

# The direct and legacy effects of drying-rewetting cycles on active and relatively resistant soil carbon decomposition

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## Abstract

Global climate change is expected to increase the frequency of drought and heavy precipitation, which could create more frequent drying-rewetting cycles (DWC) in the soils. Although DWC effects on SOC decomposition has been widely studied, the effect of DWC and the subsequent legacy effect on the decomposition of different SOC pools is still unclear. We conducted a 128-d laboratory incubation to investigate the DWC effects by using soils from old-field for 15 years (OF, representing active SOC), bare-fallow for 15 years (BF), and bare-fallow for 23 years plus extra 815-d incubation (BF+, representing relatively resistant SOC). The experiment included nine 10-d DWC of three treatments: 1) constant-moisture at 60% WHC, 2) mild DWC with 10-d drying to 40% WHC and rewetting to 80% WHC, and 3) strong DWC with 10-d drying to 20% WHC and rewetting to 100% WHC. Following DWC period, there was a 10-d stabilization period (adjusting all treatments to 60% WHC), and then a 28-d extended incubation. During DWC period, the strong DWC had strong effect on CO<sub>2</sub> release compared with the constant-moisture control, reducing the SOC decomposition from OF by 8% and BF by 10%, while increasing the SOC decomposition of BF+ by 16%. During extended period, both mild and strong DWC significantly increased SOC mineralization of OF, but decreased that of BF and BF+. This legacy effect compensated the changes in CO<sub>2</sub> release during DWC period, resulting in the minor response of SOC decomposition of OF and BF+ to the DWC during the entire incubation.

## The direct and legacy effects of drying-rewetting cycles on active and relatively resistant soil carbon decomposition

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**Abstract:** Global climate change is expected to increase the frequency of drought and heavy precipitation, which could create more frequent drying-rewetting cycles (DWC) in the soils. Although the DWC effects on soil organic carbon (SOC) decomposition has been widely studied, the effect of DWC and the subsequent

legacy effect on the decomposition of different SOC pools is still unclear. We conducted a 128-d laboratory incubation to investigate the DWC effects by using soils from old-field for 15 years (OF, representing active SOC), bare-fallow for 15 years (BF), and bare-fallow for 23 years plus extra 815-d incubation (BF+, representing relatively resistant SOC). The experiment included nine 10-d DWC of three treatments: 1) one mean constant-moisture at 60% water-holding capacity (WHC), 2) a mild DWC with 10-d drying to 40% WHC and immediately rewetting to 80% WHC, and 3) a strong DWC with 10-d drying to 20% WHC and immediately rewetting to 100% WHC. Following the DWC period (0-90 d), there was a 10-d stabilization period (adjusting all treatments to 60% WHC), and then a 28-d extended incubation under the constant moisture of 60% WHC. During the DWC period, the strong DWC had a strong effect on CO<sub>2</sub> release compared with the constant-moisture control, reducing the SOC decomposition from OF by 8% and BF by 10%, while increasing the SOC decomposition of BF+ by 16%. In addition, during the extended period, both mild and strong DWC significantly increased SOC mineralization of OF, but decreased that of BF and BF+. This legacy effect induced by DWC compensated the changes in CO<sub>2</sub> release during the DWC period, resulting in the minor response of SOC decomposition of OF and BF+ to the DWC during the entire incubation. Together, DWC could create both direct and legacy effects, and these effects vary with DWC intensity and SOC pools.

**Keywords** : Drying-rewetting; Soil carbon pool; Soil organic matter; Soil respiration; Legacy effect

**Abbreviations** : DWC – drying-rewetting cycles; OF – old-field; BF – bare-fallow; BF+ – bare-fallow + incubation; C – carbon; CO<sub>2</sub> – carbon dioxide; SOC – soil organic carbon; MBC – microbial biomass carbon; DOC – dissolved organic carbon; TC – total carbon; TN – total nitrogen; C/N – the ratio of carbon to nitrogen; F:B – the ratio of fungi to bacteria.

