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1 **Preliminary Assessment of Greenhouse Gas Emissions from a Constructed Fen on Post-**
2 **Mining Landscape in the Athabasca Oil Sands Region, Alberta, Canada**

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4
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10
11 **Abstract**

12 The ongoing reclamation of boreal forest areas that are impacted by oil sands mining involves the construction of
13 wetlands as desirable land uses. Constructed wetlands receiving peat-mining runoff are known to be major sources
14 of greenhouse gas (GHG) emissions to the atmosphere. However, the GHG dynamics of a fen that is constructed
15 with drained peat substrate and receives runoff containing effluents of salt and Naphthenic acids is not known.
16 Using a fen constructed in the post-mining landscape of the Athabasca oil sands region, a study was conducted to
17 quantify the emissions of carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) over the first two growing
18 seasons following revegetation. The specific objective of this study is to evaluate the effect of revegetation strategies
19 and other environmental characteristics on the fen GHG emissions over the growing season. Relative to a natural
20 fen, our results show significantly lower fluxes of CH₄ (p < 0.001), which correlate with higher bulk density, lower
21 organic matter, and higher pH and SO₄²⁻ concentration in the constructed fen. Revegetation did not stimulate CH₄
22 production, but increased CO₂ uptake and reduced the global warming potential of N₂O emissions by 63CO₂-e m⁻²
23 yr⁻¹ relative to the non-vegetated control. These results suggest that revegetation strategies and water chemistry
24 might be more important controls on GHG dynamics than substrate quality in a constructed fen. Although present
25 conditions in the constructed fen support a net GHG sink function, additional monitoring is required to evaluate if
26 this condition will persist in the long-term. We recommend that future reclamation projects should consider the
27 potential effects of construction materials on water chemistry prior to use in fen reclamation.

28
29 **Keywords:** Greenhouse gas emission, constructed fen, reclamation, peatlands, and Athabasca oil sands.

30 **1. Introduction**

31 Industrial development processes such as the mining of natural resources are often associated with land-use change.
32 On a global scale, changing land-use patterns have been identified as one of the major factors that affect the
33 exchange of greenhouse gases (GHG) such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O)
34 between terrestrial ecosystems and the atmosphere (IPCC 2000; Greenhalgh & Daviet 2006). The effect of land-use
35 change on GHG emissions is heightened in cases where the end-product of the changed land-use upsets the natural
36 source – sink balance of an ecosystem, contributing to enhanced GHG emissions. In Canada for instance, the
37 expansion of oil sands development has been identified as a potential major contributing component of the national
38 GHG emission budget (Environment Canada, 2014; Grant et al., 2013). Although the process of upgrading the
39 bitumen to synthetic crude oil is the primary process leading to intensive GHG emission, the land-use changes
40 associated with bitumen extraction from large areas of uplands and wetlands may also be significant sources of
41 GHG emissions (Charpentier et al., 2009; Yeh et al., 2010). Over the years, the oil sands industries have worked
42 towards the development of efficient technologies to improve GHG efficiency and reduce the footprint of the
43 industrial development processes (Flint, 2004; Wang and Naterer, 2010). The reclamation of disturbed land may
44 represent an ecological means of reducing the GHG footprint of the industry; however, this has not yet been
45 explored.

46 Research is ongoing to evaluate the possible reduction of environmental impacts of land-use change
47 through forest (upland) and wetland reclamation (Fung & Macyk 2000; Hyndman & Sobkowitz 2010; Foote 2012;
48 Audet et al. 2015). Although the regrowth of forests leads to the accumulation of carbon (C) in above and
49 belowground biomass, the choice of constructing wetlands on post-mining landscapes to sequester C could either
50 have a negative or positive feedback on the overall GHG footprint of the industry. When used for mining waste
51 water treatment, constructed wetlands (*e.g.* ponds and marshes) can act as sources of GHGs (Liikanen et al., 2006;
52 Mander et al., 2005) as these wetland types are nutrient-rich, but lacks the hydrologic regulatory function that
53 sustains the anoxic conditions required for nutrient sequestration (Holden, 2005). In contrast, peatlands sequester
54 more carbon and nutrients than wetlands with mineral soil (*e.g.* marshes), and therefore have greater potential to act
55 as a net sink of GHGs (Roulet, 2000) relative to other wetland types. However, the C sequestration potential of
56 peatland depends on hydrology, vegetation type, litter substrate quality, water chemistry and other edaphic variables

