a}	0.04 (0.05)^{a}	8.55 (5.1)^{a}	78.7 (8.1)^{a}	10.4 (1.3)^{a}	0.31 (0.18)^{a}	12.7 (5.2)^{a}	1.21 (0.55)^{a}
MLT	7.8 (0.1)^{b}	1022 (426)^{b}	0.10 (0.18)^{a}	45.4 (2.4)^{b}	71.3 (8.8)^{a}	9.87 (2.3)^{a}	0.38 (0.17)^{ab}	0.83 (0.58)^{b}	0.10 (0.05)^{b}
SDL	7.8 (0.2)^{b}	1211 (566)^{b}	0.04 (0.03)^{a}	44.9 (3.8)^{b}	71.1 (8.3)^{a}	9.30 (1.8)^{a}	0.35 (0.15)^{ab}	0.81 (0.57)^{b}	0.12 (0.08)^{b}
SMLT	7.8 (0.2)^{b}	1043 (527)^{b}	0.04 (0.03)^{a}	44.5 (4.0)^{b}	73.8 (11.6)^{a}	9.42 (2.2)^{a}	0.32 (0.13)^{a}	0.85 (0.58)^{b}	0.12 (0.06)^{b}
CTRL	7.8 (0.2)^{b}	1226 (656)^{b}	0.08 (0.11)^{a}	44.5 (3.2)^{b}	71.7 (8.6)^{a}	9.97 (2.5)^{a}	0.47 (0.30)^{b}	0.65 (0.49)^{b}	0.11 (0.05)^{b}

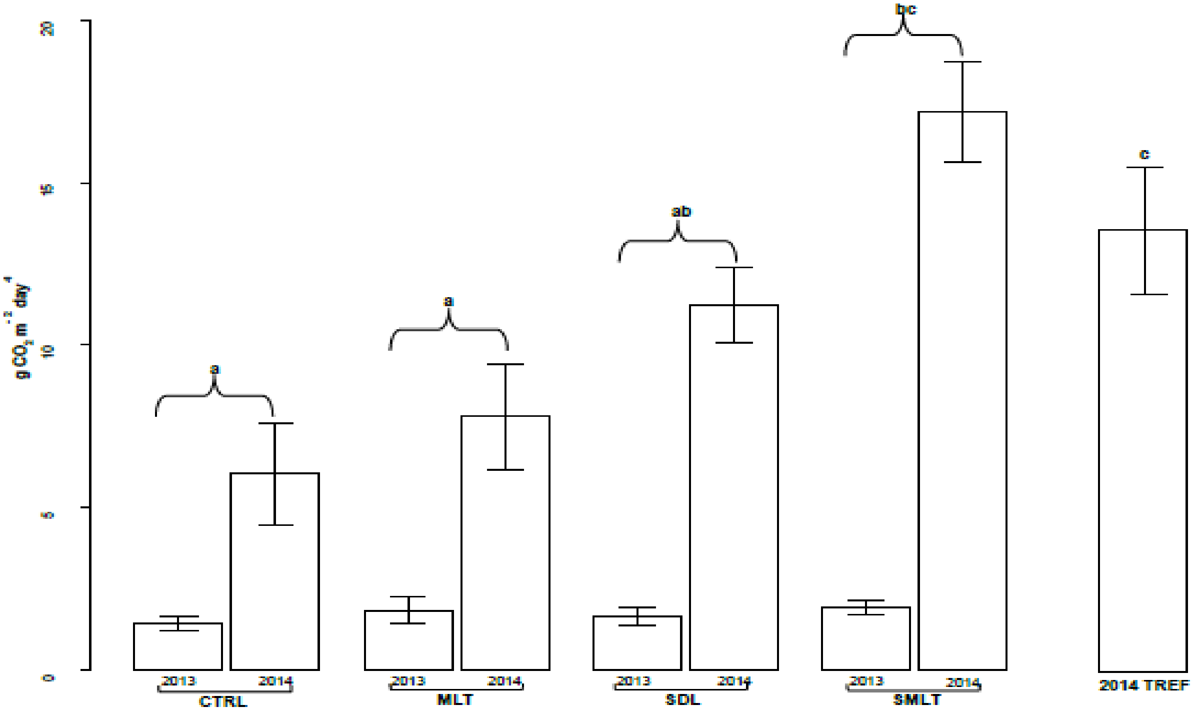
In contrast, the pore water supply rate of SO_4^{2-} was significantly higher ($F_{4,\,133} = 385.1$, $p < 0.001$) among the constructed fen plots; whereas higher concentrations of Mn^{2+} ($F_{4,\,133} = 111.8$, $p < 0.001$) and Fe^{3+} ($F_{4,\,133} = 65.6$, $p < 0.001$) were observed in REF. Peat with alkaline pH also had higher pore-water supply of SO_4^{2-} ($R = 0.78$, $p < 0.001$), whereas acidic pH was associated with higher pore-water supply of Mn^{2+} ($R = 0.68$, $p < 0.001$) and Fe^{3+} ($R = 0.80$, $p < 0.001$). The supply rate of K^+ to the rhizosphere pore-water was lower ($F_{4,\,133} = 3.11$, $p = 0.017$) in vegetated plots relative to the non-revegetated CTRL (Table 5.2).

