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Article

# Grassland Management Influences the Response of Soil Respiration to Drought

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**Abstract:** Increasing soil carbon stocks in agricultural grasslands has a strong potential to mitigate climate change. However, large uncertainties around the drivers of soil respiration hinder our ability to identify management practices that enhance soil carbon sequestration. In a context where more intense and prolonged droughts are predicted in many regions, it is critical to understand how different management practices will temper drought-induced carbon losses through soil respiration. In this study, we compared the impact of changing soil volumetric water content during a drought on soil respiration in permanent grasslands managed either as grazed by dairy cows or as a mowing regime. Across treatments, root biomass explained 43% of the variability in soil respiration ( $p < 0.0001$ ). Moreover, analysis of the isotopic composition of CO<sub>2</sub> emitted from the soil, roots, and root-free soil suggested that the autotrophic component largely dominated soil respiration. Soil respiration was positively correlated with soil water content ( $p = 0.03$ ) only for the grazed treatment. Our results suggest that the effect of soil water content on soil respiration was attributable mainly to an effect on root and rhizosphere activity in the grazed treatment. We conclude that farm management practices can alter the relationship between soil respiration and soil water content.

**Keywords:** soil respiration; soil volumetric water content; stable carbon isotopes; grassland; management practices

## 1. Introduction

Grasslands cover 26% of Earth’s ice-free land surface area, representing 70% of the world’s agricultural area [1] and containing 20% of global soil carbon stocks [2]. There is now a wide interest in improving carbon storage on land as a negative emission technology to stay below the 1.5 °C global warming limit [3]. Increasing soil carbon stocks in grasslands has a strong potential to contribute to this effort [4,5]. However, the impacts of different grassland management practices are not well understood [6], and recent reviews highlight a lack of data to clarify the mechanisms by which various management practices affect soil organic carbon (SOC) stocks [7,8]. Moreover, there are large uncertainties concerning the drivers of soil respiration ( $R_s$ ), which is the second largest terrestrial carbon flux globally [9].

Global climate change is predicted to lead to an increase in the intensity and frequency of extreme events in large areas of the world. Droughts may cause intense water stress for plants and soil organisms [10]. In other areas, increasing rainfall together with evapotranspiration and changes in precipitation patterns could potentially lead to constrained water availability during the growing season [11]. In a context where the global water cycle is predicted to change, understanding the effect of soil water content on soil respiration is critical.

Soil respiration is the result of two processes: root and rhizosphere respiration (autotrophic component), and microbial decomposition of soil organic matter (heterotrophic component). Both plants [12] and micro-organisms [13] are sensitive to changes in soil water availability, resulting in soil respiration being sensitive to both soil water content [14,15] and the frequency and intensity of precipitation [16]. However, our understanding of the effect of drought on soil respiration remains relatively limited, notably due to differential responses of the autotrophic and heterotrophic components and the difficulties associated with quantifying them [17,18].

Usually, studies addressing the effects of soil water content on heterotrophic soil respiration are carried out in the laboratory in the absence of plants. Partitioning soil respiration into its autotrophic and heterotrophic components can be achieved with minimal soil disturbance in the field using the stable isotopes of carbon [17–21], although this can prove challenging in dry conditions where low CO<sub>2</sub> efflux may be below the detection limits of instrumentation [17]. Nonetheless, even when the rate of autotrophic and heterotrophic respiration cannot be determined, the <sup>13</sup>C isotopic signature ( $\delta^{13}\text{C}$ ) of CO<sub>2</sub> emitted from the soil ( $\delta^{13}\text{C}_{\text{R}_s}$ ) can provide qualitative information on the dynamic soil respiration components. This is because the  $\delta^{13}\text{C}$  of emitted CO<sub>2</sub> becomes more enriched as the contribution of microbially derived Soil Organic Matter (SOM) becomes an increasing part of total soil respiration [22,23].

Understanding the effect of management practices on the drought response of soil respiration and the plant and microbial processes driving it is critical to develop agricultural systems that are resistant to extreme climatic events. Here we compared the impact of changing soil water content during a severe drought on soil respiration and the  $\delta^{13}\text{C}$  of CO<sub>2</sub> emitted from soils ( $\delta^{13}\text{C}_{\text{R}_s}$ ), roots, and rhizosphere ( $\delta^{13}\text{C}_{\text{R}_a}$ ), and root-free soils ( $\delta^{13}\text{C}_{\text{R}_h}$ ) in a permanent pasture grazed by dairy cows and a permanent pasture under a mowing regime with no grazing mammals. These two management systems are among the main types of grassland management in Europe [24] and have a strong potential to store carbon below ground and partly offset greenhouse gases emissions [25]. The main objective was to determine whether these two different grassland management practices influence the response of soil respiration and its components to drought.