57 such as soil temperature. Thus, a constructed fen, the minerotrophic variant of peatlands, could present an ecological
58 option of GHG emission reduction technology, with the dual benefit of reclaiming lost landscape functions and
59 contributing towards a reduction of the industry's GHG emission footprint.

60 In the Athabasca oil sands region, constructed fens are one of the land-use types targeted in ongoing
61 landscape reclamation projects (Price et al. 2010a; Wytrykush et al. 2012; Pollard et al. 2012). Relative to the
62 characteristics of natural fens, the modification of physicochemical properties in a constructed fen has resulted in an
63 array of uncertainties regarding its functional potentials. For instance, the fragmentation of donor-peat during
64 transfer and placement in a constructed fen alters the vertical stratification of peat layers (Nwaishi, et al. 2015a) that
65 have been observed to control self-regulated hydrology and redox processes in natural analogues (Strack &
66 Waddington 2007; Nwaishi, et al. 2015b). The stratified peat layers also support different microbial groups that
67 mediate biogeochemical transformation (Krab et al. 2010; Andersen et al. 2013; Elliott et al. 2015). Achieving
68 hydrologic conditions that are self-regulated within the constructed fen may require several years, and the effects
69 this will have on the redox processes that produce GHG fluxes is not known. Similarly, the displacement of
70 microbial niches through peat transfer to the constructed fen (*e.g.* exposure of catotelm microbial communities such
71 methanogens and sulfur reducers to the upper oxic layers) can be expected to suppress methanogenesis for quite a
72 long time (Schimel and Gullledge, 1998).

73 The management practices used in salvaging the donor-peat were reported to have degraded the substrate
74 quality and modified the nutrient balance, as they involved draining and aeration of the donor-peat for
75 approximately two years before transfer to the constructed fen (Nwaishi, et al. 2015a). It is anticipated that this may
76 result in GHG emissions in the short term. Drained peatlands can easily change from a CO₂ sink to a source and
77 potentially emit N₂O as the increased humification of the peat accelerates nitrogen (N) cycling (Freeman et al. 1992;
78 Martikainen et al. 1993; Moore & Roulet 1993). Rewetting of drained peat during reclamation could also shift the C
79 balance towards a CH₄ source (Beyer and Höper, 2014; IPCC, 2013; Vanselow-Algan et al., 2015). However, this
80 will depend on the stability of the recreated hydrology and substrate quality (Waddington and Day, 2007; Wilson et
81 al., 2009).

82 Vegetation species can also affect GHG fluxes in peatlands. For example, the presence of vascular plants
83 have been reported to enhance CH₄ production in peatlands (Joabsson et al., 1999; Mahmood and Strack, 2011;

84 Trinder et al., 2009). Thus, it is essential to understand how different revegetation strategies used in constructed fens
85 affect the dynamics of GHG fluxes. Notwithstanding the enhancement of CH₄ production by vascular plants, the
86 presence of elevated concentrations of inorganic compounds such as sulfate (SO₄²⁻) and iron III oxide (Fe³⁺) have
87 been reported to suppress methanogenesis in vascular plant-dominated sites due to difference in energy yields of the
88 anaerobic respiratory processes (Roden & Wetzel 1996; Dise & Verry 2001; Gauci et al. 2004). Location of the
89 constructed fen at the hub of industrial activities that release elevated concentrations of reactive S (~ 20 kg S ha⁻¹
90 yr⁻¹ (Proemse et al., 2013, 2012) will favour sulfate-dependent anaerobic methane oxidation (Schink, 1997).
91 Moreover, the tailing sand used in constructing the surrounding upland slopes contain elevated concentrations of
92 Mn⁴⁺, Fe³⁺ and SO₄²⁻ (Rowland, et al., 2009), which can serve as alternative electron acceptors for anaerobic
93 respiration and subsequently affect GHG emissions from the constructed fen (Fedorak et al., 2002).

