

CO₂ efflux from soils with seasonal water repellency

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Abstract. Soil carbon dioxide (CO₂) emissions are strongly dependent on pore water distribution, which in turn can be modified by reduced wettability. Many soils around the world are affected by soil water repellency (SWR), which reduces infiltration and results in diverse moisture distribution. SWR is temporally variable and soils can change from wettable to water-repellent and *vice versa* throughout the year. Effects of SWR on soil carbon (C) dynamics, and specifically on CO₂ efflux, have only been studied in a few laboratory experiments and hence remain poorly understood. Existing studies suggest soil respiration is reduced with increasing severity of SWR, but the responses of soil CO₂ efflux to varying water distribution created by SWR are not yet known.

Here we report on the first field-based study that tests whether SWR indeed reduces soil CO₂ efflux, based on *in situ* measurements carried out over three consecutive years at a grassland and pine forest sites under the humid temperate climate of the UK.

Soil CO₂ efflux was indeed very low on occasions when soil exhibited consistently high SWR and low soil moisture following long dry spells. Low CO₂ efflux was also observed when SWR was absent, in spring and late autumn when soil temperatures were low, but also in summer when SWR was reduced by frequent rainfall events. The highest CO₂ efflux occurred not when soil was wettable, but when SWR, and thus soil moisture, was spatially patchy, a pattern observed for the majority of the measurement period. Patchiness of SWR is likely to have created zones with two different characteristics related to CO₂ production and transport. Zones with wettable soil or low persistence of SWR with higher proportion of water filled pores are expected to provide water with high nutrient concentration resulting in higher microbial activity and CO₂ production. Soil zones with high SWR persistence, on the other hand, are dominated by air filled pores with low microbial activity, but facilitating O₂ supply and CO₂ exchange between the soil and the atmosphere.

The effects of soil moisture and SWR on soil CO₂ efflux are strongly co-correlated, but the results of this study support the notion that SWR indirectly affects soil CO₂ efflux by affecting soil moisture distribution. The appearance of SWR is influenced by moisture and temperature, but once present, SWR influences subsequent infiltration patterns and resulting soil water distribution, which in turn affects respiration. This study demonstrates that SWR can have contrasting effects on CO₂ efflux. It can reduce it in dry soil zones by preventing their re-wetting, but, at the field soil scale and when spatially-variable, it can also enhance overall CO₂ efflux. Spatial variability in SWR and associated soil moisture distribution therefore need to be considered when evaluating the effects of SWR on soil C dynamics under current and predicted future climatic conditions.

1 Introduction

Soil is the most important reservoir of terrestrial carbon (C), storing four times more C than plant biomass (Stocker et al., 2013), but large amounts of C are released back to atmosphere mainly as carbon dioxide (CO₂) formed by microbial decomposition of soil organic matter (SOM) as well as biological activity of roots and microfauna (Bond-Lamberty and Thomson, 2010; Rey, 2015). Soil moisture is one of the most important environmental factors regulating the production and transport of CO₂ in terrestrial ecosystems (Maier et al., 2011; Moyano et al., 2012). It influences not only soil organic C bioavailability and regulates access to oxygen (O₂) (Moyano et al., 2012; Yan et al., 2016), but also C mass transport (Davidson et al., 2012).

Soil C models consider changes in soil moisture conditions, but they use functions that represent a response of soil respiration (i.e. CO₂ efflux) to average soil water content (SWC) and do not account for within-soil moisture variability, which is a characteristic of most soils (Rodrigo et al., 1997; Moyano et al., 2013; Yan et al., 2016). Most soils are very heterogeneous, with moisture distribution and water movement being variable and dependent on a number of factors (e.g. texture, structure, SOM content) that determine soil hydrological properties. Soils prone to development of soil water repellency (SWR) are particularly susceptible to spatially highly variable soil moisture distribution and irregular wetting (Dekker and Ritsema, 1995; Doerr et al., 2000; Ritsema and Dekker, 2000). SWR is a common feature of many soils worldwide, and is expected to become even more widespread and severe under a warming climate (Goebel et al., 2011). SWR affects soil-water relations by restricting infiltration, which results in large areas of soil remaining dry for long periods even after substantial rainfall events (Keizer et al., 2007). It often leads to enhanced preferential flow where water moves along pathways created by cracks, root channels

and zones of less repellent soil, leaving other areas completely dry (Urbanek et al., 2015). Irregular water infiltration in water-repellent soil often creates distinct zones with water filled pores, concentrated dissolved organic C (DOC), and nutrients, adjacent to dry regions with air-filled pores (Wallach and Jortzick, 2008; Urbanek and Shakesby, 2009; Müller et al., 2014). Several studies have investigated microbial activity in water-repellent soils, mainly to determine whether the microbial exudates and proteins can cause the development of hydrophobic surfaces in soils (White et al., 2000; Feeney et al., 2006; Lozano et al., 2014). SWR has also been reported to reduce soil microbial activity and it has been considered as one of the factors protecting SOM from microbial decomposition by separation of the microorganisms from their food and water sources (Piccolo and Mbagwu, 1999; Piccolo et al., 1999; Bachmann et al., 2008). Goebel et al. (2007) demonstrated that SWR affects the distribution and continuity of the liquid phase in the soil matrix, therefore restricts the accessibility of SOM and the availability of water, O₂ and nutrients to the microorganisms. Using laboratory-based studies, they observed lower respiration rates from soils in a water-repellent state, and decreasing CO₂ efflux with increasing severity of SWR (Goebel et al., 2005; Goebel et al., 2007). In a review of this topic Goebel et al. (2011) highlighted the importance of SWR in SOM decomposition especially during extreme climatic events such as drought, suggesting that it reduces the total soil CO₂ efflux. The small number of existing laboratory-based studies suggest reduced soil respiration when soil is water-repellent, but a thorough field study investigating temporal changes in SWR and their effect on soil CO₂ efflux is still lacking.

The aim of the current study is to investigate soil CO₂ efflux responses to SWR under undisturbed *in-situ* conditions. We test the hypothesis that the presence of SWR reduces soil respiration under natural field conditions. The study sites selected were humid-temperate grassland and pine forest in the UK, which were anticipated to exhibit substantial temporal and spatial variability in SWR (Doerr et al., 2006), a common feature of water-repellent soils in general (Doerr et al., 2000).

20 **2 Materials and methods**

2.1 Experimental design

A forest and a grassland site, both subject to humid-temperate conditions, were chosen because of their likely high susceptibility to develop seasonal SWR in view of their sandy texture and permanent vegetation cover, which are characteristics known to be conducive to SWR development (Doerr et al., 2000). Both study sites consisted of six study plots

with adjacent grass and bracken cover, arranged along a 20-m transect (Fig. 1). At each study site twelve PVC collars for CO₂ measurements (two collars per study plot) were installed, and respiration was measured on vegetated and bare soil respectively. Bare soil measurements were conducted on soil from which the vegetation and litter layer inside the collar was temporarily removed for the duration of the CO₂ efflux measurement to assess the contribution of different layers to the total soil respiration, and put back after the measurement. The sites were monitored from May till November in three consecutive years (2013-2015). Continuous measurement of SWC and soil temperature was conducted, while CO₂ efflux and persistence of SWR measurements were taken during site visits at approximately monthly interval.