## 2. Materials and Methods

### 2.1. Site Description

The site is located at the national long-term experimental observatory *Système d’Observation et d’Expérimentation pour la Recherche en Environnement-Agroecosystems, Biogeochemical Cycles and Biodiversity (SOERE-ACBB)* near Lusignan, western France (46°25′12.91″ N; 0°07′29.35″ E). The soil is classified as a Dystric Cambisol [26]. It developed from loamy parent material of unknown origin over red clay [27]. The soil profile can be divided into two main domains: Upper soil horizons are characterized by a loamy texture, classified as Cambisol, whereas lower soil horizons are clayey rubefied horizons, rich in kaolinite and iron oxides, classified as a Paleo-Ferralsol [28].

The two experimental paddocks of about 3 ha in surface area were converted to permanent grasslands in 2005. Since then, one paddock was grazed by a herd of dairy cows, and the other paddock was managed by periodically mowing and harvesting the biomass. The timing for harvest and mowing are made so as to maximize above-ground production and rarely happen at the same time. Following this guideline, harvest happened 2 days before our experiment started, while grazing had happened 2 weeks before the start of the experiment.

The plant community in the mown grassland was a mixture including *Dactylis glomerata* L. (cocksfoot) cultivar Ludac, *Festuca arundinacea* Schreb (tall fescue) cultivar Soni, and *Lolium perenne* L. (rye-grass) cultivar Milca. In the grazed grassland, *Trifolium repens* L. (white clover) cultivar Menna was added to the multispecies mixture. The mown grassland was cut four times a year with biomass exported, and nitrogen (N) fertilizer was applied at rates comprised between 120 and 310 kg N ha<sup>-1</sup> year<sup>-1</sup>. Fertilizer application rates were adjusted to maintain the nitrogen nutrition index between 0.9 and 1.0, that is, close to non-limiting nitrogen nutrition to near maximum plant production [28]. Grazing in the grazed paddock took place from March to December with 50 days per year using 15 to 20 livestock unit per hectare. Grazed grasslands did not receive nitrogen fertilization but nitrogen losses were returned by dung and urine and through the presence of leguminous species.

In 2011, six years after management conversion to permanent grasslands, bulk density was identical in the mown and grazed treatments, averaging 1.4 g cm<sup>-3</sup> in the top 300 mm. Carbon stocks in 2014 ranged between 55 (mown grassland) and 64 (grazed grassland) t ha<sup>-1</sup> in the first 300 mm. Nitrogen stocks were also similar in both grasslands, with 1.7 and 1.9 t ha<sup>-1</sup> for the mown and grazed grasslands, respectively. No significant changes had occurred during the first 9 years (2014) after the conversion to permanent grasslands (A. Crème, personal communication).

## 2.2. Experimental Design

The experiment took place in early June 2017, at the beginning of a heat wave that affected large parts of Europe, including western France. At the beginning of a period of 10 days with no precipitation, we created a large range of soil water contents by applying a large artificial rainfall event. The responses of soil respiration ( $R_s$ ) of the <sup>13</sup>C isotopic signature of CO<sub>2</sub> respired from the whole soil ( $\delta^{13}CR_s$ ), and its autotrophic (live roots and rhizosphere,  $\delta^{13}CR_a$ ) and heterotrophic (root and rhizosphere-free soil,  $\delta^{13}CR_h$ ) components to changes in soil water content were compared for the grazing and mown systems.

One month before the start of the experiment, 10 rectangular plots of 1 × 2 m were positioned at random locations in each paddock, and four PVC collars (100 mm diameter, 30 mm depth) were fully inserted at random locations within each plot. On each day of measurement, a plot was randomly selected in each paddock (mown and grazed) and a set of measurements of  $R_s$ ,  $\delta^{13}CR_s$ ,  $\delta^{13}CR_a$ ,  $\delta^{13}CR_h$ , soil volumetric water content ( $\theta_s$ ), and soil temperature ( $T_s$ ) were taken from each collar.

A full set of measurements were taken on the four collars in one plot of each paddock before water was applied (day 0). Two hundred liters of water per plot was progressively applied over the course of 12 h to prevent run-off and pooling to the nine remaining plots in each paddock, equivalent to 100 mm of rainfall. Measurements were then made from the four collars in one randomly selected plot of each paddock every morning for the next 8 days, starting about 24 h after the end of watering to avoid the potential short-term burst of CO<sub>2</sub> emissions (the so-called “Birch effect”) observed when dry soils are rewetted.

One plot in the grazed paddock was selected to characterize short-term changes in soil respiration due to the application of water. An additional set of four PVC collars were inserted in a 1 × 2 m area adjacent to the selected plot but received no water addition, constituting a control treatment. Measurements of  $R_s$ ,  $\theta_s$ , and  $T_s$  were taken 2 h and 1 h before water application on each of the eight collars. Water was then applied on one of the plots over the course of 1 h by spreading 100 mm evenly on the surface area using a hand watering can. Measurements of  $R_s$ ,  $\theta_s$ , and  $T_s$  were then carried out on the four watered and four dry collars every hour for 12 h.

