

The influence of drought intensity on soil respiration during and after multiple drying-rewetting cycles



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ABSTRACT

Global climate change is projected to intensify soil drying-rewetting (DRW) events with extended drought, especially in arid and semiarid ecosystems. However, the extent to which the soil DRW with intensified drought can alter soil respiration (R_s) in forests is still under debate, and subsequent legacy effects on R_s are not well understood. Here, we conducted a 180-d soil incubation experiment to investigate how soil DRW with different drought intensities alter the R_s in poplar (*Populus simonii*) and Mongolian pine (*Pinus sylvestris* var. *mongolica*) plantations. The incubation experiment included four 30-d cycles of 1) constant moisture treatment (control), 2) DRW with 10-d drying and 20-d rewetting (DRW₁₀₋₂₀) or 3) DRW with 20-d drying and 10-d rewetting (DRW₂₀₋₁₀), and then an extended 60-d incubation under constant moisture. During the four DRW cycles, the direct C release with respiration of Mongolian pine soils (27 g C m⁻² in DRW₁₀₋₂₀ and 140 g C m⁻² in DRW₂₀₋₁₀, respectively) decreased to a much lower extent than that of poplar soils (228 g C m⁻² in DRW₁₀₋₂₀ and 498 g C m⁻² in DRW₂₀₋₁₀, respectively). R_s did not significantly change during the extended 60-d incubation in the DRW₁₀₋₂₀ treatment compared to control treatment. However, the respired CO₂ were increased by 68 g C m⁻² in the poplar soils and 19 g C m⁻² in the Mongolian pine soils in the DRW₂₀₋₁₀ treatment, which approximately compensated for 14% of the decreases of total respiration during four DRW cycles. This legacy effect induced by the DRW with intensified drought was attributed to the higher amount of remaining substrates and soil microbial biomass. Our study highlights that DRW can cause both direct and legacy effects on R_s , but the effects vary with drought intensity and forest type.

1. Introduction

Soil water is one of the most important factors regulating carbon cycles of terrestrial ecosystems (Moyano et al., 2013; Wu et al., 2010). Extreme rainfall and drought following longer intervals between rainfall events have been forecast to increase as a consequence of human-induced global warming (Knapp et al., 2008; Min et al., 2011). Considering the changes of global hydrological cycle, soil drying and rewetting (DRW) cycle is expected to have intensified drought in future (Evans and Wallenstein, 2012; Muhr et al., 2010). Furthermore, soil DRW cycle is especially frequent in the semi-arid region with irregular rainfall events, high temperature, and dry climate conditions (Austin et al., 2004; Chatterjee and Jenerette, 2011; Fierer and Schimel, 2002;

Wang et al., 2014).

Because soil respiration (R_s) is the second largest source of terrestrial carbon flux (Bond-Lamberty and Thomson, 2010), it is critical to quantify how R_s responds to soil drying and rewetting. Soil drought can decrease R_s (Muhr et al., 2010; Shi and Marschner, 2015) by reducing soil substrate diffusion and accessibility for microorganisms (Voroney and Heck, 2015) and by causing the dormancy or death of soil microorganisms (Pulleman and Tietema, 1999). Conversely, the rewetting of dry soils will lead to pulses of R_s , the so-called “Birch effect” (Kim et al., 2012; Xu et al., 2004). Therefore, soil drying and rewetting is considered as one of the most important environmental factors that regulate soil carbon balance in grassland (Fierer and Schimel, 2002; Wu et al., 2010), cropland (Beare et al., 2009) and forest (Muhr et al., 2010,

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2008; Pulleman and Tietema, 1999) ecosystems (Moyano et al., 2013). Although numerous studies have been conducted on the impacts of soil DRW on R_s , whether cumulative soil CO_2 loss increases or decreases with DRW is still under debate (Baumann and Marschner, 2013; Beare et al., 2009; Butterly et al., 2010; Guo et al., 2012; Harrison-Kirk et al., 2013; Jin et al., 2013; Miller et al., 2005; Shi and Marschner, 2013).

The changes in cumulative soil CO_2 loss are believed to depend on the frequency and drought intensity of soil DRW (Borken and Matzner, 2009; Fierer and Schimel, 2002; Muhr et al., 2010) and to vary by ecosystem type (Sawada et al., 2016). The impacts of soil DRW frequency on respiration have been well evaluated (Fierer and Schimel, 2002; Wu and Brookes, 2005; Xiang et al., 2008). Total CO_2 loss increased with the number of DRW cycles in oak forest soils but not in grassland soils (Fierer and Schimel, 2002). The cumulative CO_2 loss in one cycle decreased with the increasing numbers of DRW cycles for a loamy sand soil (Yu et al., 2014), and the pulse of R_s decreased upon frequent rewetting (Baumann and Marschner, 2013; Butterly et al., 2009; Shi and Marschner, 2015). Currently, the effects of intensified DRW on cumulative soil CO_2 loss are not yet well studied. Soil bacterial growth upon rewetting could be changed by the intensified drought, thereby altering the magnitude of respiration pulses and total CO_2 loss (Meisner et al., 2015). Furthermore, although the legacy effects (Monger et al., 2015) of drought on the soil carbon cycle are relatively well recognized (Anderegg et al., 2015; Göransson et al., 2013; Rousk et al., 2013), there is little information available on the legacy effects of DRW on total CO_2 loss, especially regarding the intensified drought. Thus, the direct and legacy effects of DRW on R_s remain unclear, and it is necessary to study the changes in R_s during each DRW cycle and after the DRW cycles cease.

