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Validating laboratory insights into the drivers of soil rewetting respiration pulses with field measurements

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Abstract. Improved understanding of the mechanisms driving heterotrophic CO_2 emissions after rewetting of a dry soil may improve projections of future soil carbon fate. While drying and rewetting (DRW) under laboratory conditions has demonstrated that heterotrophic CO_2 emissions depend on DRW features and soil and environmental conditions, these laboratory insights have not been validated in field conditions. To this aim, we collated mean respiration rates over 48 hours after rewetting from two data sources: 37 laboratory studies reporting data for more than three DRW cycles (laboratory respiration, LR), and six field datasets recording hourly heterotrophic respiration and soil moisture (field respiration, FR). LR and FR were explained by six predictors using random forest algorithms and partial dependence plots. Results indicated that the most important driver of LR and FR were SOC and temperature, respectively. Both LR and FR increased with increasing SOC and temperature. LR increased with soil dryness before rewetting, but this trend was less clear in FR. LR decreased with soil moisture increments at rewetting, while FR increased with soil moisture increments. LR was higher in soils from humid climates than from arid climates, but this effect was not observed in FR. We concluded that laboratory insights could be partly validated with current datasets. Caution should be taken when extending laboratory insights to predicting fluxes in ecosystem.

1 Introduction

Drought intensity and frequency are increasing, exposing ecosystems to more frequent and intense soil drying and rewetting (DRW) events (IPCC, 2022). These DRW events can influence the size and turnover of soil carbon pools. During soil drying, less soil carbon is released because microbial growth and respiration decline as substrate availability decreases, and physiological stress at low matric potential ensues (Brangarí et al., 2021; Manzoni et al., 2012; Schimel, 2018). Upon rewetting, large amounts of CO_2 are released as microbial activity resumes (Barnard et al., 2020; Birch, 1958; Meisner et al., 2013), significantly contributing to annual carbon release (Manzoni et al., 2020). Understanding the drivers of CO_2 emissions after rewetting is therefore important to quantify soil carbon balances and predict them under changing climate.

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With the rigour offered by laboratory environments, controlled drying-rewetting (DRW) experiments have helped to isolate several drivers of respiration rates after rewetting. For example, rewetting induces higher rates of respiration following exposure to more intense (lower soil moisture), extended (longer), and pronounced (larger differences in water content between dry and moist samples) drought treatments (Fischer, 2009; Lado-Monserrat et al., 2014; Li et al., 2023a; Manzoni et al., 2020; Meisner et al., 2017; Miller et al., 2005; Tiemann and Billings, 2011). In contrast, repeated cycles of drought result in progressively smaller pulses of respiration (Miller and Berry, 2005). Moreover, the respiration rates measured in laboratory incubations increase with soil organic carbon content (SOC) (Harrison-Kirk et al., 2013) and incubation temperature (15~45 °C) (Andrews et al., 2023), and varied with climate background (Sawada et al., 2017) and soil sampling depth (Brangarí et al., 2022). However, this knowledge is based on laboratory studies, and extending insights derived from these laboratory DRW experiments to predict respiration rates after rewetting in field conditions is challenging (Canarini et al., 2017; Rousk and C. Brangari A, 2022).

It remains nearly untested whether laboratory studies of respiration responses to DRW can capture patterns occurring in the field. Soils for laboratory incubations are usually air dried and sieved, which may modify some essential field conditions such as soil structure, in particular soil aggregates and soil porosity, which in turn affects substrate availability to microbes and abundance of microbial groups (Kainiemi et al., 2016; Kaiser et al., 2015; Kan et al., 2022; Meyer et al., 2019). Moreover, laboratory studies might have altered the microbial communities (Blaud et al., 2017) due to soil preparations, thus the links between community composition and local climate, resuLting in masking the climate legacy effects on respiration. Laboratory studies could also overestimate the effects of SOC on respiration due to the fact that soil sieving can release SOC protected in aggregates, thereby increasing the proportion of bio-available SOC over stable SOC in the field soils. Laboratory studies may reduce the temperature effects of respiration. This is because temperature sensitivity of the respiration of SOC in macro-aggregates is larger than in micro-aggregates, and micro-aggregates in seived soils are more abundant compared to field soils (Kan et al., 2022). As laboratory studies usually keep incubation temperature constant and centred around 20 to 25°C, the effects of drying and rewetting intensity in the field may not be fully captured. This is because in the field soil moisture usually co-varies with soil temperature, and soil temperature affects the respiration response to moisture (Moyano et al., 2013). Moreover, soil sieving for laboratory studies reduces the heterogenous distribution of microbial hotspots and carbon resource in the field, which could alter the respiration response to drying and rewetting depending on the reaction surfaces being increased or decreased in the sieved soils. Given the above concerns, there is a need to validate if insights achieved in laboratory experiments can be extended to field conditions (Rousk and C. Brangari A, 2022).

To fill this knowledge gap, we first collated data on mean respiration rates during the two days after rewetting from both laboratory DRW experiments and field studies. We investigated how the respiration rate after rewetting could be explained by SOC content, incubation temperature (in situ soil temperature for field respiration), soil dryness, rewetting intensity, aridity index (ratio of precipitation to potential evapotranspiration), and soil sampling depth for laboratory respiration or soil moisture sensor depth for field respiration. Next, we compared the respiration rate responses to changes in these six drivers in laboratory

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and field conditions using partial dependence plots. These sets were used to address the question: are the drivers of respiration rates at rewetting the same in laboratory and field conditions?