## 2.3. Measurements of Soil Respiration and $\delta^{13}C$ of Respired CO<sub>2</sub>

Except for the short-term watering experiment, where  $R_s$  was measured using a closed dynamic chamber system (EGM-4, PP systems, Amesbury, MA, USA), measurements of  $R_s$  were taken using two custom-built open chamber systems with four chambers each, adapted from Midwood et al. [29] and Midwood and Millard [30]. The chambers were placed on the collars set in the soil, and CO<sub>2</sub>-free

air was supplied to the chambers using mass flow controllers (model FMA5510, Omega Engineering Ltd., Stamford, CT, USA). The air was pumped out of the chambers using diaphragm pumps (TD-3, Brailsford and Co. Inc., Antrim, NH, USA), and the flow rate was controlled by mass flow controllers and adjusted to  $15 \text{ mL min}^{-1}$  lower than the inflow of  $\text{CO}_2$ -free air to avoid any air ingress from the atmosphere. The  $\text{CO}_2$  concentration of the air leaving the chamber was measured using an infrared gas analyzer (IRGA) (Li840, LiCor Biosciences, Cambridge, UK), and the entire system was controlled by a datalogger (CR1000 and SDM-CV04, Campbell Scientific Ltd., Logan, UT, USA), allowing adjustment of the inflow of  $\text{CO}_2$ -free air to the measured  $\text{CO}_2$  concentration to obtain a constant target concentration value in the chamber. After an equilibration period of 90 min and when the chamber's  $\text{CO}_2$  concentrations were constant at 440 ppm, approximately 500 mL of respired air was collected in pre-evacuated air-tight bags (Tedlar<sup>®</sup> Keika Ventures, Chapel Hill, NC, USA) and the gas samples were analyzed for  $\delta^{13}\text{C}$  values. All gas samples were analyzed for  $\delta^{13}\text{C}$  values using a tuneable diode laser (TDL, TGA100A; Campbell Scientific Inc., Logan, UT, USA). The rate of  $R_s$  was calculated from the measured  $\text{CO}_2$  concentration in the chamber and the flow rate of the  $\text{CO}_2$ -free air delivered to the chamber.

Measurements of the isotopic signatures of the  $\text{CO}_2$  respired by the heterotrophic (root- and rhizosphere-free soil,  $\delta^{13}\text{C}R_h$ ) and autotrophic (roots and rhizosphere,  $\delta^{13}\text{C}R_a$ ) components of the soil were made by adapting the technique described by Snell et al. [23]. After  $R_s$  had been measured and the soil surface efflux sampled, roots and soils were collected. The collars were removed, and a soil core was extracted using a 100 mm diameter steel tube hammered into the soil to a depth of 250 mm. The soil from the core was broken up loosely and the roots removed by hand.

Shifts in  $\delta^{13}\text{C}R_h$  have been shown to change exponentially with time after a soil core is extracted and broken up [19,23]. Thus, to obtain values of  $\delta^{13}\text{C}R_h$  that are representative of the isotopic signature of the carbon pool used as a substrate before disturbance (time zero), one needs to work as rapidly as possible. To be as consistent as possible between samples, each operation was kept to a constant time. The process of breaking up the soil core in a tray and removing the roots by hand was kept close to 90 s. Subsequently, a subsample of root-free soil was placed in an air-tight bag, flushed quickly three times with nitrogen gas to purge atmospheric air from the soil, then filled with approximately 500 mL of  $\text{CO}_2$ -free air and allowed to incubate at the ambient temperature until the time from sampling the soil core reached 3 min. The air in the bag was then sampled and analyzed for  $\delta^{13}\text{CO}_2$ .

The roots were then cleaned of most of the remaining soil attached to them and placed in a separate air-tight bag. The bags were evacuated and then filled with approximately 500 mL of  $\text{CO}_2$ -free air and allowed to incubate at ambient temperature for between 20 and 60 min, after which the air in the bags was sampled for measurements of  $\delta^{13}\text{CO}_2$ . The roots were then dried at  $105^\circ\text{C}$  for 24 h and weighted to give an estimate of the root biomass in each soil core.

