


Article

Soil Type and a Labile C Addition Regime Control the Temperature Sensitivity of Soil C and N Mineralization More than N Addition in Wetland Soils in China

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Received: 24 August 2020; Accepted: 25 September 2020; Published: 29 September 2020



Abstract: Wetlands store a large amount of carbon (C) and many are vulnerable to potential global warming. It is critical to quantify the temperature sensitivity of soil nitrogen (N) and C mineralization in response to external labile C or N addition in different types of wetland. Through incubation experiments, the effects of temperature and the addition of N or C on soil C and N mineralization were tested using soils from the Sanjiang Plain wetland (SW), Zoigé alpine wetland (ZW), Yellow River estuary wetland (YW), and Baiyangdian Lake (BL). Our findings showed that temperature, available C and wetland type were dominant factors in the regulation of soil C loss, with soil C in SW and ZW being less stable and poorly resistant to increases in temperature. The response of net N mineralization to N addition showed regional differences. A lack of long-term effects of the deposition of N on soil mineralization suggested that there may be a particular N addition threshold level for changed C and N mineralization. It is predicted that an increase in labile C supply due to elevated carbon dioxide (CO₂) and its interactions with wetland types will increase CO₂ efflux more than N deposition in wetland soils.

Keywords: soil CO₂ efflux; net N mineralization; N or C addition; temperature sensitivity; wetland soil type

1. Introduction

The decomposition of soil organic matter (SOM) releases carbon dioxide (CO₂) and nitrogen compounds into the soil and atmospheric environment, which is mainly influenced by the soil microenvironment, the properties of the substrate and nutrient availability to the microbes in the soil [1–3]. Ongoing global changes such as global warming and nitrogen (N) deposition possibly affect the mineralization of SOM by altering the factors discussed above. For instance, enhanced temperature can change the microbial abundance and increase microbial activity to enhance CO₂ flux, resulting in global warming by producing a positive feedback loop [2,3]. Higher soil temperatures and N deposition may be associated with greater increases in available N in the soil, subsequently leading to an increase in plant growth, as many terrestrial ecosystems are N limited, and thus enhancing the sequestration of CO₂ and altering the quantity and quality of substrate input to the soil [4–6]. The changes in nutrient availability in the soil will, in turn, influence SOM mineralization and regulate the C balance in terrestrial ecosystems.

Temperature is known to regulate almost all microbial-mediated processes in soil, such as C and N mineralization, etc. [2,3,7]. A number of studies have reported increases in SOM mineralization with increased temperatures [7–9]. Substrate availability is an important factor affecting SOM decomposition [10].

Labile C, such as dissolved organic carbon (DOC) and microbial biomass C mainly composed of low molecular weight organic compounds, acts as an energy source and a substrate to drive the growth and activity of microbes. It is used in early decomposition processes and is depleted first; the more complex C dominates the later stages [11]. Consequently, adding labile C to soil can lead to greater soil microbial biomass and respiration. Furthermore, increased microbial biomass stimulates microbial demand for N and the immobilization of available N [12]. Thus, in N-limited soil, as N mineralization is characterized by soil microbe immobilization, N addition increases the availability of N in soil and promotes microbial growth, which may enhance the decomposition and mineralization of soil organic C (SOC) and organic N, producing a positive excitation effect [13,14]. In contrast, it has also been reported that the addition of N has neutral [15] and negative [16–18] effects on SOM mineralization, depending on the ecosystem type and soil properties.

Although studies on the correlation between CO₂ efflux and net N mineralization rate have been widely conducted, the results were complex and contradictory, and largely depended on C and N availability and the soil C:N ratio [19–21]. When the organic matter input into the soil has a lower C:N ratio, microorganisms are less limited by N, which will lead to more mineralization of organic N than immobilization, resulting in a positive relationship between soil C to N mineralization [22]. However, substrates containing a higher C:N ratio often promote microbial communities which are severely N-limited and show more N immobilization to meet their needs in N-limited soils, leading to a negative correlation between soil C and N mineralization [23].

