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Infilled ditches are hotspots of landscape methane flux following peatland re-wetting

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1 **Abstract.** Peatlands are large terrestrial stores of carbon, and sustained CO₂ sinks, but over
2 the last century large areas have been drained for agriculture and forestry, potentially
3 converting them into net carbon sources. More recently, some peatlands have been re-wetted
4 by blocking drainage ditches, with the aims of enhancing biodiversity, mitigating flooding
5 and promoting carbon storage. One potential detrimental consequence of peatland re-wetting
6 is an increase in methane (CH₄) emissions, offsetting the benefits of increased CO₂
7 sequestration. We examined differences in CH₄ emissions between an area of ditch-drained
8 blanket bog, and an adjacent area where drainage ditches were recently infilled. Results
9 showed that *Eriophorum vaginatum* colonisation led to a ‘hotspot’ of CH₄ emissions from the
10 infilled ditches themselves, with smaller increases in CH₄ from other re-wetted areas.
11 Extrapolated to the area of blanket bog surrounding the study site, we estimated that CH₄
12 emissions were around 60 kg CH₄ ha⁻¹ yr⁻¹ prior to drainage, reducing to 44 kg CH₄ ha⁻¹ yr⁻¹
13 after drainage. We calculated that fully re-wetting this area would initially increase emissions
14 to a peak of around 120 kg CH₄ ha⁻¹ yr⁻¹, with around two thirds of the increase (and 90% of
15 the increase over pre-drainage conditions) attributable to CH₄ emissions from *Eriophorum*
16 *vaginatum*-colonised infilled ditches, despite these areas only occupying 7% of the landscape.
17 We predicted that emissions should eventually decline towards pre-drainage values as the
18 ecosystem recovers, but only if *Sphagnum* mosses displace *Eriophorum vaginatum* from the
19 infilled ditches. These results have implications for peatland management for climate change
20 mitigation, suggesting that restoration methods should aim, if possible, to avoid the
21 colonisation of infilled ditches by aerenchymatous species such as *Eriophorum vaginatum*,
22 and to encourage *Sphagnum* establishment.

24 **Key Words**

25 Methane, carbon, peatland, blanket bog, re-wetting, restoration, *Eriophorum*, *Sphagnum*

26 **Introduction**

27 Peatlands are characterised by an anoxic catotelm, where water-logged conditions and high
28 acidity inhibit biological processes, suppressing decomposition and enabling organic material
29 to accumulate (e.g. Freeman et al., 2001; Belyea and Baird, 2006). Since the end of the last
30 ice age, peatlands have acted as a net sink for carbon dioxide (CO₂) wherever decomposition
31 has been slower than litter formation. The carbon stock of peatlands in the Northern
32 hemisphere is estimated to be 473-621 Pg C (Yu et al., 2010), representing 40% of global soil
33 carbon, despite only covering 3% of the Earth's terrestrial surface.

34 The conditions within peatlands that favour CO₂ sequestration also favour the production of
35 methane (CH₄), a powerful greenhouse gas, through the anaerobic decomposition of organic
36 matter by methanogenic microbes (Denman et al., 2007). According to the most recent
37 assessment report of the Intergovernmental Panel on Climate Change (IPCC), CH₄ has a 100
38 year global warming potential 28 times that of CO₂, rising to 34 if feedbacks from CH₄-
39 driven warming on oceanic and terrestrial CO₂ release are taken into account (see Myrhe et
40 al., 2013, and references therein).

41 CH₄ may enter the atmosphere via diffusion, ebullition, or plant-mediated transport. CH₄
42 may be oxidised by methanotrophic microbes as it diffuses through the aerobic acrotelm,
43 such that almost all of the CH₄ produced may be removed before reaching the atmosphere
44 (Calhoun and King, 1997). Alternatively, CH₄ may effectively by-pass oxidation if

45 transported via ebullition (i.e. transport in biogenic gas bubbles; Baird et al., 2006) or through
46 gas-transporting plant stems (aerenchyma). The difference between CH₄ production and
47 oxidation in the ecosystem determines the net flux of CH₄ between terrestrial ecosystem and
48 the atmosphere. Estimates of annual global CH₄ emissions from peatlands to the atmosphere
49 range from 38 to 157 Tg CH₄ year⁻¹ (Petrescu et al., 2010).

50 Over recent centuries, large areas of Northern peatlands have been damaged through drainage
51 and extraction, most notably in Northern Europe and European Russia, but also in parts of
52 North America and Asia (e.g. Joosten, 2010). In these areas the water level is typically
53 lowered by the digging of drainage channels or ditches. Draining peatlands can promote
54 decomposition by increasing the depth of the anaerobic zone, exposing stored organic matter
55 to oxidation and potentially resulting in high rates of CO₂ emission. On the other hand, this
56 expansion of the aerobic zone also increases rates of CH₄ oxidation (Sundh et al., 1995).

57 More recently, re-wetting of some drained peatlands has taken place via the blocking of
58 drainage ditches, in an attempt to return these ecosystems to a near-natural state, and thereby
59 to enhance their biodiversity and conservation value (Carroll et al., 2011). A variety of
60 methods have been tried, including the use of dams (made from peat blocks or plastic
61 sheeting), in-filling with materials such as wood brash or bales of locally harvested
62 vegetation such as *Calluna vulgaris*, or 're-profiling' the ditch by transferring peat material
63 into the ditch from adjacent areas (Armstrong et al., 2009). The expectation in all cases is
64 that restoring water-logged conditions will promote the functioning of peatlands as net CO₂
65 sinks, or at least reduce net CO₂ emissions, but there is a risk that increased CH₄ emissions
66 may reduce, or negate, the benefits of peatland restoration in terms of the net greenhouse gas
67 balance. A number of studies have quantified the direct effects of blocking drainage ditches

68 on CH₄ emissions. Waddington and Day (2007) found evidence to suggest that CH₄
69 emissions increased 4.6 times following restoration of a cutover peatland site in Bois-des-Bel,
70 Canada. Tuittila et al. (2000) observed an increase in CH₄ flux with the colonisation of
71 *Eriophorum vaginatum* after restoration of a cut-over peatland in Southern Finland. Best and
72 Jacobs (1997) observed a 3.4 fold increase in CH₄ production seven years after raising the
73 water level in a grass dominated peatland in the Netherlands, while Wilson et al. (2008)
74 found that CH₄ fluxes increased from near-zero in an area of bare cutaway peat to between 4
75 and 39 g CH₄ m⁻² yr⁻¹ in re-wetted areas, depending on the plant community present.
76 Mesocosm studies also indicate that rewetting blanket bog peat leads to an increase in CH₄
77 flux to the atmosphere (Dinsmore et al., 2009; Green et al., 2011).

