Changes in the temperature sensitivity of SOM decomposition with grassland succession: implications for soil C sequestration

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Introduction
It has become clear that the decomposition of soil organic matter (SOM) is positively correlated with temperature (Hartley and Ineson 2008; Zimmermann and Bird 2012; Zimmermann et al. 2012). The index of temperature sensitivity (Q¹₀) has been widely used to depict the response of SOM decomposition to diurnal or seasonal temperature changes and increasingly to predict the response of soil carbon (C) sequestration in terrestrial ecosystems under warmer climate scenarios (Kirschbaum 1995; Luo et al. 2001; Knorr et al. 2005; Davidson and Janssens 2006; Conant et al. 2011).

However, the temperature sensitivity of SOM decomposition remains a highly debated topic, particularly in relation to the extent to which regional or global convergence of Q¹₀ occurs (Zhou et al. 2009; Mahecha et al. 2010; Sierra 2012), and also regarding whether Q¹₀ differs among soils and soil fractions (e.g., labile vs. recalcitrant) (Fang et al. 2005, 2006; Karlu et al. 2010a; Plante et al. 2010; Wetterstedt and Agren 2011; Tucker et al. 2013). Landscapes (e.g., grassland or forest) comprise mixed series of ecosystems in stage of succession or degradation. Understanding whether SOM decomposition and Q¹₀ vary with ecosystem succession is important to evaluating soil C sequestration. To date, the variability of

Abstract
Understanding the temperature sensitivity (Q¹₀) of soil organic matter (SOM) decomposition is important for predicting soil carbon (C) sequestration in terrestrial ecosystems under warming scenarios. Whether Q¹₀ varies predictably with ecosystem succession and the ways in which the stoichiometry of input SOM influences Q¹₀ remain largely unknown. We investigate these issues using a grassland succession series from free-grazing to 31-year grazing-exclusion grasslands in Inner Mongolia, and an incubation experiment performed at six temperatures (0, 5, 10, 15, 20, and 25°C) and with four substrates: control (CK), glucose (GLU), mixed grass leaf (GRA), and Medicago falcata leaf (MED). The results showed that basal soil respiration (20°C) and microbial biomass C (MBC) logarithmically decreased with grassland succession. Q¹₀ decreased logarithmically from 1.43 in free-grazing grasslands to 1.22 in 31-year grazing-exclusion grasslands. Q¹₀ increased significantly with the addition of substrates, and the Q¹₀ levels increased with increase in N:C ratios of substrate. Moreover, accumulated C mineralization was controlled by the N:C ratio of newly input SOM and by incubation temperature. Changes in Q¹₀ with grassland ecosystem succession are controlled by the stoichiometry of newly input SOM, MBC, and SOM quality, and the combined effects of which could partially explain the mechanisms underlying soil C sequestration in the long-term grazing-exclusion grasslands in Inner Mongolia, China. The findings highlight the effect of substrate stoichiometry on Q¹₀ which requires further study.
Q_{10} with ecosystem succession has not been extensively evaluated.

Inner Mongolian grasslands in China (about 150 million ha) have an enormous capacity to sequester atmospheric CO_2 through rational land-use practices, especially through grazing exclusion (GE) (He et al. 2008, 2012). Soil C storage demonstrated an initial rapid increase following GE introduction and was maintained at a steady state after two decades of GE (He et al. 2008). Understanding the stability of accumulated SOM (decomposition rate and Q_{10}) under long-term GE would be useful for accurately evaluating long-term soil C sequestration. The “carbon quality-temperature (CQT)” hypothesis predicts that the temperature sensitivity of SOM decomposition increases with biochemical recalcitrance, and this hypothesis has been tested on a variety of substrates and scales (Fierer et al. 2005; Conant et al. 2008b; Craine et al. 2010). Therefore, we assumed that Q_{10} should decrease with grassland succession as GE period increases because the C associated with sand and silt fractions in surface soil increase logarithmically (He et al. 2009). Moreover, the higher N:C ratio of SOM should enhance Q_{10} and soil C mineralization due to greater N limitation in grassland ecosystems (Billings and Ballantyne 2013).