Wetland soils are of particular interest when considering sensitivity to global warming, storing 25–30% of the terrestrial SOC pool in 5–8% of the terrestrial area [24]. The response of soil mineralization to global change (rising temperatures and increased N deposition) may differ among various wetland types, as the litterfall quality and quantity and soil physical and chemical properties vary widely. How soil mineralization alters with wetland type and climate change is worthy of comprehensive assessment. We studied four wetland soils from different regions in China to investigate soil CO₂ flux and net N mineralization rate in response to temperature, wetland type, and N or C addition, thereby determining the mechanisms affecting mineralization. We hypothesized that (1) the temperature sensitivity of soil mineralization in different wetland types would respond to N or C addition differently, and the addition of N or C would lead to high temperature sensitivity of mineralization (compared with the control); (2) the correlation between soil CO₂ flux and N mineralization would be negative, as N mineralization is characterized by soil microbe immobilization in N-limited soil, while external N or C addition would shift the relationship from negative to positive, which may be driven mainly by the excitation effect.

2. Materials and Methods

2.1. Experimental Sites

The soil samples were collected from four wetlands in different geographical locations in China: Sanjiang Plain wetland (SW), Zoigê alpine wetland (ZW), Yellow River estuary wetland (YW) and Baiyangdian Lake (BL) (Figure 1). SW, located in China's northeast corner (47°35' N, 133°31' E) is the largest natural peat wetland, where the thickness of the peat layer is generally about 3–17 m [25]. The mean annual temperature and precipitation in this region are approximately 1.9 °C and 600 mm, respectively. *Calamagrostis angustifolia* and *Carex lasiocarpa* are dominant plant communities in SW. ZW, in the eastern region of the Tibetan Plateau, is recognized as one of the world's largest alpine wetlands. The Tibetan Plateau covers nearly one-quarter of China's land area and is regarded as "the roof of the world" and "the third pole". It has the highest average altitude in the world (3452 m above sea level) and is sensitive to climate change [26]. The mean annual temperature and precipitation in this area are approximately 1.4 °C (min: −10.6 °C in January, max: 10.8 °C in July) and 700 mm, respectively [22]. The soil here is peaty, acidic, and rich in organic C, with a peat layer thickness of 2–5 m, on average [27]. *Carex muliensis* and *C. lasiocarpa* are dominant plant communities in peatland. In general, long cold winters and short, cool summers characterize the climate in SW and ZW, given their relatively high

latitude and high altitude, respectively. YW and BL are in Shandong Province and Hebei Province, respectively. BL is an inland lake; both are in the south of North China, where the mean annual temperature is 12.1 °C and the climate is warm. *Phragmites australis* is a dominant plant in YW and BL. It varies geographically due to soil substrate quantity and quality, nutrient availability, location, altitude and soil climate, i.e., temperature and precipitation across the four wetland ecosystems (Tables 1 and 2). The soil SOC, labile C, and C:N ratio were higher in SW and ZW than in YW and BL, but the pH and microbial biomass C:N were similar in the four wetlands (Table 2).

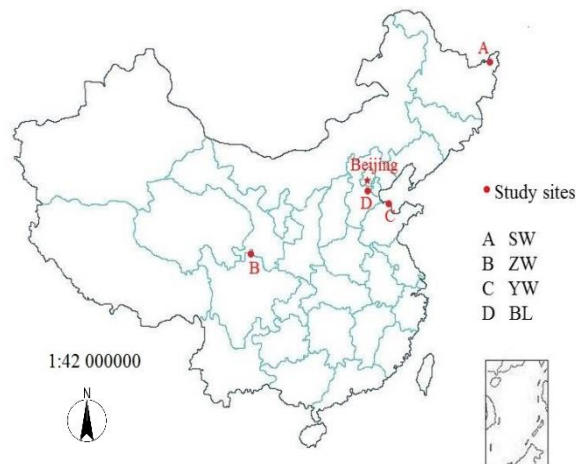


Figure 1. Locations of Sanjiang Plain wetland (SW), Zoigê alpine wetland (ZW), Yellow River estuary wetland (YW), Baiyangdian Lake (BL) from different regions of China.

