

Long-term collar deployment leads to bias in soil respiration measurements

Xiaoliang Ma¹  | Shengjing Jiang¹  | Zhiqi Zhang² | Hao Wang³  | Chao Song³  | Jin-Sheng He^{1,2} 

¹State Key Laboratory of Herbage Improvement and Grassland Agro-Ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, China

²Institute of Ecology, College of Urban and Environmental Sciences, Peking University, Beijing, China

³State Key Laboratory of Herbage Improvement and Grassland Agro-Ecosystems, College of Ecology, Lanzhou University, Lanzhou, China

Correspondence

Jin-Sheng He

Email: jsh@pku.edu.cn

Funding information

National Natural Science Foundation of China, Grant/Award Number: 32130065 and 32192461

Handling Editor: D J (Johan) Kotze

Abstract

1. Accurate measurements of soil respiration (R_s) are critical for understanding how soil carbon will respond to environmental changes. However, a commonly used method for R_s measurements, the collar deployment method, may introduce artefacts that cause bias in R_s measurements. Our objective was to quantify the effect of long-term collar deployment on R_s and to unravel potential causes due to changes in the soil environment.
2. A field experiment (2017–2019) including short-term (2–3 days before the measurement) and long-term collar deployment (lasting three consecutive growing seasons) was conducted to assess the methodological effect on R_s in an alpine grassland of the northeastern Tibetan Plateau. Soil incubation was used to further explore the mechanisms underlying the effects of collar deployment.
3. The effect of long-term collar deployment on R_s varied over time. In the first one and a half growing seasons, no significant difference in R_s was noted under short- and long-term collar deployment. This may be attributed to the negative effects of lower root biomass inside long-term collars and the positive effects of higher temperature and pulse input of dead roots following collar deployment. Under the long-term collar, R_s decreased rapidly in the middle of the second growing season and remained low until the end of the experiment, resulting in an 18.2% decrease relative to short-term collar deployment in the third growing season. Higher soil bulk density and lower root and microbial biomass inside long-term collars may explain the decrease in R_s and temperature sensitivity (Q_{10}). Soil incubation experiments revealed that the soil organic carbon (SOC) decomposition rate and Q_{10} were significantly reduced after long-term collar deployment.
4. Long-term collars led to substantial underestimates of R_s after more than 2 years. Our findings suggest that such potential artefacts should be considered when interpreting R_s data based on long-term collar deployment. Long-term collars should be relocated every 1–2 years to avoid artefacts if feasible. Alternatively, periodic measurements using short-term collars are recommended to quantify the magnitude of collar artefacts.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Methods in Ecology and Evolution* published by John Wiley & Sons Ltd on behalf of British Ecological Society.

KEYWORDS

alpine grassland, carbon cycle, chamber collar, soil respiration, temperature sensitivity

1 | INTRODUCTION

The terrestrial carbon (C) cycle is a major source of uncertainty in the global carbon budget. Reducing uncertainty requires improving the accuracy of estimating C flux between the atmosphere and terrestrial ecosystems (Friedlingstein et al., 2019). Soil respiration (R_s) is the second largest C flux between the atmosphere and terrestrial ecosystems. At an estimated 75–100 Pg C year⁻¹, it is roughly nine times larger than anthropogenic C emissions (Schlesinger & Bernhardt, 2013). R_s is known to play a key role in regulating atmospheric carbon dioxide (CO₂) concentration (Bond-Lamberty & Thomson, 2010; Raich et al., 2002). A slight change in R_s may cause a significant alteration in atmospheric CO₂ concentration and soil C pools (Schlesinger & Andrews, 2000).

Although methods for measuring R_s have been continually developed and refined during the past few decades, no instrument has been developed to measure R_s in situ without disturbing the soil. Consequently, many questions remain regarding the potential artefacts of various methodologies. The collar deployment method is one of the most widely used methods for R_s measurement (Görres et al., 2016; Healy et al., 1996). The installation of a collar with sufficient time before measurements also helps reduce disturbance to the soil (Hutchinson & Livingston, 2001). However, this method has shortcomings. First, collar insertion may cut off lateral plant roots and the mycelia of mycorrhizal fungi, which can directly affect autotrophic respiration (R_a) (Heinemeyer et al., 2011). Second, collar deployment usually requires cutting of vegetation, and long-term cutting of vegetation may affect topsoil temperature, evaporation, moisture, and soil physicochemical properties, which in turn may affect heterotrophic respiration (R_h) (Heinemeyer et al., 2011). Variations in collar-related protocols (e.g. collar coverage area, height, or installation depth) are now understood to cause biased measurements (Li et al., 2019). Moreover, many R_s measurements require the insertion of a permanent collar into the soil (Luo & Zhou, 2010). The effects of collar insertion on R_s can last for a long time and change over time. Therefore, such potential artefacts need to be considered when interpreting previous data and future studies (Batubara et al., 2019; Heinemeyer et al., 2011; Jovani-Sancho et al., 2017; Menyailo et al., 2015; Mills et al., 2011; Wang et al., 2005). These would considerably affect the accuracy of long-term R_s data and its component fluxes (Subke et al., 2006), especially their autotrophic, root-derived components (Heinemeyer et al., 2011). Despite its potential significance, the effect of long-term collar deployment on R_s , potentially through its impacts on the soil biological, physical and chemical environment, has not yet been evaluated.

Currently, much field data have been compiled for synthesis and modelling. Analyses of long time-series R_s data have become increasingly important, highlighting the need to evaluate potential

sources of bias associated with long-term measurement methods. The potential artefact of collar deployment on R_s measurements may propagate into other important properties associated with R_s . For example, temperature sensitivity (Q_{10}) of R_s may be affected by collar deployment. Long-term deployment of collars may lead to persistent disruption of vegetation growth, decreases in biomass production, and substrate availability (Hartley et al., 2007; Kirschbaum, 2004). This can suppress the Q_{10} of grassland R_s (Gershenson et al., 2009), especially when considering different Q_{10} of R_s component fluxes as shown for grasslands by Heinemeyer et al. (2012). Although Q_{10} is a key parameter for projecting the future terrestrial carbon budget and chamber-based methods are widely used for estimating it, the effect of long-term collar deployment on the Q_{10} of R_s has not been thoroughly investigated.