94 In view of the underlying uncertainties surrounding the biogeochemical functioning of constructed fens,
95 especially in relation to GHG emissions, we conducted a study to quantify the emissions of CO₂, CH₄ and N₂O over
96 the first two growing seasons following construction of a fen system near Fort McMurray, Alberta. Here, the fen
97 reclamation concept involves recreating hydrologic conditions that will support the re-establishment of fen
98 vegetation communities on a peat substrate that was transferred from a lease site (an area slated for mining) to the
99 constructed landscape (Pollard et al., 2012; Price et al., 2010b). The specific objectives of the study are: 1) to
100 evaluate the effect of different vegetation re-introduction strategies on average seasonal GHG emissions, and how
101 these relates to baseline fluxes measured in a natural reference system; and 2) to relate the quantified emissions to
102 edaphic factors (e.g. substrate quality) and environmental variables (soil temperature, moisture content, water table,
103 and water chemistry) that control GHG exchange between terrestrial ecosystems and the atmosphere.

104

105 **2. Materials and methods**

106 *2.1 Study sites*

107 The constructed fen is located on an oil sands lease area (56°55.944' N 111°25.035' W), about 50 km north of Fort
108 McMurray, Alberta. A natural fen that is further away from active industrial development, but within the oil sands
109 region (56°56.298' N 111°32.898' W) was used as a reference site (REF) for this study. Detailed descriptions of both

110 sites have been previously reported (Nwaishi et al. 2015a). Briefly, the natural fen is a treed rich fen, dominated by
111 shrubs, mosses and sedges, while the constructed fen is a 3 ha fen watershed built between the spring of 2010 and
112 winter of 2013. Revegetation constructed fen was initiated in the spring of 2013 using the following strategies: 1)
113 seedlings only treatment (SDL); 2) moss layer transfer (MLT); 3) seedlings with moss layer combination (SMLT);
114 and 4) a control treatment with no vegetation (CTRL). Among the vascular plants re-introduced in the constructed
115 fen, *Carex aquatilis* and *Juncus balticus* dominated the SDL and SMLT plots. However, the SMLT plots comprise a
116 more diverse plant community than the SDL plots. To quantify vegetation establishment over the study period,
117 percentage canopy cover was visually estimated within a 1 meter sq. quadrat that was systematically placed in each
118 sub-treatment plot. These cover estimations, which were conducted at the end of each growing season (*i.e.*
119 September of 2013 and 2014) indicates that over the two growing seasons, the average vegetation cover increased
120 by 62% in the CTRL, 86% in the MLT plots, and above 90% in the SDL and SMLT plots (A. Borkenhagen,
121 unpublished, Colorado State University). In the natural fen, monitoring was conducted on six plots (2 x 2 m), and in
122 the constructed fen, study plots were replicated six times for each of the four treatments (*i.e.* 24 study plots). It is
123 worthy to note that field measurements for this study were only extended to the natural fen in the second growing
124 season (May to August, 2014).

