



Nonlinear decoupling of autotrophic and heterotrophic soil respiration in response to drought duration and N addition in a meadow steppe

Bo Meng^{1,2} · Raúl Ochoa-Hueso³ · Junqin Li¹ · Shangzhi Zhong⁴ · Yuan Yao¹ · Xuechen Yang¹ · Scott L. Collins² · Wei Sun¹

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Abstract

Soil respiration (SR_{TOT}) and its main components, soil heterotrophic (SR_H) and autotrophic respiration (SR_A), were monitored in response to within-season drought events of increasing duration and soil N enrichment in a semiarid meadow steppe. The experiment consisted of the combination of five drought periods (0 days, 15 days, 30 days, 45 days, and 60 days) and two N addition levels (0 and 10 g N m⁻² year⁻¹ applied as urea). Soil respiration decreased after 30 days of drought, with the response being driven by soil heterotrophs. Moreover, N addition increased the sensitivity of soil respiration to soil water content, which we attributed to greater plant C inputs and soil microbial C and N content in the N addition treatment. Our results highlight the role of SR_H as a key regulator of C fluxes in nutrient-poor semiarid meadow steppe in response to extreme within-season drought and the role of soil N availability in modulating this response.

Keywords Extreme drought · N addition · Soil respiration · Autotrophic respiration · Heterotrophic respiration

Introduction

Soil respiration represents one of the main C fluxes between the biosphere and the atmosphere (60 Gt annually), greatly exceeding the amount of C emitted as a result of human activities (8–9 Gt C annually) (Stockmann et al. 2013). Thus, any alteration of this critical component of the global C cycle may have far-reaching interactions with global warming (Goulden et al. 1996; Grace 2004). There are two major components of soil respiration: autotrophic respiration (SR_A)

contributed by plant roots and prototrophic microorganisms, and heterotrophic respiration (SR_H) contributed by soil microorganisms and micro- and macrofauna found in the bulk and rhizosphere soils (Hanson et al. 2000; Kuzyakov 2006), both of which are sensitive to changes in environmental conditions and nutrient supply (Moinet et al. 2016; Nguyen et al. 2018; Yan et al. 2010). Understanding the relative response of the two soil respiration components to changing environmental conditions and soil eutrophication is critical to forecast future changes in global C cycling in terrestrial ecosystems (Adams et al. 1990; Grace 2004), including grasslands. Grasslands cover more than one-third of the global terrestrial area and store 20–30% of terrestrial C, most of it in the soil (Conant et al. 2017; O'Mara 2012; Scurlock and Hall 1998). Thus, understanding how autotrophic and heterotrophic components in grassland soils respond to human-induced environmental impacts is critical to predict and model C fluxes that will impact the pace of climate change in the future (Adams et al. 1990; Wang and Fang 2009).

Soil respiration in grasslands is tightly controlled by the availability of water (Borken et al. 2006; Li et al. 2018). Climate change is increasing both the likelihood and the intensity of drought events as a result of the intensification of the global hydrological cycle (Dai 2013; Trenberth et al. 2014). It

✉ Wei Sun
sunwei@nenu.edu.cn

¹ Key Laboratory for Vegetation Ecology, Ministry of Education, Institute of Grassland Science, Northeast Normal University, Changchun 130024, Jilin Province, China

² Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

³ Department of Biology, IVAGRO, University of Cádiz, Campus de Excelencia Internacional Agroalimentario (ceiA3), Campus del Río San Pedro, 11510, Puerto Real, Cádiz, Spain

⁴ Grassland Agri-Husbandry Research Center, College of Grassland Science, Qingdao Agricultural University, Qingdao 255109, China

is well-established that greater water deficit will suppress SR_A by decreasing plant metabolic activity and thus C fixation, although the magnitude of the response will directly depend on the plastic responses (or adaptability) of plants under drought (Zhang et al. 2019a). In contrast, during drought events, soil microbial activity is co-regulated by soil water content and the supply of C substrates from rhizo-deposition (Sun et al. 2019; Williams and de Vries 2020). Thus, two respiration components might asymmetrically respond to severe reductions of soil water content (Borken et al. 2006; Sun et al. 2019). For example, Liu et al. (2002) showed that the response of SR_H to changes in precipitation was quicker than the response of total soil respiration, while Zhang et al. (2019a) observed that SR_H exhibited a higher water sensitivity due to a shift in soil microbial composition. Yet many other studies have shown SR_H to be insensitive to drought stress (Hinko-Najera et al. 2015; Moinet et al. 2016; Sun et al. 2019). Hence, we still lack a clear understanding of the coordinated response of the two soil respiration components (i.e., SR_A and SR_H) during periods of increasing within-season drought intensity and even less of the interactions between these two components under the simultaneous co-occurrence of other global change factors, such as increased availability of biologically limiting soil nutrients like N (Kuzyakov 2006).

The availability of soil nutrients, such as N, is an important driver of the metabolic activity of soil organisms and, thus, of soil respiration rates (Ochoa-Hueso et al. 2018; Zhang et al. 2014). Meta-analytical studies showed that N addition consistently increased soil respiration due to the stimulation of SR_A in grasslands around the world (Zhou et al. 2014), while the effects on SR_H are widely variable (Janssens et al. 2010; Subedi et al. 2019; Treseder 2008). For example, the frequently reported negative effects of N addition on SR_H have been attributed to (1) reductions in belowground C allocation; (2) reductions in microbial activity; (3) abiotic stabilization of soil organic matter; (4) N toxicity; and/or (5) soil acidification (Freedman et al. 2016; Janssens et al. 2010; Treseder 2008). However, greater soil N availability has also been shown to increase soil microbial biomass C and N, and the activity of hydrolytic enzymes via greater plant C inputs, with knock-on effects on soil respiration (Zeng et al. 2018; Chen et al. 2017). These inconsistencies in the microbial respiration response to N may also be due to variations in soil moisture conditions and/or precipitation variability (Chen et al. 2019; Nguyen et al. 2018). While the effects of N enrichment on soil C flux have received considerable attention (Riggs and Hobbie 2016; Song et al. 2020), we know much less about how concurrent global increases in soil N availability and precipitation variability affect soil respiration. This highlights the need for experimental studies that consider simultaneously the impacts of N enrichment and drought intensity on autotrophic and heterotrophic soil respiration to fully unravel

the potential consequences of human impacts on this globally relevant aspect of the C cycle.