In addition, most previous incubation experiments used litter-free soil, which might result in an underestimation of R_s (Butterly et al., 2010; Chen et al., 2016; Fierer and Schimel, 2002; Meisner et al., 2015; Muhr et al., 2010, 2008; Shi et al., 2015; Xiang et al., 2008). Most previous studies also used sieved or ground soil, which might lead to the disruption of soil aggregates and the release of extra CO_2 (Birch, 1958; Butterly et al., 2009; Fierer and Schimel, 2002; Sun et al., 2017; Xiang et al., 2008). Therefore, studies using undisturbed soil columns with litter cover are needed to avoid substrate limitation during DRW cycles and to better simulate the litter-soil system.

Here, we aim to study the direct and legacy effects of soil DRW on R_s . The direct effect, in our study, indicates the impacts of current DRW cycles on R_s ; the legacy effect (Monger et al., 2015) indicates the impacts of previous DRW cycles on R_s . Soil samples were collected from poplar (*Populus simonii* Carr.) and Mongolian pine (*Pinus sylvestris* L. var. *mongolica* Litv.) plantations. Both plantation types have been widely used for afforestation in Horqin Sandy Land in northeastern China since 1978 (Duan et al., 2011), and are facing changes in precipitation patterns, leading to extended summer droughts (Jiang et al., 2009). We conducted an incubation experiment of 180 days, combining four 30-d DRW cycles and one 60-d extended period, to study the respective direct and legacy effects of DRW on R_s and cumulative CO_2 loss. We analyzed R_s , soil microbial biomass C (MBC), and soil chemical properties in intact soil columns of the poplar and Mongolian pine plantations. We hypothesized that (1) the DRW with intensified drought would lead to a greater reduction in CO_2 emissions during the drying period, but there would be a greater amount of CO_2 emitted during the rewetting period; (2) the frequent and intensified DRW would lead to gradual decline of R_s due to a legacy effects.

2. Materials and methods

2.1. Site description

The study site is located at Daqinggou Ecological Station (42°58' N, 122°21' E; 260 m above sea level) of the Institute of Applied Ecology, Chinese Academy of Sciences, in the southeastern Horqin Sandy Lands,

the largest sandy land area in China. This region has a typical temperate and continental climate. The local mean annual precipitation is 450 mm, the mean annual evaporation is 1780 mm, and the mean annual frost-free period is approximately 154 days. The mean annual temperature is 6.4 °C, with the lowest monthly mean temperature occurring in January (−12.5 °C) and the highest in July (23.8 °C). The soil is classified into the Entisol order, Semiaripsamment group (according to the United States Soil Classification System), with 90.9% sand, 5.0% silt, and 4.1% clay; the soil developed from sandy parent material through wind erosion (Zhenghu et al., 2007).

2.2. Experimental design

In this study, we set up one poplar plantation stand (13 years old) and one Mongolian pine plantation stand (32 years old) at the Daqinggou Ecological Station. The tree density in the poplar plantation was 688 tree·hm^{−1}, the mean diameter at breast height (DBH) was 10.97 cm, and the mean tree height (MTH) was 12.5 m. The tree density in the Mongolian pine plantation was 896 tree·hm^{−2}, the DBH was 14.26 cm, and the MTH was 6.7 m. In November 2014, leaf litter was collected using a litter trap for co-incubation with soils. Then leaf litter was air-dried and stored for later incubation experiments. Three subsamples were oven dried at 65 °C for 48 h and then finely ground for the measurement of C, N, P, lignin, and total phenol concentration (Table S1).

Forty-eight mineral soil columns were collected in September 2015 in each stand. First, the forest floor litter was carefully removed from the surface mineral soil. Then, polyacrylic cylinders (11 cm diameter, 15 cm height) were inserted into the mineral soil to a depth of 10 cm. Subsequently, the undisturbed soil columns were carefully dug out and sealed with plastic caps. The soil columns were immediately transported to the laboratory and stored at 4 °C for one week before the experiment was started. Three soil columns per stand were randomly selected, and the soil was removed for the measurement of initial soil properties, including C, N, P and bulk density (Table S1).

The remaining 45 soil columns each from the poplar and Mongolian pine plantations were divided into 3 groups, each consisting of 15 soil columns. The first group was used as a control by keeping constant moisture conditions at 60% water holding capacity (WHC) (−50 kPa for poplar soil, −52 kPa for Mongolian pine soil). The second group was subjected to DRW with 10-d drying and 20-d constant moist in each cycle (DRW₁₀₋₂₀). The third group was subjected to DRW with an intensified 20-d drying and 10-d constant moist in each cycle (DRW₂₀₋₁₀).