Given the near-impossibility of finding wettable and water-repellent soils for comparison that otherwise display identical properties (e.g. texture, organic matter content, pH, litter type), we examined sites that displayed temporally variable behaviour, switching between water-repellent and wettable states of soil. This facilitated examining the impact of SWR on CO₂ efflux, bearing in mind that temperature and moisture themselves are known to affect SWR and CO₂ efflux. C and Nitrogen (N) contents as well as pH were determined on soil samples in the laboratory to be considered as potential factors for CO₂ efflux variability between plots and study sites.

Figure 1:

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2.2 Study sites

The study sites were located in eastern England, approximately 8 km north-west (grassland site 52°24'56.42"N 0°52'31.19"E) and 8 km east (forest site 52°27'30.82"N 0°40'50.31"E) of Thetford. The sites were subject to humid-temperate conditions with an annual mean rainfall of 665 mm spread relatively uniformly throughout the year and an annual mean temperature of 14.5°C, with monthly mean maxima of 23°C in July and August and minima of 9°C in December and January (UK Met Office, 2017a). The forest site was part of a long-term forest monitoring network established in 1995 aimed to assess the impact of the changing environment on forest and soil health (Vanguelova et al., 2010; Waldner et al., 2014; Jonard et al., 2015). Both sites have been planted with similar tree species, which were Scots Pine (88%), beech (6%) and oak (6%) (the grassland site in 1928 and the forest site in 1967), but all trees at the grassland site were felled in 1999 and the area was converted to a managed grassland. The dominant vegetation cover and soil was similar for both sites with large areas covered by either grasses (*Holcus lanatus*, *Agrostis canina*) or bracken (*Pteridium aquilinum*, *Dryopteris dilatata*). At the forest site, however, some

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moss (*Eurhynchium praelongum*, *Rhytidiadelphus sp.*) was also present at the soil surface (UK Forest Research, 2017a). The forest site was subject to minimal management, a few trees having been removed during the winter/spring of 2014 near the monitoring site. At the grassland site, mowing was conducted twice a year to control tree seedling growth. The soil type at both study sites was Ferralic Arenosol with an approximately 3-cm thick litter layer at the forest site, and 0-13 cm thick Ah horizon of organic rich sand with woody roots and occasional flints (UK Forest Research, 2017b). More information about the basic properties of the soils at the study sites was provided in Table 1.

Table 1:

2.3 *In situ* monitoring of soil CO₂ efflux, soil moisture and temperature

PVC collars (20 cm diameter, 6 cm height) for CO₂ efflux measurements were inserted to a soil depth of 4 cm leaving the remaining 2 cm protruding above the surface. This minimal insertion depth (Heinemeyer et al., 2011) ensured that the collars remained in place allowing a sealed contact with the chamber during the measurement, but minimised the isolation of soil and plant roots inside the collars from areas outside.

CO₂ efflux was measured using a Li 8100A Infrared Gas Analyser (IRGA) system with a 20-cm diameter dark chamber (LiCor Inc, Lincoln, NE, USA) placed over the installed PVC collars for the time of the measurement. The change in CO₂ concentration in the chamber was monitored over 2 minutes starting at the ambient CO₂ concentration and repeated twice for each collar at 2-minute intervals. The CO₂ efflux was determined by fitting an exponential function to the accumulation of CO₂ over time, excluding a 30-s initial phase at the start of the measurement. CO₂ efflux results where the function fitting coefficient R² was below 0.95 were excluded (less than 5% of overall measurements).

During each CO₂ efflux measurement, volumetric SWC was recorded with a Theta-Probe (ML3, Delta-T Devices) inserted at the soil surface up to 5cm depth next to PVC collar. Continuous monitoring of soil moisture and temperature at 5 and 10 cm depths was also conducted at study plots (n=4) using soil sensors (5TM, Decagon Devices, Inc.) connected to a datalogger. During each field visit, intact soil samples were collected from each plot approx. 10 cm from the CO₂ efflux measurement collars using PVC tubes (5 cm diameter, 9 cm height) to allow detailed measurements of SWC and wettability at each 2 cm depth interval under controlled laboratory conditions. In addition to this regular soil sampling, intact soil samples from within collars were also collected at the end of the measuring campaign to determine soil properties within the collar.

Meteorological data were obtained from the Santon Downham meteorological station located 500 m from the grassland site, while a dedicated rain gauge for monitoring of precipitation was installed at the forest site.

2.4 Soil sample analysis

Soil samples collected during each field visit were kept sealed in a constant temperature room (20 °C) for 24 hrs, then split into 4 depths (0-2.2, 2.2-4.5, 4.5-6.7, 6.7-9.2 cm) to determine their bulk density, SWR and SWC. Wettability of soil was determined under field moist conditions using the water drop penetration time (WDPT) test by applying 5 water drops (15 µl each) of tap water to the soil surface of each sample and recording time until their full infiltration (Doerr, 1998). 20 WDPT readings were recorded for each sample, giving a total of 120 WDPT readings per measurement event. Based on the readings, WDPT frequency distribution per event was determined by separating WDPT data into “persistence” classes (Doerr, 1998), wettability (<5 s), slight- (5-60 s), moderate- (61-600 s), strong- (601-3600 s) and extreme- (>3600 s) SWR. In addition, for determining the response of CO₂ fluxes to SWR conditions, the results were grouped into the SWR distribution based on the proportion of samples falling into the extreme SWR class per measuring event. WDPT class divisions are arbitrary, but the division chosen here is based on the reasoning that presence of soil with the highest level of SWR (i.e. extreme) has the most severe effect in terms of inhibiting water infiltration and resulting in most diverse soil water distribution.

SWC of the samples was determined gravimetrically by drying them at 105°C for 24 hrs and converting the weights into volumetric equivalents by incorporating soil bulk density values. Soil bulk density was calculated from the ratio of mass per volume of dry soil.

Total C and N contents in the soil samples were determined using a PDZ Europa ANCA GSL Elemental Analyser coupled with a 20/20 isotope ratio mass spectrometer. Samples of dried, homogenised soil were weighed in tin foil capsules and combusted over chromium oxide in helium with excess O₂ at 1000°C. The resulting gases were reacted over hot copper (600°C) and quantified using gas chromatography. Elemental composition were calculated based upon peak areas relative to the standard reference materials acetanilide and atropine. Soil pH was determined after 1:5 dilutions in deionised water and measured with the pH electrode.

2.5 Data analysis

Statistical analyses of data were performed using SPSS 22. For purpose of some data analyses the results of SWC and soil temperature have been grouped into bands representing a narrow range of values (e.g. $\pm 2^{\circ}\text{C}$ or $\pm 10\%$ SWC) in order to facilitate comparison and interpretation of the dataset (e.g. soil temperature within the 8°C band included values of $6\text{-}10^{\circ}\text{C}$).

5 Data were tested for normal distribution and homogeneity of variance, and data with non-normal distribution and/or unequal variances were transformed (square root, log) in order to carry out parametric analyses. A general linear mixed model was used to identify key factors that might be affecting soil CO_2 fluxes using a grouped results approach. Soil temperature, SWC vegetation type, surface type, C content and C:N ratio were used as fixed effect factors, while the study site, plot number and measurement date were used as random effect factors. For multiple comparisons, the ANOVA and Tukey's post-hoc test were
10 used to analyse significant differences. Significance of all test outcomes was accepted at p levels <0.05 .