To fill this knowledge gap, we conducted a field study in a semiarid meadow steppe that has historically experienced frequent within-season drought events (Liu et al. 2014). In this steppe, we experimentally manipulated the duration of period without rain (i.e., droughts of increasing duration) and N fertilization. Precipitation exclusion was simulated for 15, 30, 45, and 60 days in plots that had been N-fertilized or left unfertilized for 3 years. We hypothesized that (1) given that soil respiration is highly sensitive to changes in soil water availability (Li et al. 2018), soil respiration will decrease more quickly during the initial drought stages. We also hypothesized that (2) during the early stages of drought (15 days and 30 days), plants would allocate more C belowground (Hasibeder et al. 2015), which would cause an increase in the proportion of SR_A relative to SR_H . However, after 60 days of extreme drought, we predicted (3) a widespread decline of autotrophic and heterotrophic activity. Finally, we hypothesized that (4) N addition would consistently increase SR_H and SR_A due to severe N limitation in this grassland ecosystem (Bai et al. 2010), but also that (5) this stimulation would be limited under extreme drought stress (Dijkstra et al. 2010; Liu et al. 2009).

Materials and methods

Study site

This research was carried out at Changling Horse Breeding Farm (44° 30'–44° 45' N, 123° 31'–123° 56' E) in Western Jilin Province, Northeast China, which is a meadow steppe dominated by *Leymus chinensis* and other accompanying perennials, such as *Phragmites australis*, *Kalimeris integrifolia*, and *Chloris virgata*, with a semiarid continental climate. The main soil types in the area are chernozems with high pH (8.5–9.5), and low soil total N (0.15%) and organic C (2.0%) contents. Soil texture is 35% clay, 45% silt, and 20% sand on average (Wang et al. 2008; Yin et al. 2018). Bulk density is 1.44 g cm⁻³, and field capacity (gravimetric soil water content after excess water has drained away followed saturation) is approximately 0.255 (g g⁻¹). The mean annual temperature is 6.4 °C and the average growing season (May to September) precipitation is 411 mm over the past five decades. Mean number of consecutive days without rain during the growing season is 17.4–21.9 in this semiarid region (Liu et al. 2014), while the 90th percentile of consecutive days without rain is a month or more (Lei and Duan 2011). The study area (100 m × 100 m) was fenced to exclude grazing and mowing since 2010. Before that, the study site was lightly grazed by large livestock herds dominated by cattle and sheep and annually mowed in August.

Drought and N addition treatments

We randomly assigned different within-season drought periods (0 days [i.e., no drought], 15 days, 30 days, 45 days, and 60 days without precipitation) and two N addition treatments to 40 plots (2.5 m × 2.5 m), with a buffer zone of at least 2 m between plots. Plots were arranged in 4 blocks of 10 m × 20 m, respectively, which were similar in vegetation composition prior to the initiation of the experimental treatments. All drought treatments started on 22th May 2017 (DOY 142), for which we used rainout shelters of 3 × 3 m with transparent acrylic roofs (> 90% light permeability). Prior to the start of the experiment, all drought plots were enclosed by a 2-mm thick stainless-steel plate (10 cm aboveground and 50 cm belowground) to prevent the plots from receiving overland runoff and belowground lateral soil infiltration. Drought plots were manually irrigated to equalize annual precipitation after sampling at the end of each drought period. Irrigation after 45 days and 60 days drought events were distributed over 1 week to avoid adding one large rain pulse to the treatments. Starting in 2015, we applied 2 g N m⁻² month⁻¹ from May to September (total of 10 g N m⁻² year⁻¹) to half of the plots (Bai et al. 2010). Fertilizers were applied during rainy days to avoid toxicity of accumulated N. Given that this meadow steppe is embedded within an important agricultural region, the spill-over effects of chemical fertilizer application could be an important factor driving the N economy of this grassland; therefore, we used granular urea which is also widely used as fertilizer throughout the region.

Environmental conditions

Natural precipitation and air temperature were monitored by RG2-M sensors (Onset Computer Corporation, Bourne, MA, USA) from April to October of 2017. Simultaneous with each measurement of soil respiration, soil water content (SWC) at 0–10 cm depth was determined by oven-drying 100 cm⁻³ soil samples collected from each plot, and soil temperature (ST) was measured using a thermocouple penetration probe (LI-6400-09 TC, LI-COR, Inc.). Meanwhile, soil moisture sensors (S-SMC-M005, Decagon, Pullman, WA, USA) were employed to continuously monitor SWC throughout the study period in two representative blocks.

