Supplementary material

Comparing the temperature sensitivity of organic matter decomposition in oxic and oxygen-deprived soils

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1 Experiment 1

1.1 Study area and sample collection

Three grassland soils (0–10 cm depth) experiencing periods of oxygen (O_2) limitation which was caused by freeze-thaw were sampled with each site including four sampling points (> 200 m spacing) from Shaliuhe (SLH) and Haibei (HB) in Qinghai Province, and Argun (AGN) from Inner Mongolia (Table S1). Soils were mixed evenly and passed through a 2 mm sieve after visible roots were removed. The soils were kept at 4 °C for < 1 month before conducting experiments 1 and 2. These sites have a monsoon climate, with about 80% of annual precipitation occurring in summer (Liu et al., 2018). Heavy precipitation in summer and melting of snow and ice in spring leads to the frequent occurrence of O_2 limitation. The dominant species are *Elymus nutans Griseb*, *Kobresia humilis*, and *Leymus chinensis* in sites of SLH, HB, and AGN, respectively.

1.2 Soil incubation experiment

The experiment included three soils above, three levels of O_2 concentration at oxic (21% O_2 +79% N_2), suboxic (1% O_2 + 99% argon (Ar)), and anoxic (100% Ar). There were 9 treatments with four replicates for each treatment, resulting in 40 bottles in total including four empty bottles as blank control. Soils were incubated under three varying temperature cycles to quantify soil gas emissions and temperature sensitivity (Q_{10}) of soil organic matter decomposition (Fang et al., 2005). In this study, the temperature variation range was set to 5 to 30 to 5 °C with a 5 °C step based on the annual variation

of air temperature in the study area (Fig. S1).

Fresh soil (10 g dry weight) was added to a 100 ml brown bottle, and adjusted its moisture content to 60% of the maximum water holding capacity (WHC), and covered using porous film for a 7-day pre-incubation at 25 °C. Subsequently, the bottles were sealed with butyl rubber septa and flushed using corresponding gases (oxic, suboxic, and anoxic) for 40 min, O_2 indicator tablets (MGC, Japan) were used to confirm the anoxic state in the anoxic-treated bottles. Given the increasing dissolution of carbon dioxide (CO_2) in the pore water of soils resulting from the excessive accumulation of CO_2 in the bottles (Oren and Steinberger, 2008), the concentration of CO_2 in the headspace was kept to less than 1000 ppmv by adjusting the time lengths of sealing. Thus, the incubation time for respiration detection was set to 24, 14, 10, 6, 4, and 2 h at 5, 10, 15, 20, 25, and 30 °C (Fig. S1), respectively. At the end of each temperature point, gas samples (30 ml) were collected from the headspace using a syringe. The depleted gas was then replaced with corresponding gas to maintain identical pressure with the external environment. The concentration of CO_2, methane (CH_4), and nitrous oxide (N_2O) was determined using a gas chromatograph (Agilent 7890A, California, USA) within one week. Methane was not detected during the experiment, suggesting that alternative electron acceptors such as iron were not depleted in our soils.

Three temperature cycles were performed to test the response of Q_{10} to incubation time. After the first temperature cycle (lasting 2.5 days), soil samples were placed in an incubator (5 °C) for 90 days in the darkness, followed by the second temperature cycle, and then placed in the incubator (5 °C) for 70 days in the darkness, followed by the

third temperature change cycle. Considering the existence of long-term low temperatures during the freeze-thaw period in the study area, the incubation temperature was set to a 5 ℃ incubator between two temperature cycles. Bottles were purged with the corresponding gas for 30 minutes every 2–3 days during the period between two temperature cycles.