125 **2.2 Monitoring edaphic conditions in the study sites**

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

132 **2.3 Determination of pore-water ion supply rates**

133 Plant root simulator (PRS)TM probes (Western Ag Innovations Inc., Saskatoon, SK) were used to monitor the
134 seasonal dynamics of nutrient cations and anions in the study sites. The principles underlying the application of
135 these ion exchange resin probes in environmental research were described earlier (Qian and Schoenau, 2002), and

136 has been used successfully in reclaimed oil sand landscapes (Rowland et al. 2009; MacKenzie & Quideau 2010;
137 Percy et al. 2012). For this study, four pairs of the anion and cation probes were buried vertically in each study plot,
138 and allowed to incubate for 30 days. Burial depth was maintained within the uppermost soil layer (15 cm depth)
139 where most soil nutrients are released and roots are most active (the rhizosphere effect).

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

149 **2.4. Measurement of greenhouse gas fluxes**

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

161 Concentration of CO₂ in the chamber was determined with a portable infrared gas analyser (PP Systems,
162 USA, EGM - 4). Measurements were taken at 15 s intervals for up to 1.75 min (Munir et al., 2014; Petrone et al.,
163 2011; Solondz et al., 2008). To achieve equilibrium in CO₂ concentration between measurements, the chamber was
164 lifted from the collar until temperature and CO₂ concentrations returned to ambient levels. Two small battery-
165 operated fans were attached to the chamber to continuously circulate the air inside during the measurement. The
166 incubation for CH₄ and N₂O fluxes lasted for 30 min, and measurements were taken at time zero and at every 15 min
167 intervals. Concentrations of CH₄ and N₂O in the chamber were determined using a Gasetm DX-4015 Fourier-
168 Transform Infrared Gas Analyzer (FTIR-GA) (Gasetm Technologies Oy, Helsinki, Finland), following the
169 procedure described by Brummell et al. (2012). Briefly, the gas analyzer has an in-built pump connected to a high
170 sensitivity sample cell, which collects a complete infrared spectrum at 100 ms intervals, with a multiple spectra
171 averaged over a pre-selected measurement time (80 s). The resulting spectrum gas concentrations are then calculated
172 using a laptop operated software (CalcmTM ver. 2005.1), which employs a modified classical least squares analysis
173 algorithm to simultaneously detect and quantify ambient gases in near-real-time.

174 CO₂ (g m⁻² d⁻¹), CH₄ and N₂O fluxes (mg m⁻² d⁻¹) were calculated from the linear change in concentration
175 of these gases inside the chamber headspace with incubation time, as a function of volume, air temperature and
176 pressure inside the chamber, according to the ideal gas law. It is worthy to note that the fluxes reported for REF are
177 below canopy fluxes, which does not account for tree roots respiration as these were removed from collar sites.
178 Edaphic variables such as peat temperature, moisture content and water table were also determined simultaneous
179 with the gas flux measurements.

180 *2.5 Statistical analyses*

181 All the statistical analyses were performed with R (R Development Core Team, 2013). The data sets obtained from
182 field measurements were subjected to normality testing, and when required, appropriate transformations were
183 implemented to meet the assumptions of parametric statistics. Linear mixed effect model (function “lme”, package
184 “nlme” (Pinheiro et al., 2011) was used to test effect of different vegetation re-introduction strategies on average
185 seasonal GHG emissions, relative to a natural reference system. To test for significant differences between re-
186 vegetation strategies, a multiple comparison test was conducted on the model output using post hoc analyses
187 (Tukey) built into a general linear hypothesis function (“glht”, package “multcomp” (Bretz et al. 2015)). Pearson

188 correlation coefficients (two-tailed significance) were determined across all sites for edaphic factors (e.g. substrate
189 quality), environmental variables (soil temperature, moisture content, water table, and water chemistry) and gas
190 fluxes. After identifying the edaphic factors and environmental variables that correlated with GHGs, variation
191 partitioning (function “varpart”, package “vegan” (Peres-Neto et al., 2006)) was then used to test the proportion of
192 variation in the patterns of GHG fluxes explained by these environmental characteristics. The accepted significance
193 level for all statistical tests was $p \leq 0.05$.