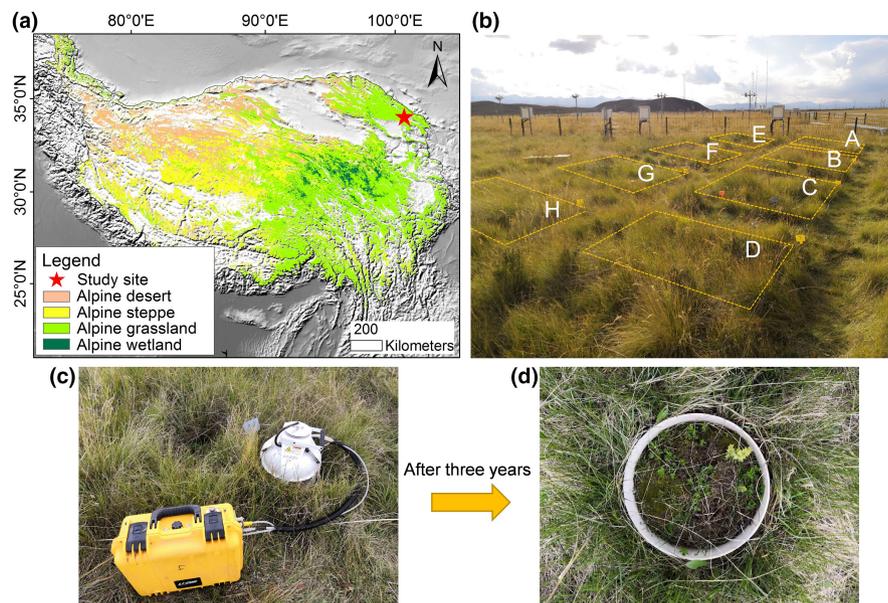
Here, a field experiment (2017–2019) was conducted in an alpine grassland of the northeastern Tibetan Plateau using a short-term collar (inserted 2–3 days before R_s measurement) and a long-term collar (lasting three consecutive growing seasons) to explore the influence of long-term collar deployment on R_s and its Q_{10} . We hypothesize that (i) during the early stage of long-term collar deployment, disturbance of root growth, turnover of dead roots and increase in soil temperature due to plants removal inside the collar are all conducive to higher respiration rates. Therefore, R_s under the long-term collar deployment would be higher than that under the short-collar treatment; (ii) in the later stages of long-term collar deployment, the reduced soil substrate availability, changes in soil physicochemical properties following severe disturbance of the vegetation, and less root growth inside the collar may contribute to a decrease in R_s and its Q_{10} .

2 | MATERIALS AND METHODS

2.1 | Site description

This study was conducted at the Qinghai Haibei Alpine Grassland National Observation and Research Station (37°36'N, 101°19'E, 3215 m a.s.l.) in the northeast Tibetan Plateau, China (Figure 1a). We did not need permission for fieldwork. The region has a continental monsoon climate, characterised by a long, cold winter and a short, cool summer. Mean annual temperature in the last 30 years was -1.2°C, with the highest temperature in July at 27.6°C, and the lowest temperature in January at -37.1°C. The mean annual precipitation is 489 mm, of which 80%–90% is concentrated during the growing season from mid-April to mid-October. The vegetation at this study site is dominated by the grass species *Stipa aliena*, *Elymus nutans*, *Helictotrichon tibeticum*, *Kobresia capillifolia* and *Carex przewalskii*. Collectively, these species account for approximately 70% of

FIGURE 1 (a) Geographical location of the study site (red star). (b) Photograph of the experimental setup. (c) Soil respiration rate (R_s) measured with a Li-8100. (d) Long-term collar installation for measuring R_s rate.



the aboveground net primary production (Ma et al., 2017). The soil texture is clay loam, and the soil is classified as Mat-Gryic Cambisol (Wang et al., 2014).

2.2 | Experimental design

The field experiment was conducted in an alpine grassland from May 2017 to October 2019 and included short- and long-term collar treatments. The long-term collar was kept in the field until the end of the experiment. The short-term collar was inserted into the soil 48–72 h before each R_s measurement and removed immediately afterwards. The locations of the collars were not fixed. Instead, each collar was placed at a random location inside the plot each time it was installed. Each plot was 2 × 2 m in size and the distance between them was 0.5 m. Each treatment was replicated eight times in 2017–2018 and six times in 2019 (Figure 1b). The collars had a diameter of 20 cm and a height of 8 cm, and were inserted 3 cm into the soil, a typical depth for this method (Heinemeyer et al., 2011; Zhou et al., 2007). To minimise the impact of spatial heterogeneity, long- and short-term collars were placed <1 m apart within each plot.

2.3 | In-situ measurements of R_s

A Li-8100 Automated Soil CO₂ Flux System (Li-Cor Inc.) was used to measure R_s (Figure 1c,d). R_s was measured 2–3 times per month from April to October in 2017–2019. All measurements were made between 9:00–11:00 AM because respiration during this time was close to the daily average R_s (Wang et al., 2014). To minimise the impact of heavy precipitation events, measurements were taken only on days with precipitation no greater than 10 mm in the previous 3 days. All plants inside the collars were cut to the ground within 24 h before each measurement. The measurements were repeated three

times for each collar, and the average was used to represent the R_s for that collar.

2.4 | Potential biotic and abiotic drivers of R_s

The physical and chemical properties of the soil inside the collars were measured as they may offer insights into the mechanisms through which collar deployment influences R_s . Soil temperature (ST, °C) and moisture (% v/v) were measured at a depth of 5 cm inside each collar. ST was measured using a thermocouple probe (LI-8100-203; Li-Cor Inc.) and soil moisture was measured using an automatic moisture sensor (Decagon Devices Inc.).

Soil samples were collected from each collar at the end of the study, and their physical and chemical properties were measured. Bulk density (BD) was measured using a bulk density sampling drill with a stainless steel cutting ring (5 cm in diameter and height). Within each collar, four random soil cores were collected using a 2 cm diameter, ethanol-disinfected auger. The soil cores were divided into four portions based on depth: 0–5, 5–10, 10–20, and 20–30 cm. The soil cores were sieved using a 2 mm sieve. The sieved roots were carefully washed in a 0.5 mm sieve, dried, and weighed to calculate root biomass (RB). Soils were collected from the same depth as the four cores in each collar, mixed in sterile bags as one sample, and then divided into three subsamples. The three subsamples were used to measure soil water content (SWC), solute concentrations, and microbial biomass carbon (MBC), respectively. Soil samples were placed in clean and resealable plastic bags and immediately transported to the laboratory in an incubator with ice packs.

Soil from one subsample was dried at 105°C for 8 h to measure total SWC. The soil from another subsample was suspended in water (soil: water ratio = 1:5) and pH and conductivity were measured. The concentrations of water-extracted nitrate (NO₃⁻-N), ammonium (NH₄⁺-N) and phosphate (PO₄⁻-P) in the soil samples were

analysed using a flow injection analyser (SmartChem 200; Westco, USA). Finally, MBC in the third subsample was measured using the chloroform fumigation-extraction method (Vance et al., 1987).