Using a grassland succession series from free-grazing to 31-year GE, we conducted an 8-week incubation experiment using six temperatures and four substrates with differing N:C ratios. The main objectives of this study were to (1) investigate whether Q_{10} decreased predictably with grassland succession consistent with the CQT hypothesis, (2) determine the influence of the N:C stoichiometry of substrates on Q_{10} and soil C mineralization, and (3) discuss soil C sequestration mechanisms in Inner Mongolian grasslands in view of SOM decomposition.

**Material and Methods**

**Study sites**

The study was conducted in a typical steppe ecosystem on the Mongolian Plateau (43°33’N, 116°40’E). The area is a typical semi-arid continental climate with a mean annual temperature of 1.1°C and average annual precipitation of 345 mm (He et al. 2008). The soil is chestnut type, that is, Calcic kastanozems. The vegetation consists predominantly of grassland plants, such as Leymus chinensis, Stipa grandis, and Cleistogenes squarrosa.

Five grasslands, with similar vegetation and topography across in 2 km × 2 km in extent, comprised a successional series of grassland restoration, where plant communities and soil properties varied predictably after eliminating the large animal disturbance by fences (Table 1). The five plots were categorized as GE0, GE4, GE7, GE11, and GE31. Plot GE0 was subjected to long-term free-grazing and was in a slightly degraded condition in terms of the aboveground community and plant diversity. Plot GE4 was established in 2008 by fencing off a section of previously free-grazing grassland. Plots GE7, GE11, and GE31 were similarly established in 2004, 1999, and 1979, respectively (He et al. 2008). We assumed that changes in vegetation and soil properties among the five plots were mainly a result of grazing intensity and the length of GE, because the five experimental plots were floristically and topographically similar, and all were distributed in the same upper basalt platform.

**Field sampling**

Field sampling was conducted in July 2011. In each plot, 4 sampling quadrats (1 m × 1 m) were established at 10-m intervals along a random transect. Aboveground biomass was clipped at ground level. Soil samples in the 0–20 cm soil layer were collected from 10 points in each quadrate. In the laboratory, we manually removed roots and visible organic debris from soil samples, and then measured soil water-holding capacity (WHC, %) and soil gravimetric moisture (%). Approximately 100 g of each soil samples was air-dried for analysis of soil properties (e.g., C, N, and pH). The remaining soil was stored at 4°C.

**Laboratory incubation and analysis**

The incubation experiment was conducted at six temperatures (0, 5, 10, 15, 20, and 25°C), using four substrates (control (CK), glucose (GLU), mixed grass leaf (GRA), and Medicago falcata leaf (MED)). GRA and MED were collected from five plots and mixed evenly. The C concentration in GLU, GRA, and MED was 40.0%, 45.1%, and 44.0%, respectively, and the N concentration was 0%, 2.0%, and 5.2%, respectively. Thus, the N:C ratios of the substrates in increasing order were GLU (0) < GRA (0.043) < MED (0.117). We added 1% of the mass of the incubated soils as GLU, GRA, and MED, which approximated a 2-year input of new SOM from roots and litter in Inner Mongolian grasslands. In total, the incubation experiment comprised 480 samples, with 5 grassland types, 6 temperatures, 4 substrates, and 4 replicates for each treatment.

First, 40 g samples of fresh soils were put into incubation bottles, and the samples were adjusted to 60% WHC. Samples were incubated at 20°C and an instant 80% humidity for 4 days and then incubated at a treatment temperature (0, 5, 10, 15, 20, or 25°C) for 3 days prior to measurement of basal soil respiration. The substrates were...
subsequently added and mixed evenly. During the 56 days incubation experiment, soil respiration rates were measured 14 times, on days 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, 42, 49, and 56.