5.3.3 Greenhouse gas fluxes

In the first growing season after re-vegetation of the constructed fen, the vascular plant plots showed the highest CO_2 uptake, while the CTRL and MLT remained a negligible source (Figure 5.1a). At the same time, CO_2 emission (ER) from the constructed fen was similar across all sites ($F_{4,\,109} = 4.86$, $p > 0.05$; Figure 5.1); whereas in the second growing season, CO_2 emission increased significantly ($F_{4,\,109} = 103.6$, $p < 0.001$; Figure 5.1b), at various rates across the sites ($F_{4,\,109} = 13.3$, $p < 0.001$). Gross ecosystem photosynthesis (Figure 5.1c) followed a similar inter-annual pattern to CO_2 emission but in the opposite direction. Thus, relative to the CTRL plot, CO_2 emissions as ER increased with vegetation productivity, with the highest rates observed in the SMLT plot ($p < 0.001$). In the second growing season, lower respiration/photosynthesis ratios were observed in the revegetated plots, especially those that received the moss transfer treatment. The release of CO_2 through ER correlated positively with organic matter content ($R = 0.24$, $p = 0.02$), peat temperature ($R = 0.21$, $p = 0.02$), Ca^{2+} ($R = 0.60$, $p < 0.01$), Mg^{2+} ($R = 0.47$, $p < 0.001$), and Fe^{3+} ($R = 0.40$, $p < 0.001$) concentrations; but negatively with bulk density ($R = -0.35$, $p < 0.001$) and SO_4^{2-} ($R = -0.32$, $p < 0.001$) concentrations.