3 Results

3.1 Meteorological and soil conditions

The average annual temperatures and precipitation during the three years of the field monitoring campaign were very close to 30-year average (1961 to 1990; UK Met Office, 2017b). The average air temperatures between three years of monitoring were
15 also similar but the precipitation patterns showed important variations (supplementary Table S1, Fig. S1, S2). Contrasting rainfall patterns occurred during summers of 2013 and 2014 with the former showing exceptionally low and scarce rainfall, the latter high total precipitation with rainfall events occurring frequently throughout the season.

The temporal and seasonal changes in meteorological conditions directly influenced soil conditions. Soil temperatures responded closely to air temperatures but, as would be expected, changes were buffered by the insulating effect of the soil
20 especially in the forest where it was less cold in the winter and less warm in the summer in comparison to the air temperature (Fig. 2). Weather conditions also resulted in drying and wetting of soil with the highest, relatively uniform SWC persisting from late autumn until early spring, contrasting with very variable SWC from late spring till early autumn. At the forest site, especially in winter, the water content in top soil layer was distinctly higher than lower down in the soil profile, while at the grassland site the differences between SWC at different depths were less pronounced. In summer, the responses to precipitation

at different soil depths were variable: typically rainfall caused an immediate increase in SWC both in the upper and lower soil. On some occasions (e.g. grassland site 8/2013, 5/2014), however, the response of SWC to rainfall at 10 cm depth was more pronounced than at 5 cm depth.

Figure 2:

5 3.2 Seasonal changes in Soil Water Repellency

SWR occurred to some degree for the majority of the warmer months (May-October) followed by a change to wettable soil conditions in the colder half of the year (November - April) (Fig. 3). However, SWR patterns varied from year to year depending on specific temperature and soil moisture. During the warmer months of 2013 and 2015, when the total precipitation was low, the majority of soil was water-repellent (WDPT >5 s; slight to extreme SWR). In 2014, during a wetter and warmer
10 summer season, SWR was very spatially variable with parts of the soil remaining wettable (e.g. grassland site 1/7/14), while the other plots showed slight to moderate SWR (WDPT 5-600 s) at the grassland site and slight to extreme SWR (WDPT 5 - >3600s) at the forest site. Only on a few occasions during the whole measurement period (e.g. 19/7/13 for the grassland site and e.g. 1/8/14 for the forest site) was soil uniformly extremely water-repellent (WDPT > 3600 s), which coincided with long dry spells lasting at least two weeks prior to the measurement dates. For most sampling events, soils showed very high spatial
15 variability in wettability with samples exhibiting the full range of WDPT values (0 - >3600 s) on a given sampling date. The WDPTs corresponded well with SWC. Thus, for the majority of cases at lower SWC, higher WDPT was observed, but it was also notable that highly variable SWC were measured when soils exhibited a range of different WDPT levels. Although the general pattern of SWR occurrence at both study sites was relatively similar, soil at the forest site showed overall higher and spatially less variable WDPT than at the grassland site. Thus, soil at the former site showed more frequent
20 occurrences of extreme SWR (especially during 2014) and also a higher proportion of soil samples being water-repellent when other samples were wettable on the same sampling event (e.g. 9/11/13, 23/3/15, 28/4/15) (Fig. 3).

Figure 3:

3.3 Seasonal variations in CO₂ fluxes

Measurements of CO₂ effluxes showed high variability between sampling dates, and between the warmer and cooler periods of each year. The lowest CO₂ effluxes were observed in early spring (e.g. 4/15, 5/15) and late autumn (11/14), but also on a few occasions during the summer (e.g. 7/13) (Fig. 4). The highest CO₂ effluxes were observed during spring and summer, which also corresponded with the highest spatial variability in efflux rates between samples. The vegetated soil plots at the forest site showed significantly higher CO₂ efflux than bare soil (Table 2).

A clear division in soil CO₂ efflux between warmer and cooler periods was observed at both study sites, highlighting soil temperature as a major factor influencing soil CO₂ efflux (Fig. 5a). CO₂ efflux values remained low at the temperatures up to 12 °C and increased with rising temperature. Beyond a maximum around 16 °C at the forest site and 20 °C at the grassland a reduction in CO₂ efflux rate was observed.

The other important factor affecting soil CO₂ efflux was soil moisture (Fig. 5b) which, together with soil temperature, can explain overall 61 % of total variation in soil CO₂ efflux. By considering soil temperature and moisture together it was clear that especially at higher temperatures (16-20 °C), low soil moisture (SWC <20 %) can be the limiting factor and can lead to reduced soil respiration. When SWC increased, soil CO₂ efflux was also higher, but reduced again at high SWC. At low soil temperatures (i.e. the 8 °C temperature band), soil moisture showed a limited effect and soil CO₂ efflux remained low irrespective of SWC.

A high variability of CO₂ efflux responses was observed even for similar mean SWC and the addition of other factors in the general model (e.g. study site, type of vegetation; Table 3) only slightly improved the explanation of the overall variability in CO₂ effluxes ($R^2=0.68$).

Figure 4:

Figure 5:

Table 2:

Table 3:

3.4 Soil water repellency and CO₂ fluxes

Given that occurrence of SWR is strongly affected by both temperature and moisture, and at the sites it was observed only when soil temperature was above 10 °C and SWC was below 25 % vol., the relationship between SWR and CO₂ efflux was considered separately from the above described model (Table 3). To consider the effect of SWR variability on CO₂ efflux, SWR data (Fig. 3) were converted into a relative fraction of extremely water-repellent soil (WDPT >3600 s) (Fig. 5c) described as SWR distribution. The zero value represents soil where extreme SWR was not detected, while a value of 1 denotes uniform extreme SWR. Values between 0 and 1 represent increasing levels of extreme SWR presence; lower indicate wettable soil with low percentage of extremely water-repellent soil, while the values closer to 1 represent soils dominated by extreme SWR with low percentage of wettable soil or at low SWR.

Soil CO₂ efflux showed a very clear association to SWR distribution. When SWR distribution had a value of 0 (i.e. the soil was wettable) soil CO₂ efflux was low, but it increased when a small fraction of soil became extremely water-repellent. The maximum soil CO₂ efflux was reached for a SWR distribution between 0.4 – 0.6. SWR distribution values >0.6 were associated with a decreased CO₂ efflux, which reached its lowest values when all soil became uniformly water-repellent (value of 1). The differences between soil CO₂ efflux for wettable/extremely SWR distribution values (0 and 1) and intermediate values (0.2-0.8) were observed mainly for time points with higher soil temperatures.

4 Discussion

4.1 Temporal variations in SWR

This study investigated, for the first time, the seasonal variability of SWR persistence in UK soils and, for the first time globally, the potential impact of reduced soil wettability on CO₂ efflux in the field. Three years of monitoring of soils under humid temperate pine forest and grassland revealed that SWR was present for most of the summer and autumn. The presence of SWR at these locations was consistent with previous studies that also reported severe SWR for UK grassland, forest and heath (Doerr et al., 2006), arable land (Robinson, 1999; Hallett et al., 2001) and on golf greens (York and Canaway, 2000), and in The Netherlands on grass-covered sand dunes under a similar climate (Dekker and Ritsema, 1996a; Ritsema and Dekker, 2000). Both investigated sites were under permanent vegetation, which is generally considered to be a state most susceptible

to SWR development (Doerr et al., 2000; Woche et al., 2005) due to the continuous input of hydrophobic substances from the vegetation and soil microbes (Doerr et al., 2000), and a low level of soil disturbance.