Soil respiration measurements and component separation

Soil respiration was measured at the end of each drought period and the end of the growing season using a portable CO₂ infrared gas analyzer (LI-6400, LI-COR Inc., Lincoln, NE, USA) with a soil CO₂ flux chamber (6400-9, LI-COR Inc., Lincoln, NE, USA) between 8:00 and 11:00 h (Jian et al. 2018). The modified clipping method was used to partition

total soil respiration (SR_{TOT}) into SR_H and SR_A, assuming that root growth will be greatly suppressed without aboveground C input (Yan et al. 2010; Zeng et al. 2018). Two types of PVC collars (10.4 cm inner diameter) differing in height (5 and 32.5 cm) were randomly installed into the ground in the fall of 2016. In each plot, three short PVC collars placed 2.5 cm deep into the soil were used to measure SR_{TOT}, and three long PVC collars driven 30 cm deep into the soil were used to measure SR_H. To measure SR_H, the aboveground portions of plants were clipped each week from the beginning of April 2017 and throughout the duration of the experiment to minimize the impacts of dead roots, whereas the aboveground portions of plants inside the short collars were removed 1 day before each SR_{TOT} measurement. SR_A was then determined by the difference between SR_{TOT} and SR_H as follows:

$$SR_A = SR_{TOT} - SR_H$$

Given that it is difficult to achieve complete removal of living roots in a short time, and the potential increase in the proportion of dead roots after clipping, the clipping approach might overestimate the contribution of SR_H. But compared with other partitioning approaches, such as component integration and excised roots (Hanson et al. 2000; Kuzyakov 2006), we considered it the most feasible method for separating soil respiration components in this semiarid grassland (Chen et al. 2009; Yang et al. 2012).

Belowground biomass and soil properties

Belowground biomass (BGB) (0–30 cm depth) was sampled in three locations in each plot using a soil corer (10 cm diameter) before each measurement of soil respiration. Then, live roots were washed to remove soil particles and oven-dried at 70 °C to constant weight (48 h).

Simultaneous with the sampling of BGB, six 4 cm diameter by 15 cm deep soil cores were collected and bulked into a single soil sample from each plot. Roots and other organic debris were removed using a 2-mm mesh stainless-steel sieve. Half of the bulk soil sample was placed on ice in a cooler for transportation back to the laboratory and stored at 4 °C until the soil microbial measurements (within 1 week of field collection). The other half was used to measure soil pH, total C, and total N content after air-drying for several days. Soil pH was measured using a pH meter (PHS-3E INESA Scientific Instrument Co., Ltd., Shanghai, P. R. China) in a 1:5 mass:volume ratio. Soil total C and N content were determined using an elemental analyzer (vario EL cube, Elementar, Langenselbold, Germany). Soil C/N ratio was calculated as total C/total N.

Soil microbial C and N

Soil microbial biomass C (MBC) and N (MBN) were estimated using the chloroform fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987). Briefly, samples of 12.5 g soil were fumigated with chloroform for 48 h in vacuum desiccators, and an additional set of 12.5 g freeze-dried soil samples was analyzed for dissolved organic C (DOC) and total extractable N (TEN). After that, non-fumigated and fumigated soils were extracted by 0.5 M K₂SO₄ (1:4). Total dissolved C and N in the filtered soil extracts were measured with a total organic C analyzer (vario TOC, Elementar, Langensfeld, Germany). MBC and MBN were determined as the difference between the total dissolved C and N extracted from the fumigated and non-fumigated soil samples. The extraction efficiency factors of 0.45 and 0.54 were applied for MBC and MBN, respectively.

Data analyses

We used an empirical model approach to estimate the relationship between soil respiration (SR_{TOT} and SR_H) and soil temperature (ST) and soil water content (SWC) (Borken et al. 2006):

$$SR = Ae^{(bST)}(1 + dSWC)$$

where A is an Arrhenius constant, b is the fitted parameter that describes the influence of ST on SR, and d is the fitted parameter that describes the sensitivity of SR to SWC. The relative change in SR by an increase in ST of 10 °C was calculated as $Q_{10} = e^{10 \times b}$.

We used two-way analysis of variance (ANOVA) to determine the univariate and interactive effects of drought and N addition on SR_{TOT}, SR_H, SR_A, and other abiotic or biotic factors. Block was included as a random factor in the analysis. We used Pearson correlation to examine the covariation among soil respiration components and all the abiotic and biotic explanatory variables measured. These analyses were performed using SPSS software 22.0 (SPSS Inc., Chicago, USA).

Finally, we used structural equation modelling (SEM) to build a more holistic, system-level understanding of the responses of soil respiration to changes in the duration of drought events and soil N availability (Grace 2004). We constructed our a priori model based on expectations from the literature and observed correlations within our dataset. In our model, N fertilization was predicted to affect plant biomass, soil N content, MBC & MBN, and DOC. These soil response variables considered in our model were consistently measured in the non-droughted plots. The effect of drought was only considered for C fluxes and was evaluated as the difference between the droughted and non-droughted plots. The response difference (RD) of soil respiration was calculated as $RD =$

$SR_{\text{drought}} - SR_{\text{non-drought}}$. This model design allowed us to simultaneously evaluate how seasonal variations in soil parameters and variations in the response of soil parameters to N addition modulated the response of C fluxes to increasing drought duration. We tested our conceptual model using the *piecewiseSEM* package (version 2.0.2) (Lefcheck, 2016) in R (3.4.0), in which a set of linear structured equations are evaluated individually. We used the *lm* function of the *stats* package to model response variables. Good fit of the SEM was assumed when Fisher's C values were non-significant ($P > 0.05$).