2.5 | Temperature sensitivity of R_s

The temperature sensitivity of R_s was quantified using in situ measurements (2017–2019) and an incubation experiment. For in situ assessments, field measurements of R_s and ST taken at a depth of 5 cm were used. For the incubation experiment, soil samples from different depths inside the long- and short-term collars were incubated using a fully automatic variable temperature soil incubation device (PRI-8800, Beijing, China) (Liu et al., 2018). Changes in CO₂ concentration in the headspace were automatically monitored using an isotope and gas concentration analyser (Picarro-G2131-i, California, USA). Specifically, for each sample, 20 g of fresh soil was adjusted to 55% water holding capacity and placed in a 150 ml polyethylene plastic bottle. A total of 48 soil samples from the six plots were used in the incubation experiment. All soil samples were placed in the PRI-8800 and pre-incubated at 20°C for 1 week. To maintain a constant soil moisture level, water loss was checked every 2 days, and the soil water content was adjusted according to weight (Liu et al., 2018). Sixteen incubation temperatures (–15 to 15°C, 2°C intervals) were established. The incubation temperature increased from –15 to 15°C. Each temperature was maintained for 3 h (equilibrium time) in a day, so that the soil sample could adapt to the changing temperature and the hysteresis of CO₂ release due to temperature changes was limited (Liu et al., 2018). The ST in each bottle was monitored every minute using a button thermometer (DS, 1922L; Maxim Integrated). The SOC decomposition rate was calculated from the slope of the CO₂ concentration versus time plot.

To describe the relationship between the soil CO₂ release rate and temperature, Q_{10} was calculated using equation:

$$R_s = A \times Q_{10}^{T/10},$$

where R_s is in situ measurement of R_s ($\mu\text{molCO}_2 \text{ m}^{-1} \text{ s}^{-1}$) or the SOC decomposition rate ($\mu\text{gCO}_2\text{-Cg}^{-1} \text{ soil day}^{-1}$) at an incubation temperature (°C); Parameter A is the basal microbial respiration rate at 0°C, which is used as an overall substrate quality index (Fierer et al., 2005; Liu et al., 2018).

2.6 | Data analysis

The data used in this study did not show a significant deviation from normal distributions; therefore, the data were not transformed for *t*-tests. Paired *t*-tests were used to compare differences in the average R_s rate, ST, and soil moisture between the short- and long-term collar treatments. Nonlinear regression of R_s and SOC decomposition rates versus temperature was performed

to calculate the Q_{10} of long- and short-term collars. Random forest (RF) (Breiman, 2001) was used to evaluate the relative importance of RB, MBC, SWC, pH, BD, DIN (nitrate + ammonium), PO₄[–]-P and ST at a depth of 0–10 cm to determine R_s . RF is a widely used machine-learning algorithm that allows the prediction of response variables from potential drivers and identification of the importance of these potential drivers. RF does not assume specific forms of function, for example, it does not assume a linear relationship as in multiple regressions, and thus is suitable for this study because how potential drivers influence R_s is still not known (Touw et al., 2013). The RF was run 100 times, and the increase in the mean square error or node purity was obtained to assess the importance of each potential driver of R_s (van Elsas et al., 2012). RF was implemented with the R package RANDOMFOREST (Breiman, 2001) and the R package RPERMUTE (Archer, 2013) was used to calculate the *p*-values of the importance of each control factor. All statistical analyses were conducted in R (version 3.6.1; R Development Core Team, 2017) and figures were produced using OriginPro 2017 (Originlab Corporation) and R (version 3.6.1). The significance level was set at *p* < 0.05.

3 | RESULTS

3.1 | R_s rate under short- and long-term collar deployment

Over the 3 years of the experiment, the differences in R_s rates between long- and short-term collars changed over time. In the first growing season, little overall difference was noted between long- and short-term collar deployments (*p* = 0.46) (Figure 2a,b). In the second and third growing seasons, the average R_s rate of the long-term collar decreased by 8.9% and 18.2%, respectively, relative to short-term collar deployment (Figure 2b). Particularly, R_s under the long-term collar decreased rapidly in the middle of the second growing season and remained low until the end of the experiment (Figure 2c,d).

3.2 | Soil variables between short- and long-term collars

The ST at a depth of 5 cm was 0.6°C higher in long-term collars during the first growing season (*p* = 0.02) but similar during the other experimental periods (*p* = 0.12) (Figure S1). Soil moisture significantly decreased by 1.6% (*p* = 0.007), 1.7% (*p* = 0.003) and 2.0% (*p* = 0.005) in the long-term collars at a depth of 5 cm during the three growing seasons, respectively (Figure S1). Relative to short-term deployments, soil RB and MBC contents inside the long-term collar significantly decreased by 47.3%–50.2% (*p* = 0.001) and 11.7%–21.8% (*p* < 0.001) at depths of 0–10 cm, respectively. The soil BD inside the long-term collar at depths of 0–10 cm increased significantly (*p* = 0.02), and soil pH (*p* = 0.03), NO₃[–]-N (*p* = 0.005), and PO₄[–]-P (*p* = 0.04) contents of the long-term collar increased significantly at depths of 0–5 cm. Soil SWC

($p = 0.02$), conductivity ($p = 0.007$) and $\text{NH}_4^+\text{-N}$ ($p = 0.002$) of the long-term collar significantly decreased at depths of 0–5 cm relative to the short-term collar (Figure 3).

3.3 | Temperature sensitivity of R_s and SOC decomposition rate

An exponential function provided an adequate fit for ST at 5 cm and R_s . The Q_{10} of the long-term collar was lower than that of the

short-term collar in the three growing seasons, and the difference gradually increased with the experimental duration (Figure 4a,d).

During the laboratory incubation experiment, the SOC decomposition rate at different incubation temperatures exhibited large variations in different soil layers after 3 years of long-term collar deployment. The mean decomposition rate of all soil layers collected inside the long-term collar was significantly lower than that collected under short-term collar deployment (all $p < 0.01$). Decomposition rates were 0.36, 0.28, 0.21 and 0.18 $\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil h}^{-1}$ for the 0–5, 5–10, 10–20 and 20–30 cm soil layers under the long-term collar deployment

FIGURE 2 (a) Soil respiration rate (R_s). (b) Mean values shown with standard error for R_s . (c) The 30-day rolling mean change in R_s in the long-term (LT) collar relative to the short-term collar (ST) during 2017–2019. (d) Relative change $[(R_{s\text{-LT}} - R_{s\text{-ST}})/R_{s\text{-ST}}]$ measured with the two methods during the growing season of 2017–2019. Error bars represent standard errors. Significant differences among the R_s for the two collars were indicated: * $p < 0.05$, ** $p < 0.01$.

