

Fourteen Annually Repeated Droughts Suppressed Autotrophic Soil Respiration and Resulted in an Ecosystem Change

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ABSTRACT

Predictions of future climate over the next 100 years show that the frequency of long periods of droughts in summer will increase in the Netherlands. This study investigated the effect of 14 annually repeated droughts on soil respiration at a Dutch heathland. Field measurements of total soil respiration (R_S) and microbial respiration (R_H) were modeled to determine annual C losses and to derive root respiration (R_A) C losses. The application of repeated droughts resulted in suppression of the total soil C loss from 392 to 332 g C m⁻² year⁻¹ in 2010–2011 and from 427 to 358 g C m⁻² year⁻¹ in 2011–2012. The R_H was the greatest contributor to heathland soil C loss (74–76%) and this was suppressed when directly exposed to drought conditions, although not significantly reduced on an annual basis. Annual R_A was suppressed by 42% (2010–2011) and 45% (2011–2012) under re-

peated drought, indicating there was a greater effect of the repeated annual drought in roots than in microbes. Field observations of photosynthesis (P_G) showed paradoxical results, with significantly greater ecosystem P_G on the drought treatment than the control treatment. Inclusion of plant activity (P_G) as a variable did not improve the fit of the models used in this study. However, other changes in plant composition and structure, such as increasing moss cover on the drought treatment, were noted to have occurred during the 14 years of annually repeated drought and these long term trends may help explain the effects of climate change (drought) on soil processes.

Key words: heterotrophic respiration; autotrophic respiration; soil C loss modeling; *Calluna vulgaris* heathland; drought; climate manipulation.

Received 5 April 2013; accepted 16 September 2013;
published online 17 October 2013

Author Contributions: GRK, AT, and DA conceived and designed the study and contributed to performing the research. GRK and EEVL analyzed data and (together with AT) wrote the paper.

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INTRODUCTION

Since 1950, global dry areas have increased by approximately 1.7% per decade and this drying trend has been linked to global increases in temperature (Dai 2011a). Within Europe, this drying trend is predicted to increase over the next 100 years, particularly in the southern regions, while in western Europe the frequency of periods

of extremely wet and dry conditions is predicted to increase (that is, indicating an increase in extreme conditions) (Dai 2011a, b). Also for the Netherlands, Blenkinsop and Fowler (2007) concluded from a multi-model study that in general the frequency of long periods of droughts during the summer is expected to increase under future climate.

A consequence of more frequent or prolonged droughts is that key ecosystem functions such as carbon (C) storage, nutrient cycling, and species composition are likely to be affected (IPCC 2007; Wessel and others 2004). Therefore, increasing drought frequency is likely to impact the C cycle more strongly in the future (van der Molen and others 2011).

Drought periods can impact ecosystem functioning in a number of ways. Plants can be affected in a physiological and structural manner, such as reducing their enzymatic activity or closing stomata to minimize water loss, with a resulting decrease in photosynthesis and respiration rates (Chaves and others 2002; van der Molen and others 2011). Reductions in leaf area after drought can also occur due to early senescence and leaf shedding, or arrest leaf expansion (Peñuelas and others 2007; van der Molen and others 2011). Plant roots may respond to drought by increasing the root to shoot ratio, root length or root area to enable increased water and nutrient uptake, although these responses vary between plant species (Liu and Stützel 2004; Rodrigues and others 1995). Microbial processes in the soil are also affected, with reductions of soil water films inhibiting the diffusion of substrates, reducing the soil C mineralization rate, and decreasing subsequent CO₂ release (Davidson and Janssens 2006; Jensen and others 2003).

The production of CO₂ in soils (that is, soil respiration “R_S”) is almost entirely from two sources. The first source is autotrophic respiration (R_A), which is associated with the activity of roots and rhizosphere organisms, and the second source is heterotrophic respiration (R_H), which is associated with both the bacterial and fungal decomposition of organic matter and soil faunal activity (Davidson and Janssens 2006; Hanson and others 2000).

Root exclusion methods, for example trenching and gap analysis, are accepted methods of quantifying ecosystem R_S and R_H (Díaz-Pinés and others 2010; Hanson and others 2000; Jassal and Black 2006). The resulting R_S and R_H data are modeled to derive R_A and to calculate total annual C losses. As organic matter decomposition is temperature

dependent, most R_S models relate the efflux of CO₂ from soils to temperature in an exponential function (Davidson and Janssens 2006; Sierra and others 2011). Organic matter decomposition and plant activity are also affected by moisture availability and therefore many models also take soil water content or precipitation effects into account (Davidson and others 2006; Raich and Schlesinger 1992). Increasingly, measures of plant activity, such as plant metabolism or litter production are also included within R_S models to link the above-ground and belowground processes within ecosystems (Bahn and others 2010b; Metcalfe and others 2011; Ryan and Law 2005).

To investigate the effect of drought on annual soil C loss, R_S and R_H were measured a decade after the start of a long term climate manipulation trial in which a repeated annual drought treatment had been applied for 14 years. This climate trial was established on a Dutch *Calluna vulgaris* heathland, as part of a network of European shrubland sites that aim to investigate the effect of the IPCC predicted climate change of altered rainfall patterns, in particular decreased precipitation during the growing season (Beier and others 2004).

Calluna heathlands are known to be able to withstand quite severe summer droughts if annual rainfall is high enough to compensate (Loidi and others 2010). However, the exact response of *Calluna* root respiration (and therefore R_A) to low soil moisture is unknown. Previous heathland studies have shown that drought conditions result in decreased net C uptake by *Calluna* and subsequent decreased C allocation to the soil (Gorissen and others 2004). Periods of drought on *Calluna* heathlands with a similar moisture status have also shown a reduction in microbial processes and a concurrent reduction in soil microbial respiration (Emmett and others 2004; Sowerby and others 2008). The composition and response of the microbial community can change due to drought (Jensen and others 2003) and so it is possible that the dynamics of the heathland may change under long-term drought conditions. In this study, the effect of an increased drought frequency and the subsequent reduction in available soil moisture on the C losses associated with R_A and R_H soil respiration was quantified. Secondly, evidence was collected to support the hypothesis that a heathland community which is exposed to repeated drought conditions may adjust its soil respiration response to various environmental factors, including reduced soil moisture.

METHODS

Study Site

The investigation was undertaken at a dry heathland located at Oldebroek, the Netherlands. The dominant vascular species at the site is the perennial woody dwarf shrub *Calluna vulgaris* (L.) and the dominant non-vascular species is the moss *Hypnum cupressiforme* Hedw. The site has a nutrient-poor, well drained, acid sandy Haplic Podzol soil, and experiences high N deposition rates (10.7–37.4 kg ha⁻¹ year⁻¹). Further information about the site location, species composition and climate is provided in Table 1.

Climate Trial Experimental Design

Six experimental plots of 5 × 4 m² (with a 0.5 m buffer strip around the perimeter) were established in a homogeneous area of the heathland in 1998. Two treatments were allocated randomly: a control treatment and prolonged drought during the growing season (drought treatment). Light scaffold structures were built over all the plots, to ensure that any impact from the scaffolding (for example, shading and sheltering) occurred in all plots (Beier and others 2004).