SWR has long been known to be temporally variable and has commonly been observed when soil is warm and dry, while disappearing during prolonged cold and wet conditions (Doerr et al., 2000; Leighton-Boyce et al., 2005; Buczko et al., 2006; 5 Stooft et al., 2011). At the sites investigated here, SWR was observed from early summer (May/June) until early autumn (October). The exact timing of SWR development and also of its complete disappearance could not be precisely determined in this study due to the monthly timings of the sampling visits, but it was clearly associated with low soil moisture contents and higher soil temperatures. SWR was not observed at soil temperatures lower than 10 °C despite low SWC, suggesting not only soil moisture but also the temperature is important in SWR occurrence. SWR remained spatially and temporally variable 10 throughout the warmer seasons. Only long dry spells resulted in high persistence of SWR in all investigated soil samples, suggesting that the majority of the soil at the sites was water-repellent at that time. For the majority of the warmer season, SWR was present, but of variable severity and often spatially interspersed with a small proportion of wettable zones. The high variability of SWR can be attributed to frequent changes between sufficiently dry and wet periods, characteristic of the UK climate, which allows development and partial disappearance of SWR. During the warmer dry periods in 2013 and 2015, the 15 soil became water-repellent (WDPT >5 s), but its persistence in different soil plots varied from minutes to hours. In contrast, during summer 2014, the proportion of wettable to water-repellent samples was very high (up to 65 % at grassland site, up to 50 % at forest site), which can be attributed to the particularly rainy summer (total rainfall for summer 2014 was 50 % higher than 2013 and 20 % higher than 2015). The high spatial variability of SWR and only partial change to a wettable state during the summer 2014 was likely a consequence of spatially uneven infiltration into the soil, further enhanced by preferential water 20 flow, both caused by presence of hydrophobic surfaces. The flat topography and surface cover of litter (at the forest site) or vegetation (at the grassland site) probably restricted surface runoff and resulted mainly in spatially variable infiltration and preferential water flow (Bughici and Wallach, 2016). We believe that substantial amount of rainfall was transferred below the near-surface repellent layer via faunal burrows (Shakesby et al., 2007), roots and soil cracks (Dekker and Ritsema, 1996b; Kobayashi and Shimizu, 2007; Urbanek et al., 2015) leaving zones with high persistence of SWR near wettable soil zones.

Patchy SWR distribution was associated with variable SWC, soil zones with high SWR persistence had a lower soil moisture content while wettable and soil with low degree of SWR was moist, which is consistent with the typically observed relationship between soil moisture and SWR reported in many other studies (Doerr and Thomas, 2000; Dekker et al., 2001). The dry, water-repellent soil patches would have been not only deficient in water, but also restricted in supply of nutrients (de Jonge et al., 2009; Goebel et al., 2011), while higher nutrient and DOC concentration would be expected in the wettable zones (Müller et al. 2014).

4.2 Temporal variations in soil CO₂ efflux

Temporal fluctuations in soil temperature and moisture not only affected the presence or absence of SWR, but were likely to be responsible for the variability in soil respiration. The CO₂ efflux measurements at the study sites were conducted each year from June until November with only a few early measurements in spring during 2015. Therefore, no information was available on soil CO₂ efflux during the winter season. All early spring and late autumn measurements, however, showed lower soil CO₂ efflux than measurements during the warmer period. During the colder and typically wetter part of the year, primary productivity, soil biological activity and therefore soil respiration is typically low (Davidson and Janssens, 2006). Considering the seasonal fluctuation, but also noting the positive correlation between soil CO₂ efflux and soil temperatures, it is likely that the temperature drives soil respiration to a certain level, which is consistent with many previous studies (Gaumont-Guay et al., 2009; Yvon-Durocher et al., 2012; Karhu et al., 2014). The positive response of CO₂ efflux to increasing soil temperature reflects the greater activity of roots and decomposing microorganisms, but can also involve long-term changes in microbial population communities and higher substrate supply from photosynthesis in response to longer-term trends as expected, for example, with global warming (Davidson and Janssens, 2006; Gaumont-Guay et al., 2009). At both study sites soil CO₂ efflux increased with rising temperatures, but only until a maximum CO₂ efflux level was reached, after which a notable decrease was observed. The occasions when soil CO₂ effluxes were no longer dictated by temperature occurred during the summer when the soil was exposed not only to relatively warm, but also dry conditions for prolonged periods, suggesting that soil moisture was the restricting factor for soil CO₂ efflux. The effect was observed only at times of uniformly low SWC when persistence of SWR was consistently high. On the occasions when SWC was low, but spatially variable, soil respiration was

high and followed an increasing trend with temperature. A reduction in soil moisture availability is known to reduce microbial activity and root respiration (Or *et al.* 2007), and prolonged summer droughts have been recognised in many studies as the cause of a decrease in CO₂ efflux, primarily in heterotrophic respiration (Borken *et al.* 2006).

5 4.3 Effect of soil moisture and SWR on soil CO₂ fluxes

Soil CO₂ efflux was found to respond to changing SWC, particularly at higher soil temperatures (Fig. 5b), and the variability in CO₂ efflux remained high, especially for intermediate SWC. Only after long dry spells when soil moisture availability was low, were soil respiration rates significantly reduced. At intermediate SWC, soil CO₂ efflux was high but also very variable, most likely due to frequent wetting and drying events resulting in a heterogeneous soil moisture distribution (Gaumont-Guay
10 *et al.*, 2009). Given that both sites are very susceptible to the development of SWR we expect that high variability in CO₂ efflux at intermediate SWC can be the result of uneven soil water distribution caused by the presence of SWR.

Previous studies have already shown that SWR can protect SOM from decomposing microorganisms (Goebel *et al.*, 2005; Goebel *et al.*, 2007; Bachmann *et al.*, 2008; Lamparter *et al.*, 2009; Goebel *et al.*, 2011), which results in reduced soil respiration. These laboratory-based studies focused mainly on the severity of SWR of homogeneous soil and therefore did not
15 explore the wide range of hydrological scenarios to which natural soil is exposed in presence of SWR. Most studies exploring SWR present the results based on overall median or mean WDPT, which won't allow identification of the naturally common and important situation when SWR variability is very high. SWR distribution, as used in this study, shows the proportion of soil affected by extreme SWR which can indicate the proportion of pores with inhibited water movement and allow better prediction of hydrological behaviour. The SWR distribution presented here include the following conditions of the soil (a)
20 uniformly wettable, (b) at the intermediate SWR stages when soil is dominated by wettable soil with patches of extremely water-repellent soil or *vice versa*, and c) at high (extreme) SWR as presented in a conceptual figure (Fig. 6).

