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

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Abstract

Projected climate warming, and increasingly frequent droughts, may substantially increase carbon emissions from wet organic soils, contributing to the positive feedback 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 CO₂ 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.

Keywords: soil respiration; acclimation; water retention; warming; bryophyte; *Calluna vulgaris*; soil structure

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,

because of a limited soil moisture pool being available to buffer drying events, thus representing a potentially large C pool vulnerable to climate change.

Current predictions of climate change include severe and widespread droughts in the next 30–90 years (Christensen et al. 2007; Dai 2013), and the co-occurrence of warming and drying in wet, organic-C rich soils could lead to substantial losses of soil C, as soils are exposed to aerobic decomposition (Shaver et al. 2006; Ise et al. 2008; Fenner and Freeman 2011). Indeed, some hydro–biogeochemical soil models predict a positive feedback between soil drying and SOM decomposition in wet organic soils, leading to an increased sensitivity of these soils to future climate warming (Ise et al. 2008). This positive feedback would result from changes in soil structure leading to a decrease in the soil water holding capacity. Shrinking during soil drying can have a pronounced effect on the size and stability of the pores in organic soils, leading to a destabilization of macropores and macroaggregates (Peng et al. 2007; Borken and Matzner, 2009). Soil structure might also be altered as plants change their rooting structure in response to drought, seeking water lower in the profile, or in response to warmer temperatures. Despite soil water holding capacity being a key parameter for ecosystem functioning, there is lack of empirical information about the effects of climate change drivers on this soil physical property.

The adaptation of plant and soils processes to repeated changes in the soil water balance might attenuate the predictions of large C losses from wet soils. The analysis of different soil inventories across England and Wales revealed that wet organic soils lost carbon an order of magnitude faster than well-drained mineral soils between 1978 and 2003, and suggested that these losses are likely to have been offsetting the absorption of C by terrestrial sinks (Bellamy et al. 2005). However, two subsequent studies have not supported this finding for England and Wales (Reynolds et al. 2013) or Scotland

(Chapman et al. 2013). To reduce the large uncertainties in the predictions of C losses from wet soils, more empirical data about the relative sensitivity of soil respiration and plant primary productivity to climate change drivers is urgently needed.

We investigated the long-term impact of warming and repeated summer droughts on soil respiration from organo-mineral soils in a wet shrubland, using a whole-ecosystem climate-change experiment in North Wales, UK. Previously, Sowerby et al. (2008) showed an ongoing stimulation of soil respiration by drought during the first five years of the manipulation with no evidence of attenuation. In this work, we monitored soil respiration and plant aboveground biomass over 14 years, and measured soil water release curves (WRCs) of the topsoil (0-5 cm) after a decade of experimental manipulation, to determine if the soil structure and water holding capacity had changed. Specifically, we aimed to address the following questions:

1) Does the stimulation of soil respiration under warming and drought attenuate over the long-term? We hypothesised that, because soil C decomposition is highly constrained by water-excess conditions in these soils, drought would have a relatively higher impact than modest warming, and that, because of the potentially large availability of C substrates in these soils, the apparent acclimation to warming and drought would occur at relatively long experimental periods.

2) Do drought and warming have an impact on soil water holding capacity? We hypothesised that recurrent drought would have a greater impact on soil structure than long-term warming, and that, according to theoretical models (Ise et al. 2008), repeated drying-rewetting events would reduce soil water holding capacity.

Materials and Methods

Experimental design

Our study was conducted at the Clocaenog site in NE Wales, UK (53° 03' 19"N, 03° 27' 55"W), situated at 490m a.s.l. Mean annual air temperature is 8.2 °C, rainfall is 1700 mm, and potential evapotranspiration is 302 mm. The ecosystem is an upland Atlantic heathland dominated by *Calluna vulgaris* (L.) Hull, with *Vaccinium myrtillus* L., *Empetrum nigrum* L. and *Deschampsia flexuosa* (L.) Trin. Total aboveground biomass is around 3500 g m⁻². The soils are organic-rich with 4–10 cm of organic soil (organic matter content 89%), overlaying 18-20 cm of organic-rich mineral soil (organic matter content of 37%). They are classified as Ferric stagnopodzols in the Hafren Series in the Soil Survey of England and Wales (Cranfield University, 2014), and as Endoskeletal Histic Stagnic Albic Podzols in the IUSS Working Group WRB (2007) classification. Mean annual soil temperature is 6.7 °C.