a) NEE of CO2



b) ER

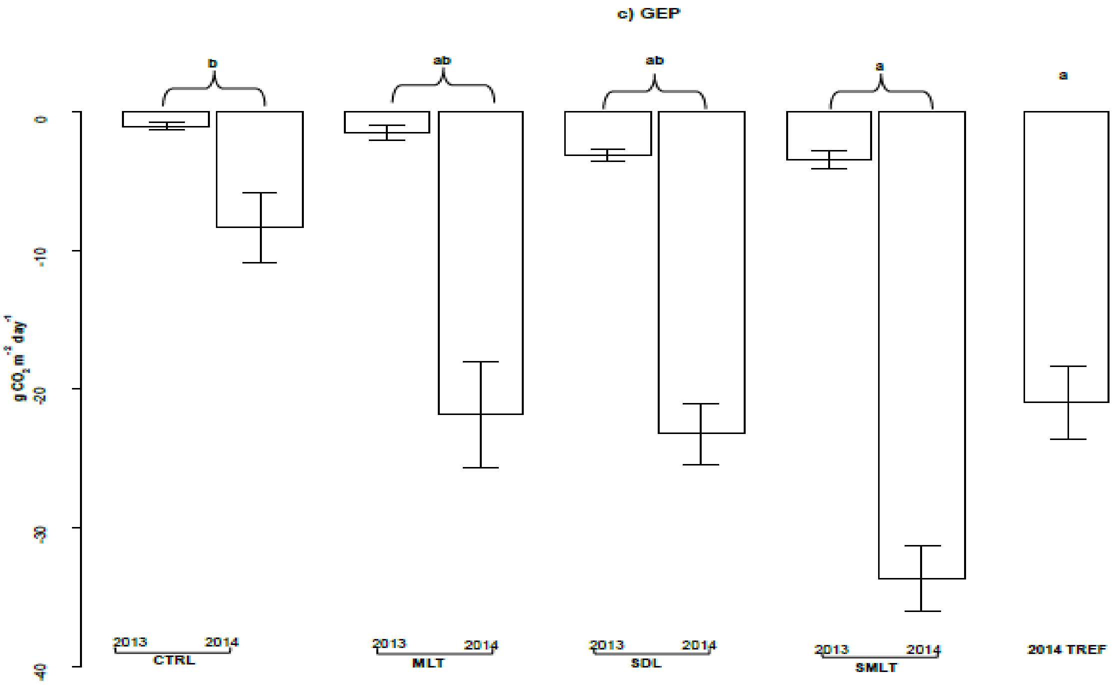


Figure 5.1: Inter-annual (± std. err) growing season's fluxes of CO$_2$ as; a) Net ecosystem exchange of CO$_2$ (NEE*ps*); b) Ecosystem respiration (ER); and c) Gross ecosystem productivity (GEP) across the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) and the Natural reference site (REF). Negative values indicate CO$_2$ removal from the atmosphere, while positive means release to the atmosphere. The characters a, b, and c are used to indicate significant differences (Tukey HSD test) among treatments.

Relative to the REF, the fluxes of CH$_4$ over the two growing seasons were significantly lower ($F_{4,\ 129} = 24.4$, $p < 0.001$; Figure 5.2a) among the constructed fen plots. The CH$_4$ fluxes measured in the entire constructed fen plots did not vary significantly ($F_{1,\ 125} = 0.10$, $p > 0.05$) over the two growing seasons; however, all the sites acted as a potential source of CH$_4$ to the atmosphere. Pearson correlation test indicated that the generally low CH$_4$ fluxes observed among the constructed fen plots were correlated with high pH ($R = –0.44$, $p < 0.001$), high concentration of SO$_4^{2-}$ ($R = –0.69$, $p < 0.001$), high bulk density ($R = –0.36$, $p < 0.001$), and low organic matter content ($R = 0.43$, $p < 0.001$), low Fe^{3+} ($R = 0.59$, $p < 0.001$), and Mn^{2+} ($R = 0.63$, $p < 0.001$) concentrations. There was no significant difference in N$_2$O fluxes among the study sites over the two growing seasons ($F_{1, 125} = 0.16$, $p > 0.05$; Figure 5.2b). However, the study sites with vascular plants (SDL, SMLT and REF) had a larger uptake of N_2O.