Results

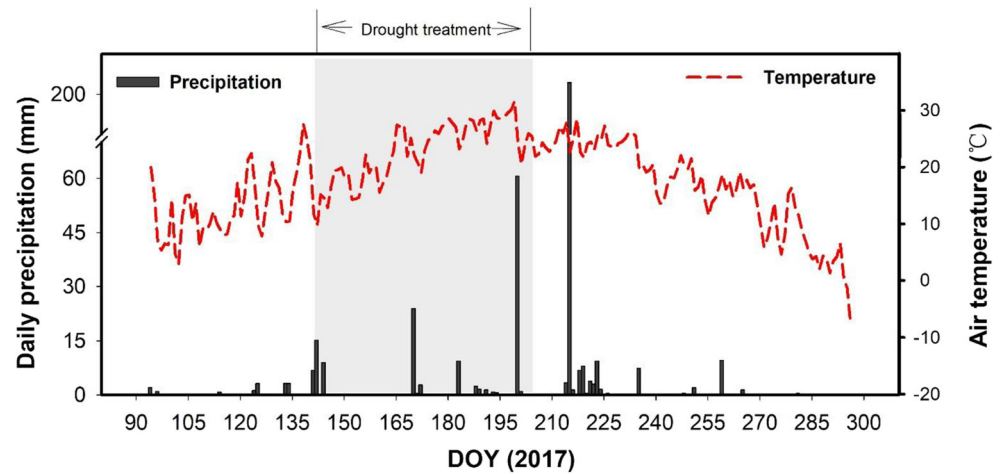
Environmental conditions

Precipitation during the growing season of 2017 was 415.4 mm, which is close to the long-term mean of 411 mm. During the whole drought period, the rainout shelters excluded about 130.6 mm of precipitation, with 24.5 mm in the first 15 days, 52 mm after 30 days, and 61.8 mm after 45 days (Fig. 1). An additional 251.4 mm of precipitation occurred after the drought treatments ended (DOY 203–253). Soil water content decreased gradually during the first 30 days of drought, and stabilized at 0.04 (g g⁻¹, 15.5% of field capacity). Experimental drought significantly reduced soil water content after 45 days ($P = 0.02$) and 60 days ($P < 0.01$). N addition had no effect on SWC (Fig. 2B). Soil temperature increased from 18.4 to 25.4 °C over the drought period. There was no difference in soil temperature between treatments (Fig. 2A).

Soil respiration and component separation

On average, drought significantly decreased SR_{TOT} by about 25% after 30 days in both unfertilized and fertilized conditions ($P < 0.05$). Although we observed a trend of N addition induced increase in SR_{TOT} and its components, differences were not statistically significant between drought and the combination of N addition and drought (Fig. 3). The SR_H exhibited a greater negative response to drought than SR_{TOT}, with 20% reduction after 15 days of drought, 34% after 30 days, 40% after 45 days, and 29% after 60 days under unfertilized conditions; and 26% reduction after 15 days of drought, 39% after 30 days, 53% after 45 days, and 41% after 60 days under fertilized conditions (all P value < 0.01). In contrast, only the 60 days drought downregulated SR_A (Fig. 3C). Additionally, we detected that SR_A/SR_{TOT} was significantly greater after 15 days ($P = 0.04$) and 30 days ($P = 0.02$) in the droughted plots than under ambient conditions (Fig. 3D). By the end of the growing season in 2017, no significant legacy effects of each treatment on SR_{TOT}, SR_H, and SR_A were detected (Fig. 3E, F, G).

Fig. 1 Daily precipitation (mm) and air temperature (°C) in 2017. The light gray shading represents the drought period (day 142–202 in 2017)



Belowground biomass and soil properties

Sixty days of extreme drought and 10 g N m^{-2} were not enough to impact root biomass, despite a trend of increasing root biomass in response to N addition. Nitrogen addition significantly increased DOC and TEN, but soil pH and soil C/N ratio were not significantly influenced (Table 1).

Moreover, DOC increased after 45 ($P = 0.04$) and 60 days of drought ($P = 0.04$).

Soil microbial C and N

The prolonged drought (45–60 days) significantly reduced MBC (20–30% in unfertilized plots, and 38–33% in fertilized

Fig. 2 Soil temperature (A, °C) and soil water content (B, g g^{-1}) at 0–10 cm soil depth for four treatments (C, ambient precipitation; D, drought; N, ambient precipitation plus N addition; ND, drought plus N addition). Data are reported as mean $\pm 1 \text{ SE}$ ($n = 4$). The non-significant effects of block are not shown in this figure. Asterisks indicate significant differences. ns: $P > 0.05$; * $P < 0.05$; ** $P < 0.01$