2 Methods

2.1 Data from laboratory incubations

To obtain data from laboratory DRW experiments, we selected studies from previous meta-analyses and data syntheses (Canarini et al., 2017; Jin et al., 2023; Li et al., 2023c; Sang et al., 2022; Zhang et al., 2020), and added recently published studies (later than May 2019) using the same search term as in Zhang et al (2020). To calculate respiration rates over two days (see below), we only included studies that reported daily or hourly resolution time series of respiration rates, or total respiration over the two days after rewetting from both DRW and moist control laboratory incubations, and that included at least three DRW cycles. These criteria led us to select 37 studies (Appendix B), which span diverse climatic zones and soil conditions (Figs. A01).

To standardize soil moisture changes during DRW events across the laboratory studies, they were scaled to the percentage of water holding capacity (WHC). Soil moisture values reported as field capacity or soil water potential at -0.33 bar were regarded as 100% WHC. Soil moisture values reported in % water-filled pore space (WFPS) were multiplied by 1.4 to convert into a value expressed as %WHC (Franzluebbers, 2020). Soil moisture values reported as soil water potentials were converted to WHC using water retention curves parameterized according to soil texture (Clapp and Hornberger, 1978; Dingman, 2015).

The respiration rate values were obtained from tables or digitized figures (The software Engauge Digitizer 12 (https://digitizer.sourceforge.net/) from the 37 studies. Next, the mean respiration rate was calculated from the integrated respiration rates over 48 hours after each rewetting event of each soil or treatment considered in a given study (denoted as laboratory respiration, LR). The chosen mean respiration rate offers a comparable response metric between lab and field datasets. This choice also avoids the issues of using response ratios (the ratio of absolute CO_2 emissions after rewetting to absolute CO_2 emissions at constant control) on interpreting driver's effects on respiration rates (Zhang et al., 2020), which might cause contrasting conclusions in previous meta-analyses (Canarini et al., 2017; Jin et al., 2023; Li et al., 2023b; Sang et al., 2022; Zhang et al., 2020). The 48 hours time frame was chosen to ensure a sufficient number of datasets. Very few studies reported high resolution respiration rates after rewetting across three drying and rewetting cycles, and most of the studies measured daily respiration or only reported mean respiration rate over two days. Six predictors were recorded, including soil dryness (the soil moisture at the end of drying (expressed as % WHC), the lower values, the larger dryness), rewetting intensity (RI: soil moisture increments at rewetting, % WHC), incubation temperature (TMP, °C), soil organic carbon content (SOC, g kg-1), soil sampling depth (cm), and the aridity index (AI: ratio of mean annual precipitation to potential evapotranspiration). The soil sampling depth refers to the deepest depth of a soil core, which could be used to indicate organic matter composition, with more microbially processed material at depth. The AI was obtained from Zomer, Xu & Trabucco (2022) for the period

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1970 to 2000, based on the coordinates of soil sampling. Larger values of AI indicate wetter climate. The obtained datasets are available in Supplement 1.

2.2 Data from field sites

To obtain respiration rates after DRW in field conditions (FR), we retrieved data from the COSORE database (Bond-Lamberty et al., 2020), which reports continuous high-resolution CO_2 emission, soil moisture, and soil temperature data from chambers located in trenched plots (to ensure only heterotrophic respiration is included in the measured rates). We included observations where soil moisture and temperature were measured in the soil surface layer (≤10 cm) because soil moisture fluctuations in deep layers are less correlated with respiration rates at the surface due to the delayed transport of CO_2 to the surface (Chu et al., 2023). After applying these criteria, six studies were left, which were located in North America (see Figs. A01). SOC content, depth of soil moisture and temperature sensors, and AI values were obtained from the COSORE datasets or other relevant papers on the same sites (Supplement 1).

2.3 Defining rewetting events in field studies

2.3.1 Identification of the end of drying periods

To obtain the FR values and the characteristics of rewetting events in the field, we first defined the end of drying periods preceding rewetting. In these drying periods, soil moisture declines or varies little, whereas it increases afterward. Based on these two criteria, the hourly soil moisture time series were progressively scanned. We calculated $\Delta\theta^-$and $\Delta\theta^+$, where $\Delta\theta^-$ is the difference between the minimum soil moisture in the previous 24 hours (θ^-_{min}) and soil moisture of the current time point (θ) (Eq.(1)), and $\Delta\theta^+$ is the difference between the maximum soil moisture in the subsequent 24 hours (θ^+_{max}) and θ (Eq.(2))

$$\Delta\theta^- = \theta^-_{min} - \theta \, , \tag{1}$$

$$\Delta\theta^+ = \theta^+_{max} - \theta \, , \tag{2}$$

where a positive $\Delta\theta^-$ indicates that soil moisture declines before the current time point, while a negative value indicates a moisture increment; small moisture increments might still be part of the drying period if due to daily fluctuations not associated with a rainfall event, as long as the fluctuations are lower than an increment tolerance threshold ($\Delta\theta_{tolerance}$); a positive $\Delta\theta^+$ indicates that soil moisture starts increasing after the current time point, possibly indicating a rewetting event. Time points when both $\Delta\theta^-$ was larger than $-\Delta\theta_{tolerance}$ and $\Delta\theta^+$ was larger than a rewetting threshold ($\Delta\theta_{rewet}$) were defined as points at the end of a drying period. The end of drying period before rewetting was then defined as a continuous sequence of at least five hourly time points fulfilling these criteria with at least 18 hours of data without gaps ahead.