## 1. Introduction

Global climate models predict increases in atmospheric carbon dioxide (CO<sub>2</sub>) concentration and surface temperature (IPCC, 2013), which are likely to alter regimes of global hydrologic cycling, including an increase in the number and severity of droughts and heavier precipitation between drought periods (Dai, 2013; Donat et al., 2016). Soil moisture is a key factor controlling the microbial decomposition of soil organic carbon (SOC) (Moyano et al., 2013). When the soil is dry, microbial activity and substrate diffusion will be restricted, and soil respiration will be reduced (Schimel, 2018). The rapid precipitation after drought usually increases mineralization of SOC, creating a large pulse of CO<sub>2</sub> (Birch, 1958; Kim, et al., 2012). The CO<sub>2</sub> pulse has been attributed to the rapid consumption of microbial necromass as available substrates and released microbial osmoregulatory substances in response to water stress (Blazewicz et al., 2014; Chowdhury et al., 2019; Warren, 2016). In addition, the sudden influx of water at rewetting can expose previously inaccessible carbon (C) to microorganisms through aggregates breakdown (Denef et al., 2001; Najera et al., 2020; Schimel et al., 2011). This rewetting-driven CO<sub>2</sub> pulse can be sustained for more than 20 days (Canarini et al., 2017) and elevated by as much as 475% relative to a constant-moisture soil (Fierer and Schimel, 2003). Therefore, soil drying-rewetting cycles (DWC) are considered an essential environmental factor regulating C cycle in terrestrial ecosystems (Borken and Matzner, 2009; Fierer and Schimel, 2002; Muhr et al., 2008; Schimel, 2018; Zhu and Cheng, 2013).

Although a large number of studies have examined the effects of DWC on soil C decomposition, DWC increased (Butterly et al., 2009; Miller et al., 2005; Shi and Marschner, 2014; Xiang et al., 2008; Yemadje et al., 2017), decreased (Shi and Marschner, 2014), or did not change (Yemadje et al., 2017) cumulative C decomposition relative to constant-moisture control in previous studies. Zhang et al. (2020) indicated in a recent meta-analysis that relative to a constant-moisture control (with the same mean value), the rewetting-driven CO<sub>2</sub> pulse can fully compensate the reduced CO<sub>2</sub> emission during the drying phase, thus DWC did not change cumulative C decomposition. They also commented that the changes in cumulative C loss depend on DWC intensity. Intensified drought usually triggers a stronger CO<sub>2</sub> pulse after rewetting (Barnard et al., 2015; Li et al., 2018), because a severer drought may lead to a higher frequency of microbial death and accumulate more compatible solutes, which can contribute largely to the CO<sub>2</sub> pulse (Barnard et al., 2020; Guo et al., 2012). Moreover, there may be a drought threshold, beyond which the inaccessible C

will be accessed, thereby contributing to the subsequent CO<sub>2</sub> pulse (Canarini et al., 2017; Homyak et al., 2018). Moreover, DWC can promote the release of old C that had been occluded in soils for more than 600 years (Schimel et al., 2011). However, due to the difficulties in separation of decomposition of SOC pools with different turnover times, few studies have evaluated the vulnerability of different SOC pools to DWC. Therefore, due to the large proportion of stable C in the total soil C pool, clarifying its response to climate change is essential for predicting future changes in the global C cycle.

Soil organic carbon can be divided into three pools with different physiochemical characteristics and turnover times (Davidson and Janssens, 2006; Lin et al., 2015). The most active fraction is annually cycling, active SOC, which mainly consists of microbial biomass and plant detritus, and has a fast turnover time from weeks to years, contributing to approximately 0~5% of the total SOC pool (Parton et al., 1987; Semenov et al., 2018). It hardly contributes much to the global C cycle due to its small size and very fast turnover time. The most inert fraction is millennially cycling, passive (inert) SOC, which is mainly composed of black carbon or humic substances and has a turnover time even more than centuries, accounting for about 10~40% of the total SOC pool (Schmidt et al., 2011). Furthermore, the more critical pool is the decadal cycling, relatively resistant (intermediate, slow) SOC, which is the dominant component of the total SOC pool, accounting for around 60~80% of the total SOC pool, and has a turnover time of decades. It is generally believed that different microbial communities use different soil substrates (Berg and McClaugherty, 2008; Xu et al., 2015), and bacteria are considered more responsible for consuming readily available substrates (Moore-Kucera and Dick, 2008), while fungi are believed to have the ability to decompose more stable SOC (Xu et al., 2015). Fungi display an overall higher resistance to drying and subsequent rewetting processes than bacteria (Barnard et al., 2015; Yuste et al., 2011). As a consequence, relatively resistant SOC may be more susceptible to DWC due to the higher resistance of fungi to drought and thus the smaller reduction in C decomposition during the drying process (de Vries et al., 2018; Zhang et al., 2020).

