Original article

Effects of temperature, glucose and inorganic nitrogen inputs on carbon mineralization in a Tibetan alpine meadow soil

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A B S T R A C T

High levels of available nitrogen (N) and carbon (C) have the potential to increase soil N and C mineralization. We hypothesized that with an external labile C or N supply alpine meadow soil will have a significantly higher C mineralization potential, and that temperature sensitivity of C mineralization will increase. To test the hypotheses an incubation experiment was conducted with two doses of N or C supply at temperature of 5, 15 and 25 °C. Results showed external N supply had no significant effect on CO2 emission. However, external C supply increased CO2 emission. Temperature coefficient (Q10) ranged from 1.13 to 1.29. Significantly higher values were measured with C than with N addition and control treatment. Temperature dependence of C mineralization was well-represented by exponential functions. Under the control, CO2 efflux rate was 425 g CO2-C m -2 year -1, comparable to the in situ measurement of 422 g CO2-C m -2 year -1. We demonstrated if N is disregarded, microbial decomposition is primarily limited by lack of labile C. It is predicted that labile C supply would further increase CO2 efflux from the alpine meadow soil.

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1. Introduction

Understanding the sensitivity of organic C mineralization in relation to soil temperature and soil C or N content is important for the reliable prediction of C dynamics under future climatic scenarios. Future changes in global climate include increases in temperature and changes in atmospheric CO2 concentrations as well as atmospheric N deposition [17]. All these changes would affect various soil processes [1,14]. It has been suggested that global warming will increase organic matter decomposition which will release CO2 into the atmosphere contributing to further warming by creating a positive feedback loop resulting in an additional increase in atmospheric CO2 [26,27]. In addition, higher soil temperatures as well as N deposits may lead to an enhanced soil N availability and subsequently to an increase in net primary production (NPP) since NPP is nutrient-limited in these particular ecosystems [19,23,34]. The changes in C and N content within litter itself will, in turn, affect decomposition rates of organic material [10,13,27]. Until now the influence in feedback processes of climatic changes on C and N mineralization are not clear.

Alpine and arctic soils are of particular interest when considering responses to potential global changes [31] since global warming is expected to be the most severe in alpine and arctic ecosystems [22,33]. Dense organic soils within these particular ecosystems contain a large stock of organic C [3,28]. The Qinghai-Tibet Plateau covers approximately 2.5 million km2 with an average altitude of more than 4000 m ASL. Approximately 35% of its area is alpine meadow [46] where low temperature causes low rates of organic matter decomposition [47]. Net N mineralization rates in these meadows reflect microbial immobilization during the growing season (−26.79 mg m -2 day -1). Most N is bound in organic forms [5]. Growth of alpine flora is nutrient-limited during the entire growing season, with particular deficiencies in mineral N at the beginning of the growing season [47]. Responses of soil respiration to temperature in alpine meadows have been rigorously measured in situ by examining the diel or seasonal changes in CO2 efflux [16]. Results showed that the soil respiration rate is to a large extent related to soil temperature at 5 cm depth [16]. These measurements were used as comparison with ecosystem respiration determined from the eddy covariance method (Halbei
Research Station is one of the locations of China Flux) [16]. Although field measurements have clear advantages when examining relationships under natural conditions, effects of soil temperature and the limitation of soil C or N content to soil microbial activity are difficult to verify since these variables co-vary in the field. Moreover, field measurements of soil organic matter decomposition are difficult to work with as they need to separate the root respiration from total soil respiration. Further move, questions such as, how soil respiration responds to elevated temperature and to changes in soil available C or N content, and to what extent of temperature sensitivity of soil organic decomposition is influenced by external C or N supply, are unclear. Answers to these questions are essential when attempting to predict CO2 flux patterns, to improve our understanding of CO2 efflux dynamics in natural ecosystems. Accordingly, an incubation experiment under controlled conditions was conducted under three (5, 15 and 25 °C) soil temperatures and external labile C or N input. Soil respiration in this experiment was compared with the field measurements. The effect of external C or N supply and elevated temperatures on mineralization dynamics were examined along with total CO2 release.

The specific aims of this study were: i) to test the effects of soil temperature and external C or N supply on C mineralization rates and to try to discern the limiting factors of C mineralization for alpine meadow soil; ii) to investigate the temperature sensitivity of C mineralization to external C or N supply; iii) to compare estimates of CO2 emissions from the laboratory with in situ field measurements; and iv) to predict the potential CO2 efflux caused by soil heterotrophic respiration under external C or N inputs. An attempt was made to test the following hypotheses by integrating the results of the aforementioned experiments: (1) High labile C or N levels in soil result in high heterotrophic respiration, and (2) high labile C or N levels result in high temperature sensitivity of C mineralization.