78 The potential to develop policy and economic instruments to support peatland re-wetting as a
79 mechanism for reducing greenhouse gas (GHG) emissions has received increasing recent
80 attention (e.g. Bain et al., 2011; Dunn and Freeman, 2011). Wetland re-wetting has now been
81 adopted as a voluntary reporting activity under the Kyoto Protocol of the United Nations
82 Framework Convention on Climate Change (UNFCCC), and new guidance provided by the
83 Intergovernmental Panel on Climate Change on methods to support GHG accounting for
84 drained and re-wetted organic soils (IPCC, 2013). In the UK, policy impetus towards peat
85 restoration was provided by the recent International Union for Conservation of Nature
86 (IUCN) Commission of Enquiry on Peatlands (Bain et al., 2011), leading to the development
87 of a pilot 'Peatland Code' to underpin a 'Payment for Ecosystem Services' scheme to provide
88 financial support to peatland restoration (Reed et al., 2013). All of these international and
89 national-scale initiatives depend, however, on robust and representative underpinning data on
90 the effects of both drainage and ditch-blocking on CO₂ and CH₄ fluxes, as the basis for
91 calculating 'emission factors' (estimates of the mean annual GHG flux per unit of land

92 surface within a particular land-use category) for use in national greenhouse gas inventories
93 and other accounting schemes. In the UK, and particularly for the upland blanket bog
94 peatlands that make up a large part of the UK peat resource (JNCC, 2011) these data are
95 largely lacking.

96 In this study, we therefore aimed to provide suitable data to support the development of
97 emission factors for GHG accounting in drained and re-wetted blanket bogs, with a focus on
98 CH₄, based on an experimental peat restoration site in North Wales, UK. We also aimed to
99 enhance current understanding of the processes that drive changes in CH₄ emissions
100 following peat re-wetting, particularly in relation to vegetation changes. We hypothesised: 1)
101 that the ditch-blocked area would emit greater amounts of CH₄ than the drained area, as
102 wetter conditions favour methanogenesis and restrict methane oxidation; and 2) that the
103 actual rate of CH₄ emission within the ditch-blocked area would vary according to the extent
104 and type of vegetation re-establishment within the site, due to the role of plants as sources of
105 labile substrate for methanogenesis, and (in the case of aerenchymatous species) in providing
106 pathways for CH₄ transport to the atmosphere. To provide a comparison with more natural
107 (i.e. pre-drainage) conditions, further data were collected at a nearby site with minimal
108 drainage impacts.

109 **Materials and Methods**

110 **Site Description**

111 Research was undertaken within the catchment of Llyn Serw (52° 58' 09" N, 3° 49' 00" W), a
112 small lake within the Migneint blanket bog, which drains to the River Conwy. The Migneint,
113 which lies within a European Special Area of Conservation, largely comprises heterogeneous

114 upland wet heathland on blanket peat, with dominant plant species being *Calluna vulgaris*,
115 *Eriophorum spp*, *Juncus spp*. and *Sphagnum spp*. The Llyn Serw study site lies at an altitude
116 of 450-460m, in an area strongly affected by historic peat drainage, which was undertaken
117 during the 20th century with the intention of enhancing grazing productivity. The site is
118 entirely comprised of *Calluna*-dominated mire with an average canopy height of 50 cm (UK
119 National Vegetation Classification (NVC) class M19 (Rodwell, 1998)). Average peat
120 thickness is around 2 m, with underlying bedrock consisting of Ordovician shales and
121 volcanic tuffs (Lynas, 1973).

122 The hillslope on which the study took place was previously drained by two sets of
123 intersecting drainage ditches (Figure 1). The first set of parallel ditches was dug in the 1920s-
124 1930s running downslope from northeast to southwest. Some vegetation recolonisation and
125 in-filling of these ditches subsequently took place, although in general they have continued to
126 function. A second, deeper set of ditches was dug in the 1970s-1980s, running diagonally
127 across the slope from southeast to northwest. The latter ditches are approximately 40 m apart
128 and flow into Llyn Serw via a single ditch flowing northwest to southeast. The area was
129 affected by a wildfire in 2003, so that the regenerating *Calluna* heathland is at a relatively
130 early growth stage. In August 2008, as part of a pilot restoration study, four of the deeper
131 southeast-northwest ditches were completely blocked with heather bales and then re-profiled,
132 whereby the upper layers of peat adjacent to the ditch were scraped over the heather bales to
133 entirely fill the ditch. The remaining two ditches (those closest to the lake) were left open as
134 a control, visible in Figure 1.

135 In addition to the Llyn Serw site, further data were collected over the same time period from
136 the nearby Nant y Brwyn catchment (52° 59' 51" N, 3°48' 0" W), located 3 km to the North

137 East. This catchment is topographically and botanically similar to Llyn Serw, but has been
138 less affected by drainage, and recent land-management activities have been minimal. The site
139 therefore served as a relatively undisturbed reference site for the drained (and now re-wetted)
140 Llyn Serw catchment. Mean annual air temperature at an automatic weather station located
141 at the Nant y Brwyn (altitude 415 m) is 5.6 °C, with monthly averages ranging from -1.2 °C
142 in December to 12.2 °C in July. The mean annual precipitation is around 2200 mm. For
143 further details of the Migneint area see also Ellis and Tallis (2001; Billett et al., 2010; Evans
144 et al., 2012).

145 **Experimental design**

146 Two sampling transects were established, each approximately 8 m in length, following the
147 slope and extending approximately 4 m either side of an unblocked and a blocked ditch from
148 the second, deeper set of ditches (Figure 1,2). Along each transect, a sequence of
149 measurement points (points A-F, in a sequence from upslope to downslope) were established,
150 within which replicate fixed collars for static chamber measurement and co-located dipwells
151 and pore-water samplers were deployed. Sampling points were also established within the
152 unblocked and blocked ditches themselves. The aim of the experimental design was to obtain
153 a set of integrated and comparable measurements, focusing primarily on CH₄ emissions, at a
154 set of points subject to varying degrees of water table drawdown, during a two year period
155 closely following the re-wetting of a part of the site.

156 **Methane flux measurements**

157 A total of 26 sets of CH₄ flux measurements were made over a 27-month period from June
158 2009 to August 2011, using the static chamber approach (Livingston and Hutchinson, 1995).

159 Sampling was intensified during the growing season, in order to capture the period of
160 anticipated higher CH₄ emissions, and reduced during winter. At sampling points A-F, two
161 replicate gas sampling collars, 30 cm in diameter, were installed in March 2009 and allowed
162 a two-month settling period before sampling commenced. The collars were inserted
163 approximately 5 cm into the ground, with 5cm above-ground. The subsurface part of the
164 collar was perforated to allow subsurface water throughflow and to prevent ponding within
165 the collar during high rainfall. When making flux measurements, we followed the method
166 described by Ward et al. (2007) for the same habitat type, using a chamber modified from a
167 garden cloche (a domed plastic cover designed to protect plants from frost) which was
168 attached to the collar using a rubber seal, to make a closed chamber with a maximum height
169 of 31 cm, and an internal volume of 19 litres. Each chamber contained a vent covered in an
170 expandable polythene skin to allow air pressure inside and outside the chamber to equilibrate.
171 During enclosure, the internal chamber air temperature was monitored using Tinytag
172 temperature loggers (Gemini Data Loggers (UK) Ltd, Chichester, UK) installed in eight of
173 the chambers, with two further loggers monitoring ambient air temperature. Sampling was
174 undertaken from adjacent boardwalk to minimise disturbance and the risk of inducing
175 ebullition. Gas samples of 30 ml were extracted through SubaSeal septa using a syringe and
176 needle. Ambient air samples were also collected during gas sampling, and assumed to
177 represent initial gas concentrations. During the first part of the study, three 30 ml gas samples
178 were extracted from the chamber headspace over an enclosure time of two hours. In 2011, in
179 order to shorten the enclosure time, increase temporal resolution and ensure detection of any
180 non-linear CH₄ concentration changes, four measurements were extracted over a 30 minute
181 period. Extracted gas samples were immediately transferred into pre-evacuated 22 ml air-
182 tight glass vials, and analysed within a week of sampling whenever possible.