A retractable curtain made of transparent polyethylene plastic was supported on the frames over

the drought plots. During the drought period, rain sensors activated the motor to extend the cover over the plots during precipitation and retract the cover when precipitation stopped. Outside the drought period, no manipulation was applied and all plots received the same precipitation amount. The annual drought treatment began in May 1999 and reduced precipitation annually by 6–29% (Table 2). Further details on the design of the treatment manipulations can be found in Beier and others (2004).

In September 2009, all vegetation was harvested from an area of 1 × 2 m² in each of the plots, to simulate the effects of heathland cutting management. The roots and soil were not disturbed. The *Calluna* and moss layers from these cut areas were separated, oven dried at 70°C and the dry weight of each component was recorded (*n* = 9). To be certain that no roots remained alive and that root decomposition did not contribute to CO₂ efflux results, any vegetation regrowth was continuously removed and the soil respiration measurements only commenced 18 months after vegetation removal. At this time, there was no *Calluna* regrowth occurring from root stock and only occasional seedling growth. The respiration data of the control treatment cut areas were compared to the heterotrophic respiration results obtained from a trenching trial conducted outside the climate trial plots

Table 1. Description of the Oldebroek Trial Location

Location	ASK Oldebroek, Oldebroekse heide, Province of Gelderland, The Netherlands						
Co-ordinates	52°24'N 5°55'E						
Elevation	25 m ASL						
Slope	2%						
Climate	Temperate, humid.						
Rainfall	1,072 mm						
Temperature	Average for		January: 2.0°C	July: 17.8°C	Annual: 10.1°C		
Plant Species	<i>Calluna vulgaris</i> , <i>Molinia caerulea</i> , <i>Deschampsia flexuosa</i> , <i>Pinus sylvestris</i> , <i>Betula pendula</i> , <i>Empetrum nigrum</i> , <i>Juniperus communis</i> , <i>Hypnum cupressiforme</i> Hedw., <i>Hypnum jutlandicum</i> Holmen et Warncke, <i>Dicranum scoparium</i> Hedw.						
Community Age	28 years since last heathland management cycle						
Soil	Haplic Podzol with mormoder humus form (L, F, H, Ae, Bh and BC horizons)						
Parent Material	Coversand, fluvioglacial deposits						
Soil chemistry	Organic horizons		Mineral horizons				
	L+F	H	Ae	Bs	1BC	2BC	C
Depth (cm)	+8.0 to +1.4	+1.4 to 0	0 to 5.5	5.5 to 13	13 to 21	21 to 27	>27
pH ¹	3.7	3.9	3.9	4.0	4.5	4.4	4.9
Soil moisture ²	24.3	32.4	20.2	18.7	10.5	8.2	7.3

¹Water extraction of 1:5 for organic horizons and 1:1 for mineral horizons.

²Soil Moisture at field content obtained following a rainfall event and reported as g cm⁻³ of dry weight soil.

Table 2. Drought Treatment Effects

Year	Site annual precipitation (mm)	Drought treatment dates	Annual precipitation reduction (%)	Soil moisture content after drought ¹	
				Control (m ³ m ⁻³)	Drought (m ³ m ⁻³)
1999	891	26 May–10 Aug	8.2	0.141	0.097
2000	1,142	24 May–25 July	18.8	0.184	0.062
2001	1,030	28 May–14 Aug	28.6	0.222	0.044
2002	1,032	3 Jul–16 Aug	23.0	0.191	0.044
2003	836	3 Jun–12 Aug	17.9	0.082	0.037
2004	1,220	25 May–28 Jul	23.0	0.202	0.041
2005	1,196	16 Jun –12 Aug	26.6	0.236	0.050
2006	1,233	30 May–7 Aug	20.1	0.134	0.042
2007	1,018	13 Jul–14 Sep	21.0	0.118	0.049
2008	874	2 Jun–29 Jul	20.1	No data	No data
2009	820	27 May–28 Jul	29.0	No data	No data
2010	1,007	22 Apr–30 May	5.9	0.164	0.150
2011	887	5 Apr–10 Jun	7.3	0.194	0.089
2012	890	20 Mar–12 Jun	18.5	0.190	0.101

¹The soil moisture results for both treatments were the average values at 4–7 cm below ground surface recorded in the final weeks of each annual drought.

(data reported in Kopittke and others 2013a). There was no significant difference between the two data sets ($p = 0.85$). Therefore, soil respiration from the cut areas was deemed to be representative of microbial soil respiration and will be henceforth referred to as heterotrophic respiration.

In April 2011, an additional four uncut control plots ('Validation' plots) were established outside the climate treatment plots. The data collected from these plots were used for the purpose of validating the control treatment derived R_S model.

In this study, the terminology 'total soil respiration' and 'heterotrophic soil respiration' refers to the observed field data from the uncut plots and cut plots, respectively. The terminology ' R_S ', ' R_H ' and ' R_A ' refers to the modeled total soil respiration, modeled heterotrophic soil respiration and calculated autotrophic respiration, respectively.

Site Meteorological and Treatment Soil Conditions

Site meteorological conditions were recorded on an hourly basis (Decagon Devices Inc.; DC, USA). Air temperature and relative humidity measurements were obtained from a central location on the site. Rainfall was measured using a Vaisala tipping bucket rain gauge (Vaisala; Vantaa, Finland) connected to a Decagon datalogger. Treatment soil conditions were recorded on an hourly basis (Decagon Devices Inc.; DC, USA). Soil moisture (m³ m⁻³) and soil temperature (°C) measurements were obtained from 4 to 7 cm below ground surface in each treatment plot (5TM Sensor, Decagon

Devices Inc., DC, USA). This paper uses sensor measurements that correspond to the soil respiration measurement periods; that is, July 2010–August 2012 in the treatment uncut areas and April 2011–August 2012 in the treatment cut areas.

Soil Respiration Measurements

Soil respiration collars of 10 cm diameter and 6 cm height were inserted approximately 1 cm into the soil surface in each plot, maintaining a buffer zone of 30 cm from the plot boundary. On the uncut areas of the plots, moss was removed from inside these collars, to ensure that only soil respiration was measured. Soil respiration measurements were obtained using the a Portable Gas Exchange and Fluorescence System (LI-6400; LICOR Biosciences, Lincoln, NE USA) in combination with a small soil CO₂ flux chamber (LI-6400-09; LICOR Biosciences) which fitted onto the collars.

Soil respiration measurements using this methodology commenced on uncut areas of the climate trial in July 2010. Initially these measurements were obtained from only one collar per plot ($n = 6$) however, after December 2010, this increased to three collars per plot ($n = 18$). A total of 30 soil respiration measurement events occurred on these uncut areas. On cut areas, soil respiration measurements commenced in April 2011. One collar was installed in the cut area of each plot ($n = 6$) and a total of 19 soil respiration measurement events occurred on these collars. On validation plots, measurements commenced in April 2011 and a total of 33 events occurred on these plots.