For incubation, leaf litter (3 g dry weight) of poplar or Mongolian pine was saturated with water for 12 h and carefully placed on the surface of the soil column in each cylinder with tweezers. Then, the cylinders were pre-incubated for 10 days at soil moisture of 60% WHC and air temperature of 25 °C to ensure stable R_s . For the drying stage of each DRW cycle, the lids of the cylinders were removed, and the soils were air-dried for 12 h each day. For the rewetting stage, soil columns were rewetted to a soil moisture of 60% WHC by adding distilled water within 1 h and the constant moisture were maintained. To maintain constant soil moisture of 60% WHC throughout the entire experimental period, soil cylinders were weighed and the distilled water were added each day. After four 30-days DRW cycles, the soil columns in each group were further incubated for 60 days under constant moist condition (60% WHC) (Fig. 1).

2.3. Measurement of R_s and soil chemical properties

R_s ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was measured using a portable LI-8100 (LI-COR Inc., Lincoln, NE, USA) every other day during the pre-incubation period (−10 to 0 d), daily during the DRW period (1–120 d), and less frequently during the extended incubation period, i.e., on the 131st, 135th, 150th, 165th and 180th days. Moreover, R_s was measured 5 h after the soil columns were rewetted in each DRW cycle. In each day the lids

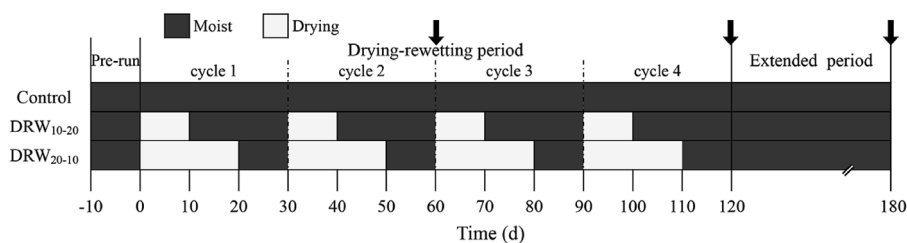


Fig. 1. A schematic overview of the experimental design showing the soil drying-rewetting treatments. Down arrows (\downarrow) indicate the time for soil sampling. Control: constant moisture treatment. DRW₁₀₋₂₀: DRW with 10-d drying and 20-d rewetting (DRW₁₀₋₂₀). DRW₂₀₋₁₀: DRW with 20-d drying and 10-d rewetting.

were removed from the soil columns for 15 min to allow air exchange before measurement, and then one chamber was placed on the soil column for 1 min to measure the increase in soil CO₂ concentration. During the pre-incubation and extended periods, the cylinders were also uncovered for 15 min every day to allow air exchange even when R_s was not measured, thereby preventing the high accumulation of CO₂ in the headspace and potential anoxia.

Soils were destructively sampled on the 60th, 120th, and 180th days (five soil columns per date) and then stored at 4 °C within one week before measurements of soil MBC, dissolved organic C (DOC) and soil inorganic nitrogen (SIN, NH₄⁺-N + NO₃⁻-N). Soil MBC was estimated using the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, three portions of soils were fumigated with chloroform for 24 h and another three replicates were unfumigated. Then the fumigated and unfumigated samples (25 g dry weight) were extracted with 100 mL 0.5 mol L⁻¹ K₂SO₄ for 30 min. The extracts were filtered and the dissolved organic carbon was determined using a Shimadzu TOC-V combustion analyzer (Shimadzu Corporation, Kyoto, Japan). Soil MBC was calculated from the dissolved organic carbon between fumigated and unfumigated samples, and the dissolved organic carbon levels in unfumigated samples were used as soil DOC. Soil samples (20 g) were extracted with 50 mL 2 mol L⁻¹ KCl, and then soil NH₄⁺-N and NO₃⁻-N were analyzed using a continuous-flow autoanalyzer (AutoAnalyzer III, Bran + Luebbe GmbH, Germany).

Total soil organic carbon (TOC) was determined via the K₂Cr₂O₇-H₂SO₄ oxidation method (Walkley and Black, 1934). Total nitrogen (TN) and total phosphorus (TP) were digested with 98% H₂SO₄ and were then measured using a continuous-flow autoanalyzer (AutoAnalyzer III, Bran + Luebbe GmbH, Germany). Litter lignin was analyzed using a modified acetyl bromide method (Iiyama and Wallis, 1990; Zhao et al., 2013). Briefly, 6 mg of litter samples were digested with 25% acetyl bromide dissolved in 5 mL acetic acid and 0.2 mL 70% HClO₄, then were incubated at 70 °C for 30 min in water bath. Lignin concentration in the solution was measured using a UV spectrophotometry at 280 nm. The total phenol concentration in litter samples were determined using the Folin-Ciocalteu method (Waterman and Mole, 1994). The total phenol in leaf litter was extracted with 60% methanol, 75 g L⁻¹ Na₂CO₃ and 0.5 mL Folin-Ciocalteu reagent, and was then measured by UV spectrophotometry at 760 nm.

