1 **Title: Sustained impact of drought on wet shrublands mediated by soil physical**

2 **changes**

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19 **Abstract**

20

21 Projected climate warming, and increasingly frequent droughts, may substantially

22 increase carbon emissions from wet organic soils, contributing to the positive feedback

23 between the terrestrial carbon cycle and climate change. Evidence suggests that, in these

soils, the stimulation of soil respiration by warming can be sustained over long periods of time due to the large availability of C substrates. The long-term response of wet organic soils to drought remains however uncertain. Organo-mineral soils might be particularly vulnerable, because of their limited soil moisture pool to buffer drought events. Using a whole-ecosystem climate-change experiment in North Wales (UK) we show that wet shrublands on podzolic (organo-mineral) soils are more vulnerable to recurrent drought than to warming, and that the drought impact does not attenuate at decadal time scales. Stimulation of soil respiration by drought was linked to major changes in soil structure that led to a 54 % reduction in water holding capacity compared to control. Bryophyte abundance was found to buffer soil moisture losses, moderating soil CO_2 efflux under warming. As there was no evidence of change in plant productivity to offset the increased soil C emissions under drought, this response may result in a positive climate feedback. The results indicate the potentially critical role that changes in sub-dominant vegetation and in soil physical properties may have in determining climate change impacts on soil C dynamics.

Introduction

Global soil respiration has increased by 0.1 Pg C yr^{-1} during the last two decades in response to a warming climate (Bond-Lamberty and Thomson 2010). Based on the kinetic theory of photosynthesis and plant/soil respiration, several models have predicted a positive feedback to climate, resulting from higher increases in respiratory fluxes than in net primary productivity (Friedlingstein et al. 2006; Denman et al. 2007). This climate feedback is, however, critically dependent on the long-term adaptation of the plant and soil processes to warming and other climate change factors, such as rising CO_2 and changing precipitation patterns (Luo 2007).

Empirical data suggests that the stimulation of soil respiration by warming decreases over time in some ecosystems. This evidence is mainly confined to well-drained mineral soils, where the initial increase in soil respiration returns to pre-warming rates within a few years (Rustad et al. 2001; Luo et al. 2001; Melillo et al. 2002; Eliasson et al. 2005; Hartley et al. 2007). Two main, non-exclusive types of mechanisms have been proposed to explain the attenuation of respiration rates over time. The first type refers to indirect effects of warming on microbial activity (*apparent thermal acclimation*, *sensu* Bradford 2013) such as the rapid depletion of the labile C pools (Kirschbaum, 2004; Knorr et al. 2005; Eliasson et al. 2005; Hartley et al. 2007), while the second type includes direct physiological adjustments of the soil microbial communities (*thermal adaptation*), through changes in the processes involved in microbial respiration and growth (reviewed in Bradford 2013).

Wet organic soils, however, may show a different long-term response given that the environmental constraints to soil organic matter (SOM) decomposition are different in these soils. Soil CO_2 efflux in wet organic soils is controlled by water-excess

conditions, which restricts oxygen diffusion to decomposition reaction sites, inhibiting the activity of phenol oxidase as well as some hydrolyzing enzymes, and promoting anaerobic decomposition (Freeman et al. 2001; Fenner and Freeman 2011). Consequently, the effect of warmer temperatures on soil respiration is greatly influenced by soil moisture (Shaver et al. 2006; Vicca et al. 2009; Xiang and Freeman 2009). As wet organic soils contain large organic C stocks, much of which becomes accessible to soil microorganisms following natural drying-rewetting events, apparent thermal acclimation might occur more slowly in comparison to soils with a low C content. Several studies have confirmed that this is true for organic arctic soils (Shaver et al. 2006; Dorrepaal et al. 2009), where warming-induced respiration stimulation does not attenuate in a range of time scales, due to the large C substrate availability (Shaver et al. 2006).