183 The additional sampling points G-H were established within the blocked drain in June 2010,
184 on bare peat and re-colonising vegetation respectively, while a point G was also established
185 on bare peat within the open drain in January 2011. Due to the flow of water and sediment,
186 *in situ* collars were not used in the open drain; instead, chamber lids were placed directly onto
187 the ditch base during sampling. Evidence of an air tight seal was demonstrated by suction
188 resistance when the chambers were removed at the end of sampling. Further CH₄
189 measurements (following the same methods) were made in a ditch in the Nant y Brwyn
190 catchment that had naturally infilled with *Sphagnum fallax*, and from a nearby area of
191 *Calluna-Sphagnum* blanket bog that was minimally influenced by drainage. These data were
192 used as a 'reference' for the Llyn Serw site, on the assumption that it will eventually
193 resemble this less disturbed site.

194 Gas analysis was carried out using a Perkin Elmer Clarus 500 Gas Chromatograph (GC).
195 CH₄ was detected using FID (flame ionisation detector) at 375°C, and the sample oven at
196 40°C, equipped with a methaniser. The calibration of the GC for CH₄ involved three
197 standard concentrations (5 ppm, 20 ppm and 50 ppm; Cryoservice, UK) and calibration was
198 accepted at $r^2 > 0.99$. Standard gas concentrations were analysed after every ten samples to
199 assess accuracy of the calibration. The flux was calculated from the time series of CH₄
200 concentrations within the chamber using linear regression (Levy et al., 2011). A flux was
201 accepted if the coefficient of determination (r^2) was at least 0.70. However Alm et al. (2007)
202 highlighted that low fluxes (particularly those close to zero) generally have a low r^2 , and
203 should not therefore be excluded, as this can lead to an over-estimate of mean fluxes.
204 Therefore, fluxes with $r^2 < 0.7$ were retained provided that the residual variance did not
205 exceed a threshold (based on an inspection of the typical variability in the dataset) of 30
206 ($\mu\text{mol mol}^{-1}$)². Mean fluxes were calculated for individual sampling points and for each

207 landscape category (e.g. drained *Calluna-Sphagnum* bog, *Eriophorum vaginatum*-colonised
208 infilled ditch) by first aggregating retained flux measurements into three time periods, namely
209 October to March, April to June, and July to September. The longer period over which data
210 were aggregated during winter was a consequence of the smaller number of measurements
211 made during this period. For each time period, individual collar mean CH₄ fluxes were
212 calculated, and used to derive a seasonal mean flux and associated standard error for each
213 landscape category. Annual mean fluxes were calculated by taking a time-weighted average
214 of the three seasonal subset means. This approach was taken in order to eliminate any
215 potential bias resulting from the greater frequency of summer versus winter measurements.

216 **Other measurements**

217 For each flux measurement, soil temperature was measured at 10 cm depth, and water table
218 depth was measured in a dipwell adjacent to each chamber on each sampling occasion. Mean
219 annual water levels were calculated per dipwell, and aggregated by landscape class, following
220 the same seasonal subset approach applied to CH₄ fluxes. Freely draining pore water was
221 collected using shallow piezometers comprising 2.5cm diameter perforated PVC pipes coated
222 with filter gauze, sampling water at a depth of 15-20 cm. The top of each piezometer was
223 covered with a polypropylene lid, with a vent hole, to avoid contamination. One piezometer
224 was installed next to each set of gas sampling collars at Llyn Serw, including the vegetated
225 and unvegetated sampling points within the infilled ditch. Samples from the intact drainage
226 ditch were collected by directly sampling the surface water with a syringe. At the Nant y
227 Brywn reference site, two piezometers were installed in the *Calluna-Sphagnum* bog, and two
228 in the *Sphagnum*-filled ditch.

229 All piezometers were emptied and allowed to refill before sampling, and samples collected
230 monthly from October 2009 until November 2010 using tubes attached to a 50 ml syringe.
231 Samples were transferred to pre-washed (10% hydrochloric acid) polyethylene bottles for
232 transfer to the laboratory, where they were analysed for pH using an Orion 720A pH meter,
233 and remaining sample filtered using 0.45 µm cellulose membrane filter (Minisart, Sartorius
234 Stedim Biotech, Germany) and stored at 4°C prior to analysis. Dissolved organic carbon
235 (DOC) was analysed with a Thermalox 5001.03 carbon analyser (Analytical Sciences
236 Limited, Cambridge, UK) using the non-purgable organic carbon (NPOC) method, whereby
237 samples were acidified to pH 2.0 and purged with oxygen to drive off any inorganic carbon
238 prior to analysis for DOC. Sulphate concentrations were measured using a Metrohm 850 Ion
239 Chromatograph equipped with a Dionex AS14A analytical column. Meteorological data were
240 obtained from the automatic weather station in the Nant y Brwyn catchment.

241 Within each of the sampling collars, the percent cover was recorded for each species, both in
242 the field and using photographs taken in June 2010 and June 2011. The number of
243 *Eriophorum vaginatum* spikelets (flower clusters, visible in Figure 3 below) was also
244 recorded from these photographs.

245 **Statistical analysis**

246 A simple linear mixed-effects model (Pinheiro and Bates, 2000) was used to analyse the CH₄
247 dataset in relation to land cover category (i.e. undrained, drained and re-wetted blanket bog,
248 active ditch, and infilled ditches with bare peat, *Eriophorum* and *Sphagnum*). Land-cover
249 category was treated as a fixed effect, and repeated measurements at each individual collar
250 location as a random effect. Mean CH₄ flux from each measurement point was analysed

251 against mean measured water table depth and *Eriophorum* cover using simple linear
252 regression.

253 **Area-weighted flux estimation**

254 Area-weighted fluxes were calculated in order to estimate the overall CH₄ emission from the
255 blanket bog landscape in the vicinity of the measurement transects, taking account of the
256 differing proportions of different landscape features, for a number of pre- and post-drainage
257 scenarios. As the basis for this landscape upscaling, we defined a rectangular area around our
258 measurement sites, with a total area of 11000 m² (Figure 1). Although the boundaries of this
259 area are essentially arbitrary, they encompass a fairly typical and homogenous ‘target area’ of
260 the drained blanket bog, much of which was ditch-blocked during the restoration. Adjacent
261 natural wetland flushes, dominated by *Juncus effusus*, were excluded. Within the target area,
262 a high-resolution (0.5 m pixel size) LiDAR digital elevation dataset (National Trust,
263 unpublished data) was used to map the ditches, and to calculate total ditch length. Average
264 width of unblocked and blocked ditches was recorded on the ground, and used to calculate
265 total ditch areas within the target area before and after ditch blocking. Note that blocked
266 ditches remained as shallow, broader features within the landscape following the infilling
267 process (Figure 2). Within these infilled ditches, the proportion of the area occupied by bare
268 peat and recolonising vegetation was also quantified, and measured CH₄ fluxes from the G
269 and H collars used to calculate emissions for each category. The *Calluna*-dominated bog
270 between the ditches was considered as a homogenous landscape component in terms of CH₄
271 fluxes, which were therefore calculated from the mean of all measured fluxes from collars A-
272 F within the drained and re-wetted areas respectively. Although ditch blocking was only
273 carried out on part of the study site, for the purposes of evaluating CH₄ fluxes from drained

274 and re-wetted sites we calculated landscape-scale emissions within the target area for the
275 original fully-drained condition, and for a fully re-wetted scenario. In addition, we applied
276 three alternative ‘long-term restored’ scenarios. The first of these assumed that the wet
277 depressions created by ditch-blocking would become fully colonised by a persistent *E.*
278 *vaginatum* dominated community. The second scenario assumed that these depressions would
279 ultimately become dominated by *S. fallax*, as has been observed at naturally infilled ditches
280 elsewhere on the Migneint. The third scenario additionally assumed that the re-wetted blanket
281 bog would eventually attain the same level of CH₄ emission as an undrained system. These
282 calculations utilised measured CH₄ fluxes from the ‘reference’ site in the nearby Nant y
283 Brwyn catchment.