2.4. Data calculation and statistical analyses

The cumulative CO₂ loss (g C m⁻²) was calculated by a linear interpolation method (Liu et al., 2009):

$$\text{Cumulative CO}_2 \text{ loss} = \sum_{i=0}^n R_i T_i$$

where n is the number of incubation days, R_i is the mean respiration rate in g CO₂ m⁻² h⁻¹ between two successive respiration measurements, T_i is the hours between two successive respiration measurements.

Data were analyzed using SPSS[®] 19.0 software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was conducted to test

the differences between the two plantations in the initial litter or soil properties. Effects of soil DRW treatments on all indicators were analyzed in poplar plantation and Mongolian pine plantation, respectively, considering the dependent differences in the initial soil and litter in the two plantations. Effects of soil DRW treatments on soil water content at the end of the drying periods, and average values of R_s during constant moisture period across the four cycles, and soil MBC, DOC and SIN, and cumulative CO₂ loss were tested using one-way ANOVA. Fisher's least significant difference (LSD) test was used for multiple comparisons among treatments if the data were homogeneous; alternatively, Tamhane's analysis was performed for heterogeneous data. Significance for all statistical analyses was accepted at the level of $p < 0.05$.

3. Results

3.1. Initial properties of leaf litter and soil

The initial properties of the leaf litter and mineral soil (0–10 cm) were different between the poplar and Mongolian pine plantations except for soil bulk density ($p = 0.26$) and litter N:P ($p = 0.06$) (Table S1). Leaf litter C, lignin and total phenol concentrations and the C:N, lignin:N and phenol:N ratios for the poplar plantations were significantly lower than those for the Mongolian pine plantations. However, leaf litter N and P concentrations were significantly higher for the poplar than for the Mongolian pine plantations. The TOC, TN, and TP were higher but the C:N and N:P ratios were lower in the poplar soils in comparison with the Mongolian pine soils.

3.2. Soil water content and respiration rate

The soil water content decreased gradually during the drying periods (Fig. S1). There was no difference in the water content at the end of the drying periods across the four DRW cycles for either poplar or Mongolian pine soils ($p > 0.05$). In the DRW₁₀₋₂₀ treatment, the average soil water content decreased from 60% WHC to an average of 36% WHC in poplar soils (equated to soil matric potentials decreasing from -50 kPa to -69 kPa) and to an average of 18% WHC for Mongolian pine soils (equated to soil matric potentials decreasing from -52 kPa to -107 kPa) at the end of the four drying periods. The intensified drought in DRW₂₀₋₁₀ treatment decreased the average soil water content to 24% WHC in poplar soils (-88 kPa) and 8% WHC (-213 kPa) in Mongolian pine soils.

The R_s during the pre-incubation period was not significantly different across treatments for either poplar or Mongolian pine soils (Fig. 2). There was a general decreasing trend of R_s in the control during the entire experimental period in poplar soils, but not for Mongolian pine soils. R_s decreased very rapidly during the first day of the drying period, and the average R_s values decreased by 72% and 58% in poplar and Mongolian pine soils, respectively. Thereafter, R_s exhibited a gradually slower decline. Although there was no significant correlation between R_s and the soil water content during the entire experimental period, the water content was significantly correlated to R_s during the drying period (Fig. S2).

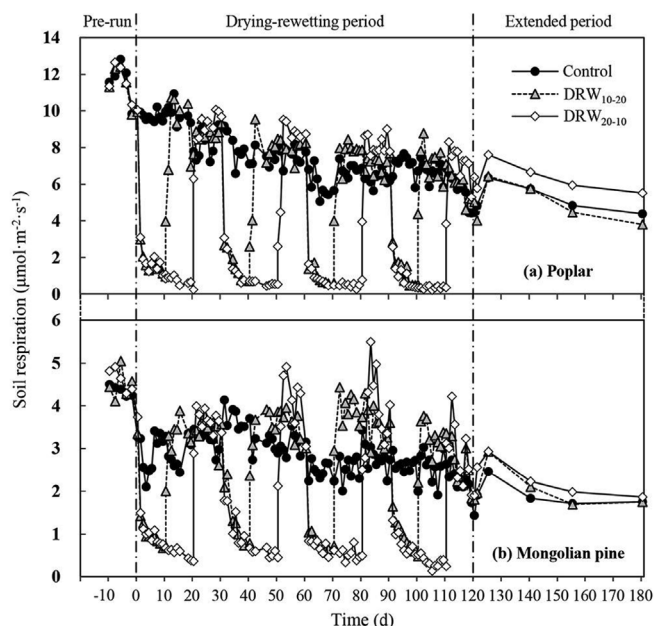


Fig. 2. Dynamics of R_s rates in (a) poplar and (b) Mongolian pine plantation soils during the whole incubation period. Control: constant moisture treatment. DRW₁₀₋₂₀: DRW with 10-d drying and 20-d rewetting (DRW₁₀₋₂₀). DRW₂₀₋₁₀: DRW with 20-d drying and 10-d rewetting.