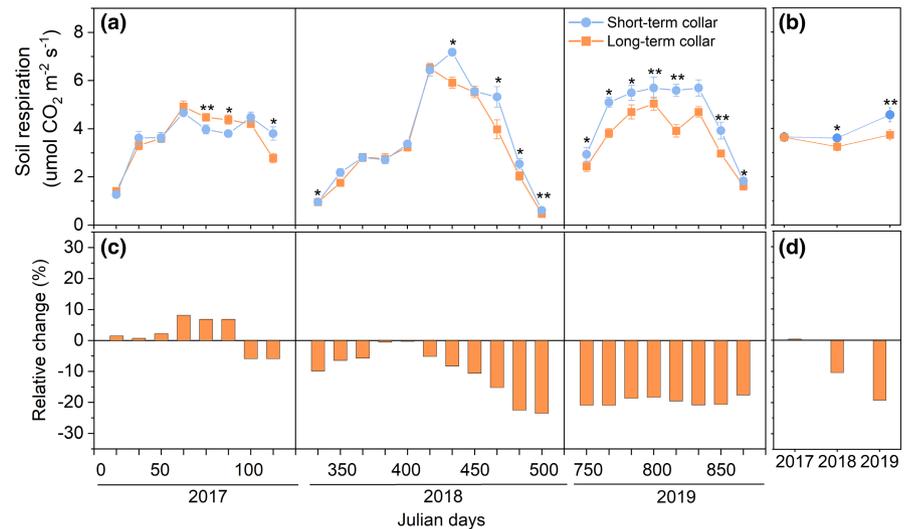
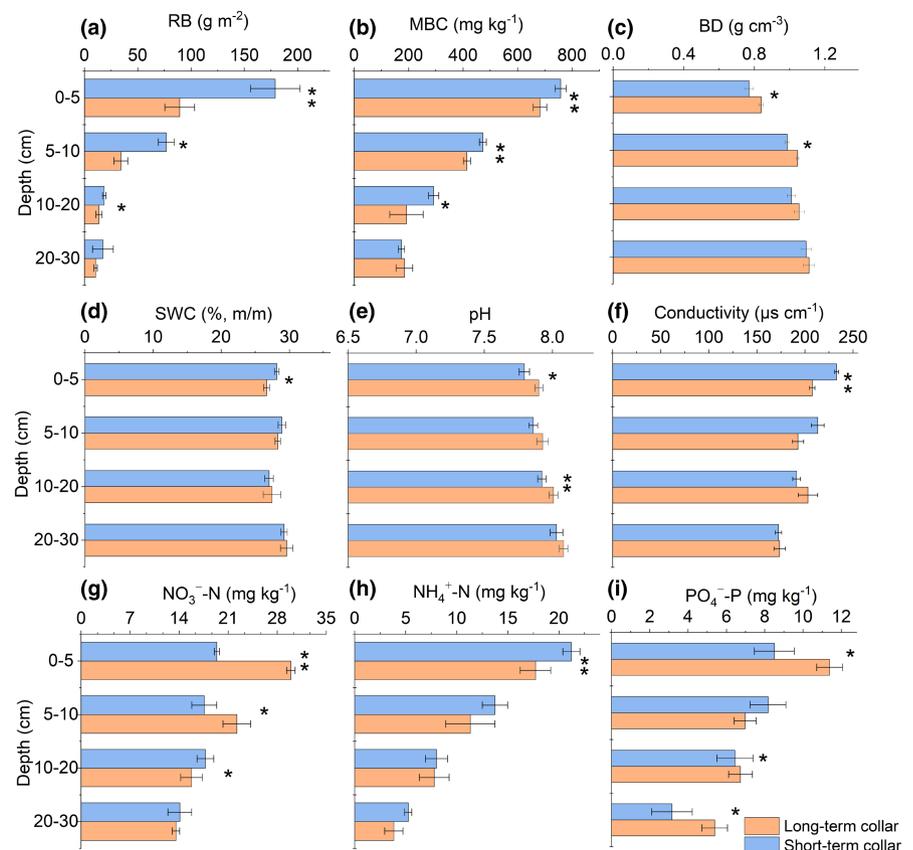


FIGURE 3 Soil biotic and abiotic variables of the different layers for the long-term installed collar (long-term collar) and short-term installed collar (short-term collar) measured at the end of the experiment in October 2019. (a) Distribution of the root biomass (RM), (b) soil microbial biomass carbon (MBC), (c) soil bulk density (BD), (d) soil water content (SWC), (e) soil pH, (f) soil conductivity, (g) soil nitrate content ($\text{NO}_3^-\text{-N}$), (h) soil ammonium content ($\text{NH}_4^+\text{-N}$) and (i) soil phosphate content ($\text{PO}_4^-\text{-P}$) in different soil layers. The error bars represent standard errors. Significant differences were indicated: * $p < 0.05$, ** $p < 0.01$.