An automatic system for measuring soil respiration rates was developed through modification of the continuous gas-flow system reported by Cheng et al. (1993). The system consisted of a Li-COR CO₂ analyzer (Li-7000), an electric water bath to control incubation temperature, an air-flow controller, soda-lime equipment to control the initial CO₂ concentration, an auto-sampler on a turn-plate, automatic transformation valves to control the sample bottle, and a data collector (Fig. 1). In practice, the system was controlled by the data collector and first automatically lowered the CO₂ concentration by using a bypass system of soda lime and then recorded the changes in CO₂ concentration as it steadily increased. Soil respiration rates were calculated from the slope of the CO₂ concentration as follows:

\[
R = \frac{C \times V \times \alpha \times \beta}{m}
\]

where \( R \) is soil respiration rate (\( \mu g CO₂ g^{-1} h^{-1} \)); \( C \) is the slope of the CO₂ concentration; \( V \) is the volume of the incubation bottle and gas tube; \( m \) is the soil weight (g); \( \alpha \) is the transformation coefficient of CO₂ mass; and \( \beta \) is the transformation coefficient of time.

Soil organic C (SOC, %) was measured using a modified Mebius method (Nelson and Sommers 1982). Soil total N (%) was measured with a modified Kjeldahl wet-digestion procedure (Gallaher et al. 1976), using a 2300

| Grassland type            | Aboveground biomass (g m⁻²) | Soil organic carbon (g kg⁻¹) | Soil total nitrogen (g kg⁻¹) | Microbial biomass C (µg kg⁻¹) | Soil pH | Land-use history                        |
|---------------------------|----------------------------|-----------------------------|-----------------------------|-------------------------------|--------|----------------------------------------|
| Free-grazing grassland (GE0) | 60.3 ± 20.6*              | 13.41 ± 0.46*              | 1.43 ± 0.10*                | 47.52 ± 0.46*                 | 8.17 ± 0.29* | Long-term free-grazing, good condition |
| 4-year grazing exclusion (GE4) | 162.3 ± 15.0b             | 15.95 ± 0.55b              | 1.60 ± 0.03b                | 42.69 ± 1.72ab                | 8.07 ± 0.11a | Grassland fenced since 2008, good condition |
| 7-year grazing exclusion (GE7) | 166.2 ± 13.3b             | 16.32 ± 2.06bc             | 1.64 ± 0.16b                | 38.06 ± 1.59b                 | 7.92 ± 0.16ab | Grassland fenced since 2004, good condition |
| 11-year grazing exclusion (GE11) | 171.6 ± 9.6b              | 18.19 ± 0.49c              | 1.72 ± 0.10a                | 39.53 ± 1.89b                 | 7.66 ± 0.19b | Grassland fenced since 1999, good condition |
| 31-year grazing exclusion (GE31) | 148.9 ± 41.3b             | 17.73 ± 2.18bc             | 1.48 ± 0.72a                | 40.02 ± 0.66b                 | 7.19 ± 0.29c | Grassland fenced since 1979, good condition |

*Data are represented as means ± 1 SD (n = 4). The same superscript letters within each column indicating no significant difference among grassland types at \( P < 0.05 \) level (ANOVA).
Kjeltec Analyzer Unit (FOSS Tecator, Höganäs, Sweden). Soil pH was determined using a pH meter and a slurry of soil mixed with distilled water (1:2.5). Microbial biomass C (MBC) of fresh soil samples was analyzed using the fumigation – extraction method (Vance et al. 1987).

Calculations and statistical analysis

In this study, we selected the data from 1, 7, and 56 days incubations, respectively, to represent the instantaneous-term, short-term, and longer-term effects of the experimental treatments on soil C mineralization. Soil C mineralization at 20°C without substrate addition was used as the base soil respiration ($C_{\text{min-20°C}}$).