We observed a rapid increase in R_s after the rewetting of dry soil (Fig. 2). The R_s of the DRW₁₀₋₂₀ and DRW₂₀₋₁₀ groups reached and even exceeded the level of the control treatment during the first two days of the constant moisture period after rewetting. Compared to the control, DRW₂₀₋₁₀ had higher mean values of R_s during the 2nd ($p = 0.042$), 3rd ($p = 0.014$) and 4th ($p = 0.046$) constant moisture periods. For the Mongolian pine soils, the mean values of R_s in both DRW₁₀₋₂₀ and DRW₂₀₋₁₀ were significantly higher than those in the control during the 2nd, 3rd and 4th constant moisture periods (all $p < 0.05$, Fig. S3b). During the extended period, the average of R_s in DRW₂₀₋₁₀ was higher than that in both control and DRW₁₀₋₂₀ treatments in poplar plantation soils ($p = 0.031$), but not for Mongolian pine soils ($p = 0.104$).

3.3. Cumulative CO₂ loss

Cumulative CO₂ loss during the pre-incubation period did not differ across treatments in either poplar or Mongolian pine soils (Table 1). However, soil DRW had significant effects on the cumulative CO₂ loss

Table 1
Cumulative CO₂ loss (g C m⁻²) during different periods among treatments in poplar and Mongolian pine plantation soils.

Forest type	Treatment	Pre-incubation period	Cycle 1	Cycle 2	Cycle 3	Cycle 4	DRW period	Extended period	Total incubation
Poplar	^a Control	119(5) a	287(28) a	233(9) a	198(10) a	200(17) a	919(61) a	324(27) ab	1362(88) a
	DRW ₁₀₋₂₀	118(4) a	207(14) b	172(7) b	163(5) b	150(5) b	691(28) b	308(21) b	1117(49) b
	DRW ₂₀₋₁₀	119(7) a	124(4) c	109(3) c	100(6) c	89(3) c	421(13) c	392(22) a	933(34) b
	One-way ANOVA								
	F values	0.02	18.52**	83.89**	40.39**	28.90**	39.02**	4.54*	13.62*
Mongolian pine	Control	44(4) a	94(4) a	99(3) a	81(2) a	71(1) a	351(5) a	117(9) a	512(11) a
	DRW ₁₀₋₂₀	45(3) a	81(5) b	85(4) b	86(4) a	72(4) a	324(13) a	125(8) a	494(21) a
	DRW ₂₀₋₁₀	47(2) a	55(3) c	60(2) c	54(1) b	42(2) b	211(4) b	136(5) a	394(7) b
	One-way ANOVA								
	F values	0.294	27.09**	40.48**	39.05**	46.40**	63.55*	1.61	21.39**

The values are mean ($n = 5$) with standard error (SE) in parentheses.

The Different lowercase letters indicate significant differences between treatments in poplar and Mongolian pine plantation at a level of $p < 0.05$, respectively. * and ** indicate significant treatment effect at a level of $p < 0.05$ and $p < 0.001$, respectively. The numerator and denominator degrees of freedom for each One-way ANOVA are 2 and 12, respectively.

^a Control, constant moist conditions at 60% water holding capacity; DRW₁₀₋₂₀, 30-d soil drying and rewetting cycle with 10-d drying and 20-d constant moist; DRW₂₀₋₁₀, 30-d soil drying and rewetting cycle with 20-d drying and 10-d constant moist.

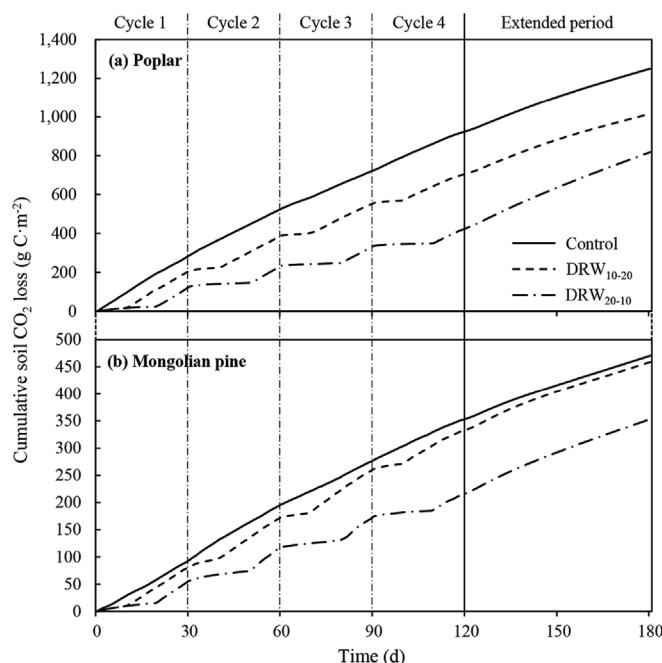


Fig. 3. The cumulative soil CO₂ loss in (a) poplar and (b) Mongolian pine plantations during incubation. DRW₁₀₋₂₀: DRW with 10-d drying and 20-d rewetting (DRW₁₀₋₂₀). DRW₂₀₋₁₀: DRW with 20-d drying and 10-d rewetting.