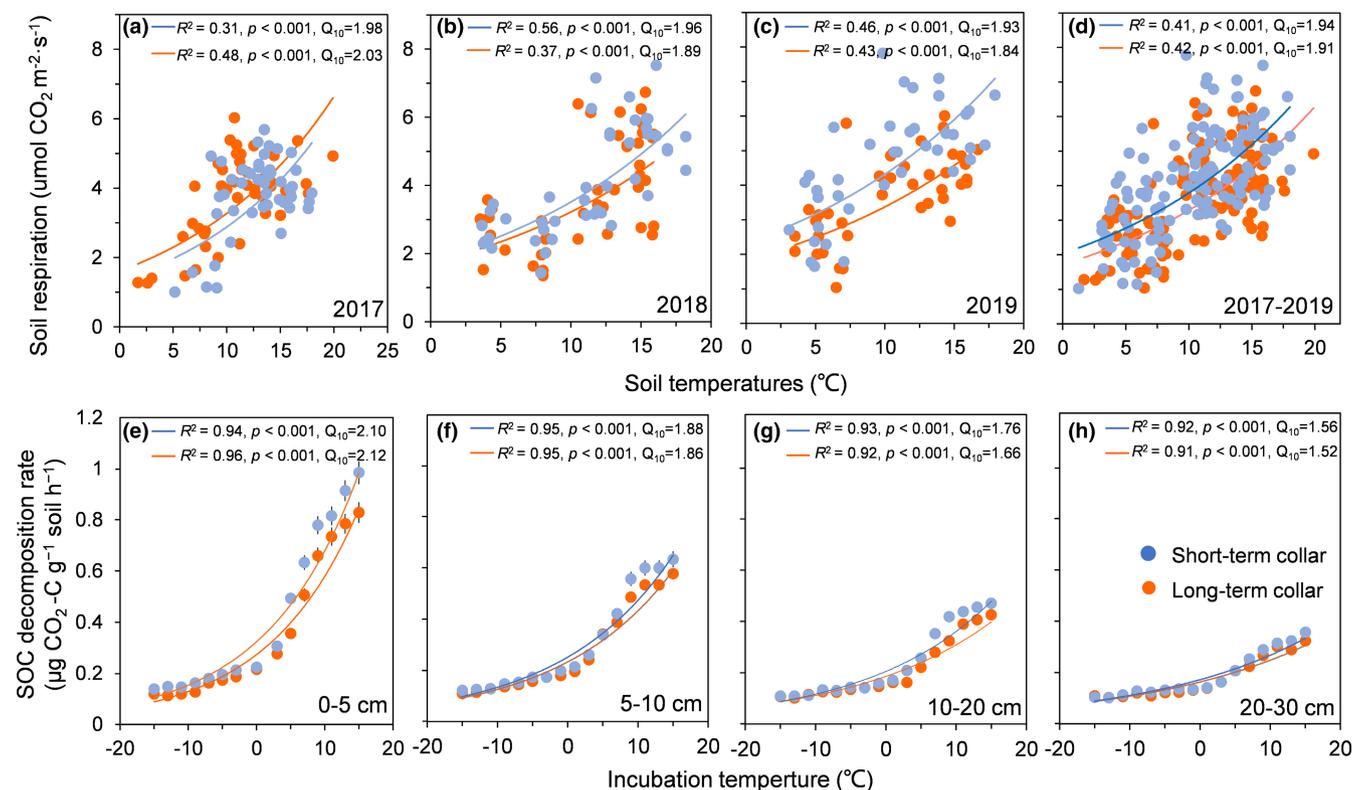


FIGURE 4 The relationships between soil respiration rate (R_s) of short-term and long-term collar measurements and temperature at 5 cm depth, and the temperature sensitivity of R_s from (a) May to September in 2017, (b) April to October in 2018 and (c) May to October in 2019 and (d) all measurements, respectively. The relationships between soil organic carbon (SOC) decomposition rate and temperature during aerobic incubation using soils from different depths for (e) 0–5 cm, (f) 5–10 cm, (g) 10–20 cm and (h) 20–30 cm, respectively, under incubation temperature from -15 to 15°C .

and 0.42 , 0.30 , 0.24 and $0.19 \mu\text{g CO}_2\text{-C g}^{-1} \text{ soil h}^{-1}$ for the same four layers under the short-term collar. Although the Q_{10} of the long-term collar was lower at different soil depths than that of the short-term collar (Figure 4e–h), differences were minor for all soil layers.

3.4 | Controlling factors for changes in R_s

RF analysis of R_s and soil variables showed that BD, RB and MBC were the main driving factors of R_s (all $p < 0.05$; Figure 5). RF analysis explained 63% of the variance in R_s .

4 | DISCUSSION

4.1 | Minor changes in R_s during the early phase of long-term collar deployment

R_s under long-term collar deployment was similar to that under short-term collar deployment during the initial one and half growing seasons (Figure 2a). This differs from previous studies where R_s measurements in grassland ecosystems were underestimated by 30%–50% during the peak of the growing season (Heinemeyer

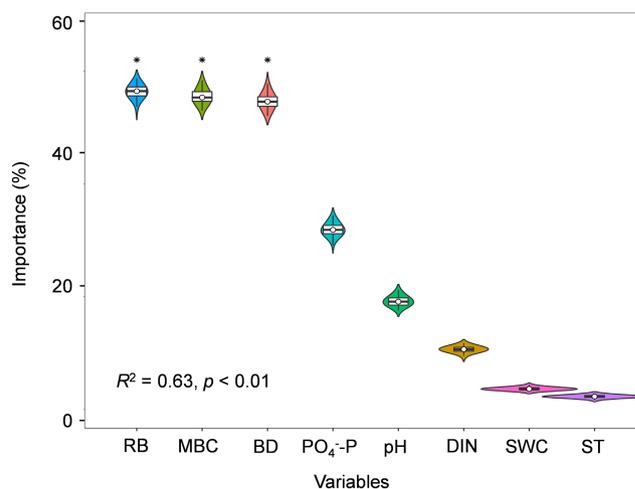


FIGURE 5 Violin plot of the relative contribution of driving factors to soil respiration (R_s). The importance of predictive variables was evaluated using the percentage increase (%) in mean square error of 100 times random forest model. The white dot is the average value of the variable. Abbreviations: BD, soil bulk density; DIN, nitrate + ammonium; MBC, soil microbial biomass carbon; pH, soil pH value; $\text{PO}_4\text{-P}$, phosphate; RB, root biomass; ST, soil temperature; SWC, soil water content. Significant differences were indicated: * $p < 0.05$.

et al., 2011). Several counteracting factors were speculated to contribute to the similarity in R_s between long- and short-term collars. First, R_s may be stimulated by higher ST inside the long-term collars. Higher temperatures inside the long-term collar may occur because plant removal inside the collar reduces plant transpiration or because cutting plants reduce shading, thus increasing solar radiation reaching the soil surface. Second, collar insertion and plant removal inside the long-term collar resulted in the pulse input of dead roots. Dead roots provide a temporary carbon source for decomposition. Previous studies on grasslands have shown that the contribution of R_h to total soil respiration increased immediately after collar insertion but declined to a stable level during the second year (Wang et al., 2021), consistent with the notion that dead roots may provide a temporary source for respiration. Third, counteracting the effects of higher ST and pulse input of dead roots reduced the live root biomass due to clipping and collar installation. Lower live-root biomass may lead to lower R_a and total root secretion in the rhizosphere, both of which may cause a decrease in R_s . Taken together, the positive and negative effects of long-term collar deployment on R_s are speculated to offset each other during the early stages of long-term collar deployment, resulting in little difference overall.

These results indicate that a permanent installation of collar for up to one and a half growing seasons may not cause substantial changes in R_s , at least in places with similar biotic and abiotic conditions as the experimental sites in this study. For the sake of accurate measurement of R_s , collar deployment is likely not an issue, although its component fluxes could be affected differently. However, the similarity in R_s is likely the result of several counteracting factors, as discussed above. Substantial differences in ST, moisture and vegetation cover were observed. As a result, even though R_s may be similar, many properties of R_s , such as Q_{10} or moisture dependence, may differ between collar treatments. Thus, caution should be exercised so that the non-significant effects of long-term collar use on R_s may not extend to other aspects of R_s .

4.2 | Decline in R_s and Q_{10} during the later phase

After the initial one and a half growing season, R_s under the long-term collar treatment started to decrease relative to the short-term collar treatment (Figure 2a). Two possible mechanisms may be responsible for the decline in R_s .