The temperature sensitivity ($Q_{10}$) of soil respiration in the 1- and 7-day incubations was calculated using the exponential equations, because 56-day incubation should be too longer to accurately evaluate $Q_{10}$ due to the respiration rates declining faster at higher temperature with faster depletion of substrate.

\[
Y = A \times \exp\left(\frac{B}{T}\right)
\]

\[
Q_{10} = \exp^{10\times B}
\]

where $Y$ is the soil respiration rate ($\mu g C g^{-1} h^{-1}$), $T$ is the temperature (°C); $A$ and $B$ are the constants.

The stimulating effects (SEs) of soil respiration were then calculated to represent the sequestration capacity of grassland soils with fresh SOM input. SEs were calculated from the accumulated C mineralization under different substrates (GLU, GRA, or MED) divided by that of CK; higher values of SEs implied lower sequestration capacity.

One-way analysis of the variance (ANOVA) was used to investigate the differences in vegetation and soil properties among different grassland types. Univariate analysis of three factors (general linear model) was used to determine the effects of grassland types, incubation temperature, and substrates on soil C mineralization, $Q_{10}$, and SEs. Logarithmic regression was used to identify the changing trend of $Q_{10}$ and SEs with the duration of GE, MBC, and SOC. Data have been represented as means ± 1 standard deviation ($n=4$). Differences were considered to be significant when $P<0.05$. All analyses were conducted using SPSS statistical software (v. 13.0, SPSS, Chicago, IL, USA).

Results

Changes in soil properties

SOC increased significantly with increasing duration of GE ($F = 9.18, P < 0.001$); however, soil N content was not significantly different among grasslands. With grassland succession as a function of duration of GE, soil pH decreased from 8.17 (GE0) to 7.19 (GE31) ($F = 15.57, P < 0.001$) and MBC decreased logarithmically ($F = 12.26, P = 0.004$).

Changes in soil C mineralization

$C_{\text{min-20°C}}$ without the addition of external substrates differed significantly among various grasslands and decreased logarithmically with increasing duration of GE.

Figure 2. Changes in soil C mineralization with grazing-exclusion duration, substrate, and incubation temperature. CK, control; GLU, glucose; GRA, mixed grass leaf; MED, Medicago falcate leaf. Data were derived from 7-day incubation and represented as mean ± SD ($n=4$).
Grassland type, incubation temperature, and added substrates significantly influenced soil C mineralization, with notable interactive effects (Fig. 2, Table 2). Moreover, accumulated C mineralization in the CK, GLU, and GRA cases increased with temperature during the 8-week incubation in all grasslands. However, under MED conditions, accumulated C mineralization increased with temperature in the first week and then was highest at 15°C (Fig. 3).

**Changes in Q10**

Grassland type and added substrate had significant effects on Q\textsubscript{10} and again showed interactions with each other (Fig. 4, Table 3). Q\textsubscript{10} values decreased significantly with the grassland succession (F = 3.87, P = 0.011 for 1 day; F = 6.32, P < 0.001 for 7 days). Q\textsubscript{10} values increased significantly with increasing N:C stoichiometry of substrates (F = 5.84, P = 0.001 for 1 day; F = 7.66, P < 0.001 for 7 days). Q\textsubscript{10} in the first day were ordered as follows: CK (1.305) < GRA (1.872) < GLU (2.263) < MED (3.222). In CK, GLU, and GRA, Q\textsubscript{10} was correlated logarithmically with C\textsubscript{min}-20°C, MBC, SOC, and duration of GE (Fig. 5, Table 4). However, no significant correlation was found with MED addition.

**Changes in SEs**

Grassland type, incubation temperature, and substrate significantly influenced SEs, with interactions among their effects (Fig. 6, Table 5). Compared with temperature and grassland type, added substrates had the strongest effect on SEs, and the effects of MED were significantly higher than those of GLU and GRA (Fig. 6).