(Fig. 3). For poplar soils, the cumulative CO₂ loss in each of the four cycles decreased in the order of control > DRW₁₀₋₂₀ > DRW₂₀₋₁₀. For Mongolian pine soils, the cumulative CO₂ loss also decreased in the order of control > DRW₁₀₋₂₀ > DRW₂₀₋₁₀ during the 1st and 2nd cycle, but there was no difference in soil cumulative CO₂ loss between the control and DRW₁₀₋₂₀ during the 3rd and 4th cycle. There were clear decreasing trends of the 30-d soil CO₂ loss with increasing cycle number in poplar soils, but not for the Mongolian pine soils. The cumulative CO₂ loss during the extended period was higher in DRW₂₀₋₁₀ compared to the control and DRW₁₀₋₂₀ in poplar soils ($p = 0.035$, $p = 0.027$, respectively), but there were no differences in soil cumulative CO₂ loss among three treatments in Mongolian pine soils ($p = 0.24$), and between control and DRW₁₀₋₂₀ in poplar plantation soils.

3.4. Soil microbial biomass carbon, dissolved organic carbon and inorganic nitrogen

Soil MBC did not differ among treatments during the DRW period

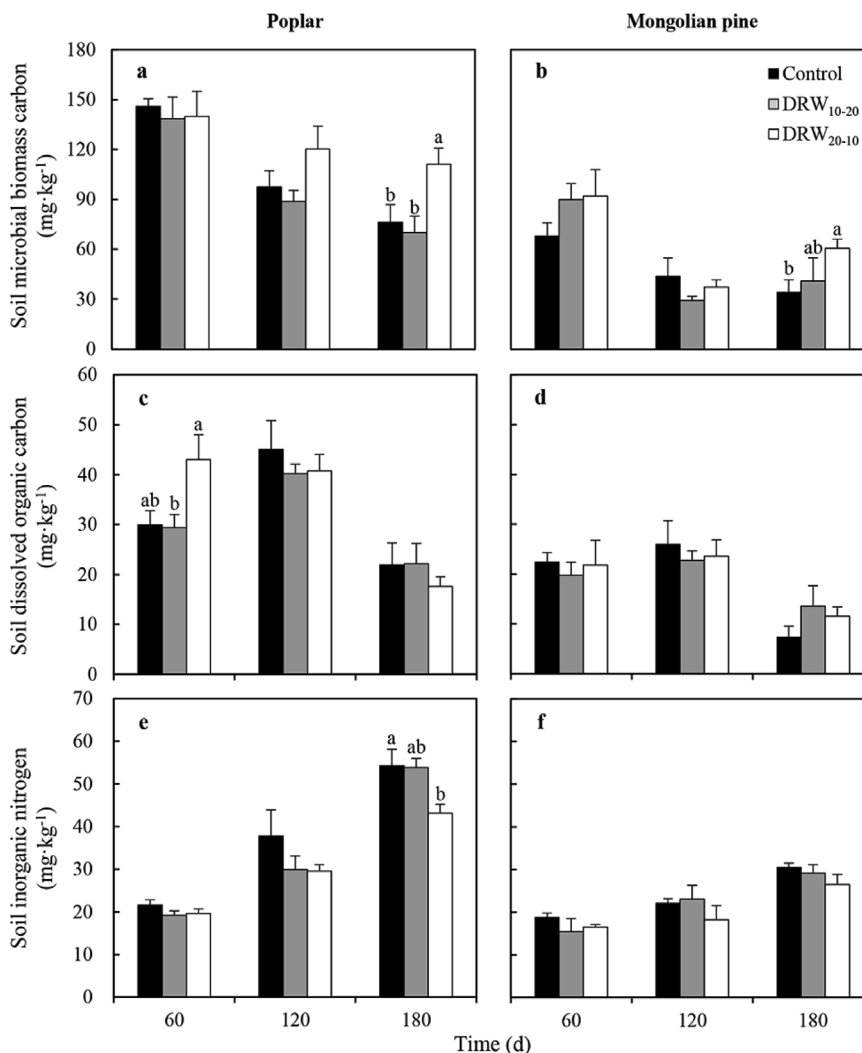


Fig. 4. Changes of soil microbial biomass carbon (a, b), soil dissolved organic carbon (c, d), and soil inorganic nitrogen (e, f) among control, DRW₁₀₋₂₀ and DRW₂₀₋₁₀ treatments in poplar and Mongolian pine plantations. The bars displayed mean ($n = 5$) and error bar presented standard error. Control: constant moisture treatment. DRW₁₀₋₂₀: DRW with 10-d drying and 20-d rewetting (DRW₁₀₋₂₀). DRW₂₀₋₁₀: DRW with 20-d drying and 10-d rewetting.