Whole-ecosystem simulation of drought and warming started in March 1999 and has been running to date. Three blocks were established at the site, each one with three experimental plots (4 × 5 m) randomly allocated to the drought, warming and control treatment, respectively. Fully automated retractable curtains (see Beier et al. 2004 for a full description) were placed over the plots to achieve air warming (warming treatment) or to exclude the rain from the plots from June to September annually (drought treatment).

The warming treatment consisted of a passive night-time warming provided by reflective aluminium curtains covering the vegetation at night. These curtains reflect 96-97 % of infrared radiation, reducing the heat loss during night by 64 % (Beier et al. 2004), and producing an increase of 0.2-2.0 °C in monthly air temperature, in the range predicted for Northern Europe for 2010-2100 under three of the four IPCC's RCP

scenarios (van Oldenborgh et al. 2013). This warming treatment increased the growing degree-days (based on lower and upper-threshold temperatures between which plant growth occurs) by 9 % on average (see Supplementary Information, Table S1). During the night, the warming curtains are retracted during rain events to prevent the reduction of rain input to the plots. However, there was a small unintended reduction in rain input brought about by the short delay in warming roofs retracting at the onset of a precipitation event (on average, annual rainfall inputs into the warming plots were a 13% lower compared to control). Nevertheless, this did not result in an overall significant reduction in soil moisture levels in the warming plots during the experiment. During 2003, soil moisture was significantly lower in the warming (annual average of $0.45 \text{ m}^3 \text{ m}^{-3}$) than in the control plots (annual average of $0.53 \text{ m}^3 \text{ m}^{-3}$; repeated measures ANOVA, treatment effect $p = 0.0143$). Likewise, during 2005 moisture levels were lower in the warming treatment (annual mean of $0.46 \text{ m}^3 \text{ m}^{-3}$) than in the control treatment (annual mean of $0.51 \text{ m}^3 \text{ m}^{-3}$; repeated measures ANOVA, treatment effect $p = 0.039$). For the rest of years, moisture levels in the warming treatment were similar (2000-2002 and 2004-2008) or slightly greater (2008 and onwards) in comparison to control.

The drought treatment consisted of waterproof polyethylene curtains triggered by a rain sensor. These curtains excluded 54 % of the rainfall from June to September, with the aim of emulating the UK summer drought of 1995, considered to be the most recent significant drought year prior to drought treatment initiation. Annual and growing season rainfall were reduced by on average 21 % and 54 % respectively (Table S1), which resulted in an average reduction of 30 % in soil moisture during the growing season. Outside the drought period (re-wetting period), drought plots are managed in the same way as the control plots.

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 CO₂ 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 CO₂ 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.

Aboveground and root biomass

Aboveground composition and biomass was monitored every year (excepting 2001, and 2004-2006) at the end of the growing season (August) using the pin-point method in three permanent 0.5 x 0.5 m² subplots per plot. In each subplot a grid of 100 pins was lowered through the vegetation. At 5 cm intervals, every touch of vegetation was recorded, indicating the species, the plant part (leaf, flower, or stem) and its status (green, dry, dead). In 2000 a calibration between pin-point measurements and plant biomass was conducted in the same site but outside the experimental plots, using a destructive sampling. Relationships between pin-point measurements and plant biomass were established for each plant species ($r^2 = 0.70$ for bryophytes; $r^2 = 0.98$ for *D. flexuosa*; $r^2 = 0.64$ for *C. vulgaris*; $r^2 = 0.65$ for *E. nigrum*; $r^2 = 0.82$ for *V. myrtillus*). Root biomass was measured in three cores per plot (15 cm depth and 5 cm diameter), collected underneath *C. vulgaris* individuals in September 2011. Cores were sliced from the top into 1cm segments until 10 cm depth, and roots in each segment were washed and extracted. Roots were dried at 70 °C for at least 48 h and weighed.