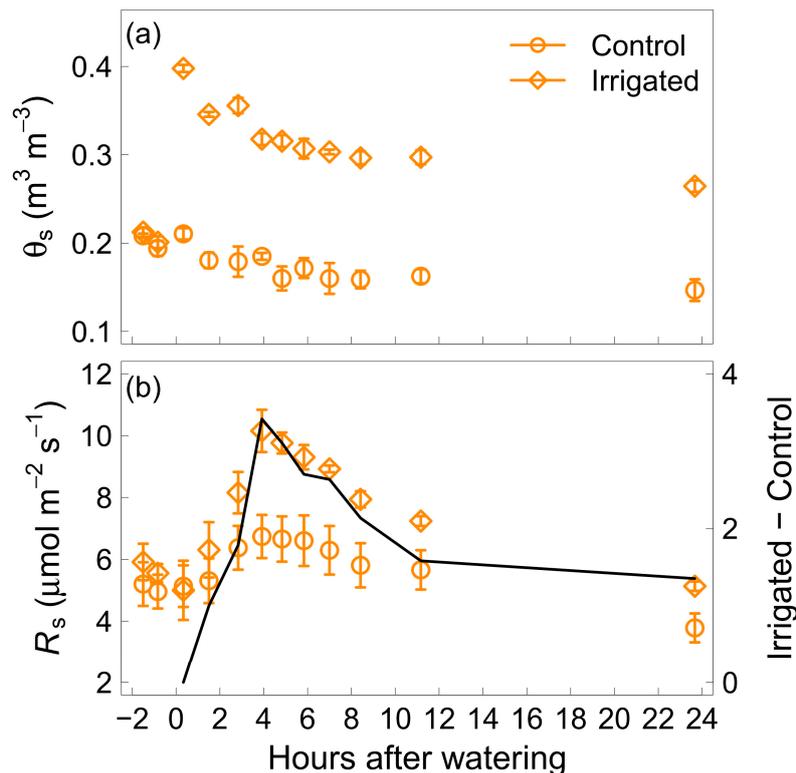
#### 2.4. Statistical Analyses

To test for the effect of soil volumetric water content ( $\theta_s$ ) on  $R_s$ ,  $\delta^{13}\text{C}R_s$ ,  $\delta^{13}\text{C}R_a$ , and  $\delta^{13}\text{C}R_h$  for the paddocks managed as grazed and mown systems, a backwards stepwise regression approach was used separately on each explained variable [31]. Each measurement of  $R_s$ ,  $\delta^{13}\text{C}R_s$ ,  $\delta^{13}\text{C}R_a$ , and  $\delta^{13}\text{C}R_h$  was treated as a sample. The full linear models included the three explanatory variables  $\theta_s$ , root biomass, and paddock management (as a factor), as well as every two-way interaction between them. Model selection was based on a comparison of Akaike's Information Criterion (AIC), the model with the lowest AIC value being the most strongly supported. As a rule of thumb, when two models presented a  $\Delta\text{AIC} < 2$ , the simpler model was selected [32]. All statistical analyses were conducted using R version 3.4.2 [33] (R Development Core Team, 2017).

### 3. Results

#### 3.1. Hourly Measurements: Short-Term Response of Soil Respiration to Water Addition

In the grazed paddock, soil volumetric water content ( $\theta_s$ ) greatly increased immediately after 100 mm of water was added (irrigated plot), approximately doubling from 0.2 to 0.4  $\text{m}^3 \text{m}^{-3}$  and becoming significantly higher than in the control plot without water addition.  $\theta_s$  then slowly decreased over the next 24 h in both plots but remained significantly higher in the irrigated plot (Figure 1a), with a difference between the irrigated and the control plot of over 0.11  $\text{m}^3 \text{m}^{-3}$  throughout the 24 h.



**Figure 1.** Soil volumetric water content ( $\theta_s$ , panel a) and soil respiration ( $R_s$ , panel b) before and over 24 h after the addition of 100 mm of water in the grazed treatment for watered plots (diamonds) and control non-watered plots (circles). The line in panel b is the difference between mean  $R_s$  in the watered plots and mean  $R_s$  in the control plot for each hour after watering. Vertical bars represent one standard error of the mean ( $n = 4$ ).