Often overlooked are the ecosystems with organo-mineral soils, which could be defined as those mineral soils with a carbon-rich surface horizon (Bol et al., 2011). This definition includes those soils having humose topsoil > 15cm thick, or peaty loam/peaty sand topsoil >15cm thick, as well as those having a peat layer <40cm thick starting at the surface or directly on bedrock (Bol et al., 2011). Organo mineral soils tend to occur within the following WRB (2006) groups: Leptosols, Umbrisols, Podzols, and Gleysols. Soils within these groups are estimated to cover 30.5 % of the European Union land area (Bol et al. 2011), thus containing a large fraction of the vulnerable soil C stocks at the European scale. In British wet shrublands (dominated by *Calluna vulgaris*) organo-mineral soils within this definition occupy 1.96 million ha (Hall et al. 2014). These wet shrublands have one of the highest soil C densities across habitats in this country due to their relatively high C concentrations and intermediate bulk density values (Reynolds et al. 2013). They might be more vulnerable to subtle drought conditions than peatlands,

4

99 because of a limited soil moisture pool being available to buffer drying events, thus

100 representing a potentially large C pool vulnerable to climate change.

101 Current predictions of climate change include severe and widespread droughts in the

102 next 30–90 years (Christensen et al. 2007; Dai 2013), and the co-occurrence of warming

103 and drying in wet, organic-C rich soils could lead to substantial losses of soil C, as soils

104 are exposed to aerobic decomposition (Shaver et al. 2006; Ise et al. 2008; Fenner and

105 Freeman 2011). Indeed, some hydro–biogeochemical soil models predict a positive

106 feedback between soil drying and SOM decomposition in wet organic soils, leading to

107 an increased sensitivity of these soils to future climate warming (Ise et al. 2008). This

108 positive feedback would result from changes in soil structure leading to a decrease in

109 the soil water holding capacity. Shrinking during soil drying can have a pronounced

110 effect on the size and stability of the pores in organic soils, leading to a destabilization

111 of macropores and macroaggregates (Peng et al. 2007; Borken and Maztner, 2009). Soil

112 structure might also be altered as plants change their rooting structure in response to

113 drought, seeking water lower in the profile, or in response to warmer temperatures.

114 Despite soil water holding capacity being a key parameter for ecosystem functioning,

115 there is lack of empirical information about the effects of climate change drivers on this

116 soil physical property.

117 The adaptation of plant and soils processes to repeated changes in the soil water balance

118 might attenuate the predictions of large C losses from wet soils. The analysis of

119 different soil inventories across England and Wales revealed that wet organic soils lost

120 carbon an order of magnitude faster than well-drained mineral soils between 1978 and

121 2003, and suggested that these losses are likely to have been offsetting the absorption of

122 C by terrestrial sinks (Bellamy et al. 2005). However, two subsequent studies have not

123 supported this finding for England and Wales (Reynolds et al. 2013) or Scotland

124   (Chapman et al. 2013). To reduce the large uncertainties in the predictions of C losses

125   from wet soils, more empirical data about the relative sensitivity of soil respiration and

126   plant primary productivity to climate change drivers is urgently needed.

127   We investigated the long-term impact of warming and repeated summer droughts on

128   soil respiration from organo-mineral soils in a wet shrubland, using a whole-ecosystem

129   climate-change experiment in North Wales, UK. Previously, Sowerby et al. (2008)

130   showed an ongoing stimulation of soil respiration by drought during the first five years

131   of the manipulation with no evidence of attenuation. In this work, we monitored soil

132   respiration and plant aboveground biomass over 14 years, and measured soil water

133   release curves (WRCs) of the topsoil (0-5 cm) after a decade of experimental

134   manipulation, to determine if the soil structure and water holding capacity had changed.

135   Specifically, we aimed to address the following questions:

136   1)   Does the stimulation of soil respiration under warming and drought attenuate

137   over the long-term? We hypothesised that, because soil C decomposition is highly

138   constrained by water-excess conditions in these soils, drought would have a relatively

139   higher impact than modest warming, and that, because of the potentially large

140   availability of C substrates in these soils, the apparent acclimation to warming and

141   drought would occur at relatively long experimental periods.

142   2)   Do drought and warming have an impact on soil water holding capacity? We

143   hypothesised that recurrent drought would have a greater impact on soil structure than

144   long-term warming, and that, according to theoretical models (Ise et al. 2008), repeated

145   drying-rewetting events would reduce soil water holding capacity.