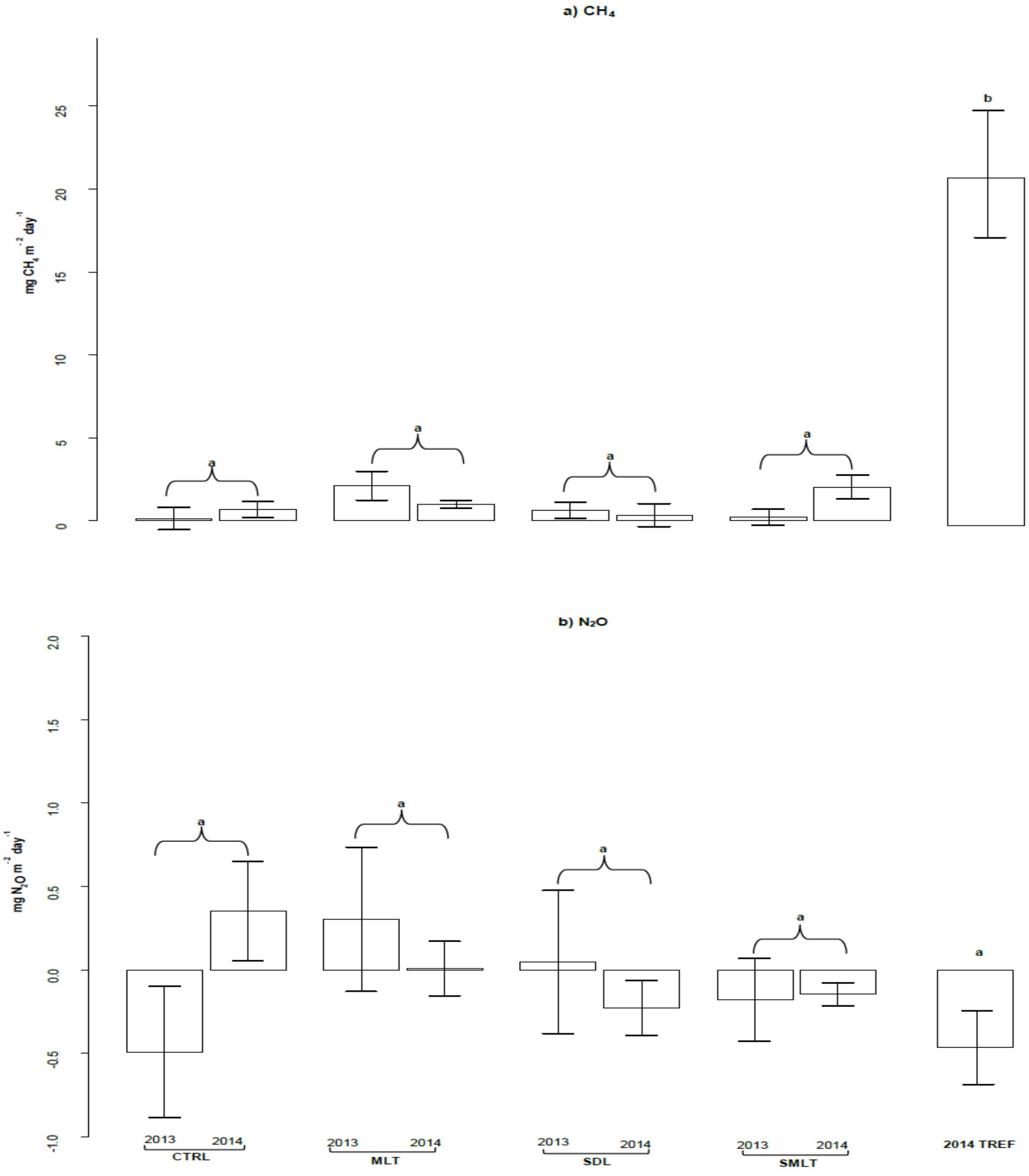


Figure 5.2: Inter-annual growing season's average (± std. err) of; a) methane (CH_4), and Nitrous oxide (N_2O) fluxes across the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) and the Natural reference site (REF). Negative values indicate removal from the atmosphere, while positive means release to the atmosphere. The characters a, b, and c are used to indicate significant differences (Tukey HSD test) among treatments.

Analysis to determine proportion of the variation in GHG fluxes that is explained by environmental characteristics showed that about 26 % of the variation was explained by the physical and chemical properties of the peat substrate (Figure 5.3). Out of this total, 16.34 % of the variation was explained by organic matter (OM), Fe and S; 7.59 % was explained by bulk density; and the remaining 2.04 % was explained by the interaction between the physical and chemical properties.

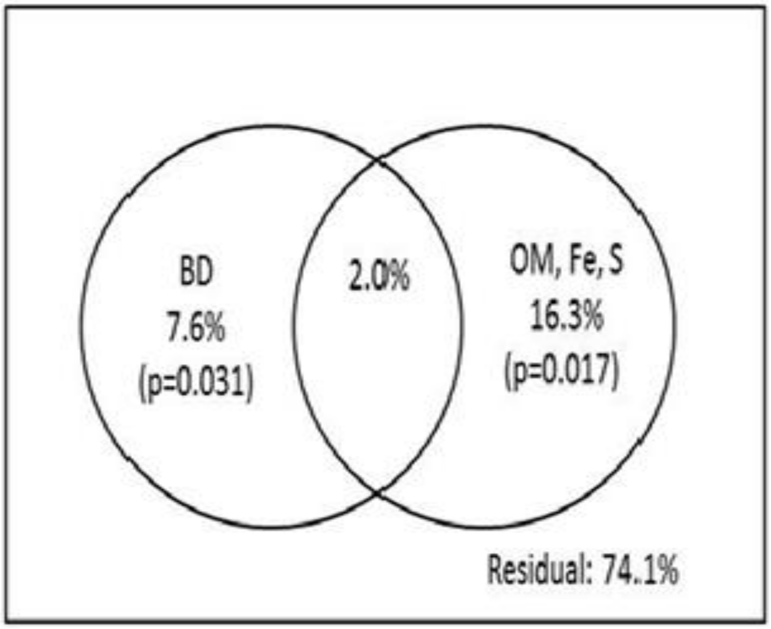


Figure 5.3: Variation partitioning Venn diagram showing the percentages of individual contributions of bulk density (BD), organic matter (OM), sulfate (S) and iron (Fe) to variation in greenhouse gas dynamics in the constructed fen. 2% represents the shared variation between environmental and water chemistry components, while the residual variation represents the percentage that is unexplained by the canonical model.