(all $p > 0.05$), but soil MBC in DRW₂₀₋₁₀ was higher than those in control and DRW₂₀₋₁₀ treatments in both poplar and Mongolian pine soils at the end of the extended period (Fig. 4a and b). Soil DOC was higher in DRW₂₀₋₁₀ compared to DRW₁₀₋₂₀ on the 60th day ($p < 0.05$), and SIN was lower in DRW₂₀₋₁₀ than in control treatment at the end of the extended period ($p = 0.04$) in poplar soils (Fig. 4c, e). For the Mongolian pine soils, the DOC and SIN concentrations were not different among the three treatments across the entire incubation period (Fig. 4d, f).

4. Discussion

4.1. Direct effects of DRW on R_s

Our results showed that R_s decreased immediately and strongly after one day of soil drying and then subsequently decreased continuously in response to drying. This pattern is consistent with findings in previous studies (Borken et al., 2006; Muhr et al., 2008; Schindlbacher et al., 2012). Positive correlations of R_s and soil water contents were observed during the drying periods (Fig. S2), indicating that soil moisture is one of the most important environmental factors that influence soil microbial activities (Blazewicz et al., 2014; Brockett et al., 2012; Cook and Orchard, 2008; Pulleman and Tietema, 1999). Soil microorganisms can

become dormant, inactive or even die due to soil drought (Bottner, 1985; De Nobili et al., 2006; Parr et al., 1981), leading to a reduction of R_s . However, R_s had a much faster decline rate at the beginning of the drying period, and a further reduction in the water content in the intensified DRW did not result in significant decreases in R_s . It suggests that soil moisture could not fully explain the observed R_s . In addition to soil moisture, other factor(s) such as soil substrates limitation due to the reduction of diffusion with drought may have contributed to the reduction of R_s during the drying periods (Xiang et al., 2008). Studies using litter-free soil incubation showed much slower decreases in R_s during the drying period compared to those observed in our study (Sawada et al., 2016; Xiang et al., 2008). The faster dehydration of leaf litter than mineral soil in columns may have played a key role in the reduction of R_s during the early drought period (Muhr et al., 2010).

The pulse of soil CO₂ release after the rewetting of dry soil has been widely observed in laboratory incubation experiments and in field observations (Muhr et al., 2010, 2008; Pulleman and Tietema, 1999; Wu and Brookes, 2005). Several mechanisms have been proposed to explain the increases in soil respiration upon soil rewetted (Xiang et al., 2008). In general, rewetting can cause the disintegration of soil aggregates, and thus soil organic substrates are released from soil particles and become accessible to soil microorganisms (Cosentino et al., 2006; Schimel et al., 2011). On the other hand, in order to acclimate to the

rapid change of soil water potential upon rewetting, soil microorganism can quickly consume the high solute concentration accumulated during drying period, leading towards a pronounced pulse of CO₂ (Fierer and Schimel, 2003; Parr et al., 1981; Schimel et al., 2007). The higher mean values of R_s during moist periods were generally observed in DRW treatments compared to the control treatment in both poplar and Mongolian pine soils (Fig. S3), indicating that the Birch effect did result in a greater loss of soil carbon compared to the control without DRW cycle. Furthermore, the higher average of R_s in DRW₂₀₋₁₀ suggested that intensified DRW will lead to stronger pulses of R_s . This result supports our first hypothesis that there would be a greater amount of CO₂ emission induced by intensified drought after rewetting.

Soil cumulative CO₂ loss did not differ significantly between the control ($351 \pm 5 \text{ g C m}^{-2}$) and DRW₁₀₋₂₀ treatment ($324 \pm 13 \text{ g C m}^{-2}$) in the Mongolian pine soils during the four DRW cycles (Table 1). Similarly, a meta-analysis conducted by Canarini et al. (2017) showed that cumulative soil CO₂ loss did not significantly differ between the control and DRW treatment, as the decrease in R_s during the drying period can be completely compensated for by the R_s pulse after rewetting. However, compared to the control, the DRW₁₀₋₂₀ treatment significantly decreased cumulative CO₂ loss by 228 g C m^{-2} in the poplar soils, and the DW2 treatment significantly decreased cumulative CO₂ loss by 498 g C m^{-2} and 228 g C m^{-2} in the poplar and Mongolian pine soils, respectively, in the four DRW cycles. This result supports our first hypothesis that the intensified DRW could decrease soil CO₂ loss due to lower R_s during drying periods.