Furthermore, previous conditions can affect current processes, which is called the legacy effect (Monger et al., 2015). The DWC-driven legacy effect can compensate for 14% of the decrease of cumulative C decomposition during the DWC period (Li et al., 2018). However, the legacy effect of DWC on the decomposition of different SOC pools remains unclear. Thus, the direct and legacy effects of DWC on SOC pools with different turnover times remain uncertain and require further investigation.

To fill this knowledge gap, we use soils from three plots in a long-term experimental field to explore the direct and legacy effects of DWC on active and relatively resistant soil C decomposition. In our study, the direct effect represents the effect of DWC on C decomposition in the DWC period, and the legacy effect denotes the effect of previous DWC on C decomposition in the post-DWC incubation period with constant moisture. We conducted a laboratory incubation experiment of 128 days, combining three water regimes, nine 10-d DWC (in a row) and one 28-d extended period, to investigate the direct and legacy effects of DWC on active and relatively resistant soil C decomposition. We hypothesized that (i) compared to the constant-moisture control, the DWC with intensified drought would stimulate more C emission during the entire drying-rewetting period due to the strong CO<sub>2</sub> pulse after rewetting; (ii) the relatively resistant SOC (the bare-fallow and the bare-fallow+incubation soils) would be more vulnerable to DWC than the active SOC (the old-field soil) due to the higher resistance of fungi in the relatively resistant SOC to DWC; and (iii) the DWC could create a legacy effect on soil C decomposition due to the changes in microbial biomass and labile substrates.

## 2. Materials and methods

### 2.1. Site description and soil sampling

Soils were sampled from a long-term experimental field located in the Shenyang station of Chinese Academy of Sciences, Liaoning province, Northeastern China (41°32' N, 122deg23' E). The climate is typical continental monsoon, with a mean annual temperature of 7.9degC and mean annual precipitation of 604 mm (1981-2018, <https://power.larc.nasa.gov>). The long-term old-field (OF) and bare-fallow (BF) treatments were established in 2003 and were paddy fields before treatments. The OF plot received fresh organic input for

15 years (2003-2018) with freely growing weeds but no anthropogenic interference. The BF plot was kept free of organic input for 15 years (2003-2018) by frequent hand weeding. Soils from these OF and BF treatments were sampled in 2018. Another BF plot had been kept free of organic input for 23 years until soil sampling for this study (Zhang et al., 2021). Soils were collected from the plow layer (0-20 cm depth) in the long-term experimental field. Within each plot, soils were sub-sampled at five randomly selected sites and then homogenized into one sample. Prior to the incubation experiment, soil samples were air-dried, sieved to 2 mm, and homogenized thoroughly. Visible roots, plant detritus, and stones were removed carefully by handpicking.

## 2.2. Experimental design and soil incubation

The soil from the OF treatment (15 year-old) represents active SOC. The soils from the BF treatment for 15 years and BF treatment for 23 years plus an extra 815 days of laboratory incubation (BF+, the additional incubation is to further consume active organic carbon) represent relatively resistant SOC (Zhang et al., 2017). Therefore, we refer to the OF SOC as active C, the BF and the BF+ SOC as relatively resistant C in this study. Although all three soils may still contain various components with different turnover times and protection mechanisms, here we operationally used the three soils to represent active and relatively resistant SOC (Conant et al., 2008; Townsend et al., 1997), and compared the relative vulnerability of these SOC pools to the direct and legacy effects of drying-wetting cycles for two reasons. First, we measured the proportion of active C (dissolved organic carbon/total carbon, DOC/TC) and found that the proportion of active C in the OF soil is the highest, and that in the BF+ soil is the lowest (Table 1). Second, we measured the chemical composition of SOC of these three soils and found that the molecular index of SOC stability (or resistance) in the OF soil is the lowest, and that in the BF+ soil is the highest (Fig. 2). In addition, Barre et al. (2010) constrained a three-pool-model using observed soil organic C data in long-term BF soils and found that most of the organic C are stable C (turnover time of several centuries or more). Basic properties of these three soils can be found in Table 1 and Zhang et al. (2017).