146

147   **Materials and Methods**

148   Experimental design

149   Our study was conducted at the Clocaenog site in NE Wales, UK (53º 03' 19"N, 03° 27'

150   55"W), situated at 490m a.s.l. Mean annual air temperature is 8.2 ºC, rainfall is 1700

151   mm, and potential evapotranspiration is 302 mm. The ecosystem is an upland Atlantic

152   heathland dominated by *Calluna vulgaris* (L.) Hull, with *Vaccinium myrtillus* L.,

153   *Empetrum nigru*m L. and *Deschampsia flexuosa* (L.) Trin. Total aboveground biomass

154   is around 3500 g m^{-2}. The soils are organic-rich with 4–10 cm of organic soil (organic

155   matter content 89%), overlaying 18-20 cm of organic-rich mineral soil (organic matter

156   content of 37%). They are classified as Ferric stagnopodzols in the Hafren Series in the

157   Soil Survey of England and Wales (Cranfield University, 2014), and as Endoskeletic

158   Histic Stagnic Albic Podzols in the IUSS Working Group WRB (2007) classification.

159   Mean annual soil temperature is 6.7 ºC.

160   Whole-ecosystem simulation of drought and warming started in March 1999 and has

161   been running to date. Three blocks were established at the site, each one with three

162   experimental plots (4 × 5 m) randomly allocated to the drought, warming and control

163   treatment, respectively. Fully automated retractable curtains (see Beier et al. 2004 for a

164   full description) were placed over the plots to achieve air warming (warming treatment)

165   or to exclude the rain from the plots from June to September annually (drought

166   treatment).

167   The warming treatment consisted of a passive night-time warming provided by

168   reflective aluminium curtains covering the vegetation at night. These curtains reflect 96-

169   97 % of infrared radiation, reducing the heat loss during night by 64 % (Beier et al.

170   2004), and producing an increase of 0.2-2.0 ºC in monthly air temperature, in the range

171   predicted for Northern Europe for 2010-2100 under three of the four IPCC´s RCP

172 scenarios (van Oldenborgh et al. 2013). This warming treatment increased the growing

173 degree-days (based on lower and upper-threshold temperatures between which plant

174 growth occurs) by 9 % on average (see Supplementary Information, Table S1). During

175 the night, the warming curtains are retracted during rain events to prevent the reduction

176 of rain input to the plots. However, there was a small unintended reduction in rain input

177 brought about by the short delay in warming roofs retracting at the onset of a

178 precipitation event (on average, annual rainfall inputs into the warming plots were a

179 13% lower compared to control). Nevertheless, this did not result in an overall

180 significant reduction in soil moisture levels in the warming plots during the experiment.

181 During 2003, soil moisture was significantly lower in the warming (annual average of

182 0.45 m^3 m^{-3}) than in the control plots (annual average of 0.53 m^3 m^{-3}; repeated measures

183 ANOVA, treatment effect $p = 0.0143$). Likewise, during 2005 moisture levels were

184 lower in the warming treatment (annual mean of 0.46 m^3 m^{-3}) than in the control

185 treatment (annual mean of 0.51 m^3 m^{-3}; repeated measures ANOVA, treatment effect p

186 $= 0.039$). For the rest of years, moisture levels in the warming treatment were similar

187 (2000-2002 and 2004-2008) or slightly greater (2008 and onwards) in comparison to

188 control.

189 The drought treatment consisted of waterproof polyethylene curtains triggered by a rain

190 sensor. These curtains excluded 54 % of the rainfall from June to September, with the

191 aim of emulating the UK summer drought of 1995, considered to be the most recent

192 significant drought year prior to drought treatment initiation. Annual and growing

193 season rainfall were reduced by on average 21 % and 54 % respectively (Table S1),

194 which resulted in an average reduction of 30 % in soil moisture during the growing

195 season. Outside the drought period (re-wetting period), drought plots are managed in the

196 same way as the control plots.

197

Soil respiration, temperature and moisture

Soil respiration (*in situ* rates) was measured fortnightly from March 1999 to December 2012 between 12:00 and 15:00, in three collars per plot (a total of 9 collars per treatment), using portable infrared gas analysers coupled to a soil respiration chamber (EGM-2, PP Systems until 2008 and LI-8100 automated soil CO_2 flux system onwards). These collars included both heterotrophic and autotrophic respiration. Any aboveground plant growth within the collars was removed regularly. During 2005, measurements were restricted to the spring and summer seasons due to technical limitations. In summer 2002, the diurnal cycle of soil respiration was studied once per month by measuring soil respiration at 14:00, 20:00, 00:30, 05:30 and 10:30 hours. Average diurnal soil respiration rates were found to be 87% of the respiration rates recorded during routine afternoon measurements taken every two weeks. This was used to correct the calculations of daily soil CO_2 emissions from the afternoon respiration rates (see data analysis).