4.2. Legacy effects of DRW on R_s

There was no significant difference in mean values of R_s rate during constant moisture period among treatments in the first DRW cycle. However, we observed the higher pulses and mean values of R_s rate during rewetting periods compared to the control in the cycle 2, cycle 3 and cycle 4 (Fig. 2; Fig. S3), indicating that the repeated DRW could cause a legacy effect on R_s . However, Fierer and Schimel (2002) and Shi et al. (2015) found that R_s was higher in the first DRW cycle compared to constant moisture treatment after rewetting. The inconsistent result might be due to the sieved soils used in these previous studies. Another comparative experiment conducted by Navarro-García et al. (2012) compared the different responses of R_s to DRW between sieved soils and intact soils, which suggested a slower response of R_s to DRW treatments due to the non-destroyed soil structure in the intact soils.

During the extended period under the same soil moisture among three treatments, the intensified DRW had consistently higher R_s rates and cumulative CO₂ loss than the control and DRW₁₀₋₂₀ treatments. Interestingly, the increased cumulative CO₂ loss during this 60-d extended period in the DRW₂₀₋₁₀ treatment compensated for 14% of the DRW-induced decreases. Our results confirmed that under high drought intensity, the legacy effect of DRW on R_s can last for at least 60 d for the two types of plantations in this study. This result is in contrast to the findings of Canarini et al. (2017), who showed that R_s (as well as DOC and MBC) was no longer affected approximately 5 days after rewetting. A higher MBC at the end of the extended period (180 d) in DRW₂₀₋₁₀ treatment compared to the control was found in this study (Fig. 4a and b), supporting the finding that the increase in soil microbial biomass contributed to the legacy effect. Additionally, we found a higher plant litter mass remaining after the DRW treatments compared to the control (Li et al., 2017). Thus, compared to the control, the higher amount of plant litter as a substrate and the higher level of microbial biomass in the DRW treatments both contributed to the higher R_s during the 60-d extended periods.

4.3. The differences between the two plantations

The consistently higher R_s measured in the poplar than in the Mongolian pine soils is consistent with the higher quality (i.e., higher N,

lower lignin, and lower phenol levels; Table 1) of the poplar litter, as well as the higher soil C and nutrient (i.e., N and P; Table 1) levels and the higher microbial biomass in the poplar than in the Mongolian pine soils. The responses of R_s to soil DRW were also more sensitive in the C-rich poplar soils compared to the C-poor Mongolian pine soils, which is consistent with a previous finding that C-rich soils would lose more C during DRW cycles. In addition, the fluctuation of soil water content was more drastic in the Mongolian pine soils, which could enhance soil aggregations, exposing occluded particulate materials and increasing R_s (Navarro-García et al., 2012).

Unlike the poplar soils, significant differences in cumulative soil CO₂ loss between the control and DRW₂₀₋₁₀ were only found in the first two DRW cycles, and no significant differences were observed during the 3rd and 4th DRW cycles or during the extended period for the Mongolian pine soils. Overall, the impact of soil DRW with mild drought on the total respiration of the Mongolian pine soils was less than 5% and thus can be considered negligible. There appeared to be an acclimation process of the microbes in the Mongolian pine soils in response to the increasing number of DRW cycles. Therefore, assuming a situation where only one or two DRW cycles were included in an experiment, extrapolating the results using only these DRW cycles (effect size > 10%) may overestimate the overall impacts of repeated DRW on R_s .

Mongolian pine and poplar are common species used in afforestation in Northeast China. Although R_s was consistently higher in the poplar than in the Mongolian pine soils, the higher C content in the poplar than in the Mongolian pine soils indicates that poplar soils have a greater capacity to sequester C than do Mongolian pine soils. In addition, our study showed that under high drought intensity in the future, poplar soils would exhibit a greater reduction in R_s than would Mongolian pine soils. Assuming an extreme case that Horqin Sandy Land would experience soil DRW with longer drought in the future and there would be 12 DRW cycles per year, a poplar plantation would store approximately 4.7 more metric tons of C per hectare than a Mongolian pine plantation through the reduction in R_s . This is a very rough estimate without consideration of how plant production and respiration or other environmental factors may change with DRW. However, afforestation projects aiming to maximize its benefit must fully consider the importance of global change and plant-species-dependent responses.

5. Conclusion

This study investigated the direct and legacy effects of DRW under different drought intensities on R_s in two semiarid forest plantation soils. A much stronger reduction in R_s was observed under high drought intensity and for poplar soils, while R_s acclimated to DRW under mild drought (i.e., R_s returned to the control level) in the Mongolian pine soils. Under higher drought intensity, the pulse of R_s during the rewetting periods could not compensate for the large reduction in R_s during the drying periods, leading to the direct effect of a net reduction in R_s of $140\text{--}498 \text{ g C m}^{-2}$. The effect on R_s as well as on MBC was observed in the DRW with intensified drought in the poplar soils, which persisted for at least 60 d and was much longer than in previous studies, while the legacy effect of DRW on R_s was weaker in the Mongolian pine soils. Our study highlighted that future long-term extreme drought followed by precipitation may have a significant and persistent effect in reducing R_s , but the effect may vary with forest type.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2018.09.018>.

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