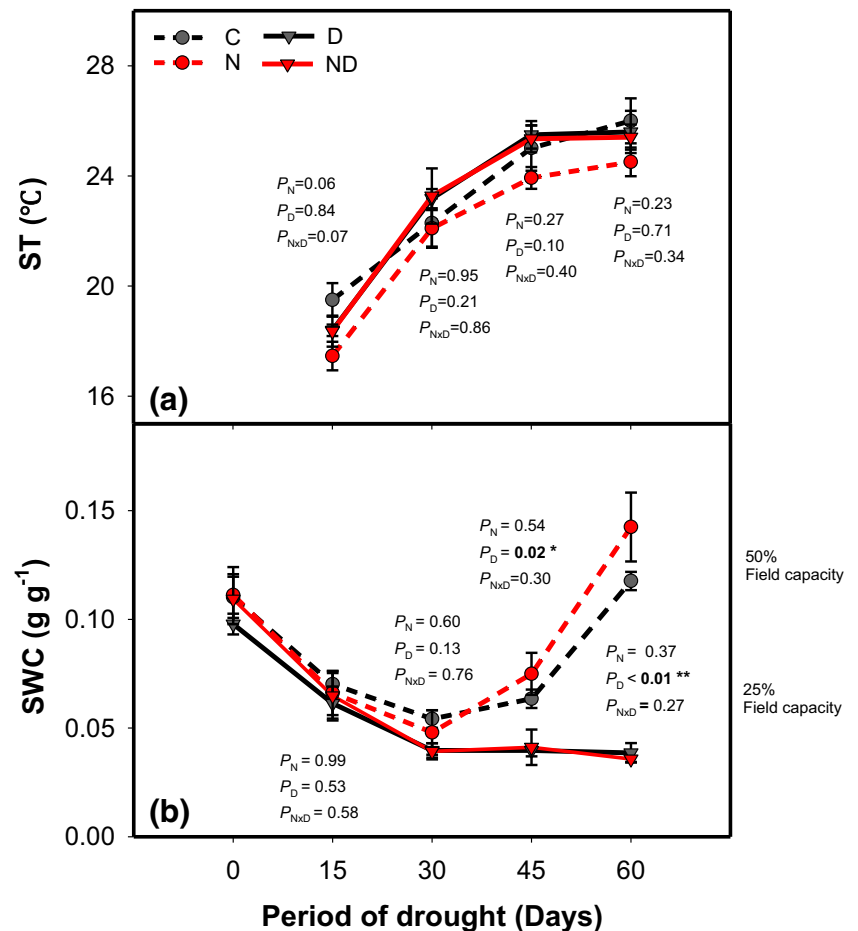
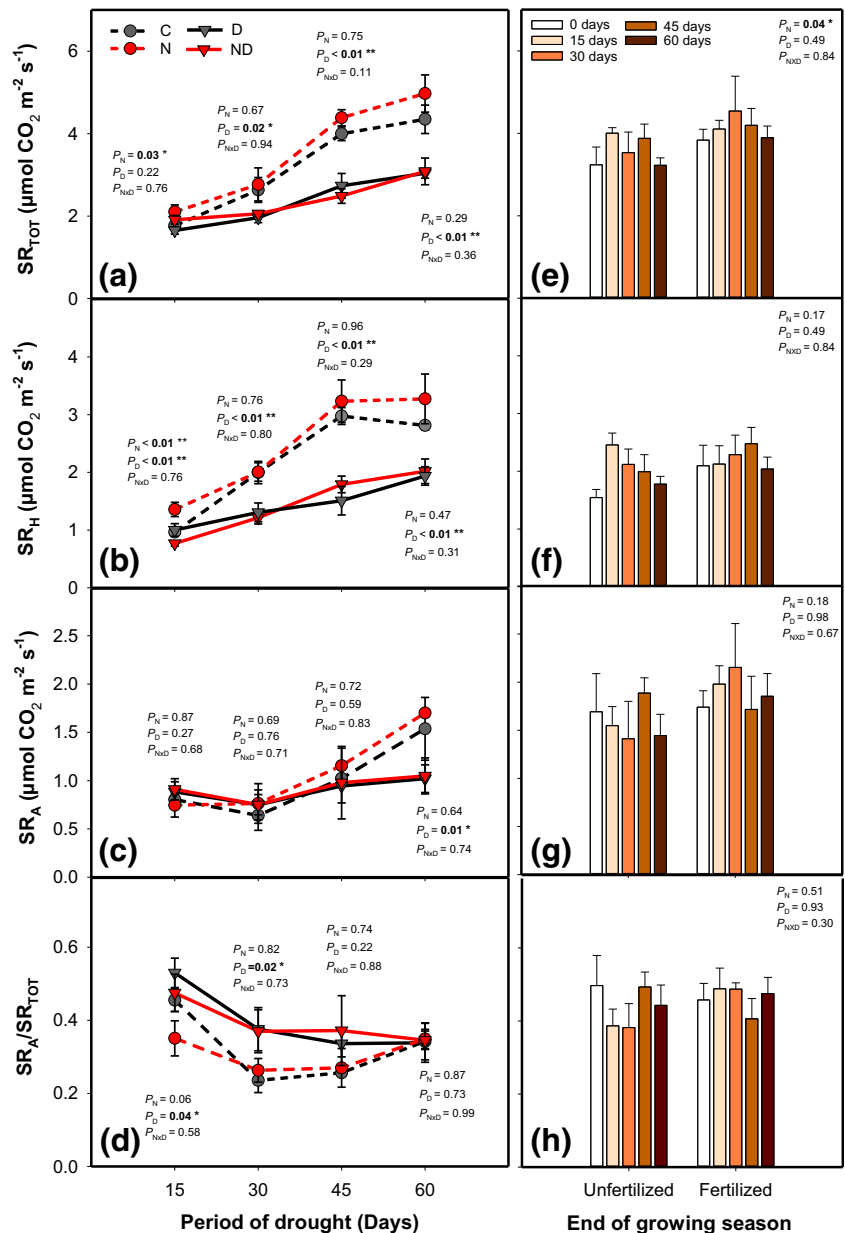


Fig. 3 Responses of total soil respiration (SR_{TOT} ; A and E), heterotrophic soil respiration (SR_H ; B and F), autotrophic soil respiration (SR_A ; C and G), and SR_A/SR_{TOT} (D and H) to the four treatments (C, ambient precipitation; D, drought; N, ambient precipitation plus N addition; ND, drought plus N addition) during the experimental drought period through the end of the growing season in 2017. Data are reported as mean \pm 1 SE ($n = 6$). Asterisks indicate significant differences. ns: $P > 0.05$; * $P < 0.05$; ** $P < 0.01$



plots) and MBN (30–42% in unfertilized plots, and 38–52% in fertilized plots) (Table 1). Nitrogen addition increased MBC by 23% ($P < 0.01$) and MBN by 42.1% ($P < 0.01$) after 60 days under ambient precipitation, but no significant interactions between drought and N addition occurred (Table 1).

Controlling factors of soil respiration

We observed significant positive correlations between SR_{TOT} and SWC and ST (both $P < 0.01$, Fig. 4). Nitrogen addition increased the water sensitivity of SR_{TOT} and SR_H by 26% and 36%; however, Q_{10} decreased from 2.31 to 1.68 and 2.45 to 1.73, respectively

(Fig. 4 and Table S1). DOC and MBC and MBN were positively correlated with SR_H , while soil C/N ratio and SR_H were negatively correlated (Table S2). BGB and SR_A were positively correlated (Table S2).

Our integrative SEM analysis showed that the response of SR_{TOT} to drought was co-regulated by changes in SR_H and SR_A . However, the increasing divergence of SR_H in droughted plots as compared to control plots over periods of increasing within-season drought duration was driven by increasing differences in MBC, particularly under N fertilization conditions. Our results also indicated a direct negative effect of drought duration on SR_A (Fig. 5).