The incubation experiment consisted of a nine 10-d DWC period and a 28-d extended period, with a 10-d stabilization period between them (Fig. 1). In the DWC period (0-90 d), soils were subjected to three water regimes, including one constant-moisture control (mean moisture content: 60% WHC), one mild DWC treatment (80-40% WHC), and one strong DWC treatment (100-20% WHC), each with three replicates. After the 90-d DWC period, all treatments were adjusted to constant moisture of 60% WHC for ten days to a consistent state (90-100 d). All soils were kept at 60% WHC during the subsequent 28-d extended period (100-128 d).

For each soil, a subsample of 60 g (dry weight basis) was placed in a 1-L incubation jar at 60% WHC with deionized water, and the soils were pre-incubated at 22degC for 10 days in the dark. To achieve soil drying, 45 g and 20 g silica gel were placed in the incubation jars for 100-20% WHC and 80-40% WHC treatments, respectively (Fig. S1). These two weights were chosen because our pre-experimental data showed that these two weights of silica gel could accelerate the loss of soil moisture to the expected levels (i.e. 20% and 40% WHC) in 10 days. Inside each jar, 10 ml 0.5 M NaOH solution was used to trap CO<sub>2</sub> from SOC decomposition. At the end of each drying period, the NaOH solution and silica gel were replaced, and the soil samples were rapidly rewetted by adding deionized water with a syringe. The trapped CO<sub>2</sub>-C in the NaOH solutions were immediately measured in the form of total inorganic C using a Lotix combustion TOC analyzer (Teledyne Tekmar, USA), and the silica gel was dried in an oven at 105degC overnight to regenerate it. After replacing the silica gel and NaOH solution, the jars were sealed immediately with gas-tight lids and kept at 22degC in the dark. A previous study proved that silica gel does not absorb CO<sub>2</sub> (Harrison-Kirk et al., 2013). Three blanks without soil were incubated at the same condition to quantify the amount of C in the air and initial NaOH solution. Prior to measuring the total inorganic C, the NaOH solutions in the DWC treatments were adjusted back to 10 ml with deionized water.

After the 90-d DWC period, all soils were adjusted to a constant moisture content of 60% WHC and maintained for 10 days (stabilization period, 90-100 d). Note that we did not measure respiration for the stabilization period. On day 100, we did a destructive soil sampling. Specifically, 8 g subsamples were used

to determine dissolved organic carbon (DOC) and microbial biomass carbon (MBC), 10 g subsamples were stored at -80degC until the measurement of microbial biomass, and 10 g subsamples were incubated for another 28 d (extended period, 100-128 d) in which the CO<sub>2</sub>-C in the NaOH solutions were measured on day 103, 107, 114, 121 and 128.

In total, we incubated 30 jars ((3 x 3 + 1) x 3) consisting of three soils (OF, BF and BF+ soils), two DWC treatments (100-20% and 80-40% WHC) and one constant-moisture control treatment (60% WHC), one blank, and three replicates for each treatment.

### 2.3. Soil property analyses

Soil total C and N (nitrogen) contents were measured using an elemental analyzer (EA 3000, Euro Vector, Milan, Italy). The pH was measured in a 1:2.5 soil-water ratio with a pH meter (PHS-3C, LEICI, China). The particle size distribution was examined using the hydrometer method (Bouyoucos, 1962). The WHC was measured by placing a 100-cm<sup>3</sup> cutting ring filled with thoroughly wetted soil for 24 h on another cutting ring filled with corresponding air-dried soil and allowed to drain for 8 h.

Soil MBC content was measured using the chloroform fumigation extraction method (Vance et al., 1987). Briefly, a soil sample was divided into two sets (4 g per set), one set was fumigated with chloroform for 24 h in the dark, and another set was unfumigated. Then the fumigated and unfumigated samples were extracted with 20 ml 0.5 M K<sub>2</sub>SO<sub>4</sub> in a 1:5 ratio. The extracts were filtered through 0.45 µm syringe filter (Siyan Biotechnology, China) and were determined in the form of total organic C using a Lotix combustion TOC analyzer (Teledyne Tekmar, Mason, OH, USA). Soil MBC was calculated from the K<sub>2</sub>SO<sub>4</sub>-extractable organic C between the fumigated and unfumigated samples with a conversion factor of 0.45. Total organic C in unfumigated samples represents DOC.