Soil gases were calculated using equation S1：

$$R = V \times (C_s - C_b) \times \frac{273.15}{(273.15 + T)} \times \frac{M}{m_{\text{soil}} \times t_{incubation}} \quad \text{(S1)}$$

Where, R is soil gases emission rate (μg g^{-1} soil h^{-1}); V is the headspace volume of the bottle (l); C_s and C_b are the gas concentration (ppmv) in the soil sample and the blank bottle, respectively; T is the incubation temperature (°C); 22.4 (l mol^{-1}) is the molar volume of the gas under the standard state; M is the molecular weight; m_{soil} is the dry weight of the soil sample (g); $t_{\text{incubation}}$ is the time lengths of sealing incubation (h).

The Q_{10} was calculated using an exponential equation as follows:

$$R_s = a \times e^{bT} \quad \text{(S2)}$$

$$Q_{10} = e^{10b} \quad \text{(S3)}$$

where, R_s was the soil CO_2 release rate (μg C g^{-1} soil h^{-1}); a and b were obtained from the fitted models; T is the incubation temperature (°C).

2 Experiment 2

2.1 Experimental design

The experimental soils and treatments were the same as in experiment 1 without the temperature cycles. Fresh soils (10 g dry weight) were incubated for 94 days at 60%

of water holding capacity at 25 °C after 7 days pre-incubation. The gases of the headspace were flushed by the corresponding gas every 2–3 days to avoid the accumulation of gases in the bottle and soil moisture was maintained by adding Milli-Q water. Soils were destructively sampled on Days 6, 35, and 94 to measure water-extractable organic carbon (WEOC) and total nitrogen (WETN), ferrous iron (Fe^{2+}), ultraviolet absorbance at 254 nm ($SUVA_{254}$), and the activity of L-leucine aminopeptidase (LAP).

2.2 Soil physicochemical analyses

The WEOC and WETN were extracted by shaking 10 g of fresh soil in 30 ml of Milli-Q water for 12 h. The suspension after centrifuging at 4000 rpm for 10 min was filtered through pre-rinsed 0.45-μm Whatman GF/F filters. An aliquot of the extracts was used to measure WEOC and WETN concentration using a multi N/C analyzer (multi N/C analyzer 2100S, Analytik Jena, Germany). Another aliquot was used to detect the ultraviolet absorbance between 200 and 800 nm using an ultraviolet and visible spectrometer (Shimadzu UV-2550). The $SUVA_{254}$ ($L\ mg^{-1}\ C\ m^{-1}$) was calculated by absorbance at 254 nm divided by the corresponding WEOC concentration.

Soil Fe(II) contents were measured by the ferrozine-ultraviolet absorbance method (Stookey, 1970). Briefly, the mixture was filtered after shaking the mixture of 5 mL of HCl (0.5 M) and 0.2 g of fresh soil for 12 h. The filter was added with 5 mM ferrozine solution, and then to determine Fe(II) at 562 nm using a UV-Vis spectrometer (Shimadzu UV-2550).

The activities of LAP were analyzed using fluorogenic substrates (Marx et al. 2001). Briefly, 1 g of fresh soil was thoroughly mixed with 40 ml of ultrapure water. A 200 µl aliquot of soil suspension was added into each well of 96-well microplates, to which 50 µl 7- Amino-4-methylcoumarin (MUC)-substrates were added. The microplate was incubated for 3 h at 25° C in the darkness. Fluorescence was detected using an automated fluorometric plate reader (BioTek, USA) at an excitation of 360 nm and emission of 450 nm.

References

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Oren, A., Steinberger, Y., 2008. Coping with artifacts induced by $CaCO_3$–CO_2–H_2O equilibria in substrate utilization profiling of calcareous soils. Soil Biology and Biochemistry 40, 2569–2577.

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Table S1 Site information and soil physicochemical properties in the site of Shaliuhe (SLH) and Haibei (HB), and Argun (AGN) (mean ± standard error, n = 3).