Over the 14 years, soil temperature and moisture were also monitored. At the plot level, soil temperature was measured in 0–5 cm soil depth by Reference Thermistor sensors (Probe 107, Campbell Scientific, Logan, UT, USA). Soil moisture (0–10 cm depth) was measured fortnightly using a theta probe (ML-2, Delta-T, Cambridge, UK) and a soil moisture meter (HH2, Delta-T) until 2009. In 2009, Time Domain Reflectometer (TDR; CS616, Campbell Scientific, Logan, UT, USA) probes were inserted into the soil at 5 cm and 20 cm, for a continuous monitoring (hourly recordings) of soil moisture. Rainfall at the plot level was also recorded using open funnels with fortnightly collection to estimate the mean reduction in rainfall due to the treatment curtains.

221

222   Aboveground and root biomass

223   Aboveground composition and biomass was monitored every year (excepting 2001, and

224   2004-2006) at the end of the growing season (August) using the pin-point method in

225   three permanent 0.5 x 0.5 m^2 subplots per plot. In each subplot a grid of 100 pins was

226   lowered through the vegetation. At 5 cm intervals, every touch of vegetation was

227   recorded, indicating the species, the plant part (leaf, flower, or stem) and its status

228   (green, dry, dead). In 2000 a calibration between pin-point measurements and plant

229   biomass was conducted in the same site but outside the experimental plots, using a

230   destructive sampling. Relationships between pin-point measurements and plant biomass

231   were established for each plant species (r^2 = 0.70 for bryophytes; r^2 = 0.98 for *D.*

232   *flexuosa*; r^2 = 0.64 for *C. vulgaris*; r^2 = 0.65 for *E. nigrum*; r^2 = 0.82 for *V.myrtillus*).

233   Root biomass was measured in three cores per plot (15 cm depth and 5 cm diameter),

234   collected underneath *C. vulgaris* individuals in September 2011. Cores were sliced from

235   the top into 1cm segments until 10 cm depth, and roots in each segment were washed

236   and extracted. Roots were dried at 70 ℃ for at least 48 h and weighed.

237

238   Soil water release curves

239   Soil water release curves were measured on 250 cm^3 soil cores, 0-5 cm deep, extracted

240   from each plot underneath *C. vulgaris* in September 2011. Water release curves

241   typically took 1 month to determine using the laboratory evaporation method (Schindler

242   et al., 2010) using a HYPROP (UMS, Munchen, Germany).

243

244   Data analysis

245   We estimated the daily average respiration rates as 87% of the rate measured at midday,

246   based on the diurnal data taken in 2002. We then calculated average rates for each

247   season, and finally multiplied by the number of days in each season to obtain the

248   seasonal cumulative CO_2 emissions. Annual emissions (R_{year}) were calculated as the

249   sum of all the seasonal emissions. For each year, the relative impact of warming and

250   drought (ΔR_{year} from control) was calculated.

251   A mixed-model analysis of variance was performed to test for significant differences

252   among treatments in R_{year} for each year separately. Treatments were assigned as fixed

253   factors and block as a random factor. Tukey's HSD multiple comparison test was

254   applied to identify differences among treatments in R_{year}. A similar analysis was applied

255   to test for differences in root biomass. To test for the general effect of the treatments on

256   R_{year} emissions and aboveground biomass over the 14 years of manipulation (time $\times$

257   treatment interaction), we applied repeated measures analysis of variance, using the

258   Greenhouse–Geisser adjustment. Water release curves were tested for homogeneity of

259   slopes among treatments using ANCOVA test, with soil water volumetric content as

260   dependent variable, soil water tension as a continuous predictor (covariate, log

261   transformed), and treatment as a categorical factor, using Statistica v10 (Stat Soft Inc.).