Soil water release curves

Soil water release curves were measured on 250 cm³ soil cores, 0-5 cm deep, extracted from each plot underneath *C. vulgaris* in September 2011. Water release curves typically took 1 month to determine using the laboratory evaporation method (Schindler et al., 2010) using a HYPROP (UMS, Munchen, Germany).

Data analysis

We estimated the daily average respiration rates as 87% of the rate measured at midday, based on the diurnal data taken in 2002. We then calculated average rates for each season, and finally multiplied by the number of days in each season to obtain the seasonal cumulative CO₂ emissions. Annual emissions (R_{year}) were calculated as the sum of all the seasonal emissions. For each year, the relative impact of warming and drought (ΔR_{year} from control) was calculated.

A mixed-model analysis of variance was performed to test for significant differences among treatments in R_{year} for each year separately. Treatments were assigned as fixed factors and block as a random factor. Tukey's HSD multiple comparison test was applied to identify differences among treatments in R_{year} . A similar analysis was applied to test for differences in root biomass. To test for the general effect of the treatments on R_{year} emissions and aboveground biomass over the 14 years of manipulation (time $\times$ treatment interaction), we applied repeated measures analysis of variance, using the Greenhouse–Geisser adjustment. Water release curves were tested for homogeneity of slopes among treatments using ANCOVA test, with soil water volumetric content as dependent variable, soil water tension as a continuous predictor (covariate, log transformed), and treatment as a categorical factor, using Statistica v10 (Stat Soft Inc.).

Results

Drought impact on soil respiration

Drought stimulated soil respiration rates by 22 % (average for all seasons and years, Fig. 1). Summer drought impact on annual soil CO₂ emissions (R_{year}) was enhanced

mainly in drier years (repeated-measures ANOVA, significant year $\times$ treatment interaction, $F = 6.2$, $p = 0.011$; Fig 2a, b), with significant increases in 2004 ($p = 0.004$), 2005 ($p = 0.045$), 2009 ($p = 0.050$), 2011 ($p = 0.007$), and 2012 ($p = 0.034$). The mean R_{year} stimulation was $80 \text{ g C m}^{-2} \text{ year}^{-1}$ (17 % of the inter-annual average in the control treatment).

Stimulation of respiration was greatest during the summer months (experimental drought period) when it was directly linked to the reduction in soil moisture (Fig. 3a), but persisted into autumn months, the rewetting period, when respiration rates were up to 70 % higher, compared to control (Fig. 3b). No decrease was detected in the magnitude of the stimulation over the years, which would have indicated apparent acclimation of the soils to experimental drought. To the contrary, the relative impact of drought during the summer months increased over the years (Fig. 3b), and since 2010 drought produced a continuous stimulation of soil respiration across the seasons (Fig. 1).

Warming impact on soil respiration

The impact of warming was lower than that of drought, with a respiration stimulation of 13.2 % (average for all seasons and years, Fig. 1). The stimulation of R_{year} by warming was only marginally significant ($0.07 > p < 0.05$, average of $49 \text{ g C m}^{-2} \text{ year}^{-1}$).

Since 2005, the stimulation of respiration by warming decreased, which was associated with an increase in the abundance of bryophytes in the warming plots (Fig. 4), which, in contrast, markedly decreased in the drought plots. This change in bryophyte abundance coincided with a shift in the soil moisture dynamics in the warming plots after the natural drought of 2005 (Fig. 5a). Prior to 2005, the warming and control plots had

similar soil moisture frequency distributions, the warming soils being slightly drier, whereas after 2005 the warming plots were consistently wetter than the control or drought, regardless of whether it was a dry year (2010, Fig. 5b) or a wet year (2011, Fig. 5b).

Soil water release curves

After a decade of experimental manipulation, all soils had a porosity between 0.85 and 0.90 m³ m⁻³, but the drought soils released water much more rapidly than the control or warming soils with increasing tension (Fig. 6). Within the range of field capacity, water release curves had the same slope, but different intercept (volumetric water content) among treatments (ANCOVA model; treatment: $F = 13518$, $p < 0.001$; treatment $\times$ soil tension: $F = 1.6$, $p = 0.2$). This meant that, in the estimated range of field capacity ($\sim$ - 2kPa), the control soils retained $\sim$ 0.7 m³ m⁻³ of water, whilst the drought and warming soils retained 54 % and 26 % less respectively. This was consistent with an increase in the median pore size from 30 μ m in the control to 74 μ m and 248 μ m in the warming and drought plots, respectively, determined from the conversion of the WRCs to effective pore size.