### 2.4. Fourier-transform infrared spectroscopy analysis

The molecular composition of SOC was characterized by Fourier transform infrared (FTIR) spectroscopy (Thermo Fisher Scientific Nicolet iS10 spectrometer, USA) according to Demyan et al. (2012). Briefly, 2 mg air-dried ground soil samples (<0.147 mm) were pressed into 100 mg potassium bromide (KBr) pellets (64 scans within the spectral range 4000-400 cm<sup>-1</sup> at a resolution of 4 cm<sup>-1</sup> were averaged). Band maxima at wavenumbers around 2930, 2850, 1635, 1430 and 1040 cm<sup>-1</sup> were selected and were assigned to a range of organic functional groups (with limited mineral interference). The peak areas of these five bands were integrated using a tangential baseline from the onset to the offset of each peak, and their relative peak areas of each band were calculated to evaluate the assignments of soil C functional groups (Table S1). The ratio of the peak areas at 1635 and 2930 cm<sup>-1</sup> (rA1635/rA2930 ratio) was defined as the resistance index and often taken as an indicator of SOC stability (Demyan et al., 2012; Ernakovich et al., 2015; Hou et al., 2019).

### 2.5. Polymerase chain reaction (PCR) amplification

The copy numbers of fungi and bacteria were measured by PCR amplification following Xia et al. (2011). DNA was extracted from 0.5 g soil using the NucleoSpin Soil DNA extraction kit (Macherey-Nagel, Duren, Germany). DNA extracts were stored at -20. The 16s rRNA gene, a molecular marker for bacteria, was amplified using 515F/907R (forward 5'-GTGCCAGCMGCCGCGG-3'; reverse 5'-CCGTCAATTCMTTTRAGT-3'). PCRs were performed in 20 µl containing 1 µl of TB Green Fast qPCR Mix, 2.5 µl of each primer (10 µM), 1 µl of DNA template and 20 µl of molecular biology quality water. The amplifications were performed in a CFX-96 (Bio-Rad) following the thermal program: (1) 95°C for 5 min; (2) 40 cycles at 95°C for 45 s, 51°C for 45 s and 72°C for 60 s; and (3) 72°C for 5 min.

The ITS gene, a molecular marker for fungi, was amplified using ITS1F/ITS2R (forward 5'-CTTGGTCATTTAGAGGAAGTAA-3'; reverse 5'-GCTGCGTTCTTCATCGATGC-3'). PCRs were performed in 20 µl containing 1 µl of TB Green Fast qPCR Mix, 2.5 µl of each primer (10 µM), 1 µl of DNA template and 20 µl of molecular biology quality water. The amplifications were performed in a CFX-96 (Bio-Rad) following the thermal program: (1) 95°C for 5 min; (2) 40 cycles at 95°C for 45 s, 55°C for 45 s and 72°C for 60 s; and (3) 72°C for 5 min. The F:B values were calculated using the ratio of the fungal-to-bacterial gene

copy numbers. qPCR cannot provide an estimation of the F:B biomass ratio because different taxa contain an unknown number of copies of the rDNA operon in their genomes. But it provides information about the differences in the relative abundance of fungi and bacteria across soil samples (Fierer et al., 2005).

## 2.6. Statistical analysis

For all response variables, significant differences ( $P < 0.05$ ) among water regimes in each soil and significant differences among soils in each water regime were assessed by one-way analysis of variance (ANOVA). The effects of water regimes (one constant-moisture control treatment at 60% WHC and two DWC treatments of 100-20% and 80-40% WHC), soil treatments (OF, BF and BF+) and their interactions on C decomposition, fungal-to-bacterial ratio (F:B), MBC and DOC were assessed by linear mixed models (LMM). For the LMM, soil treatment and water regime were considered as fixed factors, and sample identity was regarded as a random factor. All statistical analyses were performed using IBM SPSS Statistics 20 (IBM SPSS Inc.), and graphs were drawn using SigmaPlot 14.0 (Systat Software Inc.).

## 3. Results

### 3.1. Effects of long-term field treatments on soil properties

The 15-year BF and 23-year BF+ treatments significantly decreased soil C and N contents, but did not significantly affect the C/N ratio compared with the OF treatment ( $P < 0.05$ , Table 1). Soil clay content was lower in the BF and BF+ treatments in comparison with the OF treatment, and soil silt content was higher in the BF+ treatment than that of the OF treatment. Furthermore, the BF and BF+ treatments significantly decreased soil DOC content, and the BF+ treatment significantly reduced DOC/TC ratio compared with the OF treatment. It implies lower active C (DOC) content in the BF and BF+ soils due to the long-term removal of plant inputs. Although soil pH of the BF and BF+ treatments and soil WHC of the BF+ treatment were significantly lower than those of the OF treatment, the differences among soil pH did not exceed 0.3 units and the differences among soil WHC did not exceed 1%.