Table 1. Research overview of four study sites. SW: Sanjiang Plain wetland; ZW: Zoigê alpine wetland; YW: Yellow River estuary wetland; BL: Baiyangdian Lake.

| Study Sites | Location | Altitude (m) | Monthly Average Temperature (°C) | | Mean Precipitation (mm) | Main Soil Types | Dominant Plant Communities |
|-------------|---------------------|--------------|----------------------------------|-------|-------------------------|---------------------------------|---|
| | | | Max | Min | | | |
| SW | 47°35′ N, 133°31′ E | 56 | 22 | −21 | 600 | Peat soil (Umbric Gleysol) | <i>Calamagrostis angustifolia</i> and <i>Carex lasiocarpa</i> |
| ZW | 33°34′ N, 102°56′ E | 3452 | 10.9 | −10.7 | 700 | Peat soil (Umbric Gleysol) | <i>Carex muliensis</i> and <i>C. lasiocarpa</i> |
| YW | 37°34′ N, 118°48′ E | 17 | 27.3 | −5.2 | 552 | Fluvo soil (Calcaric Fluvisols) | <i>Phragmites australis</i> and <i>Suaeda salsa</i> |
| BL | 38°43′ N, 115°38′ E | 8.8 | 25.5 | −3.1 | 525 | Marsh soil (Calcaric Fluvisol) | <i>Phragmites australis</i> |

Table 2. Initial physical-chemical properties in the top 15 cm at the four sampling sites (Mean \pm SE). SW: Sanjiang Plain wetland; ZW: Zoigê alpine wetland; YW: Yellow River estuary wetland; BL: Baiyangdian Lake.

| Physical-Chemical Properties | SW | ZW | YW | BL |
|--|---------------------|---------------------|--------------------|--------------------|
| Water content (%) | 398 \pm 12.46 | 125 \pm 3.74 | 25 \pm 1.34 | 23 \pm 0.94 |
| pH | 5.22 \pm 0.58 | 6.16 \pm 0.04 | 5.96 \pm 0.06 | 6.85 \pm 0.88 |
| NO ₃ ⁻ -N (mg kg ⁻¹) | 10.08 \pm 1.02 | 9.59 \pm 1.89 | 7.60 \pm 0.06 | 4.10 \pm 0.05 |
| NH ₄ ⁺ -N (mg kg ⁻¹) | 88.16 \pm 5.56 | 63.45 \pm 5.22 | 11.79 \pm 0.09 | 19.85 \pm 1.20 |
| TC (g kg ⁻¹) | 325.72 \pm 10.79 | 260.89 \pm 11.19 | 4.23 \pm 0.03 | 11.57 \pm 0.37 |
| DOC (mg kg ⁻¹) | 857.53 \pm 25.14 | 800.50 \pm 22.35 | 32.33 \pm 4.01 | 68.73 \pm 5.02 |
| Total N (g kg ⁻¹) | 17.29 \pm 0.26 | 12.03 \pm 0.05 | 0.64 \pm 0.03 | 1.87 \pm 0.04 |
| C: N | 18.89 \pm 0.41 | 21.68 \pm 0.34 | 6.60 \pm 0.12 | 6.19 \pm 0.24 |
| Microbial biomass C (mg kg ⁻¹) | 497.44 \pm 12.63 | 332.18 \pm 12.41 | 110.18 \pm 12.85 | 103.77 \pm 6.57 |
| Microbial biomass N (mg kg ⁻¹) | 89.74 \pm 2.36 | 59.18 \pm 5.56 | 26.84 \pm 1.55 | 22.52 \pm 0.96 |
| Microbial biomass C: N | 5.54 \pm 0.09 | 5.61 \pm 0.11 | 4.09 \pm 0.06 | 4.61 \pm 0.08 |
| Labile C (mg kg ⁻¹) | 1354.97 \pm 27.56 | 1133.73 \pm 24.62 | 142.72 \pm 12.47 | 172.47 \pm 12.73 |

2.2. Soil Samples and Analyses

Surface litter was first carefully removed from the ground and twelve soil cores, 0–15 cm in depth, were randomly collected at each location from 12 random points along 3 arbitrarily laid transects using 3.5 cm diameter corers. The four soil cores from each region were mixed together in the laboratory, and composite samples were replicated three times. The composite samples were passed through a 2 mm sieve to remove any roots and stones. These sieved samples were used to measure the wetland soil physicochemical characteristics, including soil water content, pH, and inorganic N among others, with the remaining samples stored at 4 °C for the incubation experiment.