Plant biomass

Aboveground biomass differed significantly across years, but never among treatments (repeated measures ANOVA: treatment: $F = 0.283$, $p = 0.73$; year: $F = 7.63$, $p < 0.0001$; treatment $\times$ year: $F = 0.509$, $p = 0.94$, Fig S1a). There was a trend of greater root biomass in warming plots compared to control (Tukey HSD test, $p = 0.044$), while root

biomass in the drought and control plots were not significantly different (Tukey HSD test, $p = 0.192$; Fig. S1b).

Discussion

Warming and drought impact on soil respiration

Wet shrublands with organo-mineral (podzolic) soils have one of the highest soil C densities across habitats in the UK, but a limited soil moisture pool to buffer drought events in comparison to peatlands. Therefore, they represent a type of ecosystem with a high vulnerability to the projected increases in the frequency of severe droughts. Our results clearly show that, as we hypothesised, soil respiration in wet shrublands is more sensitive to repeated modest drought than to subtle warming, and that the drought impact does not attenuate at a decadal time scale.

Several other experiments have shown that soil respiration or net ecosystem exchange is relatively more sensitive to changes in the precipitation regime than to soil warming in different types of ecosystems (Garten et al. 2009; Liu et al. 2009; Suseela et al. 2012), although in those studies, conducted in well-drained mineral soils, drought conditions typically suppressed respiration. In our study, drought increased summer and autumn CO₂ emissions by a 22 % and a 27 % (on average), respectively, with maximum increases above 60 %. The stimulation of soil respiration during the summer months, when temperature and the supply of plant photosynthates are not limiting, was linearly linked to the reduction in soil moisture, illustrating the importance of the water-excess as an environmental constraint to decomposition in these soils, and contrasting to the response of other mesic and dry shrubland ecosystems across Europe (Sowerby et al. 2008).

Both autotrophic and heterotrophic respiration may be enhanced by the release of water-excess conditions. Although our measurements did not include the separation of each of the respiration components there is an indication that respiration changes are likely driven by heterotrophic processes, as root biomass was not significantly greater in the drought plots (Fig. S1b). This is consistent with the finding that plant aboveground biomass did not change among treatments (Fig. S1a), suggesting high resilience of plant productivity to the climate treatments. In addition, a ^{14}C pulse-labelling experiment (Gorissen et al. 2004) revealed that C translocation belowground was reduced by 40 % in the drought treatment.

The stimulation of the heterotrophic respiration in the drought treatment could be explained by the observed increase in microbial biomass (Sowerby et al. 2005) in combination with changes in some key microbial processes as the aerobic metabolic pathways are favoured against the anaerobic pathways. The increased oxygen availability activates a *biogeochemical cascade*, leading to a decline in inhibitory phenolics and to a stimulation of extracellular hydrolase enzymes, which accelerates SOM decomposition (Fenner and Freeman, 2011). The observed response could be also partly explained by changes in the composition of the soil microbial community. Similar experimental manipulations have shown that in dry shrublands, where summer drought suppressed respiration, a shift towards a higher fungal dominance takes place (Yuste et al. 2011). In our hydric shrubland however, the diversity of fungi was reduced during the summer months in the drought treatment (Toberman et al. 2008) which might also imply a decrease in the fungi to bacteria ratio in the community. Such change could have important implications for SOM decomposition, given that fungi and bacteria differently influence the processes of macroaggregate formation and SOM protection

(Six et al. 2006). Further works to explore the mechanisms underlying these increases in soil respiration are in progress.

Soil respiration was less stimulated by our experimental warming, with an average stimulation of a 13.2 % of the respiration rates, and an average increase of the annual emissions of $49 \text{ g C m}^{-2} \text{ year}^{-1}$, although these annual increases were only marginally significant. The impact of warming on soil respiration reported here is very close to the average stimulation of 12 % reported by a meta-analysis of field warming experiments across ecosystems (Wu et al. 2011). In some organic soils both autotrophic and heterotrophic respiration responds similarly to soil warming (Dorrepaal et al. 2009). In our case, there was a trend for greater root density in the warming treatment, which could explain the slight increase in soil respiration, although concurrent increases in the heterotrophic component cannot be excluded.