284 **Results**

285 **Ecological Observations**

286 Over the 27 month sampling period, distinct changes in vegetation were recorded within the
287 blocked drains. Following the disturbance associated with reprofiling, the surface of the
288 blocked ditches was largely bare. Over the course of the study, vegetation cover increased,
289 notably by *E. vaginatum* which was the main colonising species on the perturbed peat on the
290 infilled ditches. By 2010, this had led to visible ‘white stripes’ across the blanket bog along the
291 former ditch lines (Figure 3). Despite this, surface flow continued to occur along parts of the
292 infilled ditches, leading to the persistence of substantial areas of bare peat (also visible on the
293 left of Figure 3) and very limited *Sphagnum* spp. recolonisation. This situation has persisted up
294 to the time of writing (summer 2013), although given that only five years have elapsed since

295 the ditch-blocking took place, it is probable that vegetation composition at the site is still in
296 transition.

297 **Water level and water chemistry**

298 Water levels were clearly lowered either side of the unblocked ditch, with mean water table
299 5.5 cm below the surface upslope of the ditch, and 14.7 cm below at the downslope sampling
300 points (Table 1). The greatest water table drawdown was observed at sampling point D,
301 immediately downslope of the open ditch, on average 25 cm below the surface (Figure 2).
302 Comparing the between-ditch sampling points at the two transects, mean water table over the
303 full measurement period was 7 cm higher around the blocked ditch compared to the
304 unblocked ditch, and this difference was consistent throughout the year (Figure 4a). Mean
305 water table depths of around 3 cm either side of the blocked ditch were close to those
306 measured at the Nant y Brwyn reference site (around 1 cm), indicating that the ditch-blocking
307 has been fairly successful in raising water levels towards natural levels, producing a shallow
308 and relatively uniform water table across the blocked-ditch transect, and reducing
309 interception of downslope flows by ditches running laterally across the hillslope.

310 Water chemistry measurements from within the ditches indicated that DOC concentrations
311 were lowest within the open ditch, and in pore water from the *S. fallax*-infilled reference
312 ditch, and highest in pore water in areas of the infilled ditch where *E. vaginatum* re-
313 colonisation had occurred (Table 1). In the blanket bog, mean porewater DOC concentrations
314 were lower ($< 50 \text{ mg l}^{-1}$) at the undrained site and downslope of the infilled ditch, and
315 higher ($> 70 \text{ mg l}^{-1}$) upslope of the infilled ditch and either side of the open ditch. Pore water
316 pH varied slightly between plots, but there was no clear relationship with vegetation cover or
317 water table. Sulphate concentrations were somewhat higher in the ditch-blocked transect,

318 particularly downslope of the ditch where water table drawdown was greatest, but were
319 similarly high at the undrained site.

320 **Methane fluxes**

321 Methane fluxes showed a high degree of both spatial and temporal variability. Data
322 aggregated into three seasonal time periods (Figure 4b-c) show a general tendency for
323 emissions to be lowest during the winter period (October to March), intermediate during
324 April to June, and highest in July to September. This seasonal pattern was consistent across
325 the undrained, drained and re-wetted *Calluna-Sphagnum* bog, and also in areas of infilled
326 ditch occupied by *E. vaginatum* or *Sphagnum* spp. Emissions from unvegetated areas (open
327 ditches and areas of bare peat in the infilled ditches) showed less consistent seasonal patterns,
328 although maximum fluxes were again recorded during the growing season.

329 Analysis using the mixed-effects model showed the *Eriophorum*-dominated infilled ditches to
330 be the only significantly different land cover category; the 95 % confidence intervals on the
331 other groups were overlapping. Because the study design was not replicated in a strict sense,
332 we place limited emphasis on significance testing of differences, but consider the trends
333 between the groups. For the *Calluna-Sphagnum* bog, average fluxes during all seasons were
334 in the order Re-wetted > Undrained > Drained, albeit with fairly high spatial variability
335 within each category (Figure 4b). Estimated mean annual fluxes ranged from 43.7 kg CH₄ ha⁻¹
336 yr⁻¹ at the drained site to 74.4 kg CH₄ ha⁻¹ yr⁻¹ at the re-wetted site. At the drained site, mean
337 CH₄ fluxes were higher upslope of the ditch compared to downslope (Table 2), corresponding
338 to greater water table drawdown in the downslope locations. Contrasts between fluxes
339 upslope and downslope of the blocked ditch were more subdued.

340 For the within-ditch measurements, by far the highest CH₄ emissions were recorded from
341 areas of *E. vaginatum*-colonised infilled ditch, with an estimated annual mean of 720 kg CH₄
342 ha⁻¹ yr⁻¹. In contrast, mean emissions from the active, infilled bare peat and *S. fallax*-
343 colonised ditch sites were much smaller but similar (ranging from 43 to 51 kg CH₄ ha⁻¹ yr⁻¹).

344 **Relationships between CH₄ fluxes and other measured variables**

345 Mean CH₄ flux measured at each individual collar showed a negative, non-linear relationship
346 with water table, but with very high variability in mean flux among collars with a mean flux
347 at or close to the ground surface (Figure 5a). A significant positive correlation was observed
348 between mean flux and estimated cover of *Eriophorum* spp. (adjusted R² = 0.70, p < 0.001;
349 Figure 5b). Comparing the number of recorded *Eriophorum* spikelets gave a considerably
350 stronger correlation (adjusted R² = 0.89, p < 0.001; Figure 5c), and was particularly effective
351 at differentiating fluxes between collars with a high percentage *Eriophorum* cover.

352 **Area-weighted flux estimates**

353 Data from the drained and drain-blocked transects at Llyn Serw, together with data from the
354 Nant y Brwyn reference site, were used to generate estimates of the overall CH₄ flux from the
355 defined 'target area' (Figure 1). Methane fluxes for Scenario 1 were calculated for a pre-
356 disturbance condition, when the entire area would have been occupied by *Calluna-Sphagnum*
357 blanket bog, as at the Nant y Brwyn. Based on the LiDAR data, a total ditch length of 1559 m
358 was estimated to be present within this 11000 m² area prior to restoration, which ground
359 observations indicated had a mean width of 0.5 m, giving a total of 7.1% of the target area
360 occupied by ditches (Scenario 2). The ditch-blocking process resulted in the formation of
361 shallower depressions with a mean width of approximately 1m. Thus, for a fully re-wetted

362 site, the proportional area occupied by the infilled ditches would increase to 14.1% of the
363 total area. As noted in the methods, this area was estimated to be equally comprised of bare
364 peat and re-colonised *E. vaginatum* after two years of restoration (Scenario 3). The three
365 alternative future scenarios (4a – re-wetted blanket bog with infilled ditches fully colonised
366 by *E. vaginatum*; 4b – re-wetted blanket bog with infilled ditches fully colonised by *S.*
367 *Fallax*; 4c – CH₄ fluxes equivalent to those from an undrained blanket bog, with infilled
368 ditches fully colonised by *S. Fallax*) essentially represent ‘worst’, ‘intermediate’ and ‘best’
369 cases in terms of CH₄ emissions. Note that these scenarios are not time-specific, since the
370 actual trajectory of ecosystem recovery at the site is unknown; in principle these states could
371 occur sequentially, or could represent alternative stable end-points.