262

263   **Results**

264   Drought impact on soil respiration

265   Drought stimulated soil respiration rates by 22 % (average for all seasons and years,

266   Fig. 1). Summer drought impact on annual soil CO_2 emissions (R_{year}) was enhanced

267   mainly in drier years (repeated-measures ANOVA, significant year $\times$ treatment

268   interaction, $F = 6.2$, $p = 0.011$; Fig 2a, b), with significant increases in 2004 ($p = 0.004$),

269   2005 ($p = 0.045$), 2009 ($p = 0.050$), 2011 ($p = 0.007$), and 2012 ($p = 0.034$). The mean

270   R_{year} stimulation was 80 g C m^{-2} year^{-1} (17 % of the inter-annual average in the control

271   treatment).

272   Stimulation of respiration was greatest during the summer months (experimental

273   drought period) when it was directly linked to the reduction in soil moisture (Fig. 3a),

274   but persisted into autumn months, the rewetting period, when respiration rates were up

275   to 70 % higher, compared to control (Fig. 3b). No decrease was detected in the

276   magnitude of the stimulation over the years, which would have indicated apparent

277   acclimation of the soils to experimental drought. To the contrary, the relative impact of

278   drought during the summer months increased over the years (Fig. 3b), and since 2010

279   drought produced a continuous stimulation of soil respiration across the seasons (Fig.

280   1).

281

282   Warming impact on soil respiration

283   The impact of warming was lower than that of drought, with a respiration stimulation of

284   13.2 % (average for all seasons and years, Fig. 1). The stimulation of R_{year} by warming

285   was only marginally significant ($0.07 > p < 0.05$, average of 49 g C m^{-2} year^{-1}).

286   Since 2005, the stimulation of respiration by warming decreased, which was associated

287   with an increase in the abundance of bryophytes in the warming plots (Fig. 4), which, in

288   contrast, markedly decreased in the drought plots. This change in bryophyte abundance

289   coincided with a shift in the soil moisture dynamics in the warming plots after the

290   natural drought of 2005 (Fig. 5a). Prior to 2005, the warming and control plots had

291 similar soil moisture frequency distributions, the warming soils being slightly drier,

292 whereas after 2005 the warming plots were consistently wetter than the control or

293 drought, regardless of whether it was a dry year (2010, Fig. 5b) or a wet year (2011,

294 Fig. 5b).

295

296 Soil water release curves

297 After a decade of experimental manipulation, all soils had a porosity between 0.85 and

298 0.90 $m^3 m^{-3}$, but the drought soils released water much more rapidly than the control or

299 warming soils with increasing tension (Fig. 6). Within the range of field capacity, water

300 release curves had the same slope, but different intercept (volumetric water content)

301 among treatments (ANCOVA model; treatment: $F = 13518$, $p < 0.001$; treatment $\times$ soil

302 tension: $F = 1.6$, $p = 0.2$). This meant that, in the estimated range of field capacity (~ -

303 2kPa), the control soils retained ~0.7 $m^3 m^{-3}$ of water, whilst the drought and warming

304 soils retained 54 % and 26 % less respectively. This was consistent with an increase in

305 the median pore size from 30 μm in the control to 74 μm and 248 μm in the warming

306 and drought plots, respectively, determined from the conversion of the WRCs to

307 effective pore size.

308

309 Plant biomass

310 Aboveground biomass differed significantly across years, but never among treatments

311 (repeated measures ANOVA: treatment: $F= 0.283$, $p = 0.73$; year: $F= 7.63$, $p < 0.0001$;

312 treatment $\times$ year: $F = 0.509$, $p = 0.94$, Fig S1a). There was a trend of greater root

313 biomass in warming plots compared to control (Tukey HSD test, $p = 0.044$), while root

314 biomass in the drought and control plots where not significantly different (Tukey HSD

315 test, $p = 0.192$; Fig. S1b).

316

317 **Discussion**

318 Warming and drought impact on soil respiration

319 Wet shrublands with organo-mineral (podzolic) soils have one of the highest soil C

320 densities across habitats in the UK, but a limited soil moisture pool to buffer drought

321 events in comparison to peatlands. Therefore, they represent a type of ecosystem with a

322 high vulnerability to the projected increases in the frequency of severe droughts. Our

323 results clearly show that, as we hypothesised, soil respiration in wet shrublands is more

324 sensitive to repeated modest drought than to subtle warming, and that the drought

325 impact does not attenuate at a decadal time scale.