First, RB and MBC were significantly lower in the topsoil inside the long-term collar (Figure 3a,b). A decrease in RB may lead to a decrease in root metabolism and R_a (Väisänen et al., 2014), given that RB is often strongly associated with R_a in grasslands (Chen et al., 2009; Yan et al., 2010). Additionally, decreased RB resulted in a reduction in the synthesis and distribution of the soil respiratory substrate. This could lead to weakening of R_h (Davidson & Janssens, 2006). In addition, the decrease in soil MBC indicated that soil microbial activity and biomass had decreased (Chen et al., 2018; Han et al., 2014; Liu et al., 2009; Mu et al., 2017; Wan & Luo, 2003), which could lead to a decrease in R_h . The incubation results also

confirmed that long-term collar deployment significantly reduced SOC decomposition rate (Figure 4e-h). Briefly, a lower RB and microbial activity may limit R_s after long-term collar deployment.

Second, changes in soil variables in the collar during the later phase of long-term collar deployment may also contribute to the decrease in R_s . Long-term collar deployment and continuous cutting of surface vegetation seriously inhibited plant and root growth. Lower plant root growth, higher topsoil pH values (Figure 3e) and decreased conductivity (Figure 3f) could jointly cause a decline in soil microbial enzyme activity (Chen et al., 2018). Furthermore, microbial activity may have been altered as the decrease in vegetation productivity led to a decrease in soil organic matter input. This was supported by the increase in soil NO_3^- -N (Figure 3g), and a decrease in NH_4^+ -N content (Figure 3h). The increase in relative competitiveness and relative abundance of nitrifying bacteria promoted soil nitrification potential and accelerated the transformation of soil NH_4^+ -N into NO_3^- -N (Schmitt et al., 2013). In the later phase of long-term collar deployment, BD of the topsoil increased significantly (Figure 3c). Typically, a decrease in soil water holding capacity due to reduced root water uptake (Figure 3d and Figure S1), and lower fine root biomass and C input are associated with increased BD (Li & Shao, 2006). These factors are known to negatively correlate with R_s (Mu et al., 2017). This further explains why R_s decreased after long-term collar deployment. In summary, long-term collar deployment could reduce soil substrate supply, and microbial activity, and change soil variables. This weakened energy flow and material circulation (Aerts & Chapin, 1999; Moe et al., 2005), which may play a role in the decrease in R_s after long-term collar deployment (Schlesinger & Andrews, 2000). RF analysis also demonstrated that RB, MBC and BD were the main driving factors of R_s in the later phases of long-term collar deployment (Figure 5).

Similar to the R_s results, Q_{10} under long-term collar deployment decreased gradually with time, as compared to short-term collars (Figure 4a-c). This was consistent with previous findings that Q_{10} decreased with times of cutting (Li et al., 2013; Wan & Luo, 2003). The decrease in Q_{10} over time was likely due to reduced soil C input caused by reduced RB and root C transfer to the soil within the long-term collar. In addition, lower RB may lead to a decrease in the priming effect. The results of soil incubation showed that the Q_{10} of the SOC decomposition rate in different layers decreased, but there was little difference after the three growing seasons (Figure 4e-g). This further suggests that the decreased Q_{10} of R_s may be mainly due to reduced R_a .

5 | CONCLUSIONS

Long-term collar deployment had a substantial influence on R_s , as previously observed for short-term collars (Heinemeyer et al., 2011) and the magnitude of the effects changed over time. R_s was 8.9% and 18.2% lower in the second and third growing seasons, respectively, after collar deployment. These findings highlight different

factors controlling R_s change in long-term collar deployment during the early and later phases. During the early phase, minor changes in R_s may be mainly explained by the turnover of cut roots and changes in soil temperature and moisture, which may compensate for each other. During the later phase, the decrease in R_s and its Q_{10} may be explained by changes in soil physicochemical properties (e.g. BD), lower RB and root-derived C input, and microbial biomass.

Our results indicate that R_s and Q_{10} previously monitored using long-term collar installation should be adjusted based on the duration of collar deployment. Whenever long-term collars are deployed in the field for R_s measurements, periodic measurements are recommended using a method that causes minimal or no disturbance to the soil as a benchmark for calibration. Although instruments capable of measuring R_s without disturbing the soil would be ideal, no such instruments exist. While surface collars do not disturb the soil, they cannot create an airtight chamber unless the surface is perfectly flat. Thus, short-term collars are believed to provide the best benchmark for adjusting data based on long-term collar deployment. Because the differences in R_s between short- and long-term collar changes over time, short-term collar measurements must cover a similar time span as the long-term collar measurements. This ensures that a time-specific calibration can be performed. Alternatively, one can shift the locations of long-term collars every 1–2 years to avoid collar artefacts. If short-term collar or other minimal disturbance methods are not feasible, researchers should be cautious in interpreting the R_s and Q_{10} data obtained from long-term collar methods.

AUTHOR CONTRIBUTIONS

J.-S.H. designed the study. Z.Z., S.J. and X.M. conducted the field experiments. X.M. performed sample and data analyses. H.W., C.S. and J.-S.H. provided comments on the manuscript. X.M. wrote the paper with input from all co-authors.

ACKNOWLEDGEMENTS

The authors thank the Qinghai Haibei National Field Research Station of the Alpine Grassland Ecosystem for their logistic support. This study was financially supported by the National Natural Science Foundation of China (grant nos 32192461 and 32130065).

CONFLICT OF INTEREST

The authors have no competing interests that might influence the results and discussion reported in this paper.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/2041-210X.14056>.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at <https://doi.org/10.6084/m9.figshare.21644966>.