Site	SLH	HB	AGN
Location (Latitude, longitude)	37°32', 100°03'	37°36', 101°19'	50°10', 119°24'
Elevation (m)	3485	3215	520
Soil pH	5.96 ± 0.00	7.78 ± 0.01	6.84 ± 0.04
Soil organic matter (SOC) (%)	6.24 ± 0.09	4.29 ± 0.06	2.54 ± 0.13
Total nitrogen (TN) (%)	0.53 ± 0.01	0.45 ± 0.01	0.22 ± 0.01
SOC/TN	13.88 ± 0.19	11.24 ± 0.22	13.60 ± 0.31

Figure captions

Figure S1 Incubation temperature and time for sampling gas.

Figure S2 (A) Soil respiration rate and (B) nitrous oxide (N_2O) emissions in soils from Shaliuhe (SLH) and Haibei (HB), and Argun (AGN). The curve was obtained by fitting the exponential equation (eq. 1). Data is means (n = 3) and standard errors.

Figure S3 (A) Soil water-extractable organic carbon (WEOC), (B) water-extractable total nitrogen (WETN), (C)WEOC/WETN ratio, (D) ferrous iron (Fe^{2+}), (E) $SUVA_{254}$, and (F) L-leucine aminopeptidase (LAP) activity in soils from Shaliuhe (SLH) and Haibei (HB), and Argun (AGN). Data is means (n = 3) and standard errors.

Figure S1

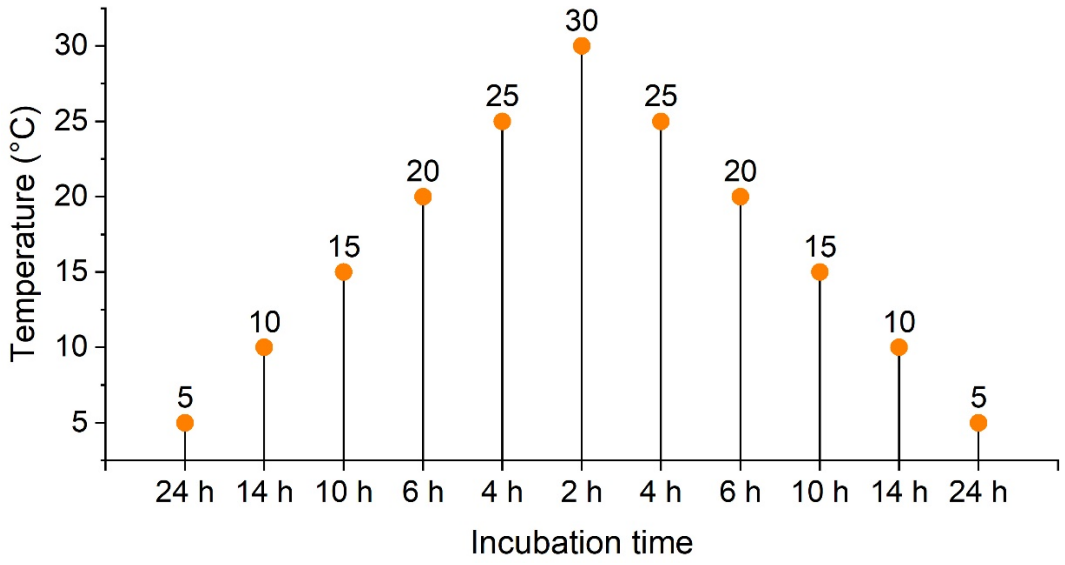

Temperature (°C)
30
25
20
15
10
5
5
10
15
20
25
30
25
20
15
10
5
24 h
14 h
10 h
6 h
4 h
2 h
4 h
6 h
10 h
14 h
24 h
Incubation time