326 Several other experiments have shown that soil respiration or net ecosystem exchange is

327 relatively more sensitive to changes in the precipitation regime than to soil warming in

328 different types of ecosystems (Garten et al. 2009; Liu et al. 2009; Suseela et al. 2012),

329 although in those studies, conducted in well-drained mineral soils, drought conditions

330 typically suppressed respiration. In our study, drought increased summer and autumn

331 CO_2 emissions by a 22 % and a 27 % (on average), respectively, with maximum

332 increases above 60 %. The stimulation of soil respiration during the summer months,

333 when temperature and the supply of plant photosynthates are not limiting, was linearly

334 linked to the reduction in soil moisture, illustrating the importance of the water-excess

335 as an environmental constraint to decomposition in these soils, and contrasting to the

336 response of other mesic and dry shrubland ecosystems across Europe (Sowerby et al.

337 2008).

14

338 Both autotrophic and heterotrophic respiration may be enhanced by the release of water-

339 excess conditions. Although our measurements did not include the separation of each of

340 the respiration components there is an indication that respiration changes are likely

341 driven by heterotrophic processes, as root biomass was not significantly greater in the

342 drought plots (Fig. S1b). This is consistent with the finding that plant aboveground

343 biomass did not change among treatments (Fig. S1a), suggesting high resilience of plant

344 productivity to the climate treatments. In addition, a ^{14}C pulse-labelling experiment

345 (Gorissen et al. 2004) revealed that C translocation belowground was reduced by 40 %

346 in the drought treatment.

347 The stimulation of the heterotrophic respiration in the drought treatment could be

348 explained by the observed increase in microbial biomass (Sowerby et al. 2005) in

349 combination with changes in some key microbial processes as the aerobic metabolic

350 pathways are favoured against the anaerobic pathways. The increased oxygen

351 availability activates a *biogeochemical cascade*, leading to a decline in inhibitory

352 phenolics and to a stimulation of extracellular hydrolase enzymes, which accelerates

353 SOM decomposition (Fenner and Freeman, 2011). The observed response could be also

354 partly explained by changes in the composition of the soil microbial community.

355 Similar experimental manipulations have shown that in dry shrublands, where summer

356 drought suppressed respiration, a shift towards a higher fungal dominance takes place

357 (Yuste et al. 2011). In our hydric shrubland however, the diversity of fungi was reduced

358 during the summer months in the drought treatment (Toberman et al. 2008) which might

359 also imply a decrease in the fungi to bacteria ratio in the community. Such change could

360 have important implications for SOM decomposition, given that fungi and bacteria

361 differently influence the processes of macroaggregate formation and SOM protection

362   (Six et al. 2006). Further works to explore the mechanisms underlying these increases in

363   soil respiration are in progress.

364   Soil respiration was less stimulated by our experimental warming, with an average

365   stimulation of a 13.2 % of the respiration rates, and an average increase of the annual

366   emissions of 49 g C m^{-2} year^{-1}, although these annual increases were only marginally

367   significant. The impact of warming on soil respiration reported here is very close to the

368   average stimulation of 12 % reported by a meta-analysis of field warming experiments

369   across ecosystems (Wu et al. 2011). In some organic soils both autotrophic and

370   heterotrophic respiration responds similarly to soil warming (Dorrepaal et al. 2009). In

371   our case, there was a trend for greater root density in the warming treatment, which

372   could explain the slight increase in soil respiration, although concurrent increases in the

373   heterotrophic component cannot be excluded.

374

375   Acclimation to warming or drought?

376   Our results supported our hypothesis that the impact of drought and warming on soil

377   respiration does not attenuate at a decadal time scale in wet shrublands. To the contrary,

378   the impact of drought on soil respiration during the summer months increased over the

379   years, which we found was linked to a fundamental change in soil structure resulting in

380   reduced soil water holding capacity. Even though the stimulation of respiration by

381   warming was subtle, it was sustained for more than a decade. The large availability of C

382   substrates in these soils might be the main reason for this lack of apparent acclimation

383   (Kirschbaum, 2004), as found for other organic C-rich soils exposed to sustained

384   warming (Shaver et al. 2006; Vicca et al. 2013). In addition, a recent work by Rousk et

385   al (2013) showed that the rates of microbial growth and respiration where similar across

386 treatments when soils were sieved and incubated under standard conditions of moisture

387 and temperature, suggesting no physiological adaptation to the treatments. In the

388 longer-term, it is likely that respiration rates will decrease as the progressive depletion

389 of the labile C pool takes place or as other nutrients, particularly N, become more

390 limiting (Beier et al. 2008).