### 3.2. Molecular index of SOC stability

Fourier-transform infrared (FTIR) spectroscopy of three soils generally featured common peaks but showed different intensities (Fig. S2). The rA1635 of BF+ soil was significantly lower than that of OF soil, and the order of the rA2930 of the three soils was OF > BF > BF+ ( $P < 0.05$ , Fig. 2a, b and Table S1). The order of the resistance index (ratio of rA1635/rA2930) of the three soils was BF+ > BF > OF ( $P < 0.05$ , Fig. 2c). The trend of resistance index (rA1635/rA2930) indicated that the molecular index of SOC stability (or resistance) was highest in BF+ soil, followed by BF soil, and finally OF soil.

### 3.3. Dynamics of soil carbon decomposition

During the DWC period, soil C mineralization rates declined rapidly after the first cycle (0-10 d) for all water regimes, and then decreased slowly with the increasing number of DWC (Fig. 3a-c). For OF soils, cumulative soil C mineralization in 60%, 80-40%, and 100-20% WHC treatments were 28.0, 27.1, and 25.7 mg CO<sub>2</sub>-C g<sup>-1</sup> SOC, respectively. For BF soils, cumulative soil C mineralization in 60%, 80-40%, and 100-20% WHC treatments were 24.0, 22.3, and 21.6 mg CO<sub>2</sub>-C g<sup>-1</sup> SOC, respectively. For BF+ soils, cumulative soil C mineralization in 60%, 80-40%, and 100-20% WHC treatments were 10.8, 11.9, and 12.5 mg CO<sub>2</sub>-C g<sup>-1</sup> SOC, respectively (Fig. 3d-f, Fig. 4a). Compared to the constant-moisture control treatment (60% WHC), the 100-20% WHC treatment decreased cumulative C mineralization of OF and BF soils by 8% and 10%, respectively, but increased that of BF+ soil by 16%. The 80-40% WHC treatment did not significantly change cumulative C mineralization of all three soils relative to the constant-moisture control treatment (Fig. 4a and Table S2). There was also an interactive effect of water regime and soil treatment on cumulative C mineralization during the DWC period ( $P = 0.001$ , Table S3).

During the extended period, cumulative soil C mineralization of 60%, 80-40%, and 100-20% WHC treatments in OF soil were 5.0, 5.9, and 5.8 mg CO<sub>2</sub>-C g<sup>-1</sup> SOC, respectively. For BF soil, cumulative soil C mineralization in 60%, 80-40%, and 100-20% WHC treatments were 5.8, 3.8, and 4.0 mg CO<sub>2</sub>-C g<sup>-1</sup> SOC, respectively (Fig.

4b and Table S2). For BF+ soil, cumulative soil C mineralization in 60%, 80-40%, and 100-20% WHC treatments were 4.9, 2.0, and 1.3 mg CO<sub>2</sub>-C g<sup>-1</sup> SOC, respectively (Fig. 4b and Table S2). Compared to the constant-moisture control treatment, both DWC treatments (80-40% and 100-20% WHC) increased the cumulative C mineralization in OF soil but decreased it in BF and BF+ soils. Specifically, the 80-40% WHC treatment increased C mineralization by 18% in OF soil and decreased it by 35% and 59% in BF and BF+ soils, respectively; The 100-20% WHC treatment increased C mineralization by 16% in OF soil and decreased it by 31% and 74% in BF and BF+ soils, respectively (Fig. 4b and Table S2). There was also an interactive effect of water regime and soil treatment on cumulative C mineralization during the extended period ( $P = 0.001$ , Table S3).

During the entire incubation period (0-90 d and 100-128 d), the 80-40% and 100-20% WHC treatments decreased cumulative C mineralization of BF soil by 12% and 14%, respectively, but did not significantly change cumulative C mineralization of OF and BF+ soils compared with the constant-moisture control treatment (Fig. 4c and Table S2).