The thresholds to include time points as the end of drying periods were calculated as percentages of the 5th to 95th percentile range of soil moisture at a given respiration chamber, to avoid the influence of extreme values. The rewetting threshold ($\Delta\theta_{rewet}$) was defined as 10% of the soil moisture range—if soil moisture increases by more than $\Delta\theta_{rewet}$ we assume that a

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rewetting event is occurring and the point is a part of a drying period. The increment tolerance threshold ($\Delta\theta_{tolerance}$) was set to 2% of the soil moisture range—if there is no soil moisture increase larger than $\Delta\theta_{tolerance}$ in previous 24 hours, the point is retained as part of the drying period. For datasets with strong daily fluctuations (named as "d20190517_MAURITZ" and "d20190617_SCOTT_WKG" in the COSORE datasets as well as supplement 2), we set $\Delta\theta_{rewet}$ =25% of the soil moisture range and $\Delta\theta_{tolerance}$ =12.5% of the soil moisture range. Based on these definitions, both $\Delta\theta_{rewet}$ and $\Delta\theta_{tolerance}$ differ across locations, reflecting the different soil moisture regimes at the different field sites.

2.3.2 Defining rewetting events

The last points of each drying period were regarded as the start of 48-hour long candidate rewetting events. Candidate rewetting events were considered as rewetting events if datasets covered a period longer than 36 hours after the end of drying and included at least five respiration measurements. In some cases, multiple rewetting events occurred within 48 hours after one end of drying period. We included such rewetting events as one rewetting event when multiple rewetting events occurred within 24 hours at the end of drying period (Fig. 1, second peak). This is because soil moisture remains at high levels due to the subsequent rain events. Otherwise, such rewetting events were excluded because the respiration within 48 hours could be highly impacted by the multiple rewetting events, thus being not comparable to other rewetting events.

The soil moisture values at the end of drying periods were defined as soil dryness (fraction, %) (Fig. 1), and the largest soil moisture increments within the next 48 hours were defined as rewetting intensities (fraction, %) (Fig. 1). The mean temperature and the mean respiration rates during the rewetting events were obtained from the measured time series of temperature and respiration.

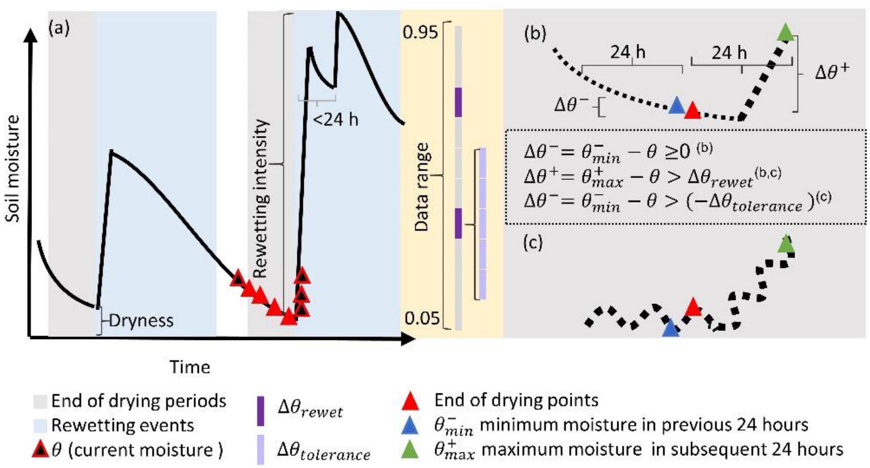

Figure 1: (a) Examples of the end of drying periods (in grey) and rewetting events (in light blue) for time series of soil moisture data in the field datasets. (b, c) two examples of the points to be selected within the end of the drying period.

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2.4 Data analysis

Random forest is an ensemble of decision trees. By averaging over the prediction made by each decision tree, random forest models are able to provide robust predictions, for both classification and regression problems. Random forest regressions often perform remarkably well for ecological prediction as they can account for non-linear and complex relationships (Huntingford et al., 2019), so we adopted this approach to evaluate insights into the drivers of respiration during DRW events.

Random forest regressions (*randomForest* package in the R) were used to predict the two response variables—mean respiration rates over two days after rewetting in the laboratory (LR measured in μg C g soil^{-1} h^{-1}) and the field (FR measured in μmol C m^{-2} h^{-1})—by six candidate predictor variables: soil dryness (soil moisture at the end of drying, expressed as %WHC for LR and as volumetric soil moisture (fraction into %) for FR), rewetting intensity (%WHC for LR; volumetric soil moisture (fraction into %) for FR), temperature (incubation temperature for LR; soil temperature in the field for FR), SOC content, and AI, as well as soil sampling depth for LR and soil moisture sensor depth for FR. The ranges and distributions of the value of these drivers for LR and FR are shown in Fig. 2. FR, LR, and SOC were log transformed to ensure a better normality of the residuals. It should be noted that expressing respiration rates and soil moisture in different units for the LR and FR datasets will not impact the results, as we are interested in the direction of the effects and significance of each driver, rather than the specific values of the respiration rate sensitivities to changes in individual drivers.

To obtain the best random forest regression model, we built 500 decision trees for each model. To build individual trees, random forest uses a bootstrapping approach where a subset of data (bootstrap sample) is obtained from the training data by resampling with replacement. The "mtry" parameter controls the number of predictors used at each split of decision trees and induces randomness (Scornet, 2017). In our case, the number of predictors in each subset varies from 2 to 6.