5.4 Discussion

The key role of peatland vegetation in the CO_2 dynamics of natural and restored peatlands has been reported by previous studies looking at C sequestration in northern peatlands (Tuittila et al. 1999; Waddington & Warner 2001; Soini et al. 2010). Consistent with these studies, the present study highlights the importance of vegetation re-introduction to the recovery of CO_2 sink function in a constructed fen. The results show that two years post-construction, the revegetated plots, especially the SMLT plot had a larger CO_2 uptake (–16.51 g CO_2 m^{-2} d^{-1}) than the CTRL (–2.28 g CO_2 m^{-2} d^{-1}). The net CO_2 fluxes measured in this plot are within the range reported for vegetated microsites in restored northern peatlands (Wilson et al. 2013; Strack et al. 2014). Given the keystone-role of mosses in restoring the C sequestration function of disturbed peatlands (Rochefort 2000; Turetsky 2003), it was not surprising to see the highest rate of CO_2 uptake in plots that received the moss treatment (SMLT and MLT). Similar results were reported by Kivimäki et al. (2008), which suggested that in restoration, complementing vascular plant stands with *Sphagnum* mosses improves the efficiency of CO_2 uptake relative to the vascular plant-only stands. The study attributed the larger CO_2 uptake observed in the mixed plots to a lower respiration/photosynthesis ratio of mosses (Kivimäki et al. 2008). The ability of mosses (*e.g. Sphagnum* and *Tomenthypnum*) to improve the efficiency of CO_2 uptake can also be explained by the role of their morphological structure in maintaining the capillary rise of water that is essential for sustaining the peatland–atmosphere carbon and water balance (*i.e.* suitable for efficient photosynthesis) under the broad range of water table conditions present in peatlands undergoing restoration/reclamation (Goetz & Price 2015b; Goetz & Price 2015a). The correlations between CO_2 emission and edaphic conditions support the hypothesis that an increase in temperature combined with high organic matter and nutrient availability might be associated with increases in the release of CO_2 to the atmosphere.

The CH_4 flux measured in the constructed fen was significantly lower than that of the REF site (Figure 2a), which is within the range of average flux reported for northern peatlands (Olefeldt et al. 2013). Given that the donor peat was drained prior to transfer to the constructed fen, we expected a low CH_4 flux in the first growing season, following reports that enhanced oxidation of organic matter reduces the amount of labile substrates available to methanogens (Waddington & Day 2007). It was also hypothesized that in the second growing season, when vascular plants were well-established, rhizodeposition and litter addition would override the substrate limitation by providing the labile substrates required to stimulate methanogenesis (Picek et al. 2007). However, results indicate that among the constructed fen plots, there was no significant difference in CH_4 efflux between the CTRL and the vegetated plots over the two growing seasons. This finding is contrary to our hypothesis, which was based on previous peatland studies that reported the enhancement of CH_4 dynamics by the presence of vascular plants (*e.g.* sedges) due to the ability of their rooting structure to penetrate peat columns below the water table and supply labile substrates for methanogenesis (Joabsson et al. 1999; Trinder et al. 2009; Mahmood & Strack 2011).

Given that vascular plants in the constructed fen established deep and dense below-ground biomass in the second growing season (data not shown), methanogenesis suppression, rather than substrate limitation may be the dominant factor that is driving the low CH_4 flux observed among the vegetated plots in the constructed fen. In agreement with CH_4 dynamics in the reference site, suppression of methanogenesis is uncommon in most natural peatlands because the concentration of SO_4^{2-} is often below the kinetic energy threshold required to stimulate sulfate-dependent methanogenesis inhibition (Schink 1997). In contrast, the high concentration of SO_4^{2-} in the constructed fen (Table 5.2) suggests that the kinetic energy limitations present in most natural peatlands might not exist in the constructed fen, hence the lower CH_4 flux. Lower pH and higher concentrations of Fe^{3+} and Mn^{2+} at REF indicates that redox conditions are more reduced in the natural site than the constructed fen. Consequently, the alkaline pH observed in the constructed fen implies that S might maintain a stable oxidized state, leading to the accumulation of SO_4^{2-} and exacerbating methanogenesis inhibition (Yu & Rinklebe 2013). This will likely contribute significantly towards reducing the GHG budget of the constructed fen. However, an anaerobic incubation experiment is required to test the hypothesis that sulfate-dependent methanogenesis suppression is dominant in the constructed fen. Such an experiment will also be useful to identify the labile substrate threshold requirement for stimulating methanogenesis under high SO_4^{2-} concentrations.