ORCID

Xiaoliang Ma  <https://orcid.org/0000-0002-0562-4328>
 Shengjing Jiang  <https://orcid.org/0000-0002-3538-4046>
 Hao Wang  <https://orcid.org/0000-0001-9115-1290>
 Chao Song  <https://orcid.org/0000-0001-8225-4490>
 Jin-Sheng He  <https://orcid.org/0000-0001-5081-3569>

REFERENCES

- Aerts, R., & Chapin, F. S. (1999). The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. In A. H. Fitter & D. G. Raffaelli (Eds.), *Advances in ecological research* (pp. 1–67). Academic Press.
- Archer, E. (2013). *Estimate permutation p-values for importance metrics*. R package version 1.5.2.
- Batubara, S. F., Agus, F., Rauf, A., & Elfiati, D. (2019). Soil respiration and microbial population in tropical peat under oil palm plantation. *IOP Conference Series: Earth and Environmental Science*, 260, 012083. <https://doi.org/10.1088/1755-1315/260/1/012083>
- Bond-Lamberty, B., & Thomson, A. (2010). Temperature-associated increases in the global soil respiration record. *Nature*, 464, 579–582. <https://doi.org/10.1038/nature08930>
- Breiman, L. (2001). Random forests. *Machine Learning*, 45, 5–32. <https://doi.org/10.1023/A:1010933404324>
- Chen, J., Luo, Y., García-Palacios, P., Cao, J., Dacal, M., Zhou, X., Li, J., Xia, J., Niu, S., Yang, H., Shelton, S., Guo, W., & van Groenigen, K. J. (2018). Differential responses of carbon-degrading enzyme activity to warming: Implications for soil respiration. *Global Change Biology*, 24, 4816–4826. <https://doi.org/10.1111/gcb.14394>
- Chen, S., Lin, G., Huang, J., & Jenerette, G. D. (2009). Dependence of carbon sequestration on the differential responses of ecosystem photosynthesis and respiration to rain pulses in a semi-arid steppe. *Global Change Biology*, 15, 2450–2461. <https://doi.org/10.1111/j.1365-2486.2009.01879.x>
- Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 440, 165–173. <https://doi.org/10.1038/nature04514>
- Fierer, N., Craine, J. M., McLaughlan, K., & Schimel, J. P. (2005). Litter quality and the temperature sensitivity of decomposition. *Ecology*, 86, 320–326. <https://doi.org/10.1890/04-1254>
- Friedlingstein, P., Jones, M. W., O'Sullivan, M., Andrew, R. M., Hauck, J., Peters, G. P., Peters, W., Pongratz, J., Sitch, S., Le Quéré, C., Bakker, D. C. E., Canadell, J. G., Ciais, P., Jackson, R. B., Anthoni, P., Barbero, L., Bastos, A., Bastrikov, V., Becker, M., ... Zaehle, S. (2019). Global carbon budget 2019. *Earth System Science Data*, 11, 1783–1838. <https://doi.org/10.5194/essd-11-1783-2019>
- Gershenson, A., Bader, N. E., & Cheng, W. (2009). Effects of substrate availability on the temperature sensitivity of soil organic matter decomposition. *Global Change Biology*, 15, 176–183. <https://doi.org/10.1111/j.1365-2486.2008.01827.x>
- Görres, C. M., Kammann, C., & Ceulemans, R. (2016). Automation of soil flux chamber measurements: Potentials and pitfalls. *Biogeosciences*, 13, 1949–1966. <https://doi.org/10.1088/1755-1315/260/1/012083>
- Han, G., Luo, Y., Li, D., Xia, J., Xing, Q., & Yu, J. (2014). Ecosystem photosynthesis regulates soil respiration on a diurnal scale with a short-term time lag in a coastal wetland. *Soil Biology and Biochemistry*, 68, 85–94. <https://doi.org/10.1016/j.soilbio.2013.09.024>
- Hartley, I. P., Heinemeyer, A., & Ineson, P. (2007). Effects of three years of soil warming and shading on the rate of soil respiration: Substrate availability and not thermal acclimation mediates observed response. *Global Change Biology*, 13, 1761–1770. <https://doi.org/10.1111/j.1365-2486.2007.01373.x>
- Healy, R. W., Striegl, R. G., Russell, T. F., Hutchinson, G. L., & Livingston, G. P. (1996). Numerical evaluation of static-chamber measurements