We compared the performance of models with "mtry" settings ranging from 2 to 6. For each "mtry" setting, we trained the models on 80% of the data individually for LR (n = 303) and FR (n = 592). We evaluated the models' performance by estimating the variance explained (R^2) and root mean squared error (RMSE) obtained between test data (remaining 20%) and predicted values of test data from the trained random forest models. This training was repeated 50 times, and the mean values of R^2 and RMSE from these 50 iterations were used to measure the performance of models for a specific value of "mtry". The best performance was obtained when the "mtry" was set to 3, so that this value was selected for the analyses shown in the Results section.

To assess the importance of the chosen six predictor variables, we used two different goodness of fit metrics: the percentage increase in Mean Square Error (%IncMSE) and the increase in node purity (IncNodePurity) (Fox et al., 2017). %IncMSE for each predictor variable measures the increase in the model Mean Square Error (MSE) when the predictor variable is removed while keeping the values of other variables intact. IncNodePurity measures how much the splitting based on a predictor improves the homogeneity of the nodes in decision trees. The larger values of %IncMSE and/or IncNodePurity, the more important is that particular predictor variable (Breiman, 2001).

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Finally, we used partial dependence plots to understand the response of individual explanatory variables on respiration rates after DRW events for both field and lab conditions. The partial dependence plots depict the effect of one explanatory variable on the response variable (LR or FR) with the other variables held constant. The partial dependence plots were obtained using the *pdp* package in R (Greenwell, 2017).

To test if the results were sensitive to our selection of the rewetting events, we increased $\Delta\theta_{rewet}$ to 15% of the soil moisture range for the four datasets without strong daily fluctuation. The results were similar to the results obtained by setting $\Delta\theta_{rewet}$ =10% of the moisture range (not shown).

All statistical analysis was performed using R Statistical Software (version R-4.1.3) (R Core Team 2022).

3 Results

The median respiration rates within 48 hours after rewetting in the laboratory (LR) and field (FR) were 1.18 μg C g^{-1} h^{-1} and 9.85 μmol C m^{-2} h^{-1}, respectively. The 10^{th} and 90^{th} percentiles were 0. 26 μg C g^{-1} h^{-1} and 4.08 μg C g^{-1} h^{-1} for LR and 3.09 μmol C m^{-2} h^{-1} and 52.91 μmol C m^{-2} h^{-1} for FR (Fig. 2a, b). Among the different drivers we considered, temperatures in laboratory incubations were generally higher than those experienced in the field (Fig. 2e, f), soil moisture at the end of drying were lower in the laboratory than in the field (Fig. 2g, h), and field sites did not differ in AI as much as sites sampled for laboratory incubations (Fig. 2k, l). The ranges of SOC and rewetting intensity were instead comparable between laboratory and field datasets (Fig. 2c, d, i, j). Note that %WHC values are approximately four times as large as % volumetric soil moisture values, because water holding capacity is at about half of the soil saturation, which in turn corresponds to a soil moisture around 50% (e.g., 50% WHC corresponds to a volumetric soil moisture of 12.5% if soil moisture at saturation is 50% and the WHC is at 50% of soil saturation).

The random forest regressions explained 85% and 79% of the variance of log-transformed LR (RMSE=0.35) and FR (RMSE=0.36), respectively. The most two important predictors of LR were SOC and AI (Fig. 3), followed by incubation temperature, dryness and rewetting intensity. The most important predictors of FR were soil temperature and aridity index, with soil dryness, SOC and rewetting intensity follows the importance ranking. Moreover, soil sampling depth for LR and soil moisture sensor depth for FR had the lowest importance (Fig. 3).

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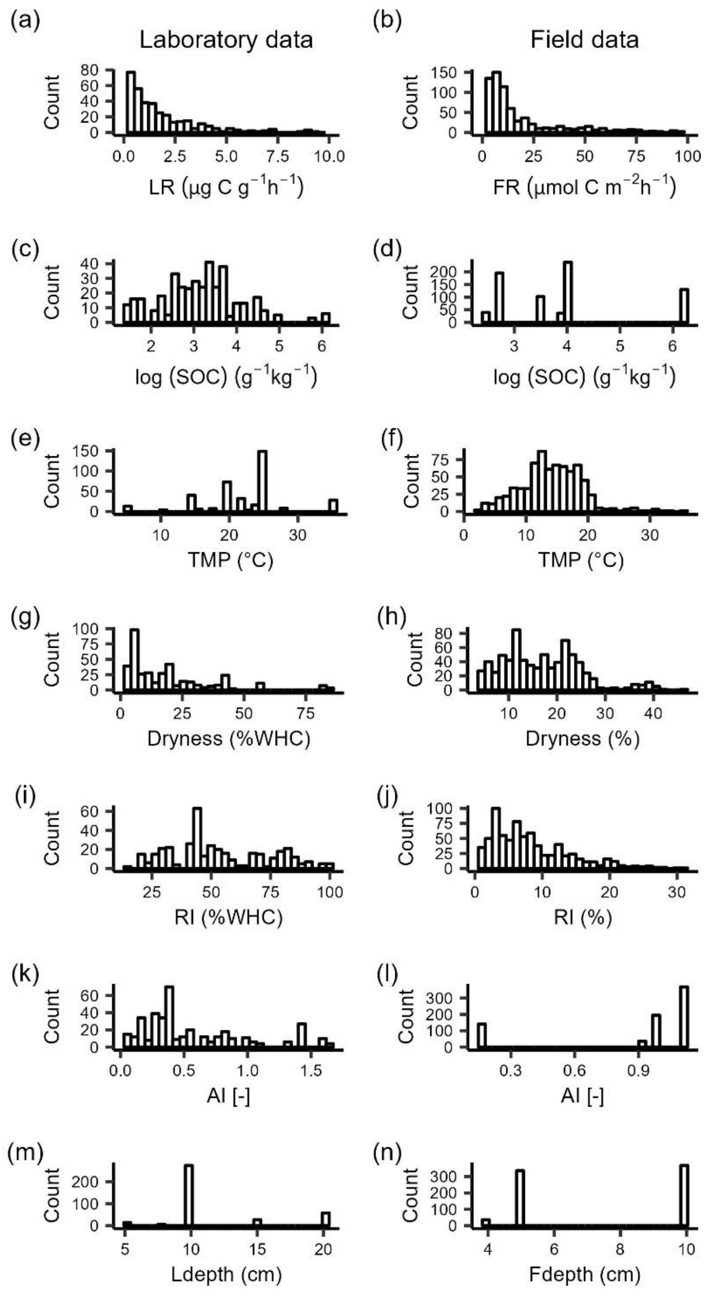