391 The impact of warming on soil efflux was attenuated after the bryophyte expansion.

392 Bryophytes acted as both a moisture reservoir and mulch, preventing water loss and

393 partially negating the stimulatory effect of warming on soil respiration, whilst in the

394 drought treatment the decline in bryophytes may further have enhanced respiration

395 losses with the soil remaining in a drier state. Because of their poorly developed

396 conduction systems and the lack of effective cuticles in many species, bryophytes can

397 trap and transport water over the entire plant surface (Turetsky, 2003). In addition,

398 because bryophyte litter decomposes slowly, a thick layer of bryophyte litter is often

399 formed on the soil surface, which buffers soil against temperature changes, thereby

400 influencing C and N mineralisation (Nilsson and Wardle, 2005; Turetsky et al. 2012).

401 Thus, the spread of bryophytes might have also reduced the impact of our experimental

402 warming on soil temperatures. Our results support recent work that suggests that

403 bryophytes might have a key role in the resilience of cold and high latitude ecosystems

404 to climate change (Turetsky et al. 2012; Lindo et al. 2013), despite their relatively small

405 contribution to total aboveground biomass.

406

407 Impacts on soil structure and water holding capacity

408 As we hypothesised, the recurrent summer droughts provoked a substantial change in

409 soil structure, which led to a decrease in the water holding capacity. Water release

410 curves were also different for the warming soils in comparison to the control treatment.

411 In organic soils, shrinking during drying reduces the stability of the aggregates,

412 macroaggregates being more affected (Borken and Maztner, 2009). The effect of

413 drying-rewetting cycles on pore size distribution depends on the intensity of the drying

414 and on the soil type; in organic-rich soils drying increases the volume of large pores and

415 decreases medium and fine pores, while in inorganic soils the volume of medium pores

416 is enhanced (Peng et al. 2007). The response of soil porosity and pore shrinkage

417 capacity to drying is hysteretic and thus depends on the intensity of previous drying

418 events, particularly in organic soils (Peng and Horn, 2007). In addition to the effects on

419 soil porosity, drying also leads to hysteretic changes in the hydrophobicity of the soil

420 surface, increasing the water repellence of the soil surface (Borken and Maztner, 2009).

421 All these effects might result in substantial changes in the processes of water

422 infiltration, distribution through the soil profile upon rewetting, and drainage from soils,

423 which could explain the observed delay in the recovery of soil moisture conditions

424 outside the experimental drought periods (Sowerby et al. 2008) and requires further

425 investigation. As a result of these physical changes, water-excess conditions are likely

426 to become less frequent, which could further intensify aerobic SOM decomposition due

427 to the greater oxygen availability and the higher exposure of SOM to changes in air

428 temperature, particularly during the summertime. Our results are in agreement with

429 some modelling work that predicts a long-term feedback between soil physical

430 conditions and SOM decomposition in organic soils, that eventually intensifies the loss

431 of soil organic C in a changing climate (Ise et al. 2008). Therefore, failing to incorporate

432   information on soil physical conditions will under-estimate feedbacks from the soil

433   system to the atmosphere.

434   In the warming plots, where we observed a trend for greater root density, the increase in

435   mean pore size could be linked to a possible shift in the distribution of root diameters

436   towards larger diameter classes. The lower soil moisture levels recorded in 2003 and

437   2005, before the bryophyte expansion, could have also contributed to the structural

438   change in the warming treatment. Additional explanatory mechanisms might involve

439   changes in soil microbial functional groups due to our treatments. For instance, soil

440   aggregation is highly influenced by fungal diversity and hyphal density (Wilson et al.

441   2009; Rillig and Mummey, 2006), and therefore changes in the relative abundance of

442   fungal species due to warming or drought might also have a pronounced effect on soil

443   aggregation and, by extension, on water holding capacity.