At both study sites CO₂ efflux was low when soil was in wettable state (Fig. 6a), which occurred under two different conditions: during early spring and late autumn when soil temperature was too low for SWR development, or during the summer when, due to frequent rainfall, SWR disappeared and high moisture was recorded. On both occasions, low CO₂ efflux was mainly
25 caused by either low temperature or high moisture content, which in any wettable soil would cause a similar response. Soil

CO₂ efflux was also low on occasions when soil was extremely water-repellent with SWR distributions close to 1 (Fig. 6c), occurring during prolonged dry spells when soil temperature was high and SWC was low. It is reasonable to expect that the reduction of CO₂ efflux was caused mainly by low SWC, which caused reduced microbial activity. Previous laboratory studies have reported low respiration rates in similar highly water-repellent soil (Goebel et al., 2007; Lamparter et al., 2009). Due to low water availability, microbial and enzymatic activity is reduced (Or et al., 2007; Moyano et al., 2012; Moyano et al., 2013;), or activity ceases entirely when extremely low matric potentials are reached and water films in soil pores become disconnected (Goebel et al., 2007). According to Or et al. (2007), diffusion rates of extracellular enzymes produced by microbes to access organic matter are proportional to the thickness of the water film surrounding soil particles, and this thickness is substantially reduced by SWR (Churaev, 2000; Goebel et al., 2011). Obstruction of microbial movement and reduction in diffusion results in physical separation of microorganisms from substrates and nutrients, which can lead to long-term starvation (Kieft et al., 1993). At the sites investigated here, such a situation was observed only on a few occasions following long dry spells, suggesting that under the current humid-temperate climate, this soil condition is not very common here. It is, however, very common in climates with distinct dry seasons or more prolonged dry periods (Doerr et al., 2003; Doerr and Moody, 2004; Leighton-Boyce et al., 2005; Stoof et al., 2011) and may become more common in the UK in the future climate predictions (IPCC, 2013). It is also an important scenario to be considered during the rewetting of extremely water-repellent soils after drought, as reported by Muhr et al. (2008, 2010) who observed a slower regeneration of CO₂ fluxes following wetting that could have been caused by SWR.

The highest CO₂ efflux was recorded at intermediate SWR distributions (SWR distributions 0.2-0.8) when SWR, and consequently soil moisture distribution, was very patchy (Fig. 6b). Variable SWR can be associated with patchy pore water distribution which creates zones of soil with water-filled pores near water-repellent soil zones. Water-filled soil pores are expected to provide a good supply of water with high nutrient concentration, which if compared to preferential flow paths, are expected to harbour larger bacterial densities (Vinther et al., 1999) and activities (Pivetz and Steenhuis, 1995) than the adjacent soil matrix. The water-repellent soil zones with air-filled pores are anticipated to provide optimal routes for gas transfer where the O₂ and CO₂ released by the decomposing microorganisms can easily be exchanged between the soil and the atmosphere (Or et al., 2007; Kravchenko et al., 2015).

In a humid temperate climate with soils susceptible to SWR, variably water-repellent soil is likely to be the most common soil condition, while in climates with distinct dry seasons or common dry spells it could represent the state of change between wettable and water-repellent taking place between wet and dry seasons or periods (Leighton-Boyce et al., 2005; Stoof et al., 2011).

5 Considering the whole soil volume examined in this study, we can reject the hypothesis that presence of SWR unequivocally reduces soil respiration also under natural, field conditions. The response of soil respiration to the presence of SWR is more complex than it has been originally anticipated and its effects are clearly more complex as discussed above.

Future studies investigating C dynamics in water-repellent soil are still needed to explore further the effect of hydrophobic soil surfaces on CO₂ fluxes. For example, further insights could be gained by more frequent or near continuous monitoring of soil
10 respiration together with SWR and soil moisture. This would allow better understanding of soil respiration during the wetting and drying processes in soils that exhibit SWR and thus restricted water infiltration. We consider the proposed conceptual figure depicted in Fig. 6 to be sufficiently simple to be applicable to a wide range of water-repellent soils. However, given the potential importance of SWR to affect soil respiration and ultimately soil C storage under changing land uses and a changing climate, further field investigations involving different soil types and environmental conditions would be valuable in
15 determining how widespread and temporally common SWR is.

Figure 6:

5 Conclusions

20 This study reports for the first time how seasonal changes in SWR distribution affect soil respiration, and demonstrates that the presence of SWR does not simply lead to a reduction in soil CO₂ efflux. The sites investigated in the UK under grassland and pine forest showed presence of SWR during warmer periods, but dominated by high spatial variability in SWR persistence. Frequent occurrence of wetting and drying events, common in humid-temperate climates, is associated with high patchiness of SWR, and only when soil is exposed to longer dry spells does it become severely and uniformly water-repellent.
25 Hydrological consequences of variable SWR distribution are unique, therefore it is necessary to recognise their distinctiveness from entirely wettable or water-repellent soils. The data collected here suggest that the response of soil CO₂ efflux is strongly

associated with soil wettability status and the distribution of water-repellent patches. Very high SWR levels were indeed associated with low soil CO₂ effluxes, caused by reduced CO₂ production by water-stressed microbial communities. However, variable SWR distribution resulted in the highest CO₂ effluxes, most likely due to microbial communities being concentrated in the water and nutrient ‘hotspots’ close to infiltration paths coupled with favourable gas exchange conditions in hydrophobicity-controlled air-filled pores. A wettable soil state was observed at the study sites at low soil temperatures or after frequently occurring rainfall events, and in both cases low CO₂ effluxes were observed during this measurement’s. The hypothesis that presence of SWR unequivocally reduces soil respiration, also under the natural field conditions, is therefore rejected.

SWR clearly has an important effect on soil respiration, but its impact is more complex than previously assumed, with its spatial variability likely to be the most influential factor. The presence of SWR can not only reduce soil respiration in affected soil zones, it can actually lead to enhanced respiration from soil zones exhibiting high spatial variability in SWR. When examining SWR, measurements should therefore not be restricted to simply recording whether soil is wettable or water-repellent with a certain persistence or severity level, but its spatial (and temporal) variability should also be inspected. This combined knowledge should then allow better predictions of soil respiration to different temperature and moisture conditions. In view of current climatic predictions and expectations that SWR will become even more widespread globally than is the case at present, it is important to include analysis of the spatio-temporal characteristics of SWR in long-term respiration studies. The comprehensive understanding of the specific effects of SWR on soil C dynamics under current conditions can be gained and a firmer foundation for prediction of C dynamics under future climatic scenarios can be established.

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List of figure captions

Figure 2: Schematic presentation of plots and CO₂ efflux measurement collars at both, the forest and grassland study sites arranged along a 20-m long transect. The dashed squares identify study plots (6) and circles - soil collars for CO₂ efflux measurements (n=12), green coloured shapes represent soil surface vegetated with grass and brown – with bracken; filled circles represent vegetated area, open circles – collars with vegetation temporarily removed for soil CO₂ efflux measurements = bare soil.

Figure 2: Temporal changes in soil moisture (—green line – SWC at 5 cm depth; —brown line – SWC at 10 cm depth) and soil temperature at 5 cm depth (—blue line) at both study sites over 3 years; a) forest site and b) grassland site. Field measurements and sampling events are marked with black circles (•).

Figure 3: Frequency distribution of soil water repellency (SWR) persistence, measured as water drop penetration time (WDPT) and soil water content (SWC) for (a) forest and (b) grassland site at 0-9 cm depth at all sampling dates. Different colours reflect WDPT classes, black circles represent mean SWC and error bars the standard deviation of the mean (n=120 per date).

Figure 4: Variations in soil CO₂ efflux for each measurement event for combined grass and bracken plots, results separated for vegetated (● filled circles) and bare (○ open circles) soil plots at a) the forest and b) the grassland study sites (n=6).