Despite reports of elevated N deposition (mean annual throughfall and bulk deposition rates of 25 N ha^{-1} yr^{-1}) within the vicinity of the constructed fen (Proemse et al. 2013), N_2O emission was mostly negative among all the study sites in both growing seasons (Figure 5.2b). The average fluxes measured in the constructed fen was similar to that of the REF, and also within the range reported for natural boreal fens (Regina et al. 1996; Wray & Bayley 2007). These results suggest that the high N_2O emissions usually reported in constructed wetlands that are receiving agricultural wastewater or peat mining runoff (Johansson et al. 2003; Liikanen et al. 2006) may not be observed in fens constructed on post-oil sands landscape, at least in the short-term. The reasons for the low N_2O emission could be explained by the physicochemical characteristics of the peat substrate and the presence of vegetation. For instance, a negative relationship has been shown for C/N ratio of soils and N_2O emissions with a ratio below 25 as the threshold for low production of N_2O (Klemedtsson et al. 2005). The average C/N ratio of 22.5 ± 0.98 reported for the constructed fen (Nwaishi et al. 2015b) is very close but still below the threshold for low N_2O emissions; hence, suggesting that other environmental characteristics such as vegetation and water chemistry could be the primary factors driving the low N_2O emission.

Higher N_2O/N_2 ratios have been shown to be dominant in acidic soils, while N_2 is usually the dominant product of denitrification under alkaline conditions (Picek et al. 2007). Given the predominant alkaline conditions in all the study sites, any N_2O produced might be instantly metabolized to N_2 by denitrifying bacteria. Other possible reasons for low N_2O emissions in the study sites might be as a result of vegetation and microbial immobilization of NO_3^-, which have been observed in the constructed fen (Nwaishi et al. 2015c). Studies investigating the emissions of N_2O from bare and vegetated peatlands have reported low and high fluxes in vegetated and bare areas respectively (Nykanen et al. 1995; Lang et al. 1995). This is in agreement with the results of this study, which observed the highest N_2O emissions from the un-vegetated CTRL plots in the second growing season. Based on the global warming potential of N_2O (*i.e.* 289 times that of CO_2 on a 100 year time scale), these results shows that relative to N_2O emissions from the CTRL plots (39 CO_2-e m^{-2} yr^{-1}), revegetation of the constructed fen reduced the GWP of N_2O to an average of –24 CO_2-e m^{-2} yr^{-1}. This value is below the range estimated for disturbed northern peatlands (Strack et al. 2014).

The variation partitioning analysis suggests that apart from the different revegetation strategies, the quality of peat substrate used in reclamation, and the resulting water chemistry could be major determinants of the GHG contribution of a constructed fen. Given the quality of the donor-peat used in the current study (Nwaishi et al. 2015b), and the low CH_4 fluxes observed in the constructed fen, results of this study are in agreement with other studies that reported low CH_4 flux for degraded peatlands undergoing restoration (Waddington & Day 2007; Agethen et al. 2015). However, Strack et al. (2014) also observed areas of high fluxes when a boreal restored site was wet. But this was not the case in the wet areas of the constructed fen, which suggests that the fen's chemistry rather than substrate quality could be the primary limiting factor to CH_4 dynamics.

5.5 Conclusions and implication of fen reclamation on greenhouse gas fluxes

The results presented in this study suggest that the dynamics of various GHGs in a constructed fen is primarily regulated by different environmental characteristics. Revegetation of the constructed fen reduced the emission of N_2O and also increased the uptake of CO_2 especially in plots where vascular plants and mosses were combined. However, inconsistent with the hypothesis, revegetation did not stimulate CH_4 production, even after two growing seasons. This could be attributed to the water chemistry of the constructed fen, which was characterised by higher pH, higher SO_4^{2-} and lower Fe^{3+} and Mn^{2+} relative to the reference fen. The results showed that in the short-term, the presence of vegetation, water chemistry and substrate quality are the most important determinants of the GHG contribution of a constructed fen. Current conditions in the revegetated plots supported a GHG sink function, which might persist until the dominant electron acceptors are exhausted. Based on these findings, it is recommended that prior to selecting reclamation materials, their ability to modify the resulting water chemistry should be considered with regards to the effect on GHG transformation processes. Also, the combination of moss and vascular plants should be considered as an ideal revegetation approach for reducing the GHG contribution of a constructed fen. Finally, continuous monitoring is essential to establish the long-term GHG dynamics of the constructed fen as conditions change over time.

Chapter Six

General Summary and Conclusion of Thesis

6.1 Summary

This thesis reported the first-time evaluation of biogeochemical processes in a constructed fen ecosystem. The overall goal of this research was to advance the knowledge of biogeochemical processes in a constructed fen ecosystem and present a functional-based approach for evaluating the functional trajectory of a constructed fen. To achieve these objectives, the first chapter of the thesis provided a general introduction, which highlighted the motivation and presented the background of the study.