Soil water content and pH were measured using the oven-drying method (at 105 °C, for 8 h) and the potential method, respectively. The NH₄⁺-N and NO₃⁻-N in soil were extracted with 2 mol L⁻¹ KCl using a rotary shaker for 1 h. After centrifugation, the supernatant was filtered through filter paper, and the filtrates were analyzed by colorimetry analysis using a continuous flow injection analyzer (Seal AutoAnalyzer 3, Seal Analytical, Norderstedt, Germany). Total N was determined using the Kjeldahl method after wet digestion with sulfuric acid. DOC was extracted from the fresh soil samples (equivalent to 20 g oven-dried) with 60 ml deionized water (1:3 soil: water ratio) on a rotary shaker for 1 h. After centrifugation (4000 rpm for 20 min), the supernatants were filtered through 0.45- μ m filter paper (Whatman, Clifton NJ, USA), and the filtrates were then analyzed for DOC using a Jena multi N/C 3100 analyzer (Germany). TC was measured by the dry combustion method (Multi N/C 2100 Analyser, Analytik Jena, Germany). Soil microbial biomass was determined using a chloroform fumigation-extraction method [28]. Soil microbial biomass C and DOC are important components of labile C [3]. The soil water content was considered in the calculation of the soil analysis results.

2.3. Soil Incubation Experiment

A 4 \times 3 \times 2 \times 2 \times 3 factorial experiment was established corresponding to four wetland types, three incubation temperatures, two levels of C or N addition, and three replicates. The two levels of C addition were 2 (C1) and 10 (C2) mg glucose (C₆H₁₂O₆)-C g⁻¹ soil, respectively. The two levels of nitrogen addition were 0.005 (N1) and 0.025 (N2) mg NH₄NO₃-N g⁻¹ soil according to the mean mineralized N level in the wetland soil, respectively. For each of the treatments, 30 g of air-dried soil was evenly distributed into 250 mL airtight Schott jars and incubated at 5, 15 and 25 °C for 28 days. Soil moisture was periodically adjusted in each soil sample to a water holding capacity of 60% throughout the incubation period, as this is commonly considered to be the most suitable for microbial respiration [29]. A small cup filled with 25 mL of 1 mol L⁻¹ NaOH was placed in the jar to trap the released CO₂. Additions of glucose and NH₄NO₃ were dissolved in 10 mL of deionized water and sprayed evenly on the soil surface with a sprayer, and 10 mL of deionized water was

sprayed on the control to reduce the impact of water on the samples. In addition, at each temperature, a series of NaOH solutions in the Schott jars without soil were also titrated in order to determine the amount of CO₂ from the atmosphere enclosed in the bottles. On the 1st, 3rd, 5th, 7th, 14th, 21st, and 28th days, the CO₂-trapped small cups were replaced with new cups. The amount of remaining NaOH was titrated with a standard HCl solution by adding BaCl₂; then, the amount of CO₂ released was calculated according to the amount of NaOH solution that had been consumed. Finally, the soil respiration rate was expressed as the total amount of CO₂ released during the incubation days divided by the number of days of incubation and soil mass. The net N mineralization rate was calculated as the difference between NH₄⁺-N and NO₃⁻-N before and after soil incubation divided by the number of days of incubation and soil mass. The relationship between soil mineralization rate and temperature was calculated as follows [30]:

$$CMR_d = CMR_0 e^{bT} \quad (1)$$

where CMR_d is the soil CO₂ flux or net N mineralization; CMR_0 is the soil CO₂ flux or net N mineralization at 0 °C; T is the incubation temperature (°C); and b is a temperature reaction coefficient. The temperature sensitivity Q_{10} was calculated as follows:

$$Q_{10} = e^{10b} \quad (2)$$

2.4. Statistical Analysis

SPSS 22.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analyses. Statistical results were significant when $p < 0.05$. A repeated-measures ANOVA (analysis of variance) was used to analyze the effects of temperature, the N or C addition, and their interactions on soil CO₂ efflux over time (the blocking factor). Differences in the temperature sensitivity response of C or N mineralization rate, labile C, and net N mineralization rate to N or C addition (three treatments) among the four wetland soils were examined using separate one-way ANOVAs and Tukey's honestly significant difference (HSD) test. Linear regressions were used to quantify the relationship between soil CO₂ efflux and the net N mineralization rate following the addition of N and C to the wetland soils.