Acclimation to warming or drought?

Our results supported our hypothesis that the impact of drought and warming on soil respiration does not attenuate at a decadal time scale in wet shrublands. To the contrary, the impact of drought on soil respiration during the summer months increased over the years, which we found was linked to a fundamental change in soil structure resulting in reduced soil water holding capacity. Even though the stimulation of respiration by warming was subtle, it was sustained for more than a decade. The large availability of C substrates in these soils might be the main reason for this lack of apparent acclimation (Kirschbaum, 2004), as found for other organic C-rich soils exposed to sustained warming (Shaver et al. 2006; Vicca et al. 2013). In addition, a recent work by Rousk et al (2013) showed that the rates of microbial growth and respiration were similar across

treatments when soils were sieved and incubated under standard conditions of moisture and temperature, suggesting no physiological adaptation to the treatments. In the longer-term, it is likely that respiration rates will decrease as the progressive depletion of the labile C pool takes place or as other nutrients, particularly N, become more limiting (Beier et al. 2008).

The impact of warming on soil efflux was attenuated after the bryophyte expansion. Bryophytes acted as both a moisture reservoir and mulch, preventing water loss and partially negating the stimulatory effect of warming on soil respiration, whilst in the drought treatment the decline in bryophytes may further have enhanced respiration losses with the soil remaining in a drier state. Because of their poorly developed conduction systems and the lack of effective cuticles in many species, bryophytes can trap and transport water over the entire plant surface (Turetsky, 2003). In addition, because bryophyte litter decomposes slowly, a thick layer of bryophyte litter is often formed on the soil surface, which buffers soil against temperature changes, thereby influencing C and N mineralisation (Nilsson and Wardle, 2005; Turetsky et al. 2012). Thus, the spread of bryophytes might have also reduced the impact of our experimental warming on soil temperatures. Our results support recent work that suggests that bryophytes might have a key role in the resilience of cold and high latitude ecosystems to climate change (Turetsky et al. 2012; Lindo et al. 2013), despite their relatively small contribution to total aboveground biomass.

Impacts on soil structure and water holding capacity

As we hypothesised, the recurrent summer droughts provoked a substantial change in soil structure, which led to a decrease in the water holding capacity. Water release curves were also different for the warming soils in comparison to the control treatment.

In organic soils, shrinking during drying reduces the stability of the aggregates, macroaggregates being more affected (Borken and Maztner, 2009). The effect of drying-rewetting cycles on pore size distribution depends on the intensity of the drying and on the soil type; in organic-rich soils drying increases the volume of large pores and decreases medium and fine pores, while in inorganic soils the volume of medium pores is enhanced (Peng et al. 2007). The response of soil porosity and pore shrinkage capacity to drying is hysteretic and thus depends on the intensity of previous drying events, particularly in organic soils (Peng and Horn, 2007). In addition to the effects on soil porosity, drying also leads to hysteretic changes in the hydrophobicity of the soil surface, increasing the water repellence of the soil surface (Borken and Maztner, 2009).

All these effects might result in substantial changes in the processes of water infiltration, distribution through the soil profile upon rewetting, and drainage from soils, which could explain the observed delay in the recovery of soil moisture conditions outside the experimental drought periods (Sowerby et al. 2008) and requires further investigation. As a result of these physical changes, water-excess conditions are likely to become less frequent, which could further intensify aerobic SOM decomposition due to the greater oxygen availability and the higher exposure of SOM to changes in air temperature, particularly during the summertime. Our results are in agreement with some modelling work that predicts a long-term feedback between soil physical conditions and SOM decomposition in organic soils, that eventually intensifies the loss of soil organic C in a changing climate (Ise et al. 2008). Therefore, failing to incorporate

information on soil physical conditions will under-estimate feedbacks from the soil system to the atmosphere.