Figure 2: Data distribution of respiration rates over 48 hours after rewetting a) in laboratory rewetting events (LR, µg C g^{-1} h^{-1}) and b) field rewetting events (FR, µmol C m^{-2} h^{-1}). LR values larger than 10 and FR values larger than 150 are not shown. Data distribution of candidate drivers of respiration rates after rewetting in the laboratory and in the field: c, d) SOC, soil organic carbon content; e and f) TMP, incubation temperature for laboratory data and soil temperature in the field for field data; g and h) dryness (soil moisture at the end of the experimental drying); f and l) RI, rewetting intensity (soil moisture increment at rewetting); g and m) AI, aridity index; h and n) Ldepth, soil sampling depth for laboratory data, Fdepth, soil moisture probe depth for field data.

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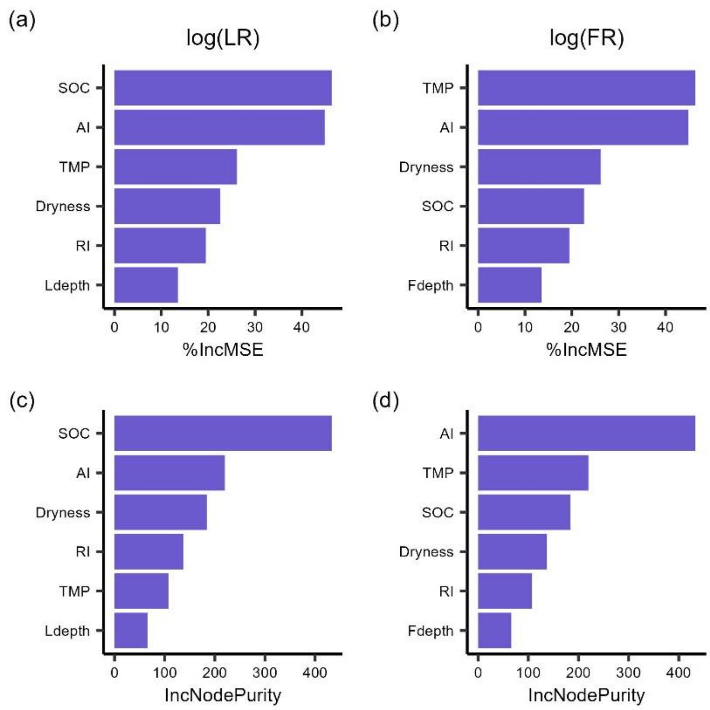

Figure 3: The importance ranking of predictors for mean respiration rates during 48 hours after rewetting, from laboratory (LR) and field (FR) measurements, based on random forest models using %IncMSE (a, b) and IncNodePurity (c, d). Predictors include soil organic content (SOC), aridity index (AI), soil dryness, rewetting intensity (RI), incubation temperature for LR and soil temperature for FR (TMP), and soil sampling depth for LR (Ldepth) and soil moisture sensor depth for FR (Fdepth).

Both LR and FR increased with SOC at where SOC contents were low (Fig. 4a, b). While LR stabilized when SOC was larger than 90 g kg^{-1}, FR continued increasing afterward (Fig. 4a). LR increased with temperature and then stabilized at 25 °C, and FR closely followed the same trend and stabilized at 20 °C (Fig. 4b). FR first increased with drying intensity up to 10% and then declined with drying intensity afterward, which is inconsistent with the observed monotonic decline of LR with drying intensity (up to 45% WHC) (Fig. 4c). FR increased with rewetting intensity while LR decreased with rewetting intensity (Fig. 4d). Differences between LR and FR trends with aridity index are difficult to assess mostly because of the narrow range of aridity index values at the field sites (Fig. 4e) and LR increased with increasing aridity index (i.e., in wetter climates). FR declined with soil moisture probe depth (0-10cm), and LR first increased and then declined with soil sampling depth (Fig. 4f).

To summarize, the increasing effects of SOC and TMP on respiration were consistent in laboratory and field conditions, and the effect of soil dryness were similar only when drying was not severe or very mild. Rewetting intensity had opposite effects in laboratory and field conditions and we were not able to draw solid conclusions for climate legacy effects (using AI as a climate index) due to the limited data range in the field datasets. The similarities between respiration rate responses to at least

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some drivers found between laboratory data and field data partly support our hypothesis that the laboratory insights could be validated under field conditions.