- of soil-atmosphere gas exchange: Identification of physical processes. *Soil Science Society of America Journal*, 60, 740–747. <https://doi.org/10.2136/sssaj1996.03615995006000030009x>
- Heinemeyer, A., Di Bene, C., Lloyd, A. R., Tortorella, D., Baxter, R., Huntley, B., Gelsomino, A., & Ineson, P. (2011). Soil respiration: Implications of the plant-soil continuum and respiration chamber collar-insertion depth on measurement and modelling of soil CO₂ efflux rates in three ecosystems. *European Journal of Soil Science*, 62, 82–94.
- Heinemeyer, A., Tortorella, D., Petrovičová, B., & Gelsomino, A. (2012). Partitioning of soil CO₂ flux components in a temperate grassland ecosystem. *European Journal of Soil Science*, 63, 249–260. <https://doi.org/10.1111/j.1365-2389.2010.01331.x>
- Hutchinson, G. L., & Livingston, G. P. (2001). Vents and seals in non-steady-state chambers used for measuring gas exchange between soil and the atmosphere. *European Journal of Soil Science*, 52, 675–682. <https://doi.org/10.1046/j.1365-2389.2001.00415.x>
- Jovani-Sancho, A. J., Cummins, T., & Byrne, K. A. (2017). Collar insertion depth effects on soil respiration in afforested peatlands. *Biology and Fertility of Soils*, 53, 677–689. <https://doi.org/10.1007/s00374-017-1210-4>
- Kirschbaum, M. U. F. (2004). Soil respiration under prolonged soil warming: Are rate reductions caused by acclimation or substrate loss? *Global Change Biology*, 10, 1870–1877. <https://doi.org/10.1111/j.1365-2486.2004.00852.x>
- Li, J.-J., Liu, L., Chen, D.-M., Xu, F.-W., Cheng, J.-H., & Bai, Y.-F. (2019). Effects of collar size and buried depth on the measurement of soil respiration in a typical steppe. *Chinese Journal of Plant Ecology*, 43, 152–164. <https://doi.org/10.17521/cjpe.2017.0280>
- Li, X., Zhang, C., Fu, H., Guo, D., Song, X., Wan, C., & Ren, J. (2013). Grazing exclusion alters soil microbial respiration, root respiration and the soil carbon balance in grasslands of the loess plateau, northern China. *Soil Science and Plant Nutrition*, 59, 877–887. <https://doi.org/10.1080/00380768.2013.862157>
- Li, Y. Y., & Shao, M. A. (2006). Change of soil physical properties under long-term natural vegetation restoration in the loess plateau of China. *Journal of Arid Environments*, 64, 77–96. <https://doi.org/10.1016/j.jaridenv.2005.04.005>
- Liu, W., Zhang, Z. H. E., & Wan, S. (2009). Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. *Global Change Biology*, 15, 184–195. <https://doi.org/10.1111/j.1365-2486.2008.01728.x>
- Liu, Y., He, N., Wen, X., Xu, L., Sun, X., Yu, G., Liang, L., & Schipper, L. A. (2018). The optimum temperature of soil microbial respiration: Patterns and controls. *Soil Biology and Biochemistry*, 121, 35–42. <https://doi.org/10.1016/j.soilbio.2018.02.019>
- Luo, Y., & Zhou, X. (2010). *Soil respiration and the environment*. Academic Press.
- Ma, Z., Liu, H., Mi, Z., Zhang, Z., Wang, Y., Xu, W., Jiang, L., & He, J.-S. (2017). Climate warming reduces the temporal stability of plant community biomass production. *Nature Communications*, 8, 15378. <https://doi.org/10.1038/ncomms15378>
- Menyailo, O. V., Matvienko, A. I., Stepanov, A. L., & Makarov, M. I. (2015). Measuring soil CO₂ efflux: Effect of collar depth. *Russian Journal of Ecology*, 46, 152–156. <https://doi.org/10.1134/S1067413615020071>
- Mills, R., Glanville, H., McGovern, S., Emmett, B., & Jones, D. L. (2011). Soil respiration across three contrasting ecosystem types: Comparison of two portable IRGA systems. *Journal of Plant Nutrition and Soil Science*, 174, 532–535. <https://doi.org/10.1002/jpln.201000183>
- Moe, S. J., Stelzer, R. S., Forman, M. R., Harpole, W. S., Daufresne, T., & Yoshida, T. (2005). Recent advances in ecological stoichiometry: Insights for population and community ecology. *Oikos*, 109, 29–39. <https://doi.org/10.1111/j.0030-1299.2005.14056.x>
- Mu, C., Zhang, T., Zhao, Q., Su, H., Wang, S., Cao, B., Peng, X., Wu, Q., & Wu, X. (2017). Permafrost affects carbon exchange and its response to experimental warming on the northern Qinghai-Tibetan plateau. *Agricultural and Forest Meteorology*, 247, 252–259. <https://doi.org/10.1016/j.agrformet.2017.08.009>
- Raich, J. W., Potter, C. S., & Bhagawati, D. (2002). Interannual variability in global soil respiration, 1980–94. *Global Change Biology*, 8, 800–812. <https://doi.org/10.1046/j.1365-2486.2002.00511.x>
- R Development Core Team. (2017). A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.r-project.org/>
- Schlesinger, W. H., & Andrews, J. A. (2000). Soil respiration and the global carbon cycle. *Biogeochemistry*, 48, 7–20. <https://doi.org/10.1023/A:1006247623877>
- Schlesinger, W. H., & Bernhardt, E. S. (2013). *Biogeochemistry: An analysis of global change*. Academic Press.
- Schmitt, A., Pausch, J., & Kuzyakov, Y. (2013). Effect of clipping and shading on C allocation and fluxes in soil under ryegrass and alfalfa estimated by ¹⁴C labelling. *Applied Soil Ecology*, 64, 228–236. <https://doi.org/10.1016/j.apsoil.2012.12.015>
- Subke, J.-A., Inglima, I., & Francesca Cotrufo, M. (2006). Trends and methodological impacts in soil CO₂ efflux partitioning: A meta-analytical review. *Global Change Biology*, 12, 921–943. <https://doi.org/10.1111/j.1365-2486.2006.01117.x>
- Touw, W. G., Bayjanov, J. R., Overmars, L., Backus, L., Boekhorst, J., Wels, M., & van Hijum, S. A. F. T. (2013). Data mining in the life sciences with random Forest: A walk in the park or lost in the jungle? *Briefings in Bioinformatics*, 14, 315–326. <https://doi.org/10.1093/bib/bbs034>
- Väisänen, M., Ylännä, H., Kaarlejärvi, E., Sjögersten, S., Olofsson, J., Crout, N., & Stark, S. (2014). Consequences of warming on tundra carbon balance determined by reindeer grazing history. *Nature Climate Change*, 4, 384–388. <https://doi.org/10.1038/nclimate2147>
- Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, 19, 703–707. [https://doi.org/10.1016/0038-0717\(87\)90052-6](https://doi.org/10.1016/0038-0717(87)90052-6)
- van Elsas, J. D., Chiurazzi, M., Mallon, C. A., Elhottová, D., Kristófek, V., & Salles, J. F. (2012). Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 1159–1164. <https://doi.org/10.1073/pnas.1109326109>
- Wan, S., & Luo, Y. (2003). Substrate regulation of soil respiration in a tall-grass prairie: Results of a clipping and shading experiment. *Global Biogeochemical Cycles*, 17. <https://doi.org/10.1029/2002GB001971>
- Wang, W. J., Zu, Y. G., Wang, H. M., Hirano, T., Takagi, K., Sasa, K., & Koike, T. (2005). Effect of collar insertion on soil respiration in a larch forest measured with a LI-6400 soil CO₂ flux system. *Journal of Forest Research*, 10, 57–60. <https://doi.org/10.1007/s10310-004-0102-2>
- Wang, Y., Liu, H., Chung, H., Yu, L., Mi, Z., Geng, Y., Jing, X., Wang, S., Zeng, H., Cao, G., Zhao, X., & He, J.-S. (2014). Non-growing-season soil respiration is controlled by freezing and thawing processes in the summer monsoon-dominated Tibetan alpine grassland. *Global Biogeochemical Cycles*, 28, 1081–1095. <https://doi.org/10.1002/2013GB004760>
- Wang, Y., Song, C., Liu, H., Wang, S., Zeng, H., Luo, C., & He, J.-S. (2021). Precipitation determines the magnitude and direction of interannual responses of soil respiration to experimental warming. *Plant and Soil*, 458, 75–91. <https://doi.org/10.1007/s11104-020-04438-y>
- Yan, L., Chen, S., Huang, J., & Lin, G. (2010). Differential responses of auto- and heterotrophic soil respiration to water and nitrogen addition in a semiarid temperate steppe. *Global Change Biology*, 16, 2345–2357. <https://doi.org/10.1111/j.1365-2486.2009.02091.x>
- Zhou, X., Wan, S., & Luo, Y. (2007). Source components and interannual variability of soil CO₂ efflux under experimental warming and clipping in a grassland ecosystem. *Global Change Biology*, 13, 761–775. <https://doi.org/10.1111/j.1365-2486.2007.01333.x>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1 Variation of (a) soil temperature and (b) moisture at depth of 5 cm in the long- and short-term collars during 2017–2019. The mean soil (c) temperatures and (d) moisture for the two collars during the growing season of 2017–2019. The \pm represents standard errors. Significant differences among soil temperatures, and moisture for the two collars were indicated: repeated measure ANOVA; *, $p < 0.05$, **, $p < 0.01$.

Figure S2 Relationships between soil respiration rates and moisture from 2017–2019.

How to cite this article: Ma, X., Jiang, S., Zhang, Z., Wang, H., Song, C., & He, J.-S. (2023). Long-term collar deployment leads to bias in soil respiration measurements. *Methods in Ecology and Evolution*, 14, 981–990. <https://doi.org/10.1111/2041-210X.14056>