Photosynthesis Measurements

The gross photosynthetic rate (P_G) was calculated as the net ecosystem exchange (NEE) rate of CO_2 flux minus the ecosystem respiration (ER) rate of CO_2 flux ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and has a negative sign. A loess smoother curve was applied to the P_G data to obtain daily estimates of plant activity to allow it to be used as an explanatory variable within the R_S models.

The CO_2 fluxes of the ecosystem were measured with a LI-6400 infrared gas analyzer (LI-COR, Lincoln, NE, USA) attached to a 288 L ultra-violet light transparent Perspex chamber ($60 \times 60 \times 80 \text{ cm}^3$) using the method described in Larsen and others (2007). Taking into account the large dimensions of the chamber and the short measuring time of 3 min, an acceptable climate inside of the chamber was assured during each measurement. One sampling location ($60 \times 60 \text{ cm}^2$) was permanently installed in the uncut area of each plot. The NEE and ER measurements occurred simultaneously at each location, with the chamber vented between measurements. The ER rates were obtained by covering the chamber with a fitted blackout-cloth to minimize any heating effect within the darkened chamber. The data were analyzed using the HMR procedure, developed for soil-atmosphere trace-gas flux estimation with static chambers (Pedersen and others 2010), implemented in an R-package (Pedersen 2011).

Data Analysis

The data were analyzed in accordance with the workflow approach described in Kopittke and others (2013a). The significance of the treatment effect on the plant biomass was investigated by a linear model ANOVA. If a treatment effect was identified, then a pairwise t test (using the Bonferroni correction factor) was undertaken whereby an effect is considered as significant if its associated

P value is ≤ 0.05 . The effect of treatment on soil respiration and on photosynthetic activity was investigated using a linear mixed effects model (Pinheiro and Bates 2000). Where the response variable was the CO_2 efflux measurement (a repeated measurement per location), the treatments formed the fixed effects and the measurement locations formed the random effects. Where mean results are referenced, the standard errors of the mean are provided in both text and graphics. For all statistical analyses, the R statistical computing program was used (R Development Core Team 2008).

Next, the observational data were used within a model selection procedure to identify a suitable model for predicting soil respiration with the best predictive performance, significant parameters and Gaussian residuals. In the selection procedure, different plausible linear and non-linear model forms and different explanatory variables (for example, air temperature, soil temperature, soil moisture, plant photosynthetic activity) were calibrated and evaluated by validation on a separate data set. The model selection procedure is described in detail in Kopittke and others (2013a) and led to the selection of generalized linear multi-level models (GLMMs) as the most suitable model form for predictive purposes of soil respiration at this heathland site. Therefore, only these GLMMs are used here (see Table 3). The models use soil temperature (T), scaled soil moisture (M), scaled biomass (B), and scaled photosynthetic activity (P) as predictor variables, and were calculated using the method provided in Kopittke and others (2013a).

Using the selected models, R_S and R_H were calculated for the length of the study period using an hourly dataset from the control and drought treatments and R_A values derived. These R_S , R_H and R_A values were then used to calculate annual soil C loss estimates for each treatment.

Table 3. The Models Used to Estimate R_S and R_H ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)

Model type	Variables	Equations for R_S model	Equations for R_H model
GLMM	T	$R_0 e^{kT}$	$R_0 e^{kT}$
	TM	$R_0 e^{kT} e^{aM}$	$R_0 e^{kT} e^{aM}$
	TB	$R_0 e^{kT} e^{cB}$	–
	TP	$R_0 e^{kT} e^{cP}$	–
	TMB	$R_0 e^{kT} e^{aM} e^{cB}$	–
	TMP	$R_0 e^{kT} e^{aM} e^{cP}$	–

The explanatory variables are soil temperature (T), relative soil moisture (M), relative *Calluna* biomass (B) and relative photosynthesis (P) as defined in Equations 2–4 in Kopittke and others (2013a). Model parameter R_0 is in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, k is in $^{\circ}\text{C}^{-1}$ and parameters a and c are dimensionless.

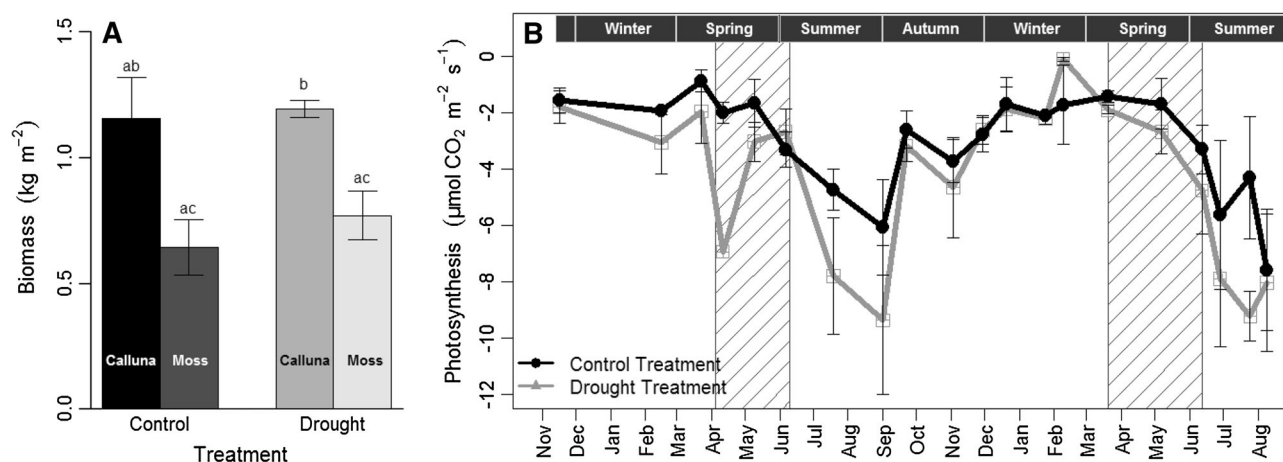


Figure 1. Measures of plant activity for the control and drought treatments, showing **A** *Calluna* biomass (kg m^{-2}) for *Calluna* and moss in each treatment ($n = 9$); and **B** C uptake by photosynthesis ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) obtained between November 2010 and August 2012 ($n = 9$). Means \pm SEM are shown and periods of rainfall exclusion (drought) are dashed.

RESULTS

Vegetation Characteristics

Mean *Calluna* biomass was not significantly different between the drought treatment ($1.19 \pm 0.04 \text{ kg m}^{-2}$) and the control treatment ($1.16 \pm 0.16 \text{ kg m}^{-2}$) due to high variability within the control plots. Similarly, mean moss biomass on the drought treatment ($0.77 \pm 0.10 \text{ kg m}^{-2}$) was not significantly different from the control ($0.64 \pm 0.11 \text{ kg m}^{-2}$). Within the drought treatment, there was significantly greater *Calluna* than moss biomass ($P = 0.015$), whereas on the control treatment, the difference between *Calluna* and moss was just above the 0.05 significance level after the Bonferroni correction ($P = 0.058$; Figure 1A).