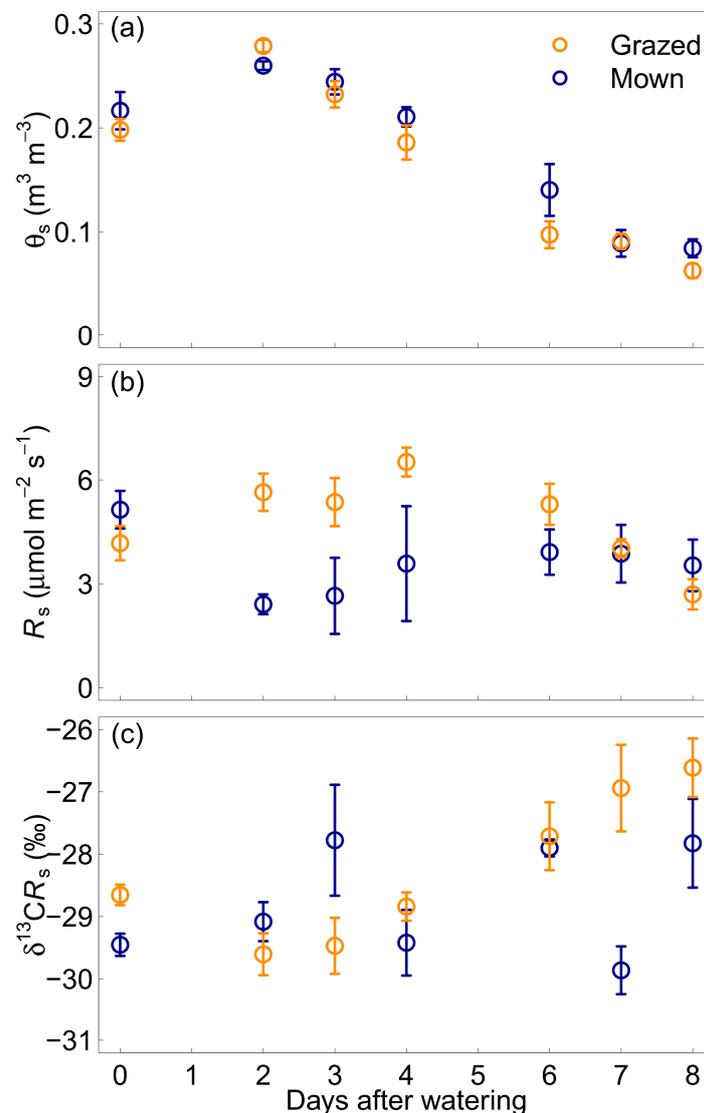
Soil respiration ( $R_s$ ) started to increase 2 h after water was added to the irrigated plot, becoming significantly higher than in the adjacent control plot and remaining higher during the 24 h measuring period (Figure 1b).  $R_s$  also varied in the control plot due to diel variation.  $R_s$  peaked for both plots 4 h after water was added. The difference between mean  $R_s$  in the irrigated and the control plot was null before and just after water was added. This difference started to increase 1 h after water addition, was highest 4 h after water addition with a value of 3.4  $\mu\text{molCO}_2 \text{m}^{-2} \text{s}^{-1}$ , and then decreased and levelled off, being similar 11 h and 24 h after water addition (Figure 1b).

#### 3.2. Daily Changes after Water Addition

To assess the effect of water addition on  $R_s$ ,  $\delta^{13}\text{CR}_s$ ,  $\delta^{13}\text{CR}_a$ ,  $\delta^{13}\text{CR}_h$ , only the measurements taken from 24 h onward after water addition were included to exclude the pulse of respiration measured shortly after water addition.

Soil volumetric water content ( $\theta_s$ ) increased after the addition of 100 mm of water on the plots and remained higher than before watering for 3 days. After the initial increase,  $\theta_s$  decreased steadily

for the whole experiment period of 8 days (Figure 2a). Individual values of  $\theta_s$  overall ranged from 0.05 to 0.31  $\text{m}^3 \text{m}^{-3}$ .



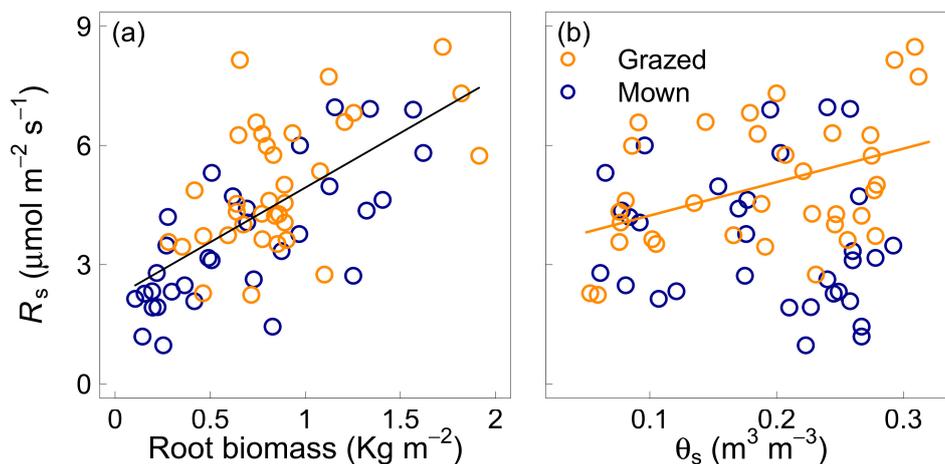
**Figure 2.** Soil volumetric water content ( $\theta_s$ , panel a), soil respiration ( $R_s$ , panel b) and  $^{13}\text{C}$  isotopic signature of soil respired  $\text{CO}_2$  ( $\delta^{13}\text{CR}_s$ , panel c) over the days after adding 100 mm of water in the grazed (orange circles) and mown (blue circles) grasslands. Vertical bars represent one standard error of the mean ( $n = 4$ ).