Figure S2

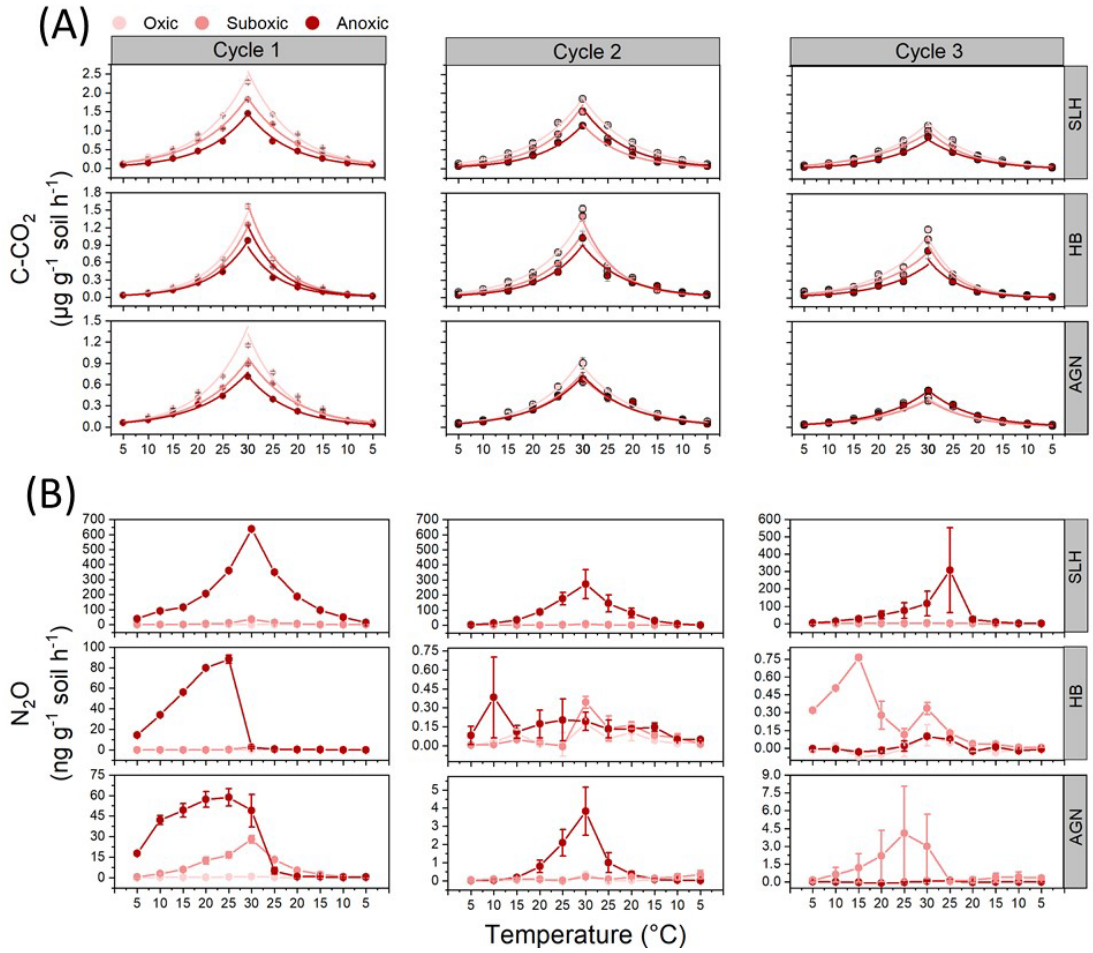
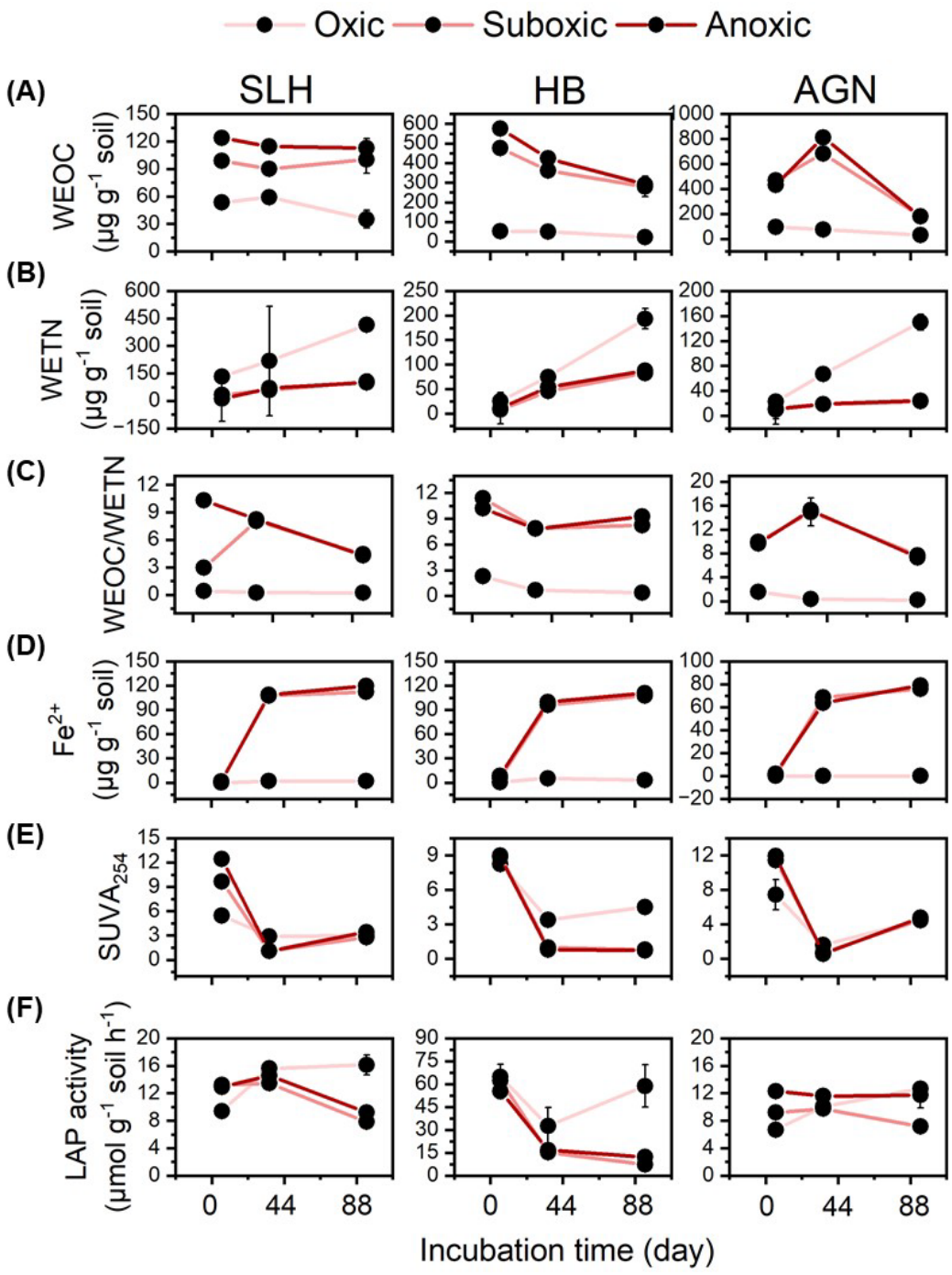

(A)
Oxic
Suboxic
Anoxic
Cycle 1
Cycle 2
Cycle 3
SLH
HB
AGN
C-CO2 (µg g-1 soil h-1)
(B)
N2O (ng g-1 soil h-1)
Temperature (°C)

Figure S3


Oxic
Suboxic
Anoxic
(A)
SLH
HB
AGN
WEOC (μg g-1 soil)
(B)
WETN (μg g-1 soil)
(C)
WEOC/WETN
(D)
Fe2+ (μg g-1 soil)
(E)
SUVA254
(F)
LAP activity (μmol g-1 soil h-1)
0
44
88
Incubation time (day)