Mean C uptake on the *Calluna* heathland was greatest (most negative CO_2 flux) during summer months ($-5.7 \pm 0.6 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) and the least during the winter months ($-1.8 \pm 0.3 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$). In a comparison of the treatments, the C uptake on the drought treatment was significantly greater than the control treatment ($P = 0.042$) during the study period. This difference between the treatments was observable in spring, summer, and early autumn. However, the large variability within the treatments resulted in the difference being significant only in the spring period, when the C uptake on the drought treatment ($-2.7 \pm 0.5 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) was significantly greater than the control ($-1.5 \pm 0.3 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$).

Soil Respiration

Overall, the greatest mean total soil respiration (uncut areas) was observed in summer months ($1.9 \pm 0.06 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) and the least in winter months ($0.6 \pm 0.4 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$). Across all measurements, there was a significant treatment difference for total soil respiration between the drought and the control treatments ($P = 0.019$; Figure 2A). In spring, when drought conditions were applied, the total soil respiration was significantly lower on the drought treatment than on the control treatment ($P < 0.001$), although this difference was more pronounced in 2012 than in 2011. There were no other seasonal differences observed between the treatments. The significant differences observed between treatments indicated that the datasets should be separated during the subsequent modeling phase.

The same trend was observed for soil respiration from the cut areas, where the greatest respiration was observed in summer months ($1.4 \pm 0.1 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) and the least in winter months ($0.6 \pm 0.06 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) (Figure 2B). Mean respiration from the cut areas in spring was greater on the control treatment than on the drought treatment and this difference was enhanced during the 2012 drought period. However, these spring respiration rates were not significantly different ($P = 0.066$) and no significant seasonal differences were observed between treatments. This absence of significant treatment differences indicated that the

datasets should not be separated during the subsequent modeling phase. To establish the sensitivity of conclusions to this decision, we also conducted all subsequent analyses while separating the data for control and drought treatments. This led to very similar results and the same conclusions. In the following sections, only the results of the analyses on the combined data are reported.

Treatment Effects

There was no significant difference between control and drought soil temperatures at 5 cm below ground surface. However, the soil temperature of the cut areas was significantly different to the uncut areas, regardless of the treatment ($P < 0.001$). This difference was greatest in spring, where mean soil temperatures on the cut areas were 2.2°C greater than on the uncut areas (Figure 3A). Mean air temperature at 20 cm above ground surface was lowest in winter ($3.0 \pm 0.03^\circ\text{C}$) and greatest in summer ($15.7 \pm 0.11^\circ\text{C}$).

There were no significant rainfall differences between the treatments in periods outside of the drought treatment application. However, there were seasonal differences between years, with less site rainfall in spring 2011 (140 mm) than in spring 2012 (212 mm) and less site rainfall in winter 2010 (127 mm) than in winter 2011 (266 mm; Figure 3D). These seasonal differences (natural drought conditions) affected the quantity of rainfall excluded by the drought treatment, leading to less precipitation exclusion in 2011 (69.5 ± 2.9 mm) than in 2012 (143.3 ± 1.7 mm).

Soil moisture of uncut areas showed a different trend between the drought and control treatment (Figure 3B, C). The driest period on the drought ($0.15 \text{ m}^3 \text{ m}^{-3}$) was observed in spring and was lower than on the control ($0.17 \text{ m}^3 \text{ m}^{-3}$). In subsequent summer months, mean soil moisture continued to be lower on the drought treatment ($0.18 \text{ m}^3 \text{ m}^{-3}$) than on the control treatment ($0.20 \text{ m}^3 \text{ m}^{-3}$), but was within the same range by the autumn months. Soil moisture on the cut and

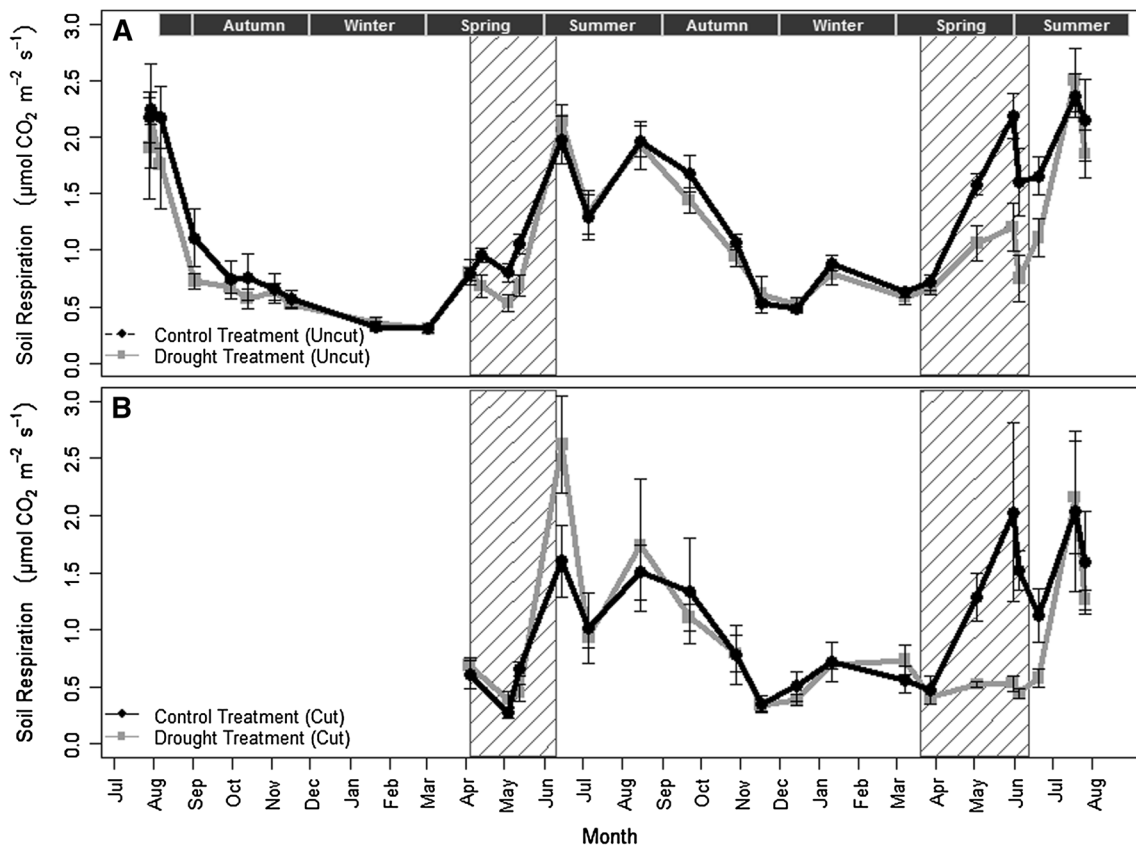


Figure 2. Soil respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) on the control and drought treatments for the **A** total soil respiration as represented by the uncut areas of the plots for July 2010–August 2012 ($n = 18$ per sampling event); and **B** heterotrophic respiration, as represented by the cut areas of the plots for April 2011–August 2012 ($n = 6$ per sampling event). Means \pm SEM are shown and the periods of rainfall exclusion (drought) are dashed.