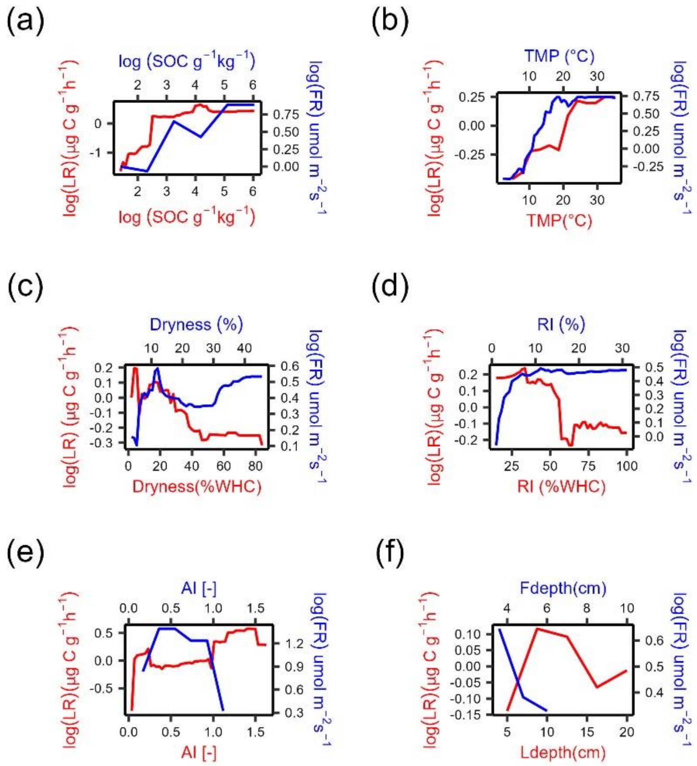

Figure 4: Partial-dependence plots for the selected predictors of absolute respiration rate over 48 h after laboratory rewetting (LR, red curve) and field rewetting (FR, blue curve) based on the random forest model. Abbreviations: SOC, soil organic carbon content; dryness (soil moisture at dry condition); RI, rewetting intensity (soil moisture increment at rewetting); TMP, incubation temperature for LR and soil temperature for FR; AI, aridity index; Ldepth, soil sampling depth for LR, Fdepth, soil moisture sensor depth for FR. The y-axes represent the marginal effect of each predictor on LR and FR while holding all other predictors constant.

4 Discussion

4.1 Validation of insights from laboratory drying-rewetting experiments using field data

Applying knowledge gained from laboratory studies conducted in controlled conditions to predict CO_2 emissions under field conditions is challenging, which motivated us to validate laboratory insights into the drivers of rewetting pulse in field conditions. To this aim, we compared the importance rankings and respiration responses to several drivers using laboratory and field datasets. Although direct/quantitative comparison of rankings between laboratory and field datasets might be affected by the different distributions of response variables (especially for temperature and soil moisture at the end of drying; Fig. 2), the qualitative comparison of respiration response shapes allowed us to validate of the drivers' effects on respiration, at least for drivers whose ranges overlap between laboratory and field datasets. In general, our results are consistent with our hypothesis that laboratory insights could be partly validated using field datasets.

Respiration rate increased with increasing SOC in both lab and field datasets (Fig. 4a). This trend is also consistent with previous studies (Canarini et al., 2017; Harrison-Kirk et al., 2013), and is probably due to the increased substrate availability with increasing SOC content. In addition, the SOC sensitivity of respiration was higher in the laboratory dataset than in the field (respiration reaches a plateau at SOC≈90 g kg^{-1}, Fig. 4a), suggesting that the sensitivity of respiration to SOC in the laboratory might be overestimated. One reason to explain the overestimation is that soil sieving may have helped to release substrates physically protected by micro-aggregates compared to intact aggregates in the field (Kpemoua et al., 2022; Zhang et al., 2022b), resulting in proportionally more bioavailable SOC for a given level of SOC content. Another reason may be that leaching of dissolved organic carbon released after rewetting does not occur in the laboratory experiments, whereas it causes carbon losses in the field (Liu et al., 2018; Rupp et al., 2021). As a result, there can be more bioavailable carbon in the laboratory experiments to fuel the respiration pulse at rewetting. If this overestimation of SOC effects on respiration obtained from laboratory could be further quantitatively confirmed, then we should expect lower carbon emission in field conditions and possibly lower sensitivity of respiration to intensified DRW cycles compared to the emissions measured in the laboratory. To conclude, the positive effects of SOC on respiration after rewetting in the laboratory could be confirmed using field data, even though laboratory studies may quantitatively overestimate the sensitivity of respiration to changes in SOC.