372 Results suggest that drainage of the blanket bog reduced CH₄ emissions by a relatively
373 modest amount, from 61 kg CH₄ ha⁻¹ yr⁻¹ to 44 kg CH₄ ha⁻¹ yr⁻¹. Re-wetting is estimated to
374 generate a large increase in overall landscape-scale flux, to approximately 117 kg CH₄ ha⁻¹
375 yr⁻¹ (for a fully re-wetted area), almost double the pre-drainage emission. Around 43% of the
376 total landscape CH₄ emission at this point derives from the estimated 7.1% of the area
377 occupied by *E. vaginatum* in infilled ditches. Of the net increase in estimated CH₄ emissions
378 from the fully re-wetted site, relative to the pre-drainage baseline, an estimated 90% is
379 attributable to the *E. vaginatum*-colonised infilled ditches.

380 Taking the ‘worst-case’ scenario of the infilled ditches becoming fully colonised by *E.*
381 *Vaginatum* (Scenario 4a), the predicted CH₄ flux would further increase to 166 kg CH₄ ha⁻¹
382 yr⁻¹. On the other hand, were the infilled ditches ultimately to become colonised by
383 *Sphagnum* (Scenario 4b), the predicted landscape CH₄ flux would reduce from current levels
384 to around 71 kg CH₄ ha⁻¹ yr⁻¹. Further assuming that CH₄ emissions from the blanket bog

385 return to pre-drainage levels (Scenario 4c), the estimated landscape flux closely approaches
386 its original level, and could even be marginally lower (on the basis that measured CH₄
387 emissions from residual, *Sphagnum*-filled ditch lines were lower than those measured from
388 undrained blanket bog).

389 **Discussion**

390

391 **Effects of re-wetting on CH₄ emissions**

392 Our results suggest that blocking ditches in a drained Welsh blanket bog has led to substantial
393 increases in CH₄ emissions in the years immediately following re-wetting. We recognise that
394 our measurements were (given logistical constraints) limited to a single study site, and thus
395 lacked true replication. Additionally, the measurement method did not permit us to quantify
396 fluxes associated with ebullition, which tend to occur as short, infrequent pulses and therefore
397 require a different, longer-term sampling technique (e.g. Baird et al., 2004). This may have
398 led to some under-estimation of the total CH₄ emission from the peatland. Nevertheless, the
399 relative changes in CH₄ flux observed in our study were generally clear, and were to a large
400 degree consistent with results from other peatland types, including re-wetted cutover sites in
401 Canada (Waddington and Day, 2007), Ireland (Wilson et al., 2009) and Finland (Tuittila et
402 al., 2000), all of which reported an overall increase in CH₄ emissions following re-wetting.
403 For the blanket bog at Llyn Serw, spatial extrapolation of the measurements suggests that
404 landscape-scale CH₄ emissions increased by a factor of 2.7 when comparing the second year
405 post re-wetting to the drained condition. By comparison, Waddington and Day (2007)
406 observed a near fivefold increase in CH₄ when comparing a re-wetted site to a drained

407 cutover site, based on measurements made a similar time after re-wetting. Their landscape-
408 scaled mean CH₄ emission from the restored site at this time was very similar to that
409 estimated in our study (127 vs 117 kg CH₄ ha⁻¹ yr⁻¹), with the greater relative difference due
410 mainly to lower emissions from their unrestored site (a largely unvegetated cutover peatland)
411 compared to our drained but largely intact blanket bog (27 vs 44 kg CH₄ ha⁻¹ yr⁻¹). The study
412 by Wilson et al. (2009) gave lower CH₄ emissions from areas of a re-wetted cutover peatland
413 dominated by an *Eriophorum/Carex* mix (32 – 43 kg CH₄ ha⁻¹ yr⁻¹), but markedly higher
414 emissions from areas occupied by tall fen species (184 – 388 kg CH₄ ha⁻¹ yr⁻¹).

415 **The role of vegetation**

416 Per unit area, our results clearly showed that the largest source of CH₄ emissions derived
417 from blocked ditches containing *E. vaginatum*, which colonised the disturbed bare peat
418 during the first two years after re-wetting. This colonisation by *E. vaginatum* after re-wetting
419 has also been observed on a similar timeframe elsewhere on the Migneint (Peacock et al.,
420 2013); three years after restoration of a peat harvesting site in Eastern Canada (Marinier et
421 al., 2004); and following restoration of a harvest site in a raised bog in Southern Finland
422 (Tuittila et al., 2000). Our results, suggesting that infilled ditches colonised by *E. vaginatum*
423 generate almost half of the total CH₄ emission from less than 10% of the re-wetted blanket
424 bog land surface, are consistent with a number of previous studies showing that CH₄ fluxes
425 tend to be highest where this species occurs (Tuittila et al., 2000; McNamara et al., 2008;
426 Green and Baird, 2012; Ström et al., 2012). Waddington and Day (2007) found that CH₄
427 emissions from a re-wetted cutover peatland were overwhelmingly derived from areas of the
428 peat surface colonised by herbaceous species, of which *E. vaginatum* was the main
429 constituent, together with ditches that had also been colonised by vascular plants, whereas the

430 ~50% of the peatland colonised by moss species acted as a marginal net CH₄ sink. For
431 blanket bogs, McNamara et al. (2008) estimated that around 75% of CH₄ emissions from a
432 semi-natural catchment were associated with *Eriophorum*, and that wet gully areas (42% of
433 which were covered by *Eriophorum* spp.) generated over 95% of all CH₄ emissions from less
434 than 10% of the landscape. Our results thus provide similar evidence of spatially
435 heterogeneous CH₄ emissions, associated with the presence of the same species, within a
436 more human-modified blanket bog landscape. The strong observed correlation between CH₄
437 emissions and *Eriophorum* spikelet numbers has particular value for upscaling, as the
438 spikelets can be detected in aerial imagery during the flowering period, and have been used to
439 map CH₄ emissions elsewhere (Kalacska et al., 2013).