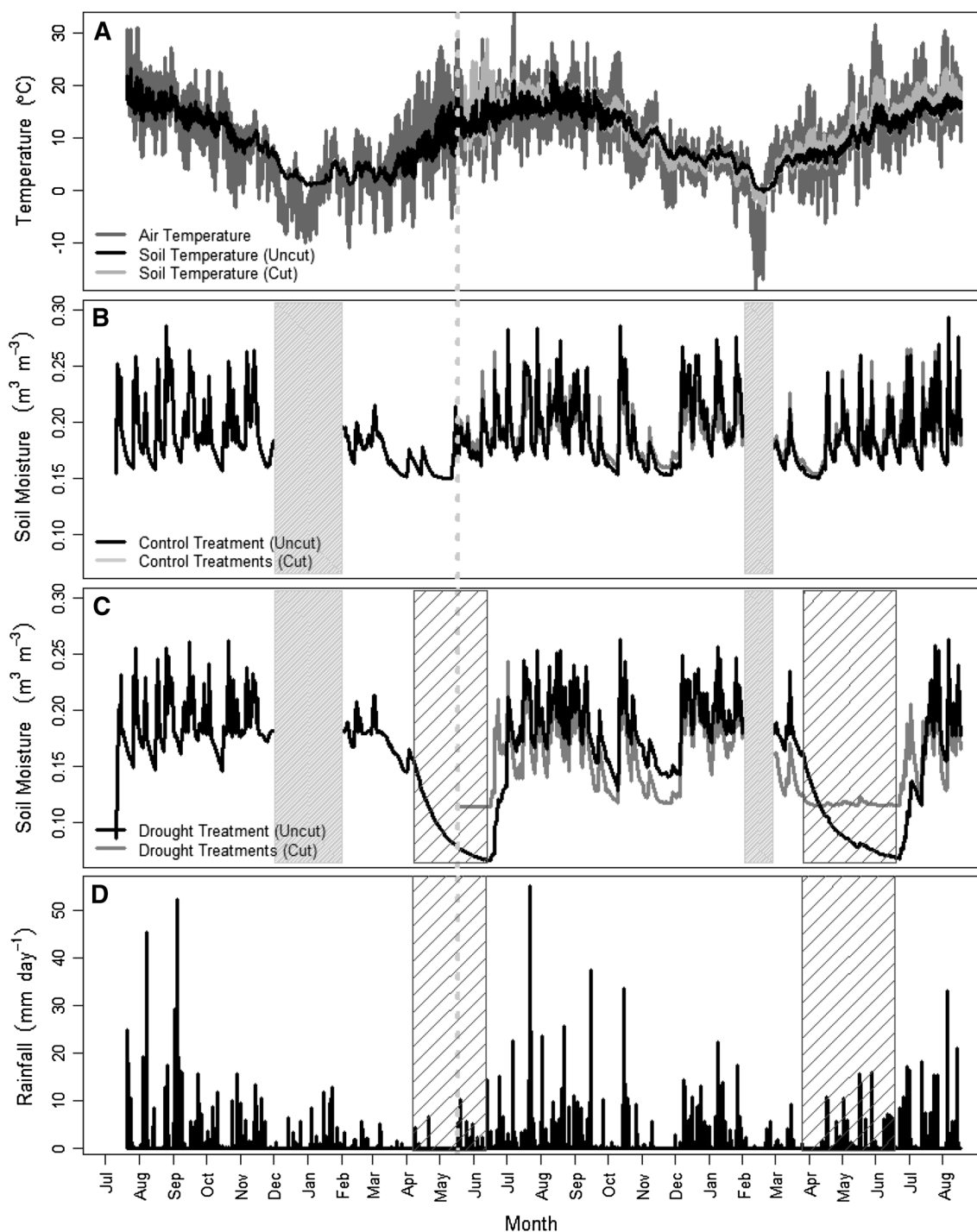


Figure 3. Environmental parameters of the uncut areas (July 2010–August 2012) and cut areas (May 2011–August 2012) showing **A** hourly temperatures ($^{\circ}\text{C}$) of the air at 20 cm above ground surface and of the soil at 5 cm below ground surface, **B** mean daily soil moisture ($\text{m}^3 \text{m}^{-3}$) at 5 cm below ground surface for the control treatment, **C** mean daily soil moisture ($\text{m}^3 \text{m}^{-3}$) at 5 cm below ground surface for the drought treatment; and **D** daily rainfall for the control treatment. The periods of rainfall exclusion (drought) are *dashed* (April–June 2011 and March–June 2012) and periods of snow or frozen soil moisture are *shaded* (December 2010–February 2011 and February 2012). The *dashed line* represents the start date of measurements on the cut areas (May 2011).

Table 4. Optimal Parameter Values of Total Soil Respiration (R_S) Models for the Control Treatments (T Model) and Drought Treatments ($T + M$ Model) and Heterotrophic Respiration (R_H) Models for the Control and Drought Treatments (T Model)

Model	Control	Drought
R_S	$R_0 = 0.29$ (0.24 – 0.35) $k = 0.12$ (0.11 – 0.13)	$R_0 = 0.23$ (0.18 – 0.31) $k = 0.11$ (0.10 – 0.12) $a = 0.74$ (0.004 – 1.484)
R_H	$R_0 = 0.32$ (0.22 – 0.48) $k = 0.08$ (0.06 – 0.10)	

The 95% confidence intervals for the parameters are shown between brackets. See text and Table 3 for model formats and parameter explanations.

uncut areas of the control plots followed similar drying patterns. However, the drought treatment cut areas did not become as dry during water exclusion periods as the uncut areas (Figure 3).

Calibration and Validation of the Total Soil Respiration (R_S) Model

All R_S model predictions generally followed the seasonal soil temperature patterns, where the lowest respiration was recorded in winter (in February). The absolute root mean square errors for the calibration data (RMSE_C) ranged between 0.40 and 0.69 and the Nash–Sutcliffe Efficiency (NSE) values ranged between 0.21 and 0.6. When T was the only variable used, the model parameters were significant for both the control and drought datasets ($P \leq 0.05$) and the NSE values were at the higher end of the observed range (0.56 and 0.45, respectively). When $T + M$ were used, the parameters were significant for only the drought model (NSE: 0.45). The other variable combinations were not significant for either the control or drought models. To insure the best models were selected, the confidence intervals were compared between models generated with the separated control and drought datasets (discussed above) and those generated from a combined control and drought dataset. This comparison showed that the confidence limits were smaller when the data sets were modeled separately and thus, this subsetting of models was selected.

The calibrated control models were used on the validation data (April 2011–August 2012). The resulting RMSE validation (RMSE_V) values were then compared to the RMSE_C values. In general, the average ratio of RMSE_V:RMSE_C in the R_S models was 1.25 and the RMSE_V ranged from 0.59 to 0.84. The R_S model which had both the lowest RMSE_C and the lowest RMSE_V value used the T as a single variable (RMSE_C = 0.52, RMSE_V = 0.60). The R_S model which performed the worst in the validation

phase included the T variable in combination with $M + P$ (RMSE_C = 0.61, RMSE_V = 0.84), indicating these models were over-parameterized.

Following the rationale described in the methodology to select the best predictive models, the model selected for R_S used only T to predict R_S for the control treatment and used $T + M$ to predict R_S on the drought treatment. The parameterized models were then used to predict soil respiration over the length of the study period (Table 4; Figure 4).