Soil respiration increased in warmer soil in both laboratory and field conditions. The observed increases were generally consistent with previous studies (Nissan et al., 2023), but the patterns can vary between studies. The observed plateaus above 20 °C (Fig. 4b) might suggest the presence of a peak of the temperature response (Niu et al., 2024), with possible declines outside the range of temperature in our data. This concave downward trend differs from the exponential increase (Andrews et al., 2023) (15 to 45 °C) and linear increase (Cruz-Paredes et al., 2023) (0 to 50 °C) found in other studies. These inconsistencies could be explained by the relatively low substrate availability in our datasets as compared to other studies, as we considered both laboratory and field respiration during multiple DRW cycles and substrate availability declines with the number of DRW cycles (Zhang et al., 2020). In addition, temperature sensitivity (Q_{10}, estimated here as the ratio of respiration rate at 20 °C over respiration rate at 10 °C) was lower in laboratory data (Q_{10}=1.2) than in field data (Q_{10}=2.3). This indicates that temperature sensitivity might be underestimated in the laboratory dataset. However, the Q_{10} value for the laboratory studies was estimated based on the random forest results, which were constrained by a temperature range limited between 20 and 25 °C (Fig. 2f), so this value could be low because of inaccurate predictions by the random forest model. This comparison would benefit from a more accurate estimation of Q_{10} from laboratory studies, which would be possible if more datasets were covering the temperature range within 10 °C to 20 °C. This lower sensitivity could be explained by sieving of soils used in the laboratory incubations. In fact, sieving breaks down macro-aggregates into micro-aggregates (Qin et al., 2019), which exhibit lower temperature sensitivity (Kan et al., 2022). Based on this, we further speculate that in the field, temperature affects C release from physically protected pools (aggregates and mineral-associated C) and thus has a more important role than bulk SOC, but this role could be weaker in the laboratory due to soil sieving. This could explain why SOC was the most important driver of

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LR while TMP was either most important or ranked second for FR (Fig.3). Taken together, the positive effects of temperature on respiration after rewetting in the laboratory could be confirmed using field data. However, correcting the bias of the temperature sensitivity of respiration due to the changed aggregate distribution after sieving may help to integrate insights from lab and field conditions.

Drier soils before rewetting drive higher respiration after rewetting in laboratory experiments but not always in field conditions. The drier the soil before rewetting, the larger LR. This trend is consistent with previous studies (Cable et al., 2008; Fischer, 2009; Manzoni et al., 2020; Patel et al., 2021; Xu et al., 2004; Yan et al., 2014), and can be explained by the greater amount of substrate accumulated in drier soils before rewetting (longer dry periods) (Schimel, 2018; Warren, 2020). It should be noted that this pattern emerges probably because soils were dried to a larger extent in laboratory conditions than they would in the field (Fig. 2g, h), resulting in large respiration pulses with a strong dependence on dryness before rewetting. In contrast, respiration in the field showed the same pattern only at intermediate values of soil moisture before rewetting (10% to 30% of volumetric soil moisture) (Fig. 4c), while it was lowest after rewetting very dry soils and relatively high after rewetting already wet soils—this pattern was not expected. In field conditions, dry soils could be rewetted slowly unless a large rainfall event occurs, which could explain why very dry soils do not always cause a large respiration pulse. Moreover, in the field, dry soil can be compacted, making substrates less accessible for microbial decomposition (Beare et al., 2009), and reducing O_2 dissolution and diffusion (Zhang et al., 2022a). The high respiration after rewetting of wet soil could instead be potentially related to anaerobic reaction pathways releasing carbon (Fairbairn et al., 2023). In addition, we speculate that soil physical properties during the dry period could play an important role in controlling respiration rate after rewetting (Navarro-García et al., 2012), but such properties are modified in the laboratory due to soil sieving before the incubations. Thus, respiration increased with prior soil dryness in laboratory conditions, but only in a narrow moisture range in the field condition. To ensure that the effects of dryness on rewetting respiration from laboratory studies are comparable to those in the field, we suggest to conduct DRW experiments using intact soil samples (Muhr et al., 2010).

The effects of rewetting intensity on respiration differed between laboratory and field conditions, as field respiration increased with increasing rewetting intensity (larger soil moisture increments after rewetting; Fig. 4d), whereas laboratory respiration decreased with rewetting intensity (Fig. 4d). The increasing trend from the field data is consistent with the idea that a larger soil moisture increment after rewetting can release more substrates that had been previously inaccessible, thus supporting a larger respiration pulse (Homyak et al., 2018; Lado-Monserrat et al., 2014; Navarro-García et al., 2012). The decreasing trend from the laboratory data could be explained by the delayed peak respiration rates due to microbial stress after large rewetting events (Li et al., 2023a; Meisner et al., 2017). For example, air-dried soils in some laboratory studies were rewetted to 50% WHC (X. Li, Leizeaga, et al., 2023), which is a very large change from the perspective of soil microbes trying to regulate turgor pressure. As the delay time for respiration can exceed two days for such large moisture increments (Li et al., 2023b), our use of respiration rates averaged over two days might underestimate the actual respiration pulse. Moreover, soil pores may become saturated in large rewetting events, resulting in oxygen limitation and thus lower respiration (Erinle et al., 2021; Keiluweit et al., 2016; Maier et al., 2011; Silver et al., 1999). Since soil moisture in the field usually declines immediately after reaching its peak, the limited oxygen supply may not be as important a driver of carbon emission in the field as in the laboratory. This may partly explain the contrasting respiration response to rewetting intensity in the lab and in the field. To summarize, laboratory insights about rewetting intensity were not validated by field datasets and more laboratory experiments are needed to test the effect of a range of soil moisture increments at rewetting and to mimic the soil moisture declines after rewetting that often occurs in field conditions.