Table 1 Drought duration (15, 30, 45, 60 days) and N addition (C, ambient precipitation; D, drought; N, ambient precipitation plus N addition; ND, drought plus N addition) effects on abiotic and biotic factors at the end of each drought period (15, 30, 45, 60 days) in 2017.

Data are reported as mean \pm 1 SE ($n = 4$). ns: $P > 0.05$; * $P < 0.05$; ** $P < 0.01$. DOC, dissolved organic C; TEN, total extractable N; BGB, belowground biomass; MBC, microbial biomass C; MBN, microbial biomass N

		Abiotic factors				Biotic factors		
		Soil pH	Soil C/N	DOC ($\mu\text{g g}^{-1}$)	TEN ($\mu\text{g g}^{-1}$)	BGB (g m^{-2})	MBC ($\mu\text{g g}^{-1}$)	MBN ($\mu\text{g g}^{-1}$)
15 days	C	9.06 \pm 0.2	22.9 \pm 2.5	283 \pm 36	17.1 \pm 2.7	1216 \pm 107	321 \pm 21	25.8 \pm 4.4
	D	9.07 \pm 0.2	23.2 \pm 2.2	319 \pm 42	17.3 \pm 1.8	1143 \pm 96	314 \pm 20	24.5 \pm 4.0
	N	9.00 \pm 0.2	21.7 \pm 2.6	368 \pm 39	24.2 \pm 1.2	1310 \pm 180	334 \pm 18	31.7 \pm 3.7
	ND	9.05 \pm 0.2	22.6 \pm 2.3	370 \pm 49	17.3 \pm 1.9	1281 \pm 165	343 \pm 42	30.4 \pm 4.4
Significance	N	ns	ns	ns	*	ns	ns	ns
	D	ns	ns	ns	ns	ns	ns	ns
	N×D	ns	ns	ns	ns	ns	ns	ns
30 days	C	9.13 \pm 0.3	22.3 \pm 2.4	318 \pm 39	18.0 \pm 0.3	1169 \pm 44	307 \pm 18	25.4 \pm 4.1
	D	9.05 \pm 0.2	23.9 \pm 2.3	346 \pm 20	17.7 \pm 1.2	1115 \pm 142	290 \pm 20	21.4 \pm 4.8
	N	9.14 \pm 0.2	17.8 \pm 0.9	467 \pm 39	21.8 \pm 1.5	1462 \pm 147	346 \pm 22	28.1 \pm 3.1
	ND	8.95 \pm 0.1	20.0 \pm 0.6	399 \pm 32	17.7 \pm 1.2	1192 \pm 175	280 \pm 23	22.3 \pm 3.5
Significance	N	ns	ns	*	*	ns	ns	ns
	D	ns	ns	ns	ns	ns	ns	ns
	N×D	ns	ns	ns	ns	ns	ns	ns
45 days	C	9.17 \pm 0.2	18.9 \pm 0.5	351 \pm 13	19.2 \pm 1.1	1431 \pm 221	352 \pm 17	31.6 \pm 2.2
	D	9.10 \pm 0.2	18.8 \pm 0.8	441 \pm 17	17.0 \pm 1.6	1705 \pm 164	282 \pm 30	22.2 \pm 4.3
	N	9.08 \pm 0.2	17.2 \pm 0.7	451 \pm 27	25.0 \pm 2.5	1456 \pm 137	416 \pm 16	38.8 \pm 4.2
	ND	9.22 \pm 0.1	19.1 \pm 1.3	475 \pm 32	17.0 \pm 1.6	2019 \pm 251	263 \pm 18	24.2 \pm 1.7
Significance	N	ns	ns	*	**	ns	ns	ns
	D	ns	ns	*	ns	ns	**	**
	N×D	ns	ns	ns	ns	ns	ns	ns
60 days	C	9.55 \pm 0.2	19.9 \pm 1.3	358 \pm 26	17.9 \pm 1.7	1327 \pm 117	358 \pm 35	32.8 \pm 4.1
	D	9.30 \pm 0.2	18.3 \pm 1.9	436 \pm 21	17.0 \pm 1.2	1571 \pm 127	250 \pm 27	19.0 \pm 4.2
	N	9.20 \pm 0.2	21.5 \pm 0.9	438 \pm 17	28.3 \pm 1.2	1683 \pm 130	440 \pm 12	46.6 \pm 3.4
	ND	9.20 \pm 0.2	19.6 \pm 1.4	468 \pm 18	17.0 \pm 1.2	2013 \pm 239	297 \pm 31	22.3 \pm 2.1
Significance	N	ns	ns	*	**	ns	**	*
	D	ns	ns	*	ns	ns	**	**
	N×D	ns	ns	ns	ns	ns	ns	ns

Discussion

In this study, we have demonstrated that increasing within-season drought duration reduced soil respiration in a meadow steppe in a nonlinear fashion, with the heterotrophic component showing a faster response to water limitation due to lower MBC later in the growing season. In contrast, greater N availability resulted in greater soil respiration. These responses were directly linked to variations in soil microbial biomass, but also to above and belowground plant biomass. Taken together, our results demonstrate soil C fluxes in this meadow steppe are sensitive to forecasted increases in the frequency and severity of drought events and soil N availability. However, they also suggest a strong capacity for recovery of these ecosystems from short-term, within-season drought, as

indicated by the lack of legacy effects on soil C flux after rainfall conditions returned to normal.