Calibration of the Heterotrophic Soil Respiration (R_H) Model

The RMSE_C values for the R_H models ranged between 0.58 and 0.61 and the NSE values between 0.29 and 0.34. The T variable was significant when applied alone on the combined control and drought dataset. When applied as $T + M$, the M parameter was just above the significance levels ($P = 0.08$). Therefore, the best R_H predictive model used only the T variable.

To insure the best models were selected, the confidence intervals were compared between models generated with the combined control and drought data sets (discussed above) and those generated separately from either the control data set or drought data set. The confidence limits were smaller when the combined dataset was used and therefore, this combined data set model was selected. The parameterized models were then used to predict soil respiration over the length of the study period (Table 4; Figure 4).

Autotrophic Soil Respiration

Autotrophic soil respiration was determined by subtracting the model predicted R_H from R_S in each treatment ($R_S - R_H = R_A$; Figure 5). The R_A component was ~ 0 on both treatments in winter. The greatest R_A was predicted to occur in the summer

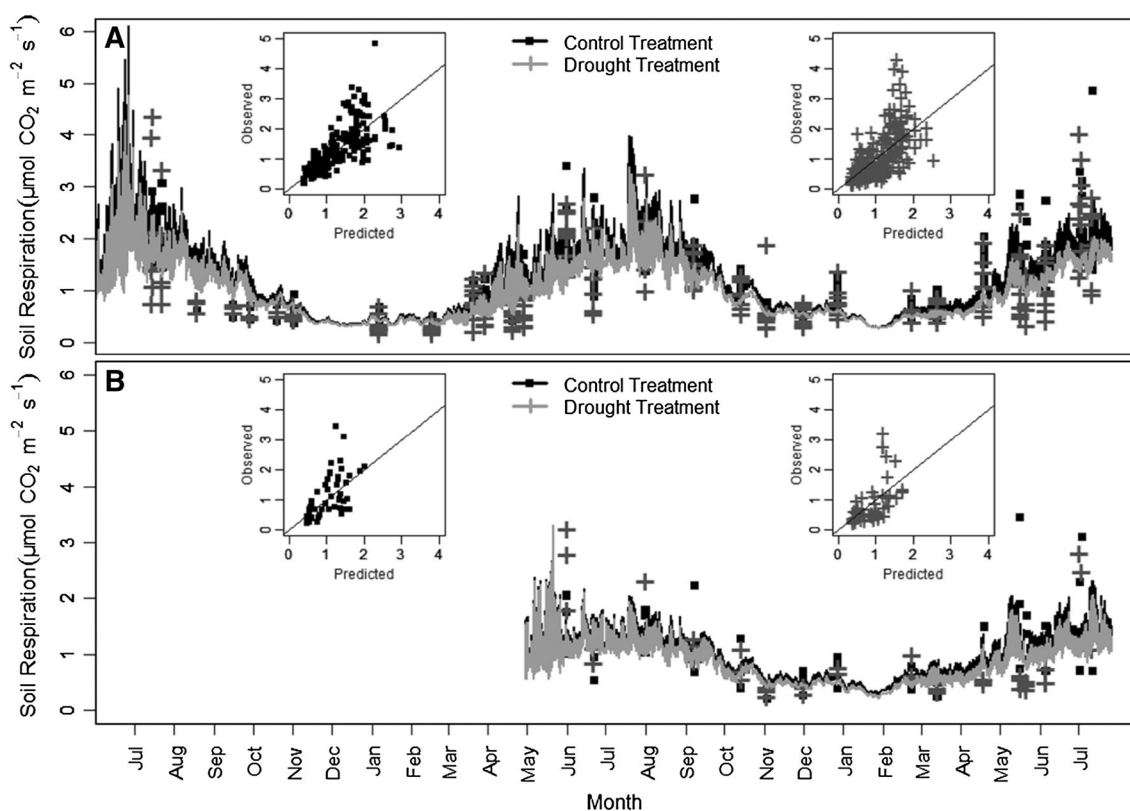


Figure 4. Predicted soil respiration for **A** total soil respiration (R_S) and **B** heterotrophic soil respiration (R_H) using the selected GLMM models. The control treatment observations are shown as squares and predictions as a *black line*. The drought treatment observations are shown as crosses and predictions as a *grey line*. The observed versus predicted respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) plots for the control treatment are *inset* on the *left* and the drought treatment are *inset* on the *right*.

months, with the 2011 maximum occurring in August when approximately 40% of soil respiration in this month was attributable to autotrophic sources on the control treatment. The peak R_A for the drought also occurred in this same time period, when approximately 32% of soil respiration in this month was attributable to autotrophic sources.

Annual C Loss Estimates

Based on model predictions, annual soil C losses were lower on the drought treatment than on the control treatment in both annual periods (Table 5). Drought treatment R_H was suppressed by the same proportion in both years; that is, the drought R_H was reduced by 5% in both 2010–2011 and 2011–2012. In contrast, R_A suppression varied between the 2 years, where drought R_A was reduced by 42% in 2010–2011 and by 45% in 2011–2012.

DISCUSSION

Soil Respiration

Drought suppressed observed total soil respiration across the entire year and this suppression was most pronounced during water exclusion periods. Outside these water exclusion periods, total soil respiration on the drought treatment continued to be less than the control, although microbial respiration (heterotrophic respiration) was not reduced. Therefore, the reduced total respiration in non-drought periods must be associated with the other source of soil C loss, that is, root respiration ($R_S - R_H = R_A$). This was also reflected in the annual C loss sums, where the drought R_A was suppressed by 42% in 2010–2011 and by 45% in 2011–2012. Drought R_H was only marginally reduced (5% suppression) in both years, suggesting that root respiration (R_A) was more affected by drought conditions than microbial activity (R_H). A similar