### Table 2. Results of univariate analysis of accumulated C mineralization ($C_{\text{min}}$) according to grassland type, incubation temperature, and substrate addition.

|                  | $C_{\text{min}}$-1 day | $C_{\text{min}}$-7 day | $C_{\text{min}}$-56 day |
|------------------|------------------------|------------------------|------------------------|
|                  | F                      | P                      | F                      | P                      | F                      | P                      |
| Grassland type (G) | 52.69                  | <0.0001                | 124.81                 | <0.0001                | 286.78                 | <0.0001                |
| Incubation temperature (T) | 587.31                | <0.0001                | 2501.55                | <0.0001                | 1253.31                | <0.0001                |
| Substrate addition (S) | 965.76                | <0.0001                | 6668.08                | <0.0001                | 4701.82                | <0.0001                |
| G $\times$ T | 6.75                   | <0.0001                | 23.12                  | <0.0001                | 26.57                  | <0.0001                |
| G $\times$ S | 6.57                   | <0.0001                | 20.86                  | <0.0001                | 60.27                  | <0.0001                |
| T $\times$ S | 144.25                 | <0.0001                | 555.77                 | <0.0001                | 220.02                 | <0.0001                |
| G $\times$ T $\times$ S | 1.69 | 0.0020 | 3.77 | <0.0001 | 5.94 | <0.0001 |
Discussion

Soil respiration with grassland succession

Soil respiration rates and MBC both decreased logarithmically with succession of grassland restoration and were significantly linearly correlated with that grazing grasslands had higher soil respiration rates, MBC, and dissolved organic C than did grazing-exclusion grasslands (Wu et al. 2012). The decrease in soil respiration with grazing exclusion mostly resulted from a decrease in MBC or from changes in microbial communities. Our results also supported the assumption that Inner Mongolian grasslands subjected to grazing exclusion have apparent C sequestration capacity as a result of depressed soil respiration (Zhou et al. 2007; He et al. 2008, 2012; Ingram et al. 2008).

These findings were not consistent with the assumption that soil respiration should increase to offset newly input SOM with grazing-exclusion grassland succession. One possible explanation is that inputs of new SOM act as a binding agents and promote the formation of soil aggregates in grazing-exclusion grasslands without large-animal trampling (Steffens et al. 2009; Wiesmeier et al. 2012; Zimmermann et al. 2012). Decreases in MBC and microbial activity and greater physical protection of SOM by increased soil aggregation (Plante et al. 2009) should be the mechanism underlying increased C sequestration in soils in these long-term grazing-exclusion grasslands in Inner Mongolia.

Temperature sensitivity with ecosystem succession

$Q_{10}$ decreased logarithmically with grassland restoration succession and was negatively correlated with the content of SOC, which is in agreement with the “C quality-temperature” hypothesis, that $Q_{10}$ should be inversely related to C quality (Conant et al. 2008a; Craine et al. 2010; Xu et al. 2012). The C associated with sand and silt fractions in the surface soil increased logarithmically as the period of GE increased (He et al. 2009), which implied greater instability of SOM and lower $Q_{10}$. This issue is still highly debated, although a large number of studies have focused on $Q_{10}$ from various soil types, soil profiles, land-use types, and latitude gradients (Pavelka et al. 2007; Vanhala et al. 2008; Karhu et al. 2010b; Schindlbacher et al. 2010; Jenkins and Adams 2011). Zhou et al. (2009) demonstrated that the global pattern of $Q_{10}$ varied from 1.40 to 2.03. Mahecha et al. (2010) found $Q_{10}$ to be globally convergent on 1.40, independent of mean annual temperature, and invariant among biomes.