3. Results

3.1. The Positive Response of Soil CO₂ Flux to Temperature and N or C Addition

In the four wetland soils, the SOM mineralization rate decreased and the cumulative CO₂ efflux increased over time during the incubation period (Figures 2 and A1). The cumulative CO₂ efflux was promoted with increases in incubation temperature (Figure 2). However, the temperature had different promoting effects on different types of wetland soils: the strongest effect occurred in the SW, for which the cumulative CO₂ efflux at 15 °C and 25 °C was respectively 62% and 124% higher than that at 5 °C for the CK treatment (Figure 2). In contrast, the cumulative CO₂ efflux at 15 °C and 25 °C was significantly increased by 40% and 86% in ZW, by 20% and 62% in YW, and by 23% and 58% in BL, respectively, and were higher than that at 5 °C for the CK treatment (Figure 2). The sensitivity responses of CO₂ efflux to temperature (expressed by Q_{10}) in soils from SW and ZW were 1.516 and 1.474, respectively, with both being significantly higher than those from YW and BL (1.201 and 1.227) for the CK treatment (Table 3).

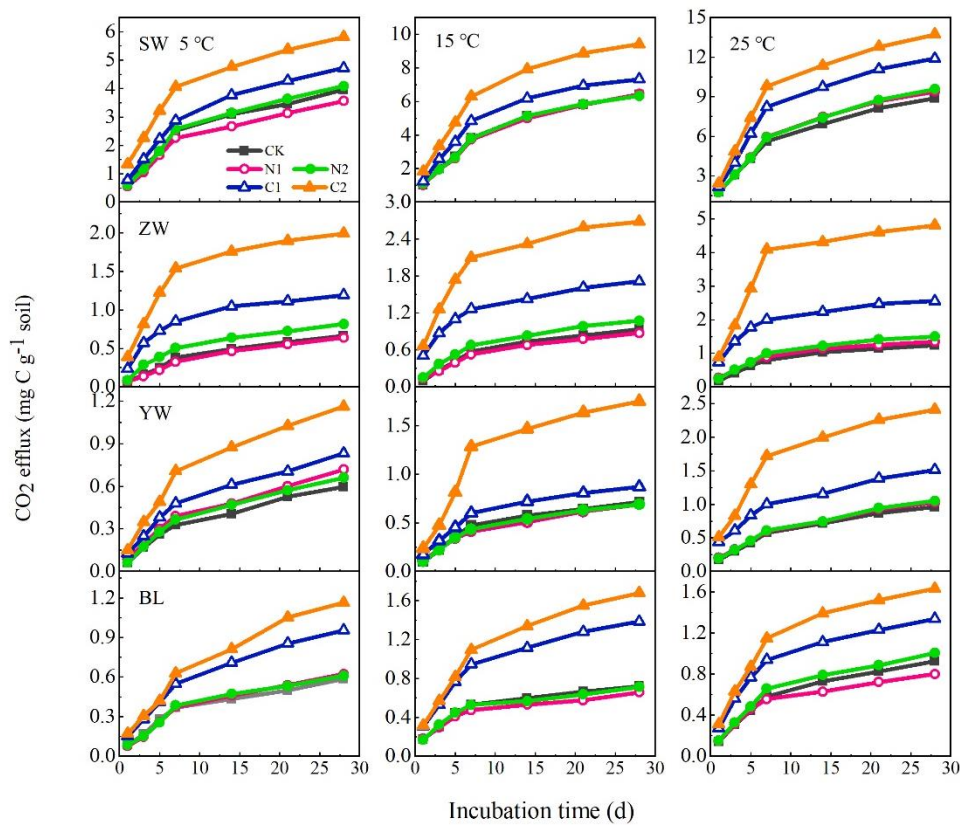


Figure 2. Cumulative CO₂ efflux four wetland soils with or without addition of NH₄NO₃ (N1 and N2) or glucose (C1 and C2) incubated at 5, 15, and 25 °C (SW: Sanjiang Plain wetland; ZW: Zoigê alpine wetland; YW: Yellow River estuary wetland; BL: Baiyangdian Lake). CK: The control without C or N addition; N1: 0.005 mg N g⁻¹ soil; N2: 0.025 mg and N g⁻¹ soil; C1: 2 mg C g⁻¹ soil; C2: 10 mg C g⁻¹ soil.