In the warming plots, where we observed a trend for greater root density, the increase in mean pore size could be linked to a possible shift in the distribution of root diameters towards larger diameter classes. The lower soil moisture levels recorded in 2003 and 2005, before the bryophyte expansion, could have also contributed to the structural change in the warming treatment. Additional explanatory mechanisms might involve changes in soil microbial functional groups due to our treatments. For instance, soil aggregation is highly influenced by fungal diversity and hyphal density (Wilson et al. 2009; Rillig and Mummey, 2006), and therefore changes in the relative abundance of fungal species due to warming or drought might also have a pronounced effect on soil aggregation and, by extension, on water holding capacity.

Implications for soil C losses and climate feedbacks

Our results suggest that in wet shrublands the impact of summer drought could lead to a substantial loss of soil C, considering that aboveground biomass was very resilient to warming or drought, and that drought did not have a clear effect on root biomass, therefore indicating that the increases in soil respiration have not been balanced by increases in C inputs to soil. Extreme climate events, such as droughts and floods, are likely to become more frequent under air warming (Dai et al. 2013). Latest IPCC projections also include increases in winter rainfall for many areas in Northern Europe (van Oldenborgh et al. 2013). Under a potential scenario of increased winter rainfall and recurrent summer droughts, understanding soil moisture dynamics is critical to predict soil C losses from these wet ecosystems. The increase in soil moisture might partially

negate the stimulation of soil respiration by warming during the winter months, as found in our warming treatment after the bryophyte expansion, while the occurrence of drought events during the summertime, when temperature and the supply of plant photosynthates are not limiting to SOM decomposition, could lead to the destabilization of large amounts of soil C.

Wet shrublands in the UK occupy landscape positions that make them very vulnerable to changes in land management (Bol et al., 2011). They usually have a mixed use (grazing, forestry, biodiversity conservation), and an increase in the intensity of some of these uses might have a substantial impact on soil C stocks. For instance, land management practises in these ecosystems often include draining to improve grazing capacity. This practise, leading to a long-term reduction in soil moisture, might provoke a substantial loss of soil C (Peacock et al. 2014), as SOM decomposition is progressively stimulated by the changes in soil physical structure.

In conclusion, our work provides compelling evidence that wet shrubland soils have a particular sensitivity to drought that does not attenuate, but increases at a decadal time scale. This was linked to a climate-change induced fundamental change in soil structure, leading to a decrease in soil water holding capacity, which illustrates the importance of including dynamic soil structural information in ecosystem models and projections of soil C losses. Indeed, in these soils the impact of drought could lead to a substantial loss of soil C that should be considered in the prediction of future C fluxes and climate feedbacks at regional and global scales. Our work also suggests that changes in sub-dominant vegetation may have a potentially critical role in determining climate change impacts on soil C dynamics in wet ecosystems.

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Figure legends

Fig. 1. Drought and warming impact on soil respiration rates (% from control) over the 14 years of climate manipulation (mean of 3 plots per treatment for each date). Average impact across all the years is indicated (mean $\pm$ standard deviation).

Fig. 2. a) Annual soil respiration (R_{year}) across the years (mean of 3 plots per treatment $\pm$ standard error) and **b)** increase in annual emissions (ΔR_{year}) by drought and warming, in relation to the control treatment (mean $\pm$ standard error).

Fig. 3. a) Relationship between the reduction in soil moisture and the stimulation of soil respiration rates (Δ Soil Respiration) in the drought treatment (average of 3 plots) during the summer months (June-September). **b)** Evolution of the drought impact over the years (average stimulation of respiration rates) during the experimental drought periods (summer) and the rewetting periods (October-December). The impact of drought during the summer increased over the experimental period (correlation line).

Fig. 4. Relationship between the impact of the experimental warming on annual soil respiration (ΔR_{year}) and the change in the bryophyte abundance, in relation to the control treatment. The direction of change over time was of increased bryophyte biomass and decreased impact of warming on soil respiration.

Fig. 5. a) Relative change in bryophyte biomass in the warming and drought plots (bars, mean + standard error, left axis), and mean annual treatment soil moisture difference from the control (lines, right axis). **b)** Annual soil moisture frequency distribution for 2004, 2010 (dry year) and 2011 (wet year).

Fig. 6. Soil water release curves (WRC's) averaged from all plots (mean $\pm$ standard error) with vertical lines indicating water content range for field capacity, at which soils would have been drained by gravity.