194

195 **3. Results**

196 *3.1 Edaphic factors and environmental variables*

197 Organic matter, bulk density and moisture content showed similar patterns of variation among the study sites (Table
198 1). As expected, the constructed fen had higher bulk density ($F = 19.79$, $p < 0.001$), and lower organic matter ($F =$
199 27.9 , $p < 0.001$) and moisture content ($F = 16.37$, $p < 0.001$) relative to the REF. Within the constructed fen, re-
200 vegetation through moss layer transfer increased organic matter and moisture content, but reduced bulk density in
201 the MLT and SMLT plots. Consequently, plots with low bulk density also had higher organic matter ($R = -0.89$, $p <$
202 0.001) and moisture content ($R = -0.83$, $p < 0.001$). Average peat temperature in the upper 0 to 15 cm depth was
203 similar among the constructed fen plots and warmer by approximately 3°C relative to the REF ($F = 11.3$, $p <$
204 0.001). Peat water table depths were within 11 cm of the surface across all sites, with deeper water tables observed
205 in the constructed fen relative to the REF ($F = 5.18$, $p < 0.001$). However, post hoc tests indicated that the water table
206 depth in the SMLT plots did not differ significantly from the shallow water table observed in the REF ($p > 0.05$,
207 Table 1).

208 *3.2 Peat pore-water chemistry*

209 The pH of the study sites ranged between slightly alkaline in the REF to strongly alkaline among the constructed fen
210 plots (Table 2, $F = 41.82$, $p < 0.001$). Similarly, electrical conductivity (EC) was significantly higher ($F = 14.29$, $p <$
211 0.001) among the constructed fen plots than the REF. In all the study sites, pH correlated positively but weakly with
212 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. In

213 contrast, the pore water supply rate of SO_4^{2-} was significantly higher ($F = 385.1$, $p < 0.001$) among the constructed
214 fen plots; whereas higher concentrations of Mn^{2+} ($F = 111.8$, $p < 0.001$) and Fe^{3+} ($F = 65.6$, $p < 0.001$) were
215 observed in the REF. Peat with alkaline pH also had higher pore-water supply of SO_4^{2-} ($R = 0.78$, $p < 0.001$),
216 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
217 < 0.001). Concentration of K^+ in rhizosphere pore-water was lower ($F = 3.11$, $p = 0.017$) in vegetated plots relative
218 to the non-revegetated CTRL (Table 2).

219 **3.3. Greenhouse gas fluxes**

220 In the first growing season after re-vegetation of the constructed fen, the highest CO_2 uptake was observed in the
221 vascular plant plots, while the CTRL and MLT remained a negligible source (Fig. 1a). At the same time, CO_2
222 release from respiration (ER) was similar across all plots in the constructed fen ($F = 4.86$, $p > 0.05$; Fig. 1); whereas
223 in the second growing season when vegetation was well established, CO_2 emission increased significantly ($F =$
224 103.6 , $p < 0.001$; Fig. 1b), at various rates across the plots ($F = 13.3$, $p < 0.001$). Gross ecosystem photosynthesis
225 (Fig. 1c) followed a similar inter-annual pattern to CO_2 emission but in the opposite direction. Thus, relative to the
226 CTRL plot, CO_2 emission as ER increased with vegetation productivity and percentage cover, with the highest rates
227 observed in the SMLT plot ($p < 0.001$). In the second growing season, lower respiration/photosynthesis ratios were
228 observed in the revegetated plots, especially those that received the moss transfer treatment. The release of CO_2
229 through ER correlated positively with organic matter content ($R = 0.24$, $p = 0.02$), peat temperature ($R = 0.21$, $p =$
230 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
231 negatively with bulk density ($R = -0.35$, $p < 0.001$) and SO_4^{2-} ($R = -0.32$, $p < 0.001$) concentrations.