Although the trend for  $\theta_s$  was identical in both paddocks,  $R_s$  showed different patterns in the grazed and mown paddocks over the days after water was added (Figure 2b). In the grazed paddock,  $R_s$  roughly followed a similar pattern to  $\theta_s$ , showing higher values after water addition for 3 days and decreasing steadily after that.  $R_s$  did not show any obvious pattern in the mown paddock and was more variable than in the grazed paddock. Similarly, the  $^{13}\text{C}$  isotopic signature of soil-respired  $\text{CO}_2$  ( $\delta^{13}\text{CR}_s$ ) presented a different pattern for the two paddocks (Figure 2c). Changes in  $\delta^{13}\text{CR}_s$  were tightly coupled with changes in  $\theta_s$  in the grazed paddock, with more depleted values 2 days after water addition and a steady change to more enriched values over the measurement period. As for  $R_s$ ,  $\delta^{13}\text{CR}_s$  did not show any obvious pattern over the days after water addition in the mown paddock.

The  $^{13}\text{C}$  isotopic signature of  $\text{CO}_2$  respired from the roots and from the root- and rhizosphere-free soil did not show clear patterns after water addition for either paddock (Figure S1).

### 3.3. Effect of Soil Volumetric Water Content

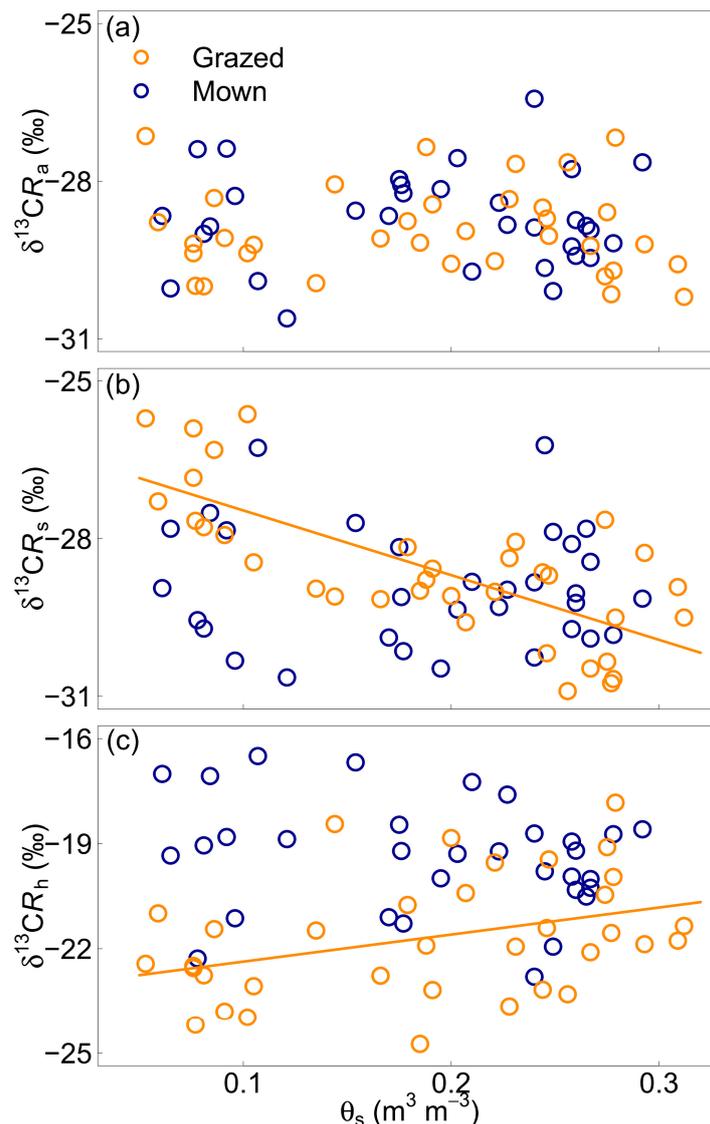
The model for  $R_s$  with the best fit selected from the backwards stepwise regression, explained 54% of the variability in  $R_s$  and incorporated the effect of root biomass ( $F = 55.74$ ,  $p < 0.0001$ ) and the interactive effect of soil water content and paddock management ( $F = 4.99$ ,  $p = 0.03$ ). The single-variate model including root biomass explained 43% of the variability in  $R_s$  (Figure 3a). Soil respiration was positively correlated with  $\theta_s$  in the grazed paddock ( $p = 0.03$ ) but was not significantly affected by  $\theta_s$  in the mown paddock ( $p = 0.2$ , Figure 3b).