440 **Potential mechanisms for increased CH₄ emissions**

441 Several mechanisms may contribute to higher CH₄ emissions in areas of *Eriophorum* cover.
442 These include the role of aerenchymatous tissue in transporting CH₄ from the anaerobic zone
443 to the atmosphere, the active production of methanogenic substrate by the plants, or simply
444 the tendency for *Eriophorum* spp. to grow within wetter, and hence CH₄-producing, areas
445 within the bog. Our results, showing a stronger relationship between mean CH₄ fluxes and
446 the presence of *Eriophorum* spp. (in particular the number of spikelets) than with mean water
447 table or other measured environmental variables (Figure 5), support previous conclusions that
448 higher fluxes are not simply due to *Eriophorum* spp. occupying wetter niches within the
449 peatland landscape, but reflect an active influence of the plant on CH₄ emissions (Greenup et
450 al., 2000; Marinier et al., 2004; Ström et al., 2012). Higher measured porewater DOC beneath
451 an *E. vaginatum*-vegetated area compared to a bare peat areas of the infilled ditch in our
452 study (Table 1) provides some support to the hypothesis that the plants increase the supply of

453 substrate for methanogenesis, and appear consistent with the results of Ström et al. (2012),
454 who found greater concentrations of acetate, (a substrate for methanogenesis) around
455 *Eriophorum* roots. Lower DOC concentrations from bare peat areas on the infilled ditch
456 suggest that these higher DOC concentrations were not solely related to their location within
457 the infilled ditches, or to decomposition of the underlying *Calluna* bales.

458 The observation that CH₄ fluxes were more closely related to the number of *Eriophorum*
459 spikelets than to percentage cover alone suggests that the vitality and productivity of the
460 plants, rather than simply their presence, may be important. However, this observation could
461 be explained by a number of factors, namely: (i) that the number of spikelets provides a better
462 proxy for aerenchymatous conduit area than our estimates of percent cover; (ii) that the
463 spikelet stems themselves act as a substantial conduit for CH₄ and oxygen exchange between
464 rhizosphere and atmosphere; and (iii) that spikelet numbers are correlated with the rate of
465 root exudate production. Further work is required to differentiate these potential influences
466 on the rate of CH₄ emission, as well as the net greenhouse gas balance implications if higher
467 CH₄ emissions are associated with more productive plants, which may offset these emissions
468 via a greater uptake of CO₂.

469 **CH₄ emissions at the landscape scale**

470 Our results demonstrate the importance of both water table (e.g. comparing fluxes from
471 undrained, drained and re-wetted *Calluna-Sphagnum* bog) and vegetation (e.g. comparing
472 fluxes from bare peat, *Eriophorum* and *Sphagnum*-filled ditches) in determining rates of CH₄
473 emission. The observed role of vegetation type supports previous attempts to use peatland
474 flora as a proxy to estimate CH₄ flux (Dias et al., 2010; Couwenberg et al., 2011; Levy et al.,
475 2012; Gray et al., 2013). Our upscaled flux estimates support the general observation that

476 peatland drainage substantially reduces total CH₄ emissions. In contrast to several previous
477 studies (e.g. Roulet and Moore, 1995; Schrier-Uijl et al., 2011; Teh et al., 2011), we did not
478 observe higher emissions from the active ditches themselves compared to the adjacent land
479 surface. This could reflect the higher ditch gradients in blanket bog (reducing both water
480 residence times and mean water depths, and thus potential for *in-situ* methanogenesis) or
481 relatively low substrate quality and nutrient levels when compared to the agriculturally-
482 drained peatlands studied by Schrier-Uijl et al. (2011) and Teh et al. (2011). On the other
483 hand, some CH₄ was emitted from the active ditch, and in general our data support the
484 inclusion of CH₄ emissions from both drained peatland surfaces and drainage ditches in GHG
485 accounting methods (IPCC, 2013), in place of the previous assumption that drained peatlands
486 do not emit any CH₄ (IPCC, 2006).

487 The positive impact of peatland re-wetting on CH₄ emissions is clear from our results, which
488 suggest that overall emissions from the re-wetted area are about 2.7 times higher than under
489 drained conditions, and 1.9 times higher than from an undrained site. However, the evidence
490 that a very high proportion of this increased emission is associated specifically with
491 *Eriophorum* colonisation of the infilled ditches highlights the importance of successional
492 changes in vegetation following peat re-wetting. *E. vaginatum* is a pioneer species, and was
493 the first to establish within the infilled ditches, and it is possible that it will be displaced, or at
494 least reduced in cover, as other species establish. If this were to happen, at least a part of the
495 increased CH₄ emissions measured in the two years after ditch-blocking could be considered
496 transient. Other factors including disturbance of the peat, and addition of labile organic
497 matter during the restoration process (in this case, heather bales) could also contribute to a
498 transient pulse of emissions. The slightly (albeit non-significantly) higher measured CH₄ flux

499 from the re-wetted *Calluna-Sphagnum* blanket bog, when compared to a botanically similar
500 undrained location, would appear to support this interpretation.

501 Two of our scenarios for landscape-scale CH₄ fluxes (Scenarios 4b and 4c, Table 3), in which
502 *S. fallax* is assumed to colonise the infilled ditches, suggest that emissions may eventually
503 reduce towards pre-drainage levels. However the validity of the assumptions underpinning
504 these scenarios, and also the time it may take to achieve a final vegetation community,
505 remain uncertain. Haapalehto et al. (2011) observed that cover of *E. vaginatum* was
506 continuing to increase 10 years after the re-wetting of a bog in Finland, suggesting the CH₄
507 emissions at our site might in fact continue to rise. For our worst-case scenario (4a), in which
508 *E. vaginatum* expands to cover the entire infilled ditch area, the landscape-scale estimated
509 CH₄ flux is almost 3.8 times higher than from the drained bog, and 2.7 times higher than the
510 from undrained bog. The establishment of *Sphagnum* within infilled ditches thus appears
511 critically important; as well as suppressing the cover of aerenchymatous species such as *E.*
512 *vaginatum*, *Sphagnum* has been shown to support methanotrophic (CH₄ consuming) bacteria,
513 reducing the release of CH₄ from the anaerobic zone to the atmosphere (Raghoebarsing et al.,
514 2005). The marked contrast in CH₄ emissions between vegetation types, and the uncertain
515 trajectory of future vegetation changes, suggests that active management to facilitate re-
516 colonisation by *Sphagnum* might be beneficial. On the Migneint, more recent re-wetting
517 activities have been undertaken using an alternative ditch-blocking method, involving the
518 ‘reprofiling’ of ditches to form shallower depressions, interspersed with peat dams and small
519 pools. Peacock et al. (2013) found that *Eriophorum* species were unable to colonise the
520 deeper pools, which instead tended to develop a *Sphagnum* cover, with probable benefits in
521 terms of CH₄ emissions.

522 Finally, it is important to emphasise that, although we observed an increase in CH₄ flux
523 following ditch-blocking at our site, this does not necessarily indicate that peatland re-wetting
524 has had a net warming effect in terms of overall GHG emissions. Waddington et al. (2010)
525 found that an increase in the CO₂ sink after restoration outweighed the increase in CH₄
526 emissions, and Wilson et al. (2013) found *Eriophorum* to be substantial sink of carbon,
527 offsetting its role as a source of CH₄ emissions. It is therefore feasible that the re-wetting of
528 our study site, and similar sites elsewhere, may be having a net cooling impact, despite
529 increasing CH₄ emissions with increasing *Eriophorum* cover.

530 **Conclusions**

531 Infilling drainage ditches increased water table elevation and landscape-scale CH₄ flux in the
532 two years following blocking. The increased CH₄ emissions observed were driven by the
533 creation of CH₄ 'hotspots' that occurred where *E. vaginatum* tussocks colonised the infilled
534 ditch. It is unknown whether this phenomenon is a long-term effect; CH₄ fluxes from the
535 ditch-blocked area were also higher than in a nearby undrained area, suggesting that at least
536 part of the observed increase may be transient. A large part of the uncertainty in attempting
537 to extrapolate these effects over longer time scales is the uncertain trajectory and time course
538 of plant succession on the blocked ditches, together with the apparent but uncertain links
539 between plant species composition and CH₄ flux. Active vegetation management may
540 therefore exert a considerable influence on the greenhouse gas balance of re-wetted
541 peatlands.