232 Relative to the REF, the fluxes of CH_4 over the two growing seasons were significantly lower ($F = 24.4$, p
233 < 0.001 ; Fig. 2a) among the constructed fen plots. The CH_4 fluxes measured in the entire constructed fen plots did
234 not vary significantly ($F = 0.10$, $p < 0.05$) over the two growing seasons; however, all the sites acted as a potential
235 source of CH_4 to the atmosphere. A Pearson correlation test indicated that the generally low CH_4 fluxes observed
236 among the constructed fen plots were correlated with high pH ($R = -0.44$, $p < 0.001$), high concentration of SO_4^{2-} (R
237 $= -0.69$, $p < 0.001$), high bulk density ($R = -0.36$, $p < 0.001$), and low organic matter content ($R = 0.43$, $p < 0.001$),
238 low Fe^{3+} ($R = 0.59$, $p < 0.001$), and Mn^{2+} ($R = 0.63$, $p < 0.001$) concentrations. There was no significant difference

239 in N₂O fluxes among the study sites over the two growing seasons ($F = 0.16$, $p > 0.05$; Fig. 2b). However, the study
240 sites with vascular plants (SDL, SMLT and REF) had a larger uptake of N₂O.

241 Analysis to determine proportion of the variation in GHG fluxes that is explained by environmental
242 characteristics showed that about 26 % of the variation was explained by the physical and chemical properties of the
243 peat substrate (Fig. 3). Out of this total, 16.3 % of the variation was explained by organic matter (OM), Fe and S was
244 16.3 %; 7.6 % was explained by bulk density; and the remaining 2.0 % was explained by the interaction between the
245 physical and chemical properties.

246

247 **4.0 Discussion**

248 The key role of peatland vegetation in the CO₂ dynamics of natural and restored peatlands has been reported by
249 previous studies looking at C sequestration in northern peatlands (Soini et al., 2010; Tuittila et al., 1999;
250 Waddington and Warner, 2001). Consistent with these studies, the present study highlights the importance of
251 vegetation re-introduction to the recovery of net CO₂ exchange function in the constructed fen. Our results show that
252 two years post-construction, the revegetated plots, especially the SMLT plot had a higher CO₂ uptake (-16.51 g
253 CO₂ m⁻² d⁻¹) than the CTRL (-2.28 g CO₂ m⁻² d⁻¹). The net CO₂ fluxes measured in this plot are within the range
254 reported for vegetated microsites in restored northern peatlands (Strack et al., 2014; Wilson et al., 2013). Given the
255 keystone-role of mosses in restoring the C sequestration function of disturbed peatlands (Rocheftort, 2000; Turetsky,
256 2003), it was not surprising to see the highest rate of CO₂ uptake in plots that received the moss treatment (SMLT
257 and MLT). Similar results were reported by Kivimäki et al. (2008), which suggested that in restoration,
258 complementing vascular plant stands with *Sphagnum* mosses improves the efficiency of CO₂ uptake relative to the
259 vascular plant-only stands. The study attributed the larger CO₂ sinks observed in the mixed plots to a lower
260 respiration/photosynthesis ratio of mosses (Kivimäki et al., 2008). The ability of mosses (*e.g.* *Sphagnum*
261 and *Tomenthypnum*) to improve the efficiency of CO₂ uptake can also be explained by the role of their
262 morphological structure in maintaining the capillary rise of water that is essential for sustaining the peatland–
263 atmosphere carbon and water balance (*i.e.* suitable for efficient photosynthesis) under the broad range of water table
264 conditions presents in peatlands undergoing restoration/reclamation (Goetz and Price, 2015a, 2015b). The

265 correlations between CO₂ emission and edaphic conditions support the hypothesis that in peatlands with lower water
266 table, increased oxygen availability and higher temperature will exacerbate organic matter mineralization thus
267 increase the release of CO₂ to the atmosphere.