**Figure 3.** Soil respiration ( $R_s$ ) as a function of root biomass (panel a) and soil volumetric water content ( $\theta_s$ , panel b) for the grazed (orange circles) and mown (blue circles) grasslands. The lines represent the fit of linear regressions. The black line in panel a indicates identical fit for the two treatments. Only significant correlations appear.

As for  $R_s$ , the model for  $\delta^{13}CR_s$  with the best fit incorporated the effect of root biomass ( $F = 4.41$ ,  $p = 0.04$ ) and the interactive effect of soil water content and paddock management ( $F = 9.65$ ,  $p < 0.01$ ). This model explained 39% of the variability in  $\delta^{13}CR_s$ . Soil volumetric water content had different effects for the two paddocks (Figure 4a), with a significant effect of  $\theta_s$  on  $\delta^{13}CR_s$  for the grazed paddock ( $p < 0.01$ ), but no significant effect for the mown paddock ( $p = 0.6$ ).

The model for  $\delta^{13}CR_h$  with the best fit incorporated the interactive effect of root biomass and paddock management ( $F = 7.63$ ,  $p < 0.01$ ) and the interactive effect of soil water content and paddock management ( $F = 6.44$ ,  $p = 0.01$ ), and explained 39% of the variability in  $\delta^{13}CR_h$ . The effect of root biomass and of  $\theta_s$  on  $\delta^{13}CR_h$  was significant only for the grazed paddock ( $p < 0.001$  for root biomass and  $p = 0.04$  for  $\theta_s$ ). The model for  $\delta^{13}CR_a$  with the best fit was a single-variate model incorporating only root biomass ( $F = 7.23$ ,  $p < 0.01$ ) and explained 10% of the variability in  $\delta^{13}CR_a$ . There was no significant effect of  $\theta_s$  on  $\delta^{13}CR_a$  or  $\delta^{13}CR_h$  (Figure 4a,c).



**Figure 4.**  $^{13}\text{C}$  isotopic signature of  $\text{CO}_2$  respired from the roots and rhizosphere ( $\delta^{13}\text{C}R_a$ , panel a), the whole soil ( $\delta^{13}\text{C}R_s$ , panel b) and the root- and rhizosphere-free soil ( $\delta^{13}\text{C}R_h$ , panel c) as functions of soil volumetric water content ( $\theta_s$ ) for the grazed (orange circles) and mown (blue circles) grasslands. The lines represent the fit of linear regressions. Only significant correlations appear.

It is noteworthy that soil temperature ( $T_s$ ) ranged from 17.6 to 29.6 °C and was negatively correlated with  $\theta_s$  ( $F = 33.84$ ,  $p < 0.0001$ ). Because of this correlation, the effect of  $T_s$  and  $\theta_s$  was analyzed separately. In the model with the best fit for each backwards stepwise regression analysis,  $\theta_s$  was replaced with  $T_s$ , and the effect was assessed by comparing the coefficient of determination ( $R^2$ ) for the models including  $\theta_s$  and  $T_s$ . Replacing  $\theta_s$  by  $T_s$  in the best models resulted in a lower coefficient of determination for all the variables.

## 4. Discussion

### 4.1. Birch Effect

It is well documented that rewetting dry soils is usually followed by a burst of respiration, which can be very short-lived [34] or last for several days [35], after which respiration rates stabilize to that of a continuously wet soil. In our study, with a flush of  $\text{CO}_2$  starting 4 h after watering and the difference between dry and wet soils coming to equilibrium over the few hours after that, the Birch effect was

observed to last less than a day. Therefore, we considered measurements taken 24 h after watering for analyzing  $R_s$  and its components to exclude the influence of the Birch effect.

#### 4.2. Soil Water Content and Isotopic Discrimination

Changes in  $\delta^{13}CR_a$  and  $\delta^{13}CR_h$  with soil water content were largely insignificant, in line with previous results where irrigated and non-irrigated plots were not found to differ significantly in their values of  $\delta^{13}CR_a$  and to marginally affect  $\delta^{13}CR_h$  [17]. This may appear surprising, particularly for the autotrophic component. Under conditions of limited water availability, plants close their stomata, improving their water use efficiency and usually leading to lower  $^{13}C$  discrimination [36,37]. Moreover, substrates respired in the roots are supplied mainly by recent photo-assimilates [38,39]. We would, therefore, have expected more enriched values for  $\delta^{13}CR_a$  with higher water stress and lower photosynthetic  $^{13}C$  discrimination. Our result suggests that the substrates for respiration of the roots were not tightly coupled with photo-assimilates. Water stress may reduce the phloem diffusion rate, therefore, increasing the time lag between assimilation and utilization of compounds in the roots and rhizosphere and resulting in photosynthesis to decrease faster than respiration rates under water stress [38,39]. We conclude that carbohydrate reserves provided substrates for the autotrophic component of soil respiration and were not exhausted during the 8 days following watering.