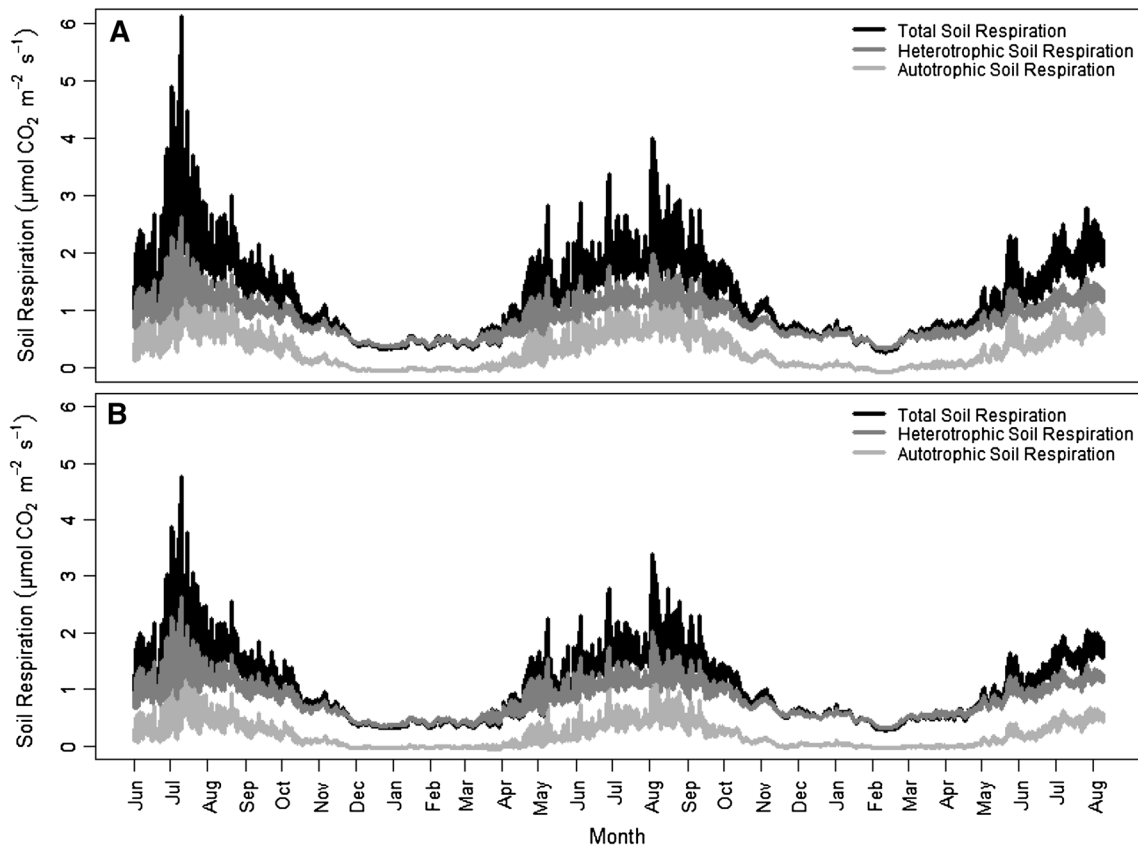


Figure 5. The predicted R_S (total soil respiration), R_A (autotrophic soil respiration) and R_H (heterotrophic soil respiration) in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for the **A** control treatment, and **B** and drought treatment over the period of June 2010–August 2012.

Table 5. Annual Predicted Total C Loss from Soil ($\text{g C m}^{-2} \text{ year}^{-1}$) for the Control and Drought Treatments After 13 Annually Repeated Droughts (August 2010–July 2011) and After 14 Annually Repeated Droughts (August 2011–July 2012)

Soil respiration ($\text{g C m}^{-2} \text{ year}^{-1}$)	2010–2011		2011–2012	
	Control	Drought	Control	Drought
R_S	392	337	427	358
R_A	96	55	112	61
R_H	296	282	315	297

response was observed on a ^{14}C allocation study undertaken in a French grassland, in which root activity was more suppressed than microbial activity when exposed to drought conditions (Sanaullah and others 2012).

At the end of the drought period, a flush of CO_2 was observed on the drought plots for both total and heterotrophic soil respiration, in particular in 2011 on the cut areas. This CO_2 flush was similar to the pulses observed on other coarse-textured soils, where rewetting after extended dry periods provided favorable conditions for biological activity

(Cable and others 2008). The relatively larger response to rewetting by heterotrophic respiration contributed to the absence of significant treatment differences on cut areas in spring, even though there was a suppression trend during the actual drought periods.

The drought-period effect (suppression and flush) was not captured in the modeled data, even when soil moisture was added as an explanatory variable; only the general suppression across all measurements was predicted. In fact, soil moisture was not a significant parameter in any of the

models assessed, with the exception of the R_S model for the drought treatment. This was unexpected as it had been hypothesized that because soil moisture affects plant and microbial activity, it would be a significant parameter in soil respiration models.

The significance of the moisture parameter is likely to be associated with the observed soil moisture ranges. During drought periods, soil of the drought uncut areas was significantly drier than on the control uncut areas or than on the cut areas of either treatment. Even under the naturally dry conditions in spring 2011 when no rain fell on either treatment, soil moisture still did not reach less than $0.1 \text{ m}^3 \text{ m}^{-3}$ for the control, although the drought treatment was below this value. This indicated that more water was removed from the drought soil than the control soil, when both treatments were exposed to the same dry conditions. It appears that plant roots were involved in this process because this moisture difference was not observed where plants were absent and soil water was free-draining (that is, on cut areas).

Three processes are hypothesized to have contributed to this difference observed between treatments when exposed to natural drought. Firstly, plant roots have been shown to respond to drought by increasing root to shoot ratio, root length, or root area to enable increased water and nutrient uptake (Chaves and others 2003). The majority of *Calluna* roots, under normal conditions, grow within the nutrient-rich, organic layer of the soil (Kopittke and others 2013a). The soil moisture results for the natural drought conditions were obtained in the mineral soil and the larger decrease in moisture recorded in the drought treatment suggests that more roots of drought plants grew below the organic layer into the mineral soil to search for moisture. Secondly, *Calluna* roots in the control treatment are experiencing an increasingly acidic soil, with pH significantly decreasing from 4.50 in 1998 to 3.85 in 2011 (Kopittke and others 2012). In contrast, the drought treatment has retarded the soil acidification trend with pH decreasing from 4.55 in 1998 to only 4.12 in 2011. *Calluna* plants are adapted to acidic soils, but rapid acidification of soil can result in decreased survival (Lawson and others 2004). Therefore, the observed acidification trend in the control treatment may have caused root damage at depth and resulted in *Calluna* plants being less able to withstand natural drought conditions.

Thirdly, a moss layer was present on both treatments and bryophytes obtain moisture by intercepting atmospheric water (dew, fog or rainfall) (Glime 2007). A delay in the rewetting of the soil

following drought was noted in previous studies at the site (Sowerby and others 2008); whereas in this investigation, significantly lower soil moisture values were observed on the drought treatment outside of the drought periods. One hypothesis may be that after drought the mosses are the first to intercept and retain rainfall; and, as bryophytes with high ground coverage have considerable water retention capacity (Michel and others 2012), this would delay the rewetting of the soil. Long term bryophyte ground cover measurements within the control and drought treatments (unpublished data collected using the method described in Kopittke and others 2013b) showed that between 1999–2000 and 2010–2011, mean moss cover increased on both the control (58–73% cover) and drought treatments (48–83% cover), with a greater increase on the drought (34% increase) than the control (15% increase). Additionally, although drought moss biomass was not significantly greater than the control, there was a thicker moss layer in the drought treatment in 2012 ($6.5 \text{ cm} \pm 0.9$) than the control ($6.0 \text{ cm} \pm 0.1$), which may indicate a greater water holding capacity per gram biomass. This greater moss cover on the drought treatment is likely to have intercepted a greater volume of precipitation, particularly if rainfall events were small and moss water holding capacity was not exceeded; thus enhancing the drought effect on *Calluna* roots.