268 The CH₄ flux measured in the constructed fen was significantly lower than that of the REF site (Fig. 2a),
269 which is within the range of average flux reported for northern peatlands (Olefeldt et al., 2013). Given that the donor
270 peat was drained prior to transfer to the constructed fen, we expected a low CH₄ flux in the first growing season;
271 following reports that enhanced oxidation of organic matter reduces the amount of labile substrates available to
272 methanogens and regenerates alternative electron acceptors that are energetically more favorable for
273 microorganisms, thus suppressing methanogenesis (Waddington and Day, 2007). But we also hypothesized that in
274 the second growing season, when vascular plants were well-established, rhizodeposition and litter addition would
275 override the substrate limitation by providing the labile substrates required to stimulate methanogenesis (Picek et al.,
276 2007). However, our results indicate that among the constructed fen plots, there was no significant difference in CH₄
277 efflux between the CTRL and the vegetated plots over the two growing seasons. This finding is contrary to our
278 hypothesis, which was based on previous peatland studies that reported the enhancement of CH₄ dynamics by the
279 presence of vascular plants (*e.g.* sedges) due to the ability of their rooting structure to penetrate peat columns below
280 the water table and supply labile substrates for methanogenesis (Joabsson et al., 1999; Mahmood and Strack, 2011;
281 Trinder et al., 2009).

282 Given that vascular plants in the constructed fen established deep and dense below-ground biomass in the
283 second growing season (data not shown), methanogenesis suppression, rather than substrate limitation may be the
284 dominant factor that is driving the low CH₄ flux observed among the vegetated plots in the constructed fen. In
285 agreement with CH₄ dynamics in the reference site, suppression of methanogenesis is uncommon in most natural
286 peatlands because the concentration of SO₄²⁻ is often below the kinetic energy threshold required to stimulate
287 sulfate-dependent methanogenesis inhibition (Schink, 1997). In contrast, the high concentration of SO₄²⁻ in the
288 constructed fen (Table 2) suggests that the kinetic energy limitations present in most natural peatlands might not
289 exist in the constructed fen, hence the lower CH₄ flux. Lower pH and higher concentrations of Fe³⁺ and Mn²⁺ at REF
290 indicates that redox conditions are more reduced in the natural site than the constructed fen. Consequently, the
291 alkaline pH observed in the constructed fen implies that S might maintain a stable oxidized state, leading to the

292 accumulation of SO_4^{2-} and exacerbating methanogenesis inhibition (Yu and Rinklebe, 2013). This will likely play a
293 key role towards reducing the GHG budget of the constructed fen; but an anaerobic incubation experiment is
294 required to test the hypothesis that sulfate-dependent methanogenesis suppression is dominant in the constructed fen.
295 Such an experiment will also be useful to identify the labile substrate threshold requirement for stimulating
296 methanogenesis under high SO_4^{2-} concentrations.

297 Despite reports of elevated N deposition (mean annual throughfall and bulk deposition rates of 25 N ha^{-1}
298 yr^{-1}) within the vicinity of the constructed fen (Proemse et al., 2013), N_2O emission was mostly negative among all
299 the study plots in both growing seasons (Fig. 2b). The average fluxes measured in the constructed fen was similar to
300 that of the REF, and also within the range reported for natural boreal fens (Regina et al., 1996; Wray and Bayley,
301 2007). These results are promising as they suggest that the high N_2O emissions usually reported in constructed
302 wetlands that are receiving agricultural wastewater or peat mining runoff (Johansson et al., 2003; Liikanen et al.,
303 2006) may not be observed in fens constructed on post-oil sands landscape, at least in the short-term. The reasons
304 for the low N_2O emission could be explained by the physicochemical characteristics of the peat substrate and the
305 presence of vegetation. For instance, a negative relationship has been shown for C/N ratio of soils and N_2O
306 emissions with a ratio below 25 as the threshold for low production of N_2O (Klemedtsson et al., 2005). The average
307 C/N ratio of 22.5 ± 0.98 reported for the constructed fen (Nwaishi et al. 2015a) is very close but still below the
308 threshold for low N_2O emissions; hence, suggesting that other environmental characteristics such as vegetation and
309 water chemistry could be the primary factors driving the low N_2O emission.