Table 3. Temperature reaction coefficient *b* and *Q*₁₀ value based on soil CO₂ flux and N mineralization rate in four wetland soils with or without N or C addition in China (Mean ± SE). SW: Sanjiang Plain wetland; ZW: Zoigê alpine wetland; YW: Yellow River estuary wetland; BL: Baiyangdian Lake. CK: The control without C or N addition; N1: 0.005 mg N g⁻¹ soil; N2: 0.025 mg N g⁻¹ soil; C1: 2 mg C g⁻¹ soil; C2: 10 mg C g⁻¹ soil.

| Wetland Type | N or C Addition | Soil CO ₂ Flux | N Mineralization Rate |
|--------------|-----------------|---------------------------|------------------------|
| | | <i>Q</i> ₁₀ | <i>Q</i> ₁₀ |
| SW | CK | 1.516 ± 0.03b(a) | 1.563 ± 0.05d(a) |
| | N1 addition | 1.520 ± 0.03b(a) | 1.243 ± 0.02c(b) |
| | N2 addition | 1.509 ± 0.02b(a) | 1.143 ± 0.03c(b) |
| | C1 addition | 1.623 ± 0.05c(b) | 0.781 ± 0.01a(c) |
| | C2 addition | 1.618 ± 0.04c(b) | 0.880 ± 0.01a(c) |
| ZW | CK | 1.474 ± 0.03b(a) | 1.552 ± 0.04d(a) |
| | N1 addition | 1.453 ± 0.03b(a) | 1.242 ± 0.03c(b) |
| | N2 addition | 1.457 ± 0.04b(a) | 1.165 ± 0.03c(b) |
| | C1 addition | 1.589 ± 0.05c(b) | 0.877 ± 0.02a(c) |
| | C2 addition | 1.609 ± 0.07c(b) | 0.871 ± 0.01a(c) |

Table 3. Cont.

| Wetland Type | N or C Addition | Soil CO ₂ Flux | N Mineralization Rate |
|--------------|-----------------|---------------------------|-----------------------|
| | | Q ₁₀ | Q ₁₀ |
| YW | CK | 1.201 ± 0.02a(a) | 1.082 ± 0.02b(a) |
| | N1 addition | 1.223 ± 0.02a(a) | 1.093 ± 0.02b(a) |
| | N2 addition | 1.219 ± 0.01a(a) | 1.078 ± 0.02b(a) |
| | C1 addition | 1.392 ± 0.02a(b) | 1.248 ± 0.03c(b) |
| | C2 addition | 1.442 ± 0.04b(b) | 1.348 ± 0.03cd(b) |
| BL | CK | 1.227 ± 0.02a(a) | 1.188 ± 0.02c(a) |
| | N1 addition | 1.221 ± 0.01a(a) | 1.223 ± 0.03c(a) |
| | N2 addition | 1.257 ± 0.03a(a) | 1.207 ± 0.03c(a) |
| | C1 addition | 1.453 ± 0.05b(b) | 1.521 ± 0.06d(b) |
| | C2 addition | 1.441 ± 0.04b(b) | 1.578 ± 0.05d(b) |

Different lowercase letters inside the parentheses indicate significant differences at $p < 0.05$ between the treatments within the same wetland soils, outside the parentheses indicate difference at $p < 0.05$ between wetland soils within the same treatments.