These hypothesized plant changes on the drought treatment may also explain the greater annual photosynthesis observed on the drought treatment. Photosynthesis occurs from both *Calluna* and mosses, whereas it is only the *Calluna* plants that have root systems contributing to R_A . Mosses have many adaptations to drying, including a high water holding capacity (up to 1,400% of their dry mass), ability to intercept atmospheric water, such as fog or dew, and can achieve maximum photosynthesis rate within 30 s of rewetting (Glime 2007). Preliminary studies in May 2012 indicated that mosses contribute significantly to P_G in this heathland (between 30 and 60% of total P_G for control), but these C uptake rates have not yet been determined for either the drought treatment or across different seasons. The greater moss cover, combined with a potentially greater water holding capacity, may have resulted in an elevated P_G on the drought treatment and a stimulation of the drought effect on *Calluna* roots (and reducing annual R_A). The drought adaptation of *Calluna* root systems to grow deeper would provide resilience to periods of low rainfall, but root activity is still likely to be reduced when exposed to prolonged drought periods (that is, reduced R_A).

Model Evaluation

The relatively high $RMSE_V$ values, the absence of the spring-time effect in model predictions and model under-estimation on some days in summer indicated that an important co-varying variable was lacking in the models parameterized so far. This is a common flux model problem which is not often discussed in published soil respiration studies, although it has been previously discussed in an eddy co-variance context (Medlyn and others 2005; Medlyn and others 2011). However, in the absence of additional appropriate explanatory variables, and because R_S and productivity increased with temperature, this study and others concluded that the soil temperature typically sufficed to explain most of the seasonal variation of R_S in ecosystems without extended periods of drought (Bahn and others 2010a; Janssens and others 2001; Reichstein and others 2003). Where extended drought periods were present, the inclusion of a soil moisture variable was found to enhance the R_S model fit.

The model validation process also identified that none of the measured plant variables explained the data variation when included in models with soil temperature. Aboveground plant activity has been shown to correlate with R_A and plant activity measures (Bahn and others 2010b; Metcalfe and others 2011; Ryan and Law 2005), however, this study and others have shown that inclusion of a plant variable does not necessarily ensure a better R_S model fit (Chen and others 2011; Kopittke and others 2013a).

Other variables which may have improved the fit of the models include the N dynamics within the root zone. Elevated N deposition rates can have direct and indirect effects on C loss rates, such as decreasing decomposition rates of recalcitrant organic matter and increasing root respiration rates (Burton and others 2012; Fog 1988). The Oldebroek site experiences high bulk N deposition rates (10.7–37.4 kg N ha⁻¹ year⁻¹; 1999–2012) and the drought treatment was shown to reduce wet N deposition rates by 8–15% when rainfall was excluded (Kopittke and others 2012). It is possible that these different N deposition regimes may affect R_A and R_H in some ecosystems; however, the control and drought treatments were both N saturated (Kopittke and others 2012) and therefore, N deposition at this site is more a characteristic of the site than a possible controlling factor of soil respiration.

A potential source of model error was the high variability between plots on the same treatment and the use of only daytime observational data to generate hourly model predictions. The high vari-

ability of soil respiration with small scale measurements is a commonly identified issue (Maestre and Cortina 2003). One way to overcome this small scale issue is to use ecosystem flux measurements; however, these eddy co-variance measurements are also recognized to have limitations relating to the non-separation of respiration components and modeling pitfalls (Gomez-Casanovas and others 2012; Medlyn and others 2005).

The modeling process identified that there was no underlying, long-term change in the drought treatment for microbial processes, as R_H was best modeled with a combined control and drought dataset. This model outcome was supported by the observational data in which heterotrophic respiration (on cut areas) was not statistically different between treatments. In contrast, there was an underlying change in processes on the drought treatment (determined using inductive reasoning from the R_S modeling, that is, $R_S - R_H = R_A$), as R_S was best modeled separately on the control dataset and the drought data set. This outcome correlated to the significant treatment differences observed for total soil respiration, photosynthesis and soil moisture on the uncut areas, as discussed in the previous section. It is hypothesized that this difference is associated with structural changes in moss cover and *Calluna* root systems.

This long-term structural change in the plant community complicates the modeling of future ecosystem processes. Models that are developed based on current observational datasets may not adequately predict the response of ecosystems to environmental changes in another decade, and so may require different models or model variables to be applied over time. A similar problem was encountered by Keenan and others (2012), where the observed rate of C uptake over 19 years at Harvard forest could not be explained by meteorological drivers alone and could only be reproduced in the model by making temporal changes in model parameters.

Annual C Loss and Links to Global Change

Our study identified that after 13–14 annually repeated droughts, 74–76% of the annual soil C loss originated from microbial decomposition of organic matter, that is, from R_H . Overall, the total annual soil C loss from R_S (drought: 332–358 g C m⁻² year⁻¹; control: 392–427 g C m⁻² year⁻¹) was much lower than the C loss identified at other European heathlands, such as at the Danish Brandbjerg heathland (672–719 g C m⁻² year⁻¹), the Danish Mols

heathland ($520 \text{ g C m}^{-2} \text{ year}^{-1}$) or the UK Cloacae-nog heathland ($580 \text{ g C m}^{-2} \text{ year}^{-1}$) (Beier and others 2009; Selsted and others 2012). These differences are likely to be associated with the ages and composition of the different heathlands; for example, our site was established on a 28-year-old *Calluna* community whereas the *Calluna* at the Brandbjerg site was approximately 5–6 years old for the first year of soil respiration measurements (Mikkelsen and others 2008; Selsted and others 2012). This decreasing C loss associated with an aging *Calluna* heathland was also observed by Kopittke and others (2013a), who noted that annual R_S C loss was greater on the more vigorous 12-year-old *Calluna* community ($650 \text{ g C m}^{-2} \text{ year}^{-1}$) than on the older 19-year-old ($461 \text{ g C m}^{-2} \text{ year}^{-1}$) or the 28-year-old community ($435 \text{ g C m}^{-2} \text{ year}^{-1}$). Our investigation found that when this 28-year-old community had been subjected to 14 prior years of annual drought treatments, the soil respiration was further reduced to $358 \text{ g C m}^{-2} \text{ year}^{-1}$ in 2011–2012.

CONCLUSION

Increased drought frequency-reduced total soil respiration on this *Calluna* heathland. Our study quantified the drought effect in terms of the C loss originating from roots (autotrophic respiration) and C loss from microbes (heterotrophic respiration) and identified that the greatest reduction occurred in root respiration. Evidence was also collected to support the hypothesis that a heathland community exposed to repeated drought conditions adjusts its soil respiration response to various environmental factors, including reduced soil moisture. This included evidence that more roots of drought plants grew below the organic layer into the mineral soil to search for moisture. Correspondingly, there was a long-term increase in the proportion of moss cover on the drought treatment compared to the control, which may have intercepted more water and enhanced the drought effect on *Calluna* roots. Field observations also indicated that plant activity (P_G) was significantly greater on the drought treatment than the control treatment, suggesting long-term changes in plant structure and composition have resulted from long-term, annually repeated droughts.

ACKNOWLEDGMENTS

This study was carried out and funded by European Commission under the FP7-Research Infrastructures Programme (Grant Agreement No. 227628) as a part of the Integrated Network on Climate

Research Activities on Shrubland Ecosystems (INCREASE) project. The authors would like to thank Sharon Mason and Louise Andresen for their contributions to this paper, the University of Amsterdam (Universiteit van Amsterdam) for making this research possible and the Royal Netherlands Army (Koninklijke Landmacht) for access to the field site.

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