444

445   Implications for soil C losses and climate feedbacks

446   Our results suggest that in wet shrublands the impact of summer drought could lead to a

447   substantial loss of soil C, considering that aboveground biomass was very resilient to

448   warming or drought, and that drought did not have a clear effect on root biomass,

449   therefore indicating that the increases in soil respiration have not been balanced by

450   increases in C inputs to soil. Extreme climate events, such as droughts and floods, are

451   likely to become more frequent under air warming (Dai et al. 2013). Latest IPCC

452   projections also include increases in winter rainfall for many areas in Northern Europe

453   (van Oldenborgh et al. 2013). Under a potential scenario of increased winter rainfall and

454   recurrent summer droughts, understanding soil moisture dynamics is critical to predict

455   soil C losses from these wet ecosystems. The increase in soil moisture might partially

456 negate the stimulation of soil respiration by warming during the winter months, as

457 found in our warming treatment after the bryophyte expansion, while the occurrence of

458 drought events during the summertime, when temperature and the supply of plant

459 photosynthates are not limiting to SOM decomposition, could lead to the destabilization

460 of large amounts of soil C.

461 Wet shrublands in the UK occupy landscape positions that make them very vulnerable

462 to changes in land management (Bol et al., 2011). They usually have a mixed use

463 (grazing, forestry, biodiversity conservation), and an increase in the intensity of some of

464 these uses might have a substantial impact on soil C stocks. For instance, land

465 management practises in these ecosystems often include draining to improve grazing

466 capacity. This practise, leading to a long-term reduction in soil moisture, might provoke

467 a substantial loss of soil C (Peacock et al. 2014), as SOM decomposition is

468 progressively stimulated by the changes in soil physical structure.

469 In conclusion, our work provides compelling evidence that wet shrubland soils have a

470 particular sensitivity to drought that does not attenuate, but increases at a decadal time

471 scale. This was linked to a climate-change induced fundamental change in soil structure,

472 leading to a decrease in soil water holding capacity, which illustrates the importance of

473 including dynamic soil structural information in ecosystem models and projections of

474 soil C losses. Indeed, in these soils the impact of drought could lead to a substantial loss

475 of soil C that should be considered in the prediction of future C fluxes and climate

476 feedbacks at regional and global scales. Our work also suggests that changes in sub-

477 dominant vegetation may have a potentially critical role in determining climate change

478 impacts on soil C dynamics in wet ecosystems.

479

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Figure legends

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678 **Fig. 1.** Drought and warming impact on soil respiration rates (% from control) over the

679 14 years of climate manipulation (mean of 3 plots per treatment for each date). Average

680 impact across all the years is indicated (mean ± standard deviation).

681

682 **Fig. 2.** **a)** Annual soil respiration (R_{year}) across the years (mean of 3 plots per treatment

683 ± standard error) and **b)** increase in annual emissions (ΔR_{year}) by drought and warming,

684 in relation to the control treatment (mean ± standard error).

685

686 **Fig. 3**. **a)** Relationship between the reduction in soil moisture and the stimulation of soil

687 respiration rates (ΔSoil Respiration) in the drought treatment (average of 3 plots) during

688 the summer months (June-September). **b)** Evolution of the drought impact over the

689 years (average stimulation of respiration rates) during the experimental drought periods

690 (summer) and the rewetting periods (October-December). The impact of drought during

691 the summer increased over the experimental period (correlation line).

692

693 **Fig. 4.** Relationship between the impact of the experimental warming on annual soil

694 respiration (ΔR_{year}) and the change in the bryophyte abundance, in relation to the control

695 treatment. The direction of change over time was of increased bryophyte biomass and

696 decreased impact of warming on soil respiration.

697

698   **Fig. 5. a)** Relative change in bryophyte biomass in the warming and drought plots (bars,

699   mean + standard error, left axis), and mean annual treatment soil moisture difference

700   from the control (lines, right axis). **b**) Annual soil moisture frequency distribution for

701   2004, 2010 (dry year) and 2011 (wet year).

702

703   **Fig. 6**. Soil water release curves (WRC's) averaged from all plots (mean ± standard

704   error) with vertical lines indicating water content range for field capacity, at which soils

705   would have been drained by gravity.