### 3.4. Soil MBC, DOC, and F:B ratio

Drying-rewetting cycles had significant impacts on MBC (Fig. 5a). The 80-40% and 100-20% WHC treatments decreased the MBC content of BF and BF+ soils. The 100-20% WHC treatment increased the MBC content of OF soil (Fig. 5a). Soil DOC content did not differ among water regimes in all three soils (Fig. 5b).

Both 80-40% and 100-20% WHC treatments increased F:B ratio in the BF+ soil (Fig. 5c). For constant moisture treatment at 60% WHC, F:B ratio was lowest in the OF soil. Both MBC and DOC had a positive linear relationship with cumulative C mineralization during the extended period across the three soils (Fig. 6).

## 4. Discussion

Our results indicated that DWC had contrasting effects on the decomposition of different SOC pools. The strong DWC (100-20% WHC) increased C release from the BF+ soil, but decreased C release from the OF and BF soils, respectively. The DWC-driven legacy effect on C release can last for at least 38 d. Both the amount of microbial biomass and labile substrates contributed to this legacy effect. When considering both the extended and the DWC periods, the 80-40% and 100-20% WHC treatments had no significant effect on SOC decomposition of OF and BF+ soils, but decreased SOC decomposition of BF soil by 12% and 14%, respectively, compared with the constant-moisture control treatment. Taken together, these results demonstrate the importance of DWC in modulating SOC dynamics and show the different vulnerability of different SOC pools to DWC.

### 4.1. Direct effects of DWC on soil carbon mineralization

As we hypothesized, the strong DWC with intensified drought had a stronger impact on C emission, showing that compared to the constant-moisture control treatment (60% WHC), the 100-20% WHC treatment decreased cumulative C mineralization during the DWC period in OF and BF soils and increased it in BF+ soil, while the 80-40% WHC treatment did not significantly change the C emission of all three soils (Fig. 4a). This is because the 80-40% WHC treatment might not cause as much osmotic shock and cell lyses as the strong DWC treatment (100-20% WHC in this study) during the drying period (Guo et al., 2012). Also, the 80-40% WHC treatment might not reach the drought threshold, and the accessible C from physical and physiological mechanisms after the mild drought (to 40% WHC) may not contribute much to the subsequent CO<sub>2</sub> pulse (Barnard et al., 2020; Slessarev et al., 2020).

Although the 100-20% WHC treatment decreased cumulative C mineralization during the entire DWC period (0-90 d) in OF and BF soils, this reduction in C emission of the 100-20% WHC treatment only occurred in two cycles and did not significantly affect C mineralization in other cycles (Table S2). This result was consistent with a meta-analysis of Zhang et al. (2020), which showed that the rewetting-driven CO<sub>2</sub> pulse can fully compensate the reduced CO<sub>2</sub> emission during the drying period.

For BF+ soil, the 100-20% WHC treatment increased cumulative C mineralization by 16% relative to the mean constant-moisture control treatment (Table S2). Since few active SOC in the BF+ soil was left after 25-year BF treatment and 815-d laboratory incubation, the microbial community in the BF+ soil is more dominated by fungi (Fig. 5c). Fungi are considered more resistant to drought and osmotic stress than bacteria due to their multicellular hyphal networks allowing remote access to water in the soil (Barnard et al., 2013). Therefore, the reduction in C mineralization of the soil with more fungi (BF+ soil) during the drying period may be smaller, and the cumulative C mineralization may be higher during the whole DWC period compared to the soil dominated by bacteria (OF soil). Moreover, DWC can result in a microbial composition shift toward more desiccation tolerant. For example, sensitive microorganisms die or become inactive during drought and are difficult to recover, thereby supplying substrates for more drought-tolerant surviving microorganisms (Meisner et al., 2021).

#### 4.2. Legacy effects of DWC on soil carbon mineralization

Both 80-40% and 100-20% WHC treatments increased C mineralization in OF soil but decreased it in BF and BF+ soils compared to the mean constant-moisture control treatment during the extended period (Fig. 4b). This result indicated that the repeated DWC could cause a legacy effect on soil C mineralization, and this legacy effect can last for at least 38 d (including 10-d stabilization period and 28-d extended period). Butterly et al. (2011) implied that this legacy effect can last for more than 35 d, and Li et al. (2018) believed that after 4 times of DWC, this legacy effect can last for at least 60 d.