2. Materials and methods

2.1. Study sites and description

Soil samples were collected from an experimental site at the Haibei Alpine Meadow Ecosystem Experimental Station (CERN, www.chinaflux.org), Chinese Academy of Sciences, located in the northeastern region of the Qinghai-Tibet Plateau (37°32’N, 101°15’E), China. The average altitude of this area is 3240 m ASL. Annual precipitation is 618 mm of which 85% is concentrated in the growing season (from May to September). Mean annual temperature is −1.7 °C. Soil type is classified as Mat Cry-gelic Cambisols [7] corresponding to Gelic Cambisol [43]. Permafrost keeps the soil cold [44]. A detailed description of soils is provided in Table 1 [53]. The study area is dominated by perennial grass Kobresia humilis Serg (Cyperaceae). Common species include grasses as Stipa aliena, Elymus nutans Griseb, Festuca ovina Linn., herbs as Saussurea superba Anth., Gentiana lawrencei Burk.var farreri T.N.Ho, Gentiana straminea Maxim., Potentilla nivea Linn., Potentilla saundersiana Royle, Scirpus distigmaticus Tang et Wang, Cyperaceae grasses as Kobresia pygmaea C.B. Clarke in Hook, and Carex sp [47]. Total surface vegetative cover is more than 95%. Rooting depth is shallow where more than 90% of root mass is concentrated within the upper 15 cm soil layer [47]. Plant growth is N limited with marked growth responses occurring in the month of August [47].

2.2. Soil sampling and preparation

Intact soil samples, of an approximate dimension of 7.5 cm diameter and 10 cm depth were obtained from five random points along arbitrarily laid transects. Five field replicates of each of these soils were collected for all soils on July 10, 2007. Samples were transported in cooled containers (4 °C) to Beijing. The five soil cores in each transect were mixed together in the laboratory where roots and stones were removed. The soil samples were then sieved first through a 2 mm sieve to homogenize samples. The sieved soil was kept at a temperature of 4 °C until incubation started shortly thereafter.

Soil moisture was measured using the gravimetric method (105 °C, 24 h). Soil pH values were measured using a glass electrode with a 1:2 soil-to-water ratio. Total N was measured by way of Kjeldahl digestion with a salicylic acid modification [29]. Total N in 0.5 M K2SO4 extracts (1:4 soil extractant) was also determined by way of Kjeldahl digestion of a salicylic acid modification whereas NH4−N and NO3−N were measured by way of steam distillation using MgO, and applying Devarda’s alloy to reduce NO3− to NH4 [4]. Dissolved organic nitrogen (DON) was calculated as the difference between total N and exchangeable inorganic N within the extracts. Microrganism biomass N was estimated by a chloroform fumigation–direct extraction technique [8]. SOC was measured following the method described by Kaledasba and Jenkinson [20]. Results of the primary soil characteristics are presented in Table 1.

2.2.1. Sample incubation

Incubation was carried out in the laboratory for 28 days. Two 2-way designs were performed (temperature × N and temperature × C). External N or C inputs were sub-factor incubated at the temperature treatments, respectively. The equivalent of 30 g of the sieved soil samples were weighed into 250 ml Schott jars. Additive N doses were 0.024 and 0.018 mg (NH4)2SO4 g−1 dry soil for each subsample, equivalent to 0.005 and 0.025 mg N g−1 dry soil. Additive C doses were 5 and 25 mg glucose (C6H12O6) g−1 dry soil for each subsample, equivalent to 2 and 10 mg C g−1 dry soil. Each subsample received 5 ml of deionized water in total without further additions (the control treatment indicated by C0) or with a single added substance (N1, N2, or C0, C2). The liquid was carefully mixed into the soil before samples were placed in Schott jars. Small caps filled with 15 ml of 1 N NaOH were placed into the jars to trap the evolved CO2. The jars were fastened airtight and incubated for 28 days at 5, 15 and 25 °C. The selection of soil temperatures were based on the range of temperatures experienced at the site. For each temperature a series of blanks was included. Moisture content of the samples was periodically adjusted to a value of 30% of the soil water content (SWC) since this is generally considered to mimic the moisture content of the field conditions. Constant soil moisture was maintained by weighing each sample once a week and adjusting the water content to the target mass. Four replicates were setup for each temperature treatment as well as each N or C additive treatment. The CO2 that evolved from the soil was measured at 1, 4, 7, 14, 21, and 28 day after incubation by way of titrating the NaOH solution against 0.1 N HCl after the addition of BaC12. A NaOH