Chapter two presented a comprehensive synthesis of the processes underlying peatland functioning in natural analogues. By considering the environmental characteristics of the constructed fen, the study built on the highlights of the synthesis to conceptualize the potential trajectory of biogeochemical processes in the constructed fen and how it can be monitored. Given the integral role and sensitivity of microbially-mediated nutrient transformation processes (*e.g.* ammonification, nitrification, methanogenesis, denitrification etc.), these were identified as potential functional-indicators of ecosystem functioning in the constructed fen. However, field experiments were required to select the most efficient candidate processes for the proposed functional-based evaluation. It was also very essential to understand how the physicochemical characteristics of the fen (a function of the donor peat quality and water chemistry of the fen) would modify the biogeochemical functioning of the constructed ecosystem and the implication of such modifications to the local and regional biogeochemical cycles.

Chapter three presented results of a field study that was designed to evaluate the impact of donor-peat management practices on the resulting peat quality and the potential implications on the biogeochemical functioning of the constructed fen. This study showed that the fragmentation of donor-peat during relocation to the constructed fen exacerbated the degradation of peat hydrophysical and chemical characteristics, which was initiated by the accelerated decomposition associated with donor-fen dewatering. Based on these this findings, it was hypothesized that fragmentation of the donor-peat might lead to nutrient limitations in the constructed fen, especially for nutrients that are usually found in the upper peat horizons (*e.g.* phosphorous). Nutrients limitation was expected to intensify nutrient immobilization and competition between plants and soil microbes. The resulting peat quality was also expected to reduce the efficiency of labile-substrate-dependent biogeochemical processes (*e.g.* methanogenesis and nitrification) in the constructed fen. Notwithstanding some limitations associated with this study, it provided crucial baseline information that would enable future studies to distinguish between inherent and acquired ecosystem characteristics in the constructed fen. The conclusions and recommendations drawn from this study would help reclamation managers to make informed decisions regarding the best management practices for preserving the quality of peat substrates slated as donor-materials for fen reclamation.

The study presented in Chapter four tested some of the hypothesis from previous Chapters that are related to nutrient cycling. The major goals of this study were to evaluate the state of nutrient cycling processing, especially as affected by the different revegetation strategies adopted in the constructed fen; and then to identify the most suitable biogeochemical processes to be used as indicators for the functional-based evaluation. Results from this study showed that over the first two growing seasons, revegetation facilitated both above-ground productivity and the cycling of below-ground nutrients, especially in plots where the transferred moss layer was combined with seedlings. The supply of labile substrates in the re-vegetated plots could have increased microbial potential activity, which was reflected in higher rates of respiration, nutrient acquisition and productivity. In agreement with the hypothesis, nutrient dynamics within the constructed fen suggest that phosphorus limitation could hamper the establishment of a diverse plant community, whereas the build-up of microbial biomass appears to be NO_3^- limited. The results also emphasized the need to track the evolution of biogeochemical processes through long-term monitoring programs, and identified the potential use of ammonification, nitrogen mineralization and phosphorus availability as functional indicators of the fen's recovery trajectory towards conditions present in the natural analogues. This study provided the first insight into a comprehensive evaluation of nutrient cycling processes in a constructed fen.

The thesis research was wrapped-up in Chapter five with a study that integrated the overall implication of the constructed fen's characteristics (both structural and functional) to GHG dynamics. This study tested the hypothesis that the inherent poor peat quality would affect the efficiency of processes like methanogenesis. However, it was expected that revegetation would supply the labile substrates required to stimulate methanogenesis in the constructed fen. In contrast, the results showed that relative to a natural fen, significantly lower fluxes of CH_4 ($p < 0.001$) were measured in the constructed fen. The low fluxes correlated with higher bulk density, lower organic matter, and higher pH and SO_4^{2-} concentration. Hence, more research is required to ascertain whether the low CH_4 flux is peat substrate or water chemistry-induced. Ongoing experiment is indicating that salinity may also be responsible for the low CH_4 efflux. Although revegetation did not stimulate CH_4 production, it increased CO_2 uptake and reduced the global warming potential of N_2O emissions by $63CO_2$-e m^{-2} yr^{-1} relative to the non-vegetated control.

These results suggest that the present conditions in the constructed fen supported a GHG sink function; but it is not known if this will persist in the long-term. This study presented, for the first time, the GHG dynamics of a constructed fen in the Athabasca oil sand region.