Compared to the mean constant-moisture treatment (60% WHC), the MBC content at the start of the extended period (100 d) was higher in the 100-20% WHC treatment for OF soil (Fig. 5a), while was lower in both 80-40% and 100-20% WHC treatments for BF and BF+ soils (Fig. 5a). Moreover, there was a positive correlation between MBC and cumulative C mineralization during the extended period across the three soils (Fig. 6a). These results suggested that the changes in microbial biomass affected the legacy effect of DWC on soil C mineralization. Additionally, DOC was also positively correlated with cumulative C mineralization during the extended period across the three soils (Fig. 6b), indicating that the amount of microbial biomass and labile substrates (i.e. DOC) both contributed to the legacy effect of DWC on soil C mineralization. Notably, the fact that both MBC and DOC were positively correlated with cumulative C mineralization during the extended period is likely due to the positive linear relationship between MBC and DOC (Fig. S4). Moreover, the DWC-driven changes of soil microbial community composition may also contribute to this legacy during the extended period (Meisner et al., 2021). Together, these results are consistent with the finding of Li et al. (2018) who showed that the legacy effect during the extended period is attributed to the amount of plant litter (as substrate) and microbial biomass.

Taking together, both mild and strong DWC treatments had a minor effect on SOC decomposition in OF and BF+ soils, respectively, but decreased SOC decomposition in BF soil by 12% and 14%, respectively (Fig. 4c). However, it should be noted that these results need to be interpreted with caution, because we did not measure the C release during the stabilization period (90-100 d) and the extended period (100-128 d, 28 days) may not be long enough. Future studies can use a longer extended period and measure microbial community composition for a more mechanistic understanding of the legacy effect of DWC on soil C release.

#### 5. Conclusion

This study explored the direct and legacy effects of DWC on active and relatively resistant SOC decomposition by using three soils from a long-term experimental field as well as an incubation experiment. Compared to the mean constant-moisture treatment (60% WHC), the strong DWC treatment (100-20% WHC) had minor effects on SOC mineralization of OF soil and BF soil in most of the cycles, but stimulated SOC mineralization of BF+ soil. This result suggests that the relatively resistant SOC is more vulnerable to DWC than active SOC during the DWC period (0-90 d). Moreover, the DWC had a legacy effect on SOC decomposition during the extended period (100-128 d), being positive for active SOC but negative for relatively resistant SOC. Both MBC and DOC may contribute to this legacy effect. However, when both direct effect and legacy effect were considered, the strong DWC did not significantly affect the cumulative SOC decomposition of OF and

BF+ soils, but had a negative effect on that of BF soil during the entire incubation period. Taken together, our study demonstrated that DWC could create both direct and legacy effects on SOC decomposition, and these effects vary with DWC intensity and SOC pools. These findings suggest that more frequent alternating drought and rapid precipitation in the future may have a minor or even negative effect on soil C loss.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Table 1** Physical and chemical properties of the soils. Values represent means of three replicates with standard deviations in parenthesis. Means within each row followed by different letters represent a significant difference ( $P < 0.05$ ). SOC, soil organic carbon; WHC, water-holding capacity; DOC, dissolved organic carbon. OF, old-field; BF, bare-fallow; BF+, bare-fallow+incubation. These soils contain little inorganic carbon and soil total C is dominated by SOC.

Parameters	OF	BF	BF+
Total C (%)	2.25 (0.26) a	1.30 (0.05) b	1.05 (0.01) b
Total N (%)	0.17 (0.00) a	0.11 (0.00) b	0.08 (0.00) c
C/N	13.6 (1.6) a	12.3 (0.4) a	13.3 (0.4) a
pH <sub>H2O</sub>	6.74 (0.01) a	6.50 (0.03) b	6.52 (0.02) b
Sand (%)	49.1 (3.3) a	50.1 (1.5) a	46.1 (1.1) a
Silt (%)	31.8 (2.8) a	36.0 (2.2) ab	40.1 (1.1) b
Clay (%)	19.1 (1.2) a	13.9 (1.0) b	13.9 (0.3) b
WHC (%)	32.7 (0.3) a	32.4 (0.1) ab	31.7 (0.3) b
DOC (mg C kg <sup>-1</sup> soil)	187.4 (5.5) a	96.4 (5.7) b	72.0 (2.7) c
DOC/TC (%)	0.84 (0.11) a	0.74 (0.04) ab	0.69 (0.03) b