#### 4.3. Soil Water Content and Components of Soil Respiration

The  $^{13}C$  signatures of  $CO_2$  respired from the roots and from the whole soil were similar in value for high water content, ranging between  $-31$  and  $-27\%$ . Probably due to the relatively low carbon content at this site, the contribution of SOM decomposition to total  $R_s$  was small and  $\delta^{13}CR_s$  was largely dominated by the signature from the roots, also in line with the observation that roots can be a dominant contributor to total  $R_s$  in many ecosystems [40–42]. Not surprisingly, soil respiration was strongly positively correlated with root biomass for both treatments.

Soil water content is a major driver of root respiration and can be the limiting factor for plant activity in dry conditions [43–45]. When the soil was wet just after water addition, the contribution of roots and rhizosphere respiration to total soil respiration was probably at its maximum and was close to 100%, leading to values of  $\delta^{13}CR_s$  and  $\delta^{13}CR_a$  being indistinguishable. With such small changes in the components of soil respiration ( $\delta^{13}CR_a$  and  $\delta^{13}CR_h$ ), changes in  $\delta^{13}CR_s$  must have been due to changes in the relative contribution of the autotrophic and heterotrophic components.

Because the autotrophic component dominated soil respiration, the enrichment of  $\delta^{13}CR_s$  with decreasing water content in the grazed paddock was likely to be due to the decreasing contribution of the root and rhizosphere component rather than an increasing contribution of the heterotrophic component. This hypothesis is in line with the results from a laboratory study showing that microbial activity in the rhizosphere of grassland species was less affected than root activity [46]. This enrichment, therefore, suggests that in the grazed treatment, soil respiration decreased with water content due to a decreasing autotrophic component.

No changes were observed in the mown treatment. Carbohydrate reserves may vary with grass species and development stage [47]. It is possible that the plant community in the mown treatment presented a rooting system with more carbon reserves and increased resilience to drought. Another explanation could be found in the fact that grazing and mowing happened at different times. Due to a farm management decision, mowing had happened shortly before the beginning of our experiment. Grasses have been observed to mobilize their root reserves and allocate them above-ground for leaf regrowth after cutting or grazing [48,49]. It is, therefore, possible that root exudation and respiration were decreased due to mowing, thereby, cancelling the positive effect of adding water on root activity observed in the grazed treatment.

#### 4.4. Consequences for Soil Carbon Sequestration

Agricultural management practices are known to influence the fate of soil carbon stocks, in interaction with climate and soil properties [5]. Although permanent grasslands are known to have a strong potential to store carbon below ground [24], soil carbon sequestration may be reversible under the effect of climate change and changes in management practices, particularly those involving soil disturbance [25]. Our results showed that different management practices and decisions are strong contributors to determine the response of soil carbon dynamics to soil water content. Although we were not able to conclude directly about which of mowing or grazing management will enhance drought resilience, we showed that the adaptation and resilience of agricultural grasslands to an intensified global water cycle will likely strongly depend on management practices and decisions. These decisions will, therefore, determine the magnitude of climate-induced changes in grasslands soil carbon stocks and should be the subject of careful evaluation.

### 5. Conclusions

Soil respiration in grazed and mown grasslands responded differently to changes in soil water content. It is possible that the unsynchronized cutting and grazing regimes partly explained this difference. Our results suggest that farm management practices and the timing of management decisions potentially alter the relationship between soil carbon dynamics and soil water content. Therefore, management choices and decisions will likely significantly contribute to driving changes in soil carbon stocks under the influence of climate change as the global water cycle changes and the intensity and frequency of droughts increase. More studies are needed to fully identify which management practices can lead to increased resilience and adaptation of grassland ecosystems to drought.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4395/9/3/124/s1>, Figure S1:  $^{13}\text{C}$  isotopic signature of  $\text{CO}_2$  respired from the roots ( $\delta^{13}\text{CR}_a$ , panel a) and of  $\text{CO}_2$  respired from root and rhizosphere free soil ( $\delta^{13}\text{CR}_h$ , panel b) over the days after adding 100 mm of water in the grazed (orange circles) and mown (blue circles) grasslands. Vertical bars represent one standard error of the mean ( $n = 4$ ).

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### Abbreviations

$R_s$	Soil respiration
$\delta^{13}\text{CR}_s$	$^{13}\text{C}$ isotopic signature of $\text{CO}_2$ respired from the whole soil
$\delta^{13}\text{CR}_a$	$^{13}\text{C}$ isotopic signature of $\text{CO}_2$ respired from roots
$\delta^{13}\text{CR}_h$	$^{13}\text{C}$ isotopic signature of $\text{CO}_2$ respired from root and rhizosphere free soil
$\theta_s$	Soil volumetric soil water content
$T_s$	Soil temperature at 100 mm depth

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