Temperature sensitivity with different stoichiometry of input SOM

Stoichiometry of input SOM (N:C ratios) has a significant influence on the $Q_{10}$ of SOM decomposition, which implies that the Michaelis–Menten equation ($R = (V_{max}[C]/(K_m + [C]))$ appropriately depicts the response of soil respiration after the addition of substrates because the canceling effect of $K_m$ on the apparent $Q_{10}$ is greatly reduced by substrate saturation. Gershenson et al. (2009) reported that substrate availability had a significant positive effect on $Q_{10}$. Compared with GLU and GRA, MED had a significantly greater effect on $Q_{10}$, which implies that substrates with higher N:C will facilitate $Q_{10}$ by reducing microbial N limitation in the process of SOM decomposition. Similarly, Billings and Ballantyne
(2013) demonstrated that any reduction in the C:N flow ratio resulting from temperature increase could exacerbate extant C limitation or shift microbes from N limitation to C limitation. Our findings supported the assumption of Billings and Ballantyne (2013) and further highlight the importance of stoichiometry of substrate.

In theory, a negative respiratory response to warming should be observed as microbes experience greater relative C limitation, although such a response may be mediated by shifts in function within the microbial community (Luo et al. 2001). The N:C ratios of added substrates significantly affected $Q_{10}$; however, it is still not clear whether $Q_{10}$ is linearly related to the N:C ratio of these substrates. Therefore, changes in the plant community and in N:C ratios of new SOM inputs should influence the stability of intrinsic SOM and soil C sequestration potential, through regulation of $Q_{10}$ by altering the stoichiometry of new inputs of SOM from root exudates in Inner Mongolian grasslands (Kong et al. 2011). If this holds true, achieving increased soil C storage by sowing legumes, as confirmed by many studies, should be re-evaluated under warming scenarios.

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**Figure 5.** Relationship between temperature sensitivity ($Q_{10}$) in 7-day incubation and duration of GE (A), $C_{min-20\degree C}$ (B), MBC (C), and SOC (D).

**Table 4.** Relationships of $Q_{10}$ with $C_{min-20\degree C}$, MBC, SOC, and duration of GE.

|                  | $C_{min-20\degree C}$ (µg C g$^{-1}$)† | MBC (µg C g$^{-1}$)‡ | SOC (g kg$^{-1}$) | Duration of GE (year) |
|------------------|---------------------------------------|----------------------|------------------|----------------------|
|                  | $R^2$ | $P$     | $R^2$ | $P$     | $R^2$ | $P$     | $R^2$ | $P$     |
| 1-day            |       |         |       |         |       |         |       |         |
| $Q_{10,CK}$*     | 0.434 | 0.002   | 0.510 | 0.001   | 0.413 | 0.003   | 0.693 | <0.001  |
| $Q_{10,GLU}$     | 0.416 | 0.002   | 0.212 | 0.041   | 0.107 | 0.160   | 0.253 | 0.024   |
| $Q_{10,GRA}$     | 0.422 | 0.003   | 0.253 | 0.028   | 0.281 | 0.020   | 0.674 | <0.001  |
| $Q_{10,Med}$     | 0.048 | 0.355   | 0.001 | 0.916   | 0.082 | 0.220   | 0.258 | 0.023   |
| 7-day            |       |         |       |         |       |         |       |         |
| $Q_{10,CK}$      | 0.608 | <0.001  | 0.408 | 0.002   | 0.541 | <0.001  | 0.684 | <0.001  |
| $Q_{10,GLU}$     | 0.555 | <0.001  | 0.455 | 0.001   | 0.310 | 0.011   | 0.646 | <0.001  |
| $Q_{10,GRA}$     | 0.426 | 0.001   | 0.374 | 0.004   | 0.375 | 0.004   | 0.726 | <0.001  |
| $Q_{10,Med}$     | 0.161 | 0.080   | 0.168 | 0.072   | 0.144 | 0.099   | 0.471 | 0.001   |