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the upper 10 cm of soil at the study site in Haibei, Qinghai-Tibet Plateau. Data (means ± SE) are shown (n = 6–8). SOC denotes soil organic carbon; DON denotes Dissolved organic nitrogen.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil characteristics</td>
<td>Values</td>
</tr>
<tr>
<td>pH (KCl)</td>
<td>8.0 ± 0.1</td>
</tr>
<tr>
<td>Bulk density (g cm−3)</td>
<td>0.70 ± 0.05</td>
</tr>
<tr>
<td>Soil moisture (%)</td>
<td>24.0</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>11.4 ± 0.3</td>
</tr>
<tr>
<td>SOC (kg m−3)</td>
<td>6.8 ± 0.3</td>
</tr>
<tr>
<td>Total soil N (kg m−2)</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>Microbial biomass N (g m−2)</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td>DON (g m−2)</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Extractable inorganic N (g m−2)</td>
<td>1.4 ± 0.4</td>
</tr>
</tbody>
</table>
solution, incubated as described above, was also titrated but without incorporating any soil (blank).

2.3. Temperature functions

The following exponential function was used to describe the temperature dependence of C mineralization:

\[ C_{\text{min}} = C_0 e^{bt} \]  

where \( C_{\text{min}} \) is the measured C mineralization rate (mg C g\(^{-1}\) soil day\(^{-1}\)); \( C_0 \) is the basal C mineralization rate at 0 °C; \( T \) is the incubation temperature (°C) and \( b \) is related to the Q10 (increase in CO\(_2\) efflux or C mineralization with a 10 °C increase in temperature) as follows:

\[ Q_{10} = e^{10b} \]

2.4. Soil heterotrophic respiration in the field

Soil heterotrophic respiration (measured in plots where plants and roots were removed and litter and roots were excluded) was measured using static chamber gas chromatograph (GC) techniques [49] throughout three years from June, 2003 to July, 2006. The chamber, which had an inner diameter of 50 cm and an inner height of 50 cm, was made of opaque fiberglass. Two small fans were mounted on the chamber ceiling to circulate the air within the chamber. To reduce the disturbance caused by installation of the chamber, the chamber was inserted into the soil (to a depth of 5 cm) 24 h before the experiment began. Soil CO\(_2\) fluxes were sampled using a 100 ml syringe at intervals of 0 min, 10 min, 20 min and 30 min. Samples were taken between 9:00 am and 11:00 am once or twice a week. Soil temperatures (at 0, 5, and 10 cm depth) were measured daily [15].

There is evidence that flux data measurements taken at a specific time of day can approximately represent daily means [6,11]. To test how flux values derived from this sampling method were in agreement with daily means, means from 9:00 am to 11:00 am were compared with whole day means based on seven diurnal flux measurements. The comparison showed that soil CO\(_2\) fluxes derived from these two sampling procedures did not differ significantly (\( t = 0.01, p > 0.05, n = 7 \)). Consequently, monthly and annual soil CO\(_2\) fluxes were calculated using the flux data from 9:00 am to 11:00 am [15] in this study. Measured data from June, 2003 to July, 2006 were used to verify the simulated results.

2.5. Calculations and statistical analyses

The General Linear Model (GLM) was used to examine the effects of temperature and the external additive C or N on C mineralization rates. Additionally, Tukey’s post hoc test (with a confidence limit of 95%) was applied to determine differences in mineralized CO\(_2\)–C under C or N dose treatments. Regress analysis was used to analyze the relationship between cumulative CO\(_2\)–C emission and external addition C doses. CO\(_2\)–C emission rate at the end of incubation (28th day) was used to test the dependence of the carbon mineralization rate to soil temperature for both the control and external C or N supply treatments respectively. One-way ANOVA was used to test the effects of external C or N addition on temperature sensitivity of carbon mineralization (Q\(_{10}\)).

The exponential equation obtained from the laboratory incubation was used to estimate C mineralization rates in the field. Soil mean temperatures (at 0, 5 and 10 cm depth) were applied as input data. Outputs were verified by the CO\(_2\)–C measured in situ. Soil heterotrophic respiration under the external N or C supply was derived in the same manner. Values of mean monthly CO\(_2\)–C evolution were summed to provide annual values. All statistical analyses were carried out using SPSS13.0.