6.2 General conclusion and recommendations

In conclusion, the major findings of this thesis are:

- Evaluation of a constructed fen can be conducted using a biogeochemical functional-based approach, which appears to be more appropriate than the traditional indicator species approach of reclamation evaluation. This also broadly support the current thinking in restoration ecology, which advocates the need to move away from the traditional view that restoration needs to reconstruct past systems, and that novel and hybrid systems – which may not be structurally equivalent to natural analogues, but support similar functions – should be accepted as possible, and desirable outcomes.
- Microbially-mediated nutrient transformation processes such as mineralization presents a potential functional indicator of a constructed fen's functioning.
- The recovery of below-ground nutrient transformation processes are constrained by poor peat quality, which is characterised by high bulk density, low organic matter and low nutrient (N and P) concentrations. However, above-ground C cycling is facilitated by revegetation of a constructed fen, especially with a combination of vascular plant seedlings and moss species transferred from a natural site.
- Revegetation of a constructed fen reduced the potential of N_2O production due to the high N demand of establishing vascular plants, which also increases CO_2 uptake.

- Substrate quality and water chemistry overrides the ability of vascular plants to stimulate CH_4 production, making the constructed fen, a net sink of CH_4. However, further research is required to determine the dominant control on low CH_4 dynamics in the constructed fen.
- If the current functional characteristics of the fen persist in the longer term, then the constructed fen could remain a net sink of nutrients and GHGs due to regulation of water chemistry by vegetation and microbial competition for nutrients.

Based on the findings, this study recommends that future reclamation projects should consider the following:

- Reclamation managers should adopt a functional-based approach for evaluation of constructed fens, targeting the processes rather than structural integrity of communities.
- Donor-peat management practices should be modified by planning dewatering and relocation of peat between fall to spring, to eliminate any chance of accelerated decomposition and preserve the inherent characteristics of the peat substrate.
- The techniques used in relocating donor-peat (e.g. *in situ* loading) should be enhanced to minimise the fragmentation of the peat material during transfer and placement in the constructed fen.
- The potential effects of construction materials on water chemistry should be considered prior to use in fen reclamation due to the subsequent impact on the biogeochemical functioning of a constructed fen.
- Given the complementary role of natural vegetation in the establishment of seedling and recovery of below-ground process in a constructed fen, future large-scale reclamation

should take adequate care in preserving the surface vegetation from a donor-fen when relocating the donor-peat.

- The combination of vascular plant seedling and moss layer transfer presents an ideal revegetation strategy with the potentials to facilitate the recovery of biogeochemical functioning in a constructed fen.
- Ecological monitoring aimed at tracking the recovery of biogeochemical functions in constructed fens should initially focus on the state of microbial-mediated below-ground nutrient cycling processes before progressing to their output (*e.g.* nutrient pools) and subsequent above-ground productivity.
- Finally, it is essential to continuously monitor the evolution of biogeochemical processes in a constructed fen. Studies like this are required to understand the long-term biogeochemical characteristics of fen such as the contributions of constructed fens to the GHG foot-print of the oil sands industry.

6.3 Future research prospects

The evaluation of biogeochemical functioning in a constructed fen is still an evolving aspect of reclamation monitoring, and thus would require a long-term monitoring that is beyond the time-frame stipulated for a doctoral research program. Given the time constraints associated with this research, future research can build on the insight from this study to advance our knowledge and understanding of ecosystem-scale biogeochemical functioning of a constructed fen. Such ecosystem-scale evaluation would explore the biogeochemical interactions (connectivity) between the fen and surrounding uplands, which is essential to the sustainability of ecohydrological functions in a constructed ecosystem, especially under climate change scenarios. Understanding the level of interactions between landscape units that constitute a constructed ecosystem (*i.e.* ponds, wetlands, uplands and slopes) would inform the design and planning of future ecosystem-scale reclamation under the integrated land management system, proposed for disturbed boreal forest areas.

Future research also needs to conduct a comprehensive biogeochemical analysis of potential donor peatlands before and after dewatering. Studies like this would provide insight on the reclamation carbon life cycle analysis, which is required to better understand the impact of reclamation practices on the GHG foot-print of the industry, and also enable stakeholders to make informed reclamation design decisions that can lead to reduction in GHG emission. Exploring the "black box" of biogeochemical processes through advanced genomic techniques is an ongoing study that is expected to explain the patterns observed in some of the processes reported in this thesis. Results from this study would also lead to the postulation of hypothesis that would inform future research questions, especially with regards to the trajectory of biogeochemical functions in a constructed fen. The below-ground component of biogeochemical function is the basis of soil function in terrestrial ecosystems. Thus, the concept of evaluating below-ground biogeochemical functioning can be extended to brownfields, oil spillage sites and general terrestrial ecosystem assessment studies, as an approach for evaluating the resilience in soil functions.

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