310 Higher $\text{N}_2\text{O}/\text{N}_2$ ratios have been shown to be dominant in acidic soils, while N_2 is usually the dominant
311 product of denitrification under alkaline conditions (Picek et al., 2007). Given the predominant alkaline conditions
312 in all the study sites, any N_2O produced might be instantly metabolized to N_2 by denitrifying bacteria. Other possible
313 reasons for low N_2O emissions in the study sites might be as a result of vegetation and microbial immobilization of
314 NO_3^- , which have been observed in the constructed fen (Nwaishi et al., 2016). Studies investigating the emissions of
315 N_2O from bare and vegetated peatlands have reported low and high fluxes in vegetated and bare areas respectively
316 (Lang et al., 1995; Nykanen et al., 1995). This is in agreement with our results, as we observed the highest N_2O
317 emissions from the sparsely-vegetated CTRL plots in the second growing season. Based on the global warming
318 potential of N_2O (*i.e.* 289 times that of CO_2 on a 100 year time scale), our results showed that relative to N_2O

319 emissions from the CTRL plots ($39 \text{ CO}_2\text{-e m}^{-2} \text{ yr}^{-1}$), revegetation of the constructed fen reduced the GWP of N_2O to
320 an average of $-24 \text{ CO}_2\text{-e m}^{-2} \text{ yr}^{-1}$. This value is below the range estimated for disturbed northern peatlands (Strack et
321 al., 2014).

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

330 **5.0 Conclusions and Implication of fen reclamation on major greenhouse gas fluxes**

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

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352 and analyses of plant root simulator probes.

353

354 **List of Tables and Figures**

355 **Table 1:** Edaphic properties (mean \pm std. dev.) of the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL),
356 Seedling + Moss (SMLT) and the Natural reference site (REF) showing organic matter, bulk density, water table,
357 gravimetric moisture content, and peat temperature. The alphabets: a, b, and c are used to indicate significant
358 differences (Tukey HSD test) among treatments.

359 **Table 2:** Average growing season's (\pm std. dev.) pore-water chemistry of the study sites showing of pH, electrical
360 conductivity (EC), and daily rates of major nutrient elements supply over the two growing seasons. The alphabets: a,
361 b, and c are used to indicate significant differences (Tukey HSD test) among treatments.

362 **Fig. 1:** Inter-annual mean (\pm std. err) growing season's fluxes of CO₂ as; a) Net ecosystem exchange of CO₂ (NEE);
363 b) Ecosystem respiration (ER); and c) Gross ecosystem productivity (GEP) across the Control (CTRL), Moss layer
364 transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) and the Natural reference site (REF). Negative values
365 indicate CO₂ removal from the atmosphere, while positive means release to the atmosphere. The alphabets: a, b, and
366 c are used to indicate significant differences (Tukey HSD test) among treatments.

367 **Fig. 2:** Inter-annual mean (\pm std. err) growing season's fluxes of; a) methane (CH₄), and Nitrous oxide (N₂O) fluxes
368 across the Control (CTRL), Moss layer transfer (MLT), Seedling (SDL), Seedling + Moss (SMLT) and the Natural

369 reference site (REF). Negative values indicate removal from the atmosphere, while positive means release to the
370 atmosphere. The alphabets: a, b, and c are used to indicate significant differences (Tukey HSD test) among
371 treatments.

372 **Fig. 3:** Variation partitioning Venn diagram showing the percentages of individual contributions of bulk density
373 (BD), organic matter (OM), sulfate (S) and iron (Fe) to variation in greenhouse gas dynamics in the constructed fen.
374 2% represents the shared variation between environmental and water chemistry components, while the residual
375 variation represents the percentage that is unexplained by the canonical model.

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