3. Results

3.1. Effect of C or N addition on C mineralization rates

The C mineralization rate decreased over time. The CO\(_2\) amount released within the first four days represented more than 50% of the total C mineralized during 28 days (Fig. 1). A rapid decrease was detected within a first 10 day incubation period for all treatments. All the curves level off after 10 days (Fig. 1). C mineralization rates were affected by glucose addition with rates significantly higher at high glucose levels.

The effects of inorganic N additions on C mineralization were not significant (Table 2). C mineralization rates were statistically insignificant between the N addition (N\(_1\) and N\(_2\)) and control treatments over time for the incubation duration (Fig. 1).

![Fig. 1](image-url)
3.2. Sensitivity of C mineralization to soil temperature and external C or N supply

The total CO₂–C that evolved during incubation was affected to a great extent by temperature treatments as well as by the interaction between additive C and temperature (Table 3). The C mineralization rates responded to temperature following a first-order kinetics. The calculated C mineralization Q₁₀ values were significantly higher under the C additive treatments than the control and N additive treatments (F = 31.01, P < 0.001), indicating the labile external C supply increased the sensitivity of soil organic matter decomposition to temperature (Table 4).

3.3. Prediction of C mineralization rates in the field

Mineralization rates were first estimated by applying the exponential model under the control treatment (Table 4) and thereafter verified by the in situ measured data. The model predicted the C mineralization rates reasonably well at most of the times from June, 2003 to July, 2006 (Fig. 2), but less well during the spring time (from March to June, 2004, 2005 and 2006) and late summer (August, 2003, 2004 and 2005) (Figs. 2 and 3). There is a hysteresis of heterotrophic respiration responding to soil surface temperature. The CO₂ evolution lags behind variation in soil surface temperature (Fig. 2). Although there is a shift in patterns of soil respiration between field measurements and modeled data, the overall estimated heterotrophic respiration was 425 g CO₂–C m⁻² year⁻¹ in the K. humilis alpine meadow ecosystem, which is comparable to the 422 g CO₂–C m⁻² year⁻¹ summed from the in situ measurements.

C mineralization rates were estimated using the exponential model under the external N or C inputs (Table 4). The calculated heterotrophic respiration was 428 g CO₂–C m⁻² year⁻¹ and 433 kg CO₂ m⁻² year⁻¹ under the N₁ or N₂ additive treatments respectively, and 586 g CO₂ m⁻² year⁻¹ and 2670 g CO₂ m⁻² year⁻¹ under the C₁ or C₂ additive treatments, respectively.

4. Discussion

4.1. Effect of external C or N addition on C mineralization

Results from the incubation experiment did not support our hypotheses to N effect. The increased CO₂ emission was due to the external labile C input, especially for high available C level, but not to the N input. External labile C increased Q₁₀ values, but external inorganic N did not affect Q₁₀. These results suggest that microbial respiration is limited by availability of soil C. Other studies showed similar results where C mineralization was not affected by N inputs [41]. An explanation for the phenomena has focus on the mechanisms of soil organic matter (SOM) decomposition. Since higher N availability neither reduces the C cost of exoenzyme production nor increases the C return from SOM [52], N may not limit decomposition kinetics even though it is a limiting nutrient for microbial growth in intertussock and wet meadow soils [42]. Recent research on enzyme activity in Antarctic dry valley soils suggested that activities for all enzymes except dehydrogenase were either unchanged or diminished by the addition of either N or C (up to 10 mg N g⁻¹ soil) with only a small amount of C (1 mg C g⁻¹ soil) [15]. This indicates that the C supply for enzyme biosynthesis is limited in the presence of a large amount of N [15]. Results from researches of Menyailo et al. [48] and Vance and Chapin [51] agree with our research, which discovered the control of respiration by easily available C and increased respiration after addition of low molecular weight organic carbon, respectively. Higher respiration might be due to the lower substrate quality (higher C:N ratio), resulting in a lower C use efficiency (higher qCO₂) [52], combined with a higher organic matter content. The biochemical processes involved in C and N mineralization require a better understanding, which make us know how the interactions between C and N regulate soil respiration.