Our experimental drought resulted in a significant reduction in SR_{TOT} after 30 days, most likely driven by changes in SWC. This is in agreement with previous experiments that suggested that soil respiration should strongly respond to water limitation (Borken et al. 2006; Sowerby et al. 2008; Vargas et al. 2012; Yu et al. 2017). However, we did not observe a rapid decrease in SR_{TOT} during the first 30 days, as we initially predicted (hypothesis 1). We detected that SR_{TOT} and SR_{A} were not altered by the 15 days of drought, which was not surprising given that 15 days drought is comparable to the historical mean number of consecutive days without rain during the growing season. However, SR_{TOT} consistently increased following the natural increase in temperature during

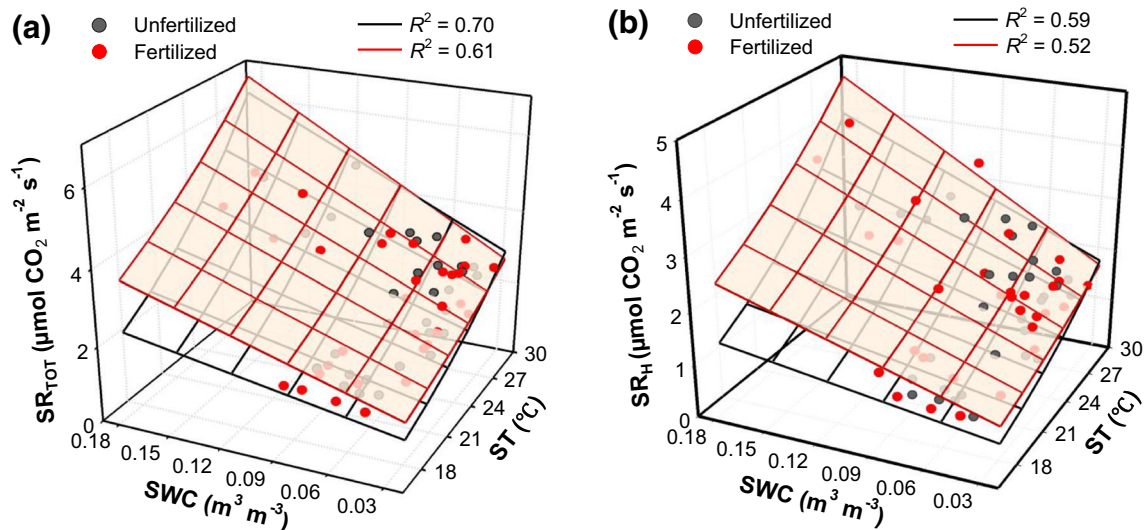


Fig. 4 Linear regressions of total soil respiration (SR_{TOT} ; A) and heterotrophic soil respiration (SR_H ; B) with soil temperature (ST) and soil water content (SWC)

the season, even in droughted plots, implying that the effects of drought need to be put in the context of natural seasonal variations of ecosystems in terms of both water availability and temperature (Schindlbacher et al. 2012).

In line with hypotheses 2 and 3, we observed a nonlinear response in the contribution of SR_A to SR_{TOT} after 60 days of drought. Our results showed that the proportion of SR_A in the

drought treatment was significantly larger after 15 days and 30 days. However, this pattern was weakened after 30 days (Fig. 3D), suggesting that the response of SR_A to drought was slower than that of SR_H . Although this result was supported by previous studies in similar ecosystems (Carbone et al. 2008; Zhang et al. 2019a; Zhao et al. 2016), studies carried out in forests and other mesic ecosystems suggested that SR_A

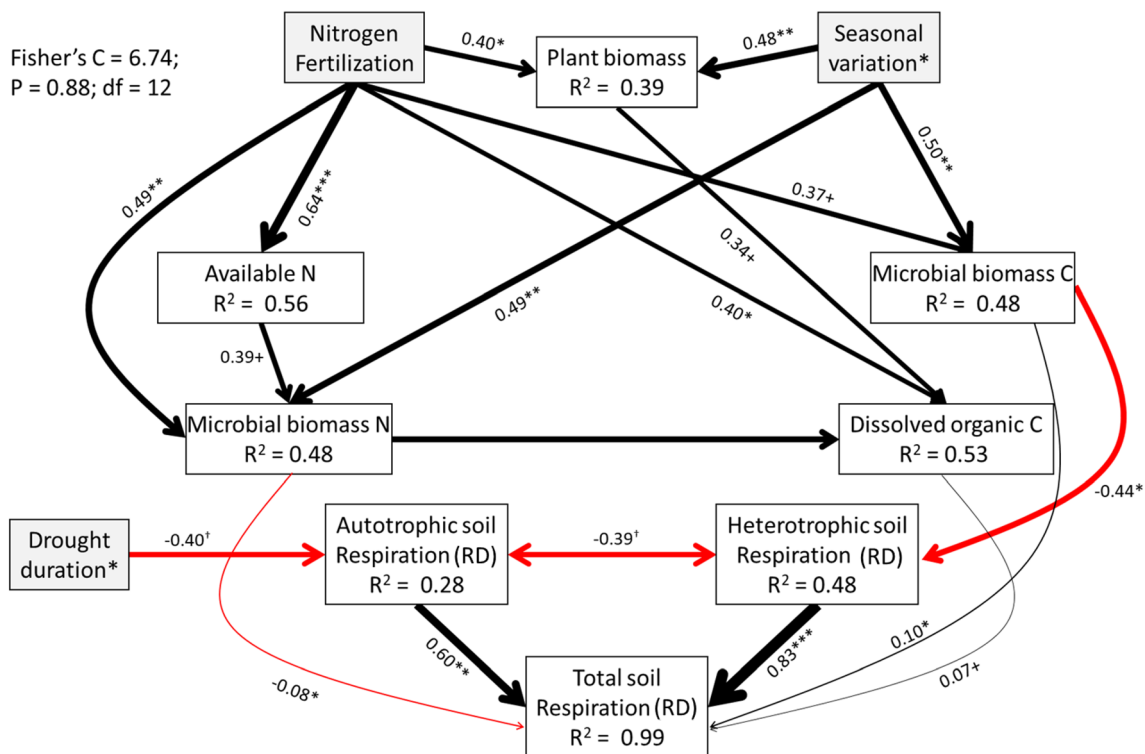


Fig. 5 Structural equation model depicting the experimental, soil, and plant drivers of soil respiration. Black lines indicate positive effects ($P \leq 0.1$), while red lines indicate negative effects ($P \leq 0.1$). The width of the arrow is proportional to the magnitude of the effect. Non-significant