Figure 5: Relationship between soil CO₂ efflux and a) soil temperature, b) soil water content (SWC) and c) soil water repellency (SWR) distribution. In figure 5a results are separated for the forest and grassland sites and soil CO₂ effluxes are represented as means with standard deviation for soil temperature grouped into 2 °C classes (+/-1 °C). In figure 5b and 5c results from the forest site and the grassland sites are combined and separated into 4 soil temperature classes. Soil CO₂ effluxes are represented as means with standard deviations. In figure 5b the results were grouped into SWC classes of 10 vol.% and in figure 5c were grouped into SWR distribution classes (±0.1) representing a relative fraction of extremely water-repellent soil. The SWR axis minimum 0 represents wettable soil, while the maximum of 1 denotes uniformly extreme SWR. Only results from the vegetated sites have been to create the figures (n=102).

Figure 6: Theoretical framework of soil water distribution at three different conditions of SWR and its potential effects on soil CO₂ production and transport.

List of table captures

Table 1: Selected properties of soil samples (n=12) retrieved from the CO₂ efflux monitoring collars after the field campaign had been completed. Presented values represent the mean (n=6) with standard deviation in the brackets.

- 5 **Table 2:** Total average CO₂ effluxes (μmol/m²/s²) from plots under bracken and grass understorey with vegetated and bare plots at the forest and grassland study sites. The asterisks indicate the statistically significant differences between groups of vegetated and bare plots (*p<0.05, ***p<0.001).

Table 3: Factors affecting soil CO₂ effluxes including the statistical significance level.

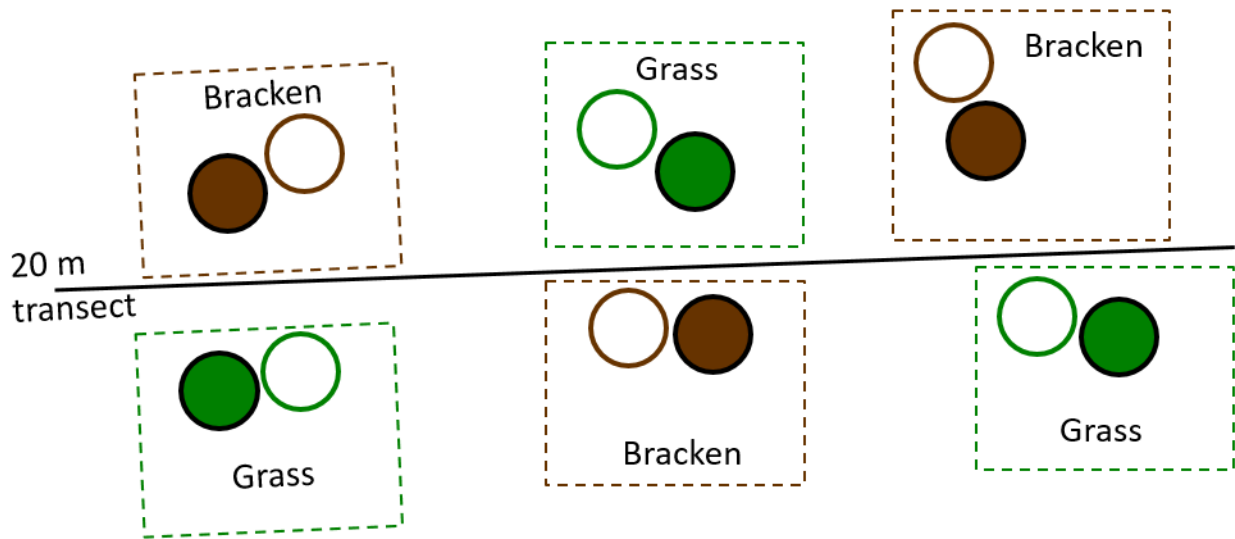


Figure 1:

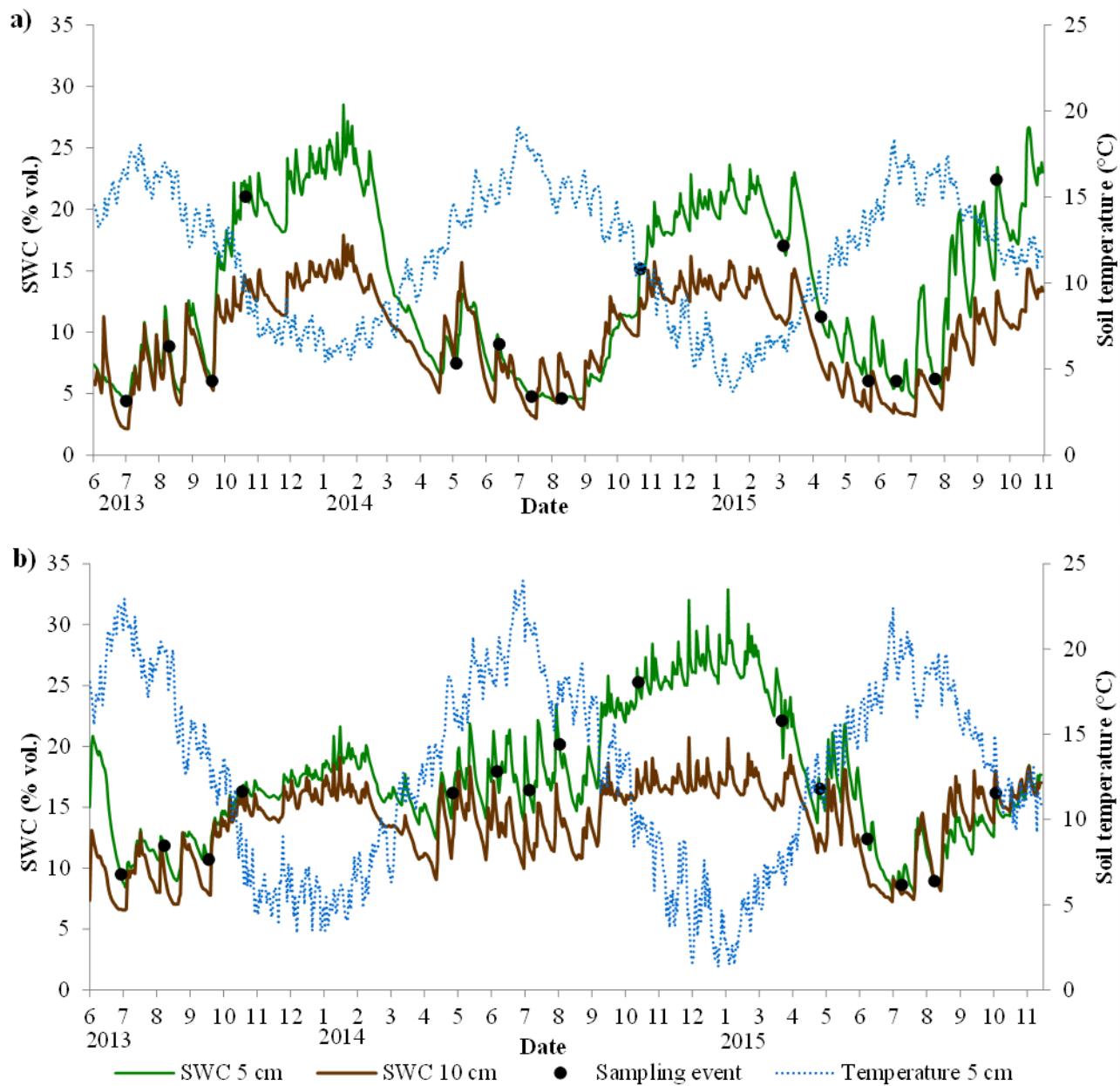


Figure 2:

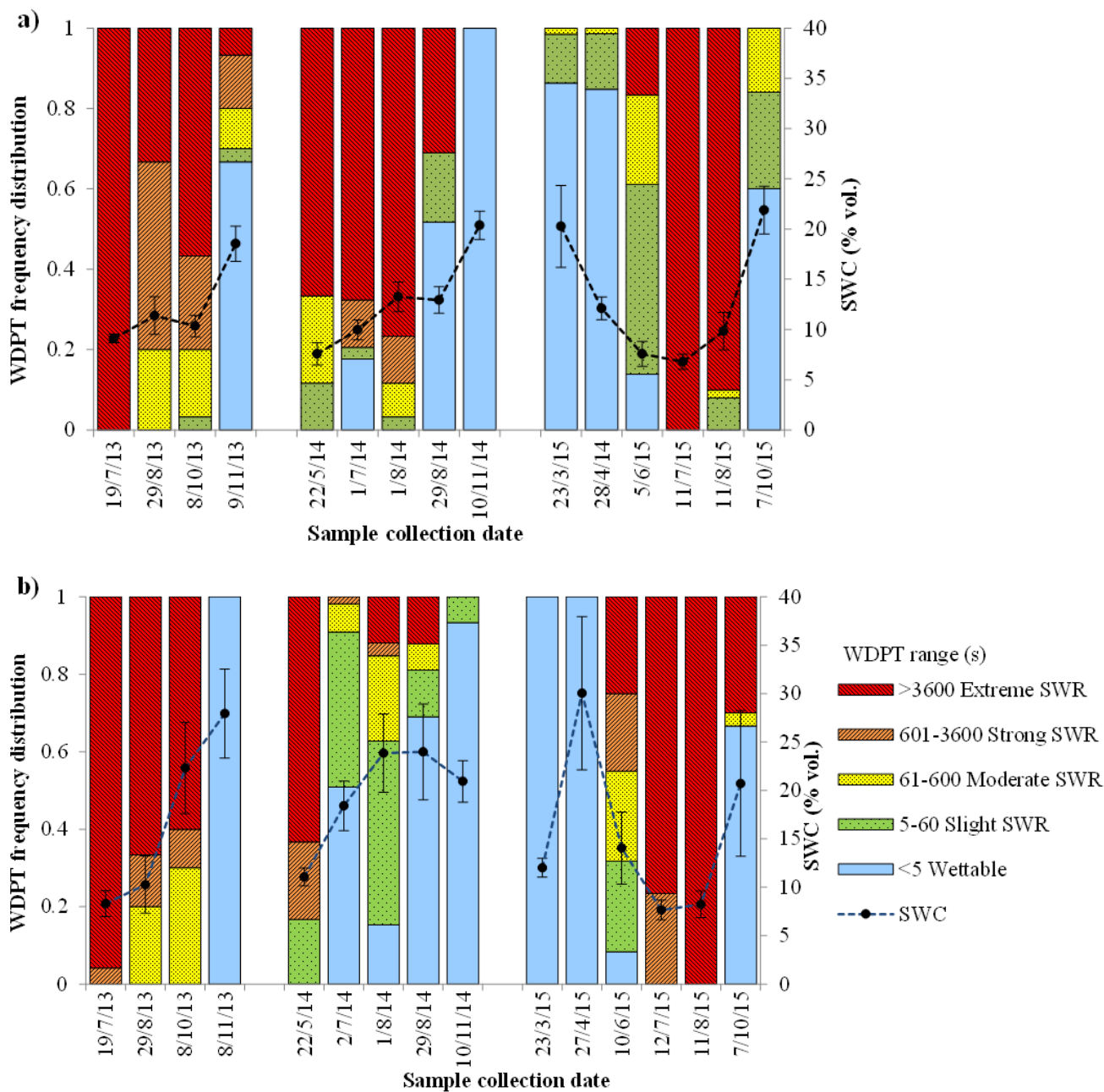


Figure 3:

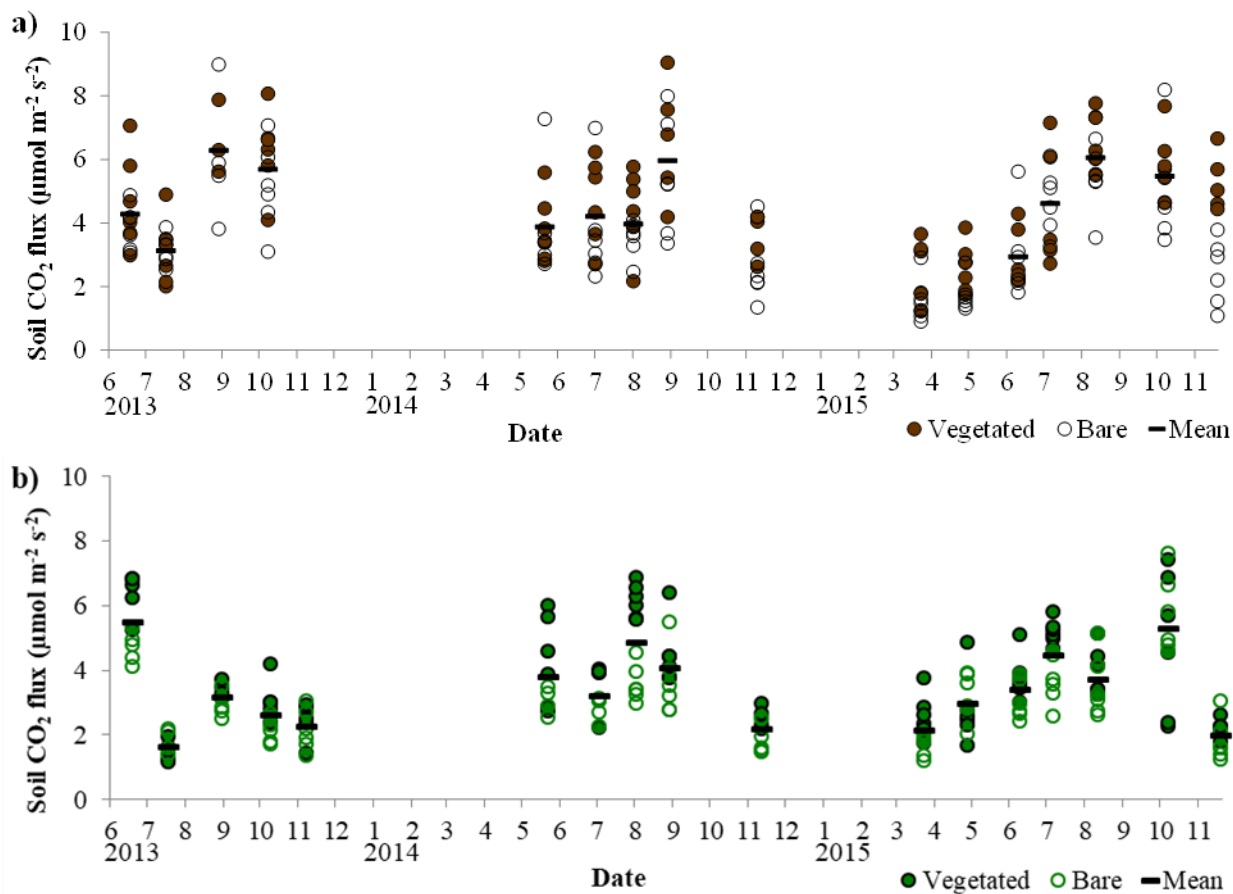


Figure 4:

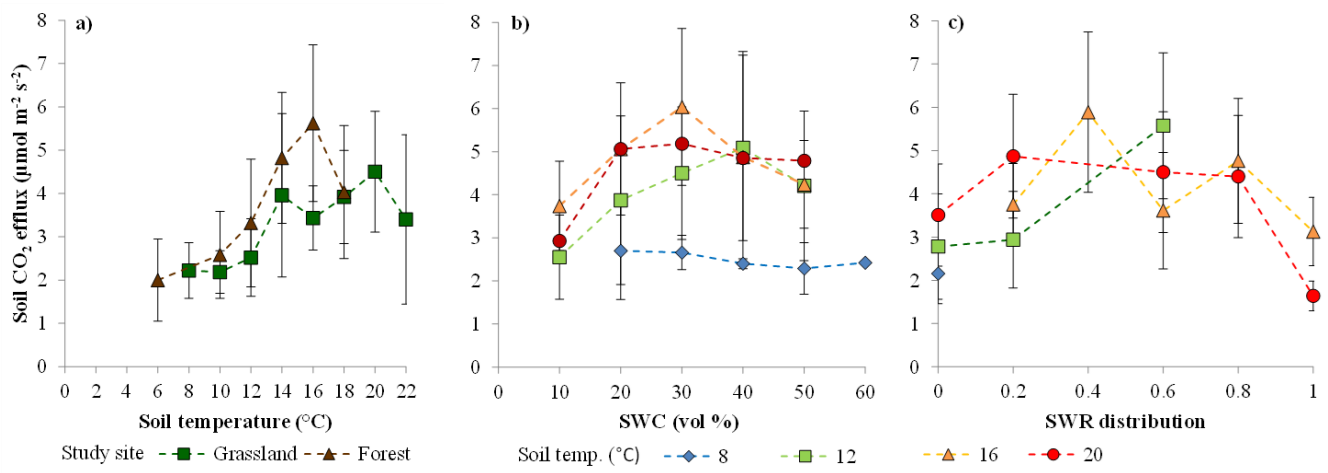
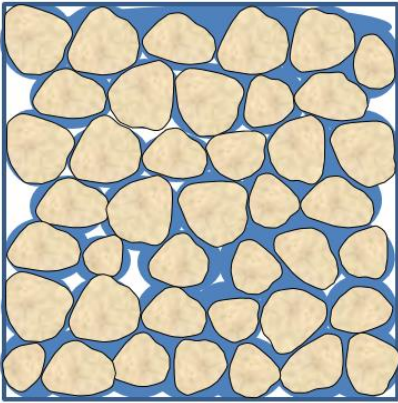


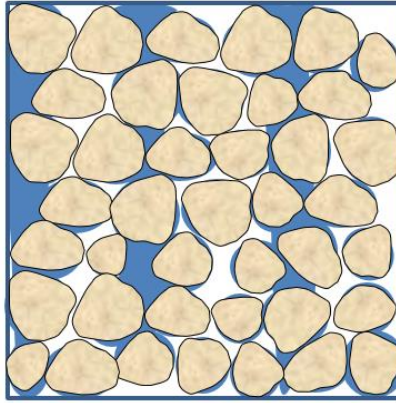
Figure 5:

a) Wettable soil



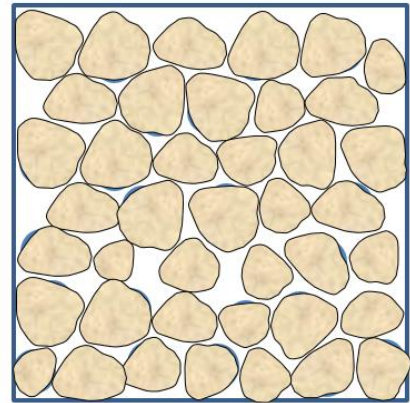
- Water distribution – uniform, unaffected by water-repellency
- Pores fully/partly filled with water
- Gas exchange – low
- Microbial respiration - low

b) Variably water-repellent soil



- Water distribution – variable; enhanced preferential water flow
- Good gas exchange, especially in air-filled tunnels
- High microbial respiration in areas with easy water/nutrient access

c) Uniformly water-repellent soil



- Limited water access
- Soil pores filled with air
- Very good gas exchange
- Low microbial respiration

Figure 6:

Table 1:

Site	Soil depth (cm)	C content (%)		C:N (-)		pH (-)		Bulk density (g cm ⁻³)	
		Bracken	Grass	Bracken	Grass	Bracken	Grass	Bracken	Grass
Forest	0-2.2	26.9 (12.1)	7.2 (6.1)	23.5 (2.0)	13.2 (6.3)	3.6	4.6	0.3	0.9
	2.2-4.5	8.3 (4.7)	2.4 (1.5)	16.3 (9.3)	9.7 (6.1)	3.7	5.2	0.7	1.2
	4.5-6.7	3.0 (2.4)	1.5 (0.8)	10.3 (2.7)	7.0 (3.4)	4.0	5.1	1.1	1.1
	6.7-9.2	1.2 (0.7)	1.6 (0.7)	6.6 (4.6)	7.2 (2.6)	4.1	5.2	1.3	1.3
Grassland	0-2.2	24.3 (6.1)	20.0 (5.3)	23.1 (6.6)	20.4 (8.6)	2.9	3.1	0.5	0.7
	2.2-4.5	8.7 (4.4)	7.4 (5.2)	13.2 (8.3)	12.2 (6.0)	3.0	3.0	1.1	1.2
	4.5-6.7	3.3 (1.3)	3.0 (2.1)	10.5 (4.5)	7.9 (8.0)	3.0	3.1	1.2	1.3
	6.7-9.2	0.8 (0.1)	1.2 (0.2)	4.9 (1.7)	5.7 (2.8)	3.2	3.1	1.5	1.8

Table 2:

Study site	Vegetation type	Vegetated plots	Bare plots
Forest	Bracken	4.57(1.9)	3.20(1.25) *
	Grass	5.14(1.89)	3.93(1.88) *
	all	4.86(1.9)***	3.57(1.63) ***
Grassland	Bracken	3.61(1.56)	3.12(1.09)
	Grass	4.04(1.55)	2.96(1.42)
	all	3.82(1.56)***	3.04(1.26) ***

Table 3:

Source	Type III sum of Squares	df	Mean square	F	Significance level
Corrected model for sqrt CO ₂ flux	23.11*	64	0.36	3.96	0.000
Intercept	24.43	1	24.43	267.72	0.000
SWC * Temp	19.85	62	0.35	3.51	0.000
Study Site	1.56	1	1.56	17.09	0.000
Vegetation type	0.84	1	0.84	9.15	0.003
Error	10.86	119	0.09		
Total	788.60	184			
Corrected total	33.96	183			

* R² = 0.68