* $Q_{10}$ calculated for soil C mineralization in 1-day and 7-day incubations.
† $C_{min-20\degree C}$ is the soil respiration rate without substrate addition under 20°C after 1-day and 7-day incubation.
‡ MBC is microbial biomass C measured using the fumigation-extraction method.
§ Logarithmic equations suited most situations, identified by the minimum Akaike Information Criterion (AIC).
SEs with grassland succession and stoichiometry of input SOM

Grassland type, incubation temperature, and added substrates influenced SEs of CO2 emission after the addition of substrates. SEs were higher in GE0 and GE31, but lower in GE4, GE7, and GE11, which implied that the soils in GE0 and GE31 had lower C sequestration for a given input of SOM. This finding provides one of underlying mechanisms for an initial rapid increase in soil C storage following GE introduction followed by relatively stable levels after 2 decades of GE (He et al. 2008; Wu et al. 2008). Temperature influences the magnitude and duration of SEs after substrates are added. SEs increased with increasing temperature in the first stage and then reached a maximum at 15°C for GLU, GRA, and MED addition. Increase in the higher N:C ratio of the substrates added led to increased SEs in the first stage, but these were of shorter duration. The possible explanations for this are as follows: (1) addition of higher N:C substrates temporarily improves SOM quality and accelerates soil respiration, but this then leads to microbial N limitation when labile SOM is depleted (Billings and Ballantyne 2013), (2) temperature affects soil microbial enzyme activity, and 15°C should be the optimum temperature at which limitation of SOM quality after substrate addition does not occur.

In conclusion, the Q_{10} of SOM decomposition decreased logarithmically with grassland restoration succession and was negatively correlated with SOC content. The stoichiometry (N:C) of new SOM input had an apparent influence on soil C mineralization and temperature sensitivity, and higher N:C ratios led to higher Q_{10} levels and stronger soil C mineralization. Our findings imply that changes in the plant community and in the N:C ratios of new SOM input from litter, root, and roots exudates would influence the stability of intrinsic SOM and soil C sequestration potential through regulation of Q_{10}. Overall, changes in Q_{10} with ecosystem succession are controlled by MBC, SOM quality, and the stoichiometry of new input SOM; these should be the underlying ecosystem-level mechanisms for soil C sequestration in grazing-exclusion grasslands.

![Figure 6. Stimulating effect of soil C mineralization according to grassland type (A), incubation temperature (B), and substrate type (C). Data with the same lowercase letters showed no significant difference at P = 0.05 level.](image)

Table 5. Univariate analysis of stimulating effects (SEs) according to grassland type, incubation temperature, and substrate addition.

|                      | SE_{1 day} | SE_{7 day} | SE_{56 day} |
|----------------------|------------|------------|-------------|
|                      | F          | P          | F           | P          | F           | P          |
| Grassland type (G)   | 21.37      | <0.0001    | 188.44      | <0.0001    | 79.36       | <0.0001    |
| Temperature (T)      | 417.12     | <0.0001    | 2979.44     | <0.0001    | 216.82      | <0.0001    |
| Substrate addition (S) | 553.31   | <0.0001    | 4740.99     | <0.0001    | 1042.76     | <0.0001    |
| G x T                | 2.97       | <0.0001    | 94.07       | <0.0001    | 32.68       | <0.0001    |
| G x S                | 5.04       | <0.0001    | 198.83      | <0.0001    | 79.40       | <0.0001    |
| T x S                | 99.79      | <0.0001    | 1648.51     | <0.0001    | 355.05      | <0.0001    |
| G x T x S            | 1.69       | 0.0082     | 87.02       | <0.0001    | 37.82       | <0.0001    |

*SE_{1 day}, SE_{7 day}, and SE_{56 day} represent the stimulating effect of substrates added in 1-, 7-, and 56-day incubations, respectively, which were calculated from the accumulated C mineralization for different substrates (GLU, GRA, and MED) divided by that of CK.
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Conflict of Interest

None declared.

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