4.2. Influence of external C or N addition on temperature sensitivity of SOM decomposition

Temperature is one of the factors that affect soil C mineralization, but temperature dependence of soil organic matter decomposition is more sensitive to changes in soil C and N content [39]. It has been found that Q₁₀ varies widely from little more than 1 to as high as 10 or greater depending upon the geographic location and ecosystem type [8]. Variation in Q₁₀ is related to soil temperature, soil moisture [2,36], and the substrate quality [10,24,30]. According to earlier studies temperature sensitivity of decomposition decreased with increasing temperatures [21,38]. A recent study [39] showed that similar amounts and qualities of microbial available C led to similar temperature dependences of SOM mineralization in the northern and southern areas of the boreal forest zone in Finland. It indicated that Q₁₀ is more sensitive to soil C and N content than to an increase in soil temperature [24,30].

C mineralization rates calculated from the exponential model exhibit hysteresis compared to the measurement in situ. It appears that the response of the measured in situ Cmin decreases behind increased soil surface temperatures, and the highest respiration occurred 12 days later than the point of the highest temperature (Fig. 2). The possible reason may be that there existed a seasonal frozen stratum.
in alpine meadow ecosystems [44]. Changes of temperature in frozen stratum are slower than changes in soil surface temperature. Therefore, the lag behind thaw in early spring and the lag behind frost in late summer may affect soil microbial activity. It is possible that the responses of microbial activity to the slow soil froze and thaw cycles result in the hysteresis effect of CO₂ evolution. A similar seasonal hysteresis pattern in soil respiration with respect to soil temperature has been observed in a boreal aspen (*Populus tremuloides*) stand [12], as well as in other temperate forests [9,25], which exhibited a lagged increase in soil respiration. The reason was attributed to high soil microbial activity in response to warming of the deeper soil layers during late summer. Recent researches reported a seasonal hysteresis on soil respiration in mixed conifer forest vegetation in California [40]. Factors influencing soil respiration, such as vegetation type and fine root as well as rhizomorph dynamics in combination with the temperature-dependent component explains the hysteresis effect [40]. Recently, the time lag between photosynthesis and CO₂ efflux were reviewed in details by Kuzyakov and Gavrichkova [50]. This review highlighted that photosynthesis affect CO₂ efflux from soil especially through growing roots. The time lag for grasses is about 12.5 ± 7.5 (SD) h [50]. Because in situ measurements of heterotrophic respiration in our study were conducted under conditions of root removal, the influence from photosynthesis was excluded. What are the main drivers of different CO₂ sources, which need to be further investigated.

### 4.3. The potential effects of climatic changes on SOM decomposition of alpine meadow soil

Increased external labile C would cause a significant increase in CO₂ evolution in alpine meadow soils. The external C₁ and C₂ treatments resulted in a CO₂–C increase of 1.4 and 6.3 times, respectively. The Tibetan Plateau is currently experiencing rapid changes such as warming, increasing CO₂ concentrations and N deposition [18,45]. These changes will have a serious impact on biogeochemical cycling, enhanced decomposition, and mineralization of soil nutrients. Fluctuations in temperature have had clear effect on the Tibetan Plateau during the past 40 years. For example, the rate of increase in temperature amounts to 0.16 °C (10 year)^{-1}, which is higher than that of other regions in China (the average rate of temperature increase in China as a whole is 0.04 °C (10 year)^{-1}) [37]. It is possible that decomposition of soil organic C in alpine meadow ecosystems will be enhanced under increasing temperatures. Our study indicated that the external labile C input increased the temperature sensitivity of SOM decomposition, and interactions between additive C and temperature would further strengthen effluence of CO₂ from C pool in the soil. Additive N may not enhance the evolution of CO₂–C directly, however, increased N availability would result in a greater C storage capacity for plants overall [13,34,35]. On the one hand, faster N cycling and higher quality of plant litter may help to promote high rates of SOM decomposition. Conversely, the C storage in plant tissues which are long-lived and slow to decompose, may also promote both plants and soil C storage as well as low rates of SOM decomposition in alpine soils [13]. Whether a shift toward promoting soil C storage occurs or not may depend upon the balance between plant allocation, decomposition rates, relatively labile plant litter and fine roots, and the recalcitrant nature of plant tissue. Long-term and systematic research is urgently needed to make predictions on alpine meadow soil.

![Fig. 2. Annual course of soil heterotrophic respiration rates measured in the field on the same *Kobresia* meadows as in this study from June 2003 to July 2006, and modeled rates based on the empirical model obtained from laboratory measurements under the control treatment, and mean soil temperature is average temperatures of 0 cm, 5 cm and 10 cm depth from June, 2003 to July, 2006.](image1)

![Fig. 3. Measured and modeled carbon mineralization rates from June, 2003 to July, 2006.](image2)
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