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Table 1. Annual mean water table depth (\pm standard error for locations with multiple dipwells), and porewater pH, DOC, SO₄ concentrations measured at 20 cm depth. 'Number of samples' corresponds to total number of pore water chemistry samples used to calculate each mean value. Water table was recorded manually from one dipwell per plot at the time of sampling.

Sampling location (plot codes)	Water table (cm)	pH	DOC (mg l⁻¹)	SO₄ (mg l⁻¹)	Number of piezometers	Number of samples
<i>Drained site (Llyn Serw)</i>						
Blanket bog, upslope (A-C)	5.5 \pm 0.7	4.91	71.4	1.00	3	28
Within ditch (G)	<i>At surface</i>	4.81	30.3	0.64	1*	10
Blanket bog, downslope (D-F)	14.7 \pm 2.6	4.84	77.0	1.86	3	21
<i>Re-wetted site (Llyn Serw)</i>						
Blanket bog, upslope (A-C)	3.0 \pm 0.5	4.94	80.7	0.90	3	30
Within ditch unvegetated (G-H)	<i>At surface</i>	4.88	35.3	0.38	1	10
Within ditch vegetated (G-H)	<i>At surface</i>	4.92	52.1	0.97	1	10
Blanket bog, downslope (D-F)	2.7 \pm 0.3	4.90	48.8	0.54	3	30
<i>Undrained reference site (Nant y Brwyn)</i>						
Blanket bog, undrained	1.0	4.67	44.1	1.91	2	17
Within ditch, <i>Sphagnum</i>	1.2	4.59	27.6	0.86	2	18

*Within-ditch sample from the open ditch was collected directly from surface water using a syringe.

Table 2. Annual mean CH₄ fluxes (\pm standard error) expressed as mg CH₄ m⁻² day⁻¹ and kg CH₄ ha⁻¹ yr⁻¹, together with the number of collars within each category for which measurements were made, and the number of individual chamber tests for which it was possible to calculate fluxes after screening.

Sampling location (plot codes)	CH ₄ (mg CH ₄ m ⁻² day ⁻¹)	CH ₄ (kg CH ₄ ha ⁻¹ yr ⁻¹)	Number of collars	Number of chamber tests
<i>Unblocked ditch (Llyn Serw)</i>				
Blanket bog, upslope (A-C)	15.1 \pm 2.0	55.3 \pm 7.3	6	111
Within ditch (G)	16.3 \pm 5.6	59.7 \pm 20.6	4	24
Blanket bog, downslope (D-F)	8.8 \pm 1.2	32.1 \pm 4.4	6	108
<i>Blocked ditch (Llyn Serw)</i>				
Blanket bog, upslope (A-C)	23.7 \pm 8.1	86.7 \pm 29.5	6	102
Within ditch unvegetated (G-H)	10.3 \pm 2.7	37.7 \pm 10.0	4	17
Within ditch vegetated (G-H)	197.0 \pm 31.1	719.5 \pm 113.5	4	28
Blanket bog, downslope (D-F)	16.3 \pm 3.9	59.6 \pm 14.1	6	98
<i>Reference site (Nant y Brwyn)</i>				
Blanket bog, undrained	16.7 \pm 2.5	61.1 \pm 9.2	8	89
Within ditch, <i>Sphagnum</i>	13.9 \pm 7.5	50.7 \pm 27.2	4	39

Table 3. Estimated area occupied by each land-cover category within the target area shown in Figure 1, and estimated CH₄ emissions, for a sequence of pre-, during- and post-drainage scenarios

	Drainage Scenario						CH ₄ flux by land-cover category (kg CH ₄ ha ⁻¹ yr ⁻¹)
	1) Pre-drainage	2) Drained	3) Recently re-wetted	4a) Long-term re-wetted 1	4b) Long-term re-wetted	4b) Long-term rewetted 3	
Area occupied by each land-cover category (%)							
Blanket bog (undrained)	100.0					85.9	61.1
Blanket bog (drained)		92.9					43.7
Blanket bog (re-wetted)			85.9	85.9	85.9		74.4
Active ditch		7.1					43.1
Infilled ditch (bare peat)			7.1				37.7
Infilled ditch (<i>Eriophorum</i>)			7.1	14.1			719.5
Infilled ditch (<i>Sphagnum</i>)					14.1	14.1	50.7
Landscape CH₄ flux by scenario (kg CH₄ ha⁻¹ yr⁻¹)	61.1	43.7	117.4	165.6	71.0	59.6	

FIGURE CAPTIONS

Figure 1. Lidar hillshade image of the Llyn Serw study site, part of the Migneint blanket bog in North Wales, UK. Two open ditches (running Southeast to Northwest) are visible as sharp linear features, whilst the infilled ditches running on the same trajectory appear as shallower, broader features. Ditches running from Northeast to Southwest are older, shallower and partly infilled, but continue to have exert some influence on water table in the Southwestern part of the site. Measurement transects are also shown, along with the ‘target area’ of relatively homogenous drained bog used for upscaling.

Figure 2. Indicative surface elevation (thinner black line, derived from LiDAR cross-sections) for the two Llyn Serw sampling transects, showing infilled and open ditches in cross-section. Ditches run diagonally across the hillslope. Static chamber sampling locations between ditches (A-E), and within ditches (G-H) are shown. Water table elevation (thicker blue line) is approximate, based on mean annual water table depth relative to the LiDAR surface at dipwells located within the bog, and observations of water table at or slightly above the surface in the infilled and open ditches respectively. The two transects are separated by a buffer section of approximately 40 m, including a blocked ditch (see Figure 1).

Figure 3. Recolonisation of *Eriophorum vaginatum* on an infilled ditch (photograph taken in July 2010, 24 months after infilling took place). Note presence of bare peat on left of ditch.

Figure 4. Seasonal and spatial variations in a) mean water table depth from undrained, drained and re-wetted *Calluna-Sphagnum* blanket bog; b) mean CH₄ flux from the same locations, c) mean CH₄ flux from active and infilled ditches, and. Error bars indicate standard

error among replicate sampling collars and dipwells within each category (note that standard errors could not be calculated from water table in undrained *Calluna-Sphagnum* bog, as only one dipwell was deployed here).

Figure 5. Mean annual CH₄ flux for each sampling collar versus a) water table, b) *Eriophorum* cover and c) number of *Eriophorum* spikelets recorded in each collar during June 2011.