Aridity index was positively correlated with respiration in the laboratory, but it was not clear in the field (Fig. 4e). With field datasets clustered in a narrow range of climate zones, this study is not able to confidently validate laboratory insights about climate legacy effects on respiration. In contrast, thanks to the wide spatial variation of soils in laboratory studies, climate legacy effects on respiration emerged in the laboratory dataset. These legacy effects were consistent with the expected lower microbial adaptation to drought in wetter climates (large values of aridity index) causing larger respiration pulses at rewetting (Tang et al., 2023; Winterfeldt et al., 2024). Moreover, climate legacy effects in the laboratory would not be easily observed if soil samples were obtained from areas with limited climatic variations (Leizeaga et al., 2021). In addition, we speculate that the closer soil structure, substrate availability and microbial characteristics to the field conditions, the easier it would be to detect climate legacy effects (Kaiser et al., 2015). That might explain why some experiments have shown climate legacy effects (Broderick et al., 2022; Hawkes et al., 2017, 2020), while others have not (Leizeaga et al., 2021). Moreover, it is possible that climate legacy effects might emerge in laboratory incubations because soil moisture is maintained at high values after rewetting, while in the field moisture values decline rapidly in dry areas with high evaporation rates, limiting the chances to detect legacy effects. Validation of climate legacy effects on respiration will need more laboratory experiments on intact soils and more globally distributed field datasets.

We initially expected that the validation of laboratory insights to the drivers of the respiration pulse induced by rewetting dry soil with field measurements could be regulated by soil sampling depth. This is because respiration sensitivity to changes in soil moisture varies with depth (Berg et al., 2017; Pallandt et al., 2022), due to vertical difference of soil properties (Hicks Pries et al., 2023; Kirschbaum et al., 2021; Slessarev et al., 2020), soil moisture memory, and microbial acclimation to DRW (Brangarí et al., 2022; Engelhardt et al., 2018; Hicks, 2023). However, soil sampling depth was not a strong predictor of the respiration pulses (Fig. 3). This may be due to the soil sieving in the laboratory mixing the entire sampled profile and thus reducing soil differences across depths. In addition, we expected an important role of soil moisture sensor depth on field respiration, as deep sensors report more buffered soil moisture variations than surface sensors, causing longer time lags of soil moisture changes and respiration changes—yet, we found negligible effects of sensor depth on the respiration pulses (Fig. 3).

4.2 Uncertainties

Some potentially important drivers of respiration after rewetting were not included in our analysis, so we could not compare their effects between laboratory and field conditions. For example, duration of the drying period and number of DRW cycles, are expected to increase and decrease respiration rates, respectively (Miller et al., 2005; Tiemann and Billings, 2011). In a test run, adding both to predict respiration in the laboratory did not increase the explained variance. Moreover, duration of soil

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drying and number of DRW cycles are not fixed in the field, where soil moisture fluctuations are driven by stochastic rain events, making the comparison with laboratory conditions difficult. Besides, soil texture, soil pH (Harrison-Kirk et al., 2014; Li et al., 2020; Singh et al., 2023), and other soil properties were not included due to lack of site-specific data.

To improve the comparison between laboratory and field conditions, a more accurate prediction of the effects of respiration drivers is needed. This requires that both laboratory and field studies cover more diverse climatic conditions and report more comprehensive information about soil properties. This need arises because the ability of random forest models (also other statistical methods) to explain variation in response variables is limited by low variation in the explanatory variables. Even among the selected drivers, some exhibit low variation both in field and laboratory studies (Fig. 2). Laboratory studies should be extended to longer periods after rewetting and should cover a wider range of soil moisture before rewetting and rewetting intensities. This would help enhance the robustness of statistical analysis on the compound role of DRW characteristics and pedo-climatic conditions on respiration after rewetting.

5 Conclusions

Testing and validation of hypotheses emerging from laboratory simulation of soil drying and rewetting are necessary for predicting respiration pulses after rewetting in field conditions. In this study, we compared the respiration response to rewetting using both laboratory datasets and field datasets. Respiration pulses increased with SOC and temperature in both these datasets, but the temperature sensitivity could not be reliably estimated due to the limited range of temperatures explored in laboratory studies. Respiration in the laboratory (but not in the field) also increased with the aridity index, suggesting climate legacy effects, but possibly also highlighting possible artifacts induced by how soil moisture is manipulated in the laboratory after the rewetting. Both soil moisture at the end of drying and rewetting intensity affected respiration differently across datasets. Higher resolution respiration data measured over a longer period, and under more varied climatic and soil conditions in both laboratory and field settings would be help to enhance the robustness of the outcome of this study. This could further help us to validate laboratory insights, and further understand and predict the CO_2 emissions under dry-rewetting events.

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Appendix A

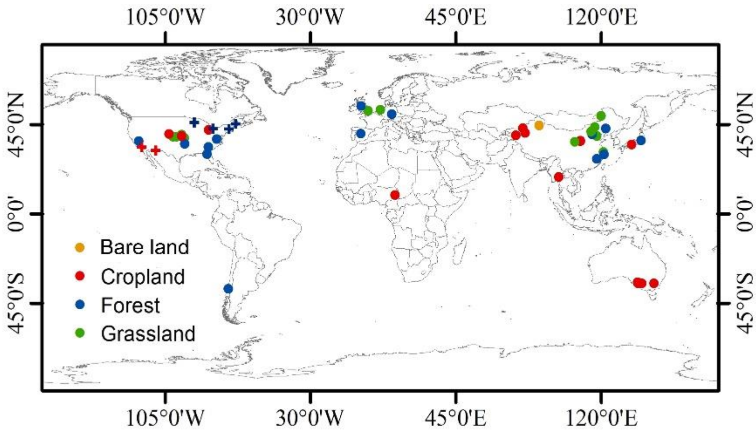

Figure A01: The data source distribution; point color shows the land-use/land cover types, point type shows that data from laboratory drying and rewetting experiments (circle) or from the field (cross)

Appendix B

Study list of laboratory data

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