Fisherta C value ($P > 0.05$) indicates good fit of the model. Grey boxes represent experimental treatments and seasonal effects. RD, response difference. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; † $P < 0.1$

may be more sensitive to changes in soil water content than SR_H (Gomez-Casanovas et al. 2012; Kopittke et al. 2014). This can be explained by greater drought adaptability of the plants living in the semiarid steppe (Stpaul et al. 2012) and greater water sensitivity of soil microbial activity at lower than at higher soil moisture conditions (Liu et al. 2009; Ochoa-Hueso et al. 2018). Furthermore, increased C allocation to roots can also directly impact the contribution from SR_A during the early drought period (Burri et al. 2013). Interestingly, Balogh et al. (2016) found that SR_A was more sensitive to drought in a dry grassland, with its relative contribution dropping from 66 to 35%. This was attributed to the greater relative contribution of SR_A in their control plots, up to twice as much, as compared to this meadow steppe system.

Consistent with the results of several previous studies in other N-poor ecosystems (Craine et al. 2001; Xu and Wan 2008; Zhang et al. 2019b), we observed a trend of N addition induced increase in SR_{TOT} and SR_H (Fig. 3). This result was further supported by the significant negative correlation between soil C/N and SR_H (Table S2), which was likely due to the N-driven increase of MBC (Table 1 and Fig. 5). Considering the potential mechanisms of SR_H suppression proposed by Janssens et al. (2010) and Treseder (2008), including decreased microbial activity and soil acidification, we posit that the reported inconsistent responses of SR_H could be explained by different ecosystem contexts. In our case, saline and alkaline soils could rule out the negative effects of acidification on microbial activity induced by N addition (Table 1). Also, N-induced stimulation of grassland primary productivity may have consistently resulted in greater availability of C for soil microorganisms due to increased plant production and litter inputs (Riggs and Hobbie 2016; Zhang et al. 2014). Moreover, the non-significant difference between drought and the combination of N addition and drought supported our hypotheses 4 and 5, demonstrating the predominance of water deficit over N enrichment as a control on soil C flux.

The use of SEM allowed us to gain a more holistic perspective on how N addition may impact the response of soil respiration to drought. For example, N addition under ambient rainfall conditions resulted in greater soil microbial biomass and activity potential, which amplified the difference in SR_H between the fertilized and unfertilized treatments as the season progressed (Fig. 5). Moreover, the SEM results further indicated that seasonal variation also played an important role in the response of soil respiration to drought via seasonal increases of MBC and plant biomass. In line with our expectations, the extended drought period directly suppressed SR_A (Fig. 5). Moreover, our results also showed that N addition could decrease the sensitivity of both SR_{TOT} and SR_H to increases in temperature (Q_{10}), which is opposite to the effects of N addition on water sensitivity. (Fig. 4 and Table S1). The sensitivity of SR to temperature and water availability is usually regulated by similar mechanisms, such as the lignin and

phenolic compounds accumulated in N-enriched soil, which is difficult to degrade by microbes when labile C is in short supply thereby decreasing SR_H (Chang et al. 2016; Davidson et al. 2006; Janssens et al. 2010). However, because this semiarid grassland is dominated by C_3 plants, higher temperature may suppress SR_A and belowground C input, an effect opposite that of increasing water availability.

The pulse of soil C flux after the rewetting of dry soil has been widely observed in laboratory incubation experiments and in field observations, suggesting that rewetting will cause rapid disintegration of soil aggregates and organic debris, such as microbial cells killed by drought, thereby accelerating soil C loss (Casals et al. 2011; Cosentino et al. 2006; Kopittke et al. 2014). Although we did not immediately measure SR_{TOT} after rewetting, our results indicate a significant soil DOC enrichment following 45- and 60-day drought treatments (Table 1), implying greater substrate availability for the C flux pulse after rewetting. However, we did not observe any legacy effect on SR_A during the post-drought period, as grassland soil C flux and its components quickly recovered even after 60 days of drought regardless of the N treatment (Fig. 3E). This rapid recovery could be attributed to a highly resilient soil microbial community in this grassland (Li et al. 2018), and the non-significant response of roots during the drought period (Table 1).

Conclusion

By experimentally manipulating drought duration and N availability, we have shown that the two main components of soil respiration, SR_H and SR_A , displayed an asymmetric response to prolonged drought in a meadow steppe ecosystem, and that higher soil N availability altered how components of soil respiration responded to drought. Our results further indicated that N addition increased the water sensitivity and reduced temperature sensitivity of SR_{TOT} in this N-poor ecosystem. Moreover, our results not only showed that SR_H was more sensitive to changes soil water availability than SR_A in the early stages of drought but they also suggested that SR_H plays a more important role than SR_A in the increased drought sensitivity induced by N addition. However, the lack of legacy effects even after 60 days of drought indicated the strong recovery potential of soil C flux in this semiarid grassland. Our results suggest that a clearer understanding of the mechanisms driving C fluxes in grasslands simultaneously subjected to drought and nutrient enrichment is urgently needed to develop sustainable ecosystem management strategies and to estimate the contribution of these ecosystems to the global C budget.

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Author contribution BM and WS designed the experiment. BM, JL, SZ, YY and XY performed the field and laboratory work. BM and ROH analyzed the data. BM, ROH, and WS wrote the manuscript. SLC provided valuable comments and suggestions on the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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