Figure 1

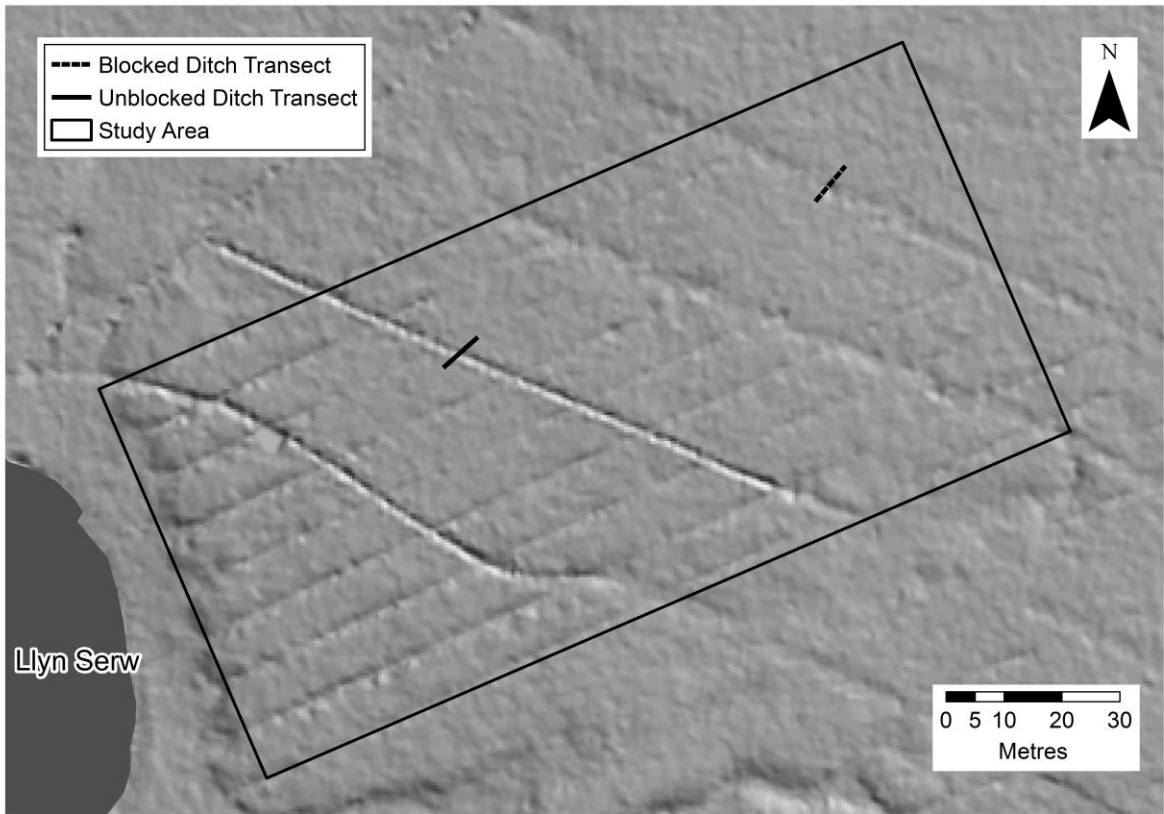


Figure 2

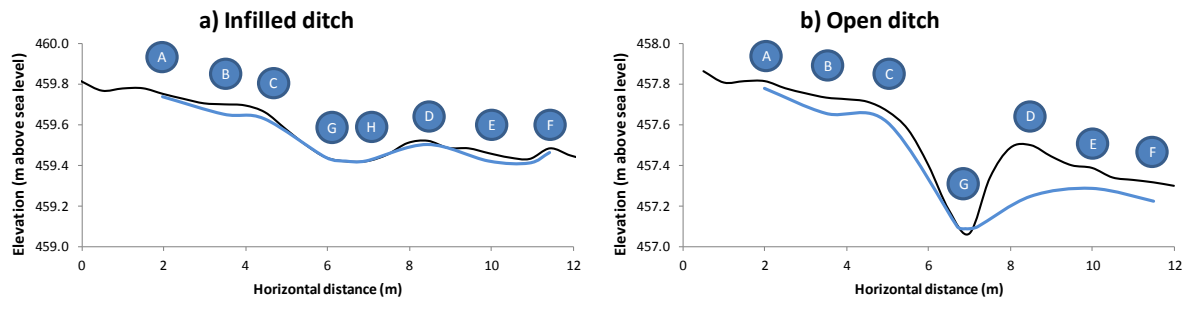


Figure 3



Figure 4

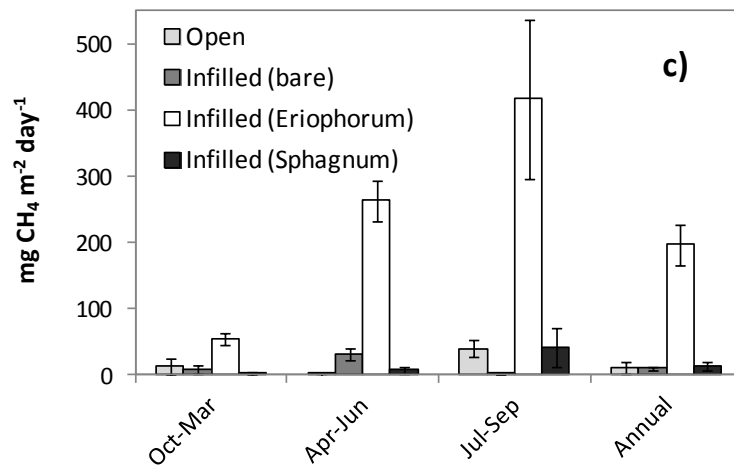
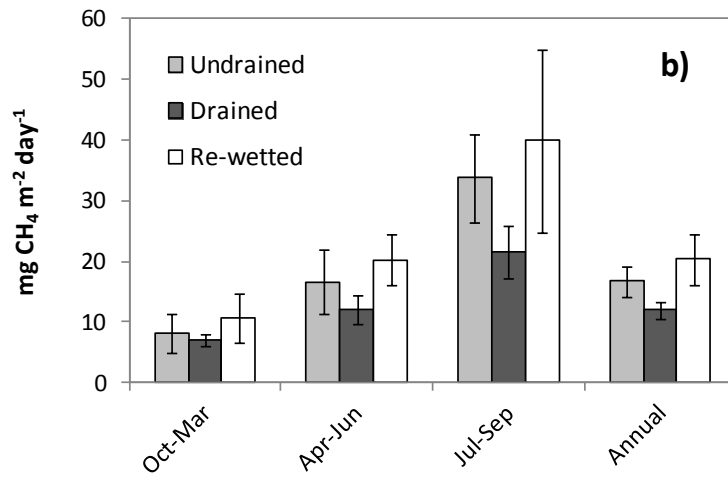
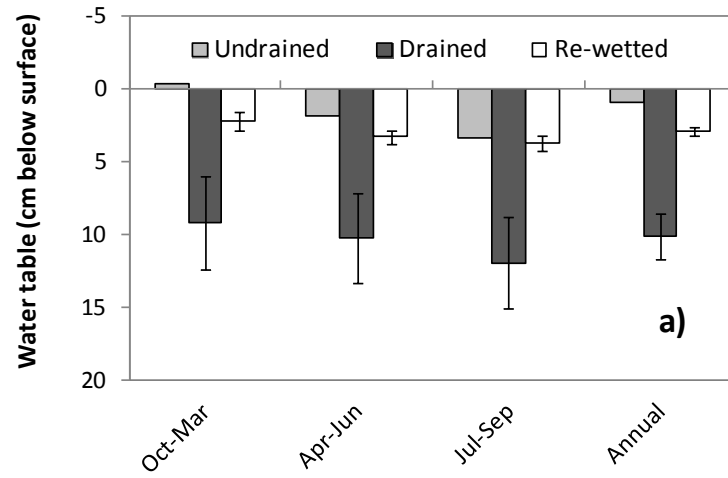


Figure 5