The addition of C (C1 and C2) significantly affected the CO₂ efflux; this was consistently greater for the C2 addition than the C1 addition. The average CO₂ efflux was increased by 0.34, 0.22, 0.09 and 0.09 mg C kg⁻¹ d⁻¹ in SW, ZW, YW and BL soils, respectively (Figure 2). Considering the CO₂ efflux in all four wetlands, a positive response of CO₂ flux to temperature and C addition was found, yet there was no significant interaction between temperature and C addition. External N addition did not significantly change in CO₂ efflux (Figure 2, Table 4). The differences in the CO₂ efflux from these four wetland soils due to temperature and N addition levels were also found to be insignificant (Table 4). The Q₁₀ value of soil CO₂ flux following labile C addition was significantly higher than that of the CK and N addition in the four wetland soils (Table 3), indicating that the labile external C supply increased the sensitivity of SOM decomposition to temperature.

Table 4. Repeated measures ANOVA analysis for the effects of temperature, N or C treatments on mean CO₂-C efflux from different wetland soil (T: temperature; N: N addition; C: C addition). SW: Sanjiang Plain wetland; ZW: Zoigê alpine wetland; YW: Yellow River estuary wetland; BL: Baiyangdian Lake.

| Subjects | Mean CO ₂ -C Efflux (mg C kg ⁻¹ Soil d ⁻¹) | | | | | | | |
|----------|--|--------|--------|--------|--------|--------|--------|--------|
| | SW | | ZW | | YW | | BL | |
| | F | p | F | p | F | p | F | p |
| T | 57.591 | <0.001 | 12.585 | <0.001 | 19.365 | <0.001 | 9.349 | <0.001 |
| N | 0.072 | 0.931 | 0.199 | 0.820 | 0.122 | 0.885 | 0.330 | 0.720 |
| C | 10.241 | <0.001 | 23.007 | <0.001 | 34.419 | <0.001 | 28.033 | <0.001 |
| T × N | 0.101 | 0.982 | 0.015 | 1.000 | 0.086 | 0.987 | 0.208 | 0.933 |
| T × C | 0.900 | 0.468 | 2.260 | 0.069 | 2.358 | 0.059 | 0.791 | 0.534 |

3.2. Different Response of Net N Mineralization Rate to Temperature and N or C Addition

The soil net N mineralization rate increased at higher incubation temperatures in YW and BL, a result consistent with their CO₂ efflux, whereas it decreased with higher incubation temperatures in SW and ZW (Figure 3). The Q₁₀ values of the net N mineralization rates in response to temperature in SW and ZW were 1.563 and 1.552, respectively, i.e., significantly higher than those in YW and BL (Table 3).

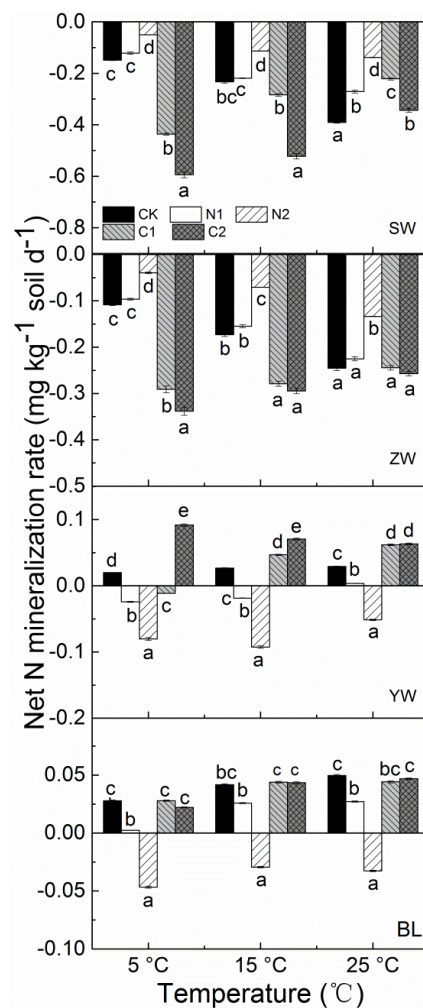


Figure 3. Net N mineralization rate under different temperature, N or C treatments from different wetland soil (SW: Sanjiang Plain wetland; ZW: Zoigê alpine wetland; YW: Yellow River estuary wetland; BL: Baiyangdian Lake) (CK: The control without C or N addition; N1: 0.005 mg N g⁻¹ soil; N2: 0.025 mg N g⁻¹ soil; C1: 2 mg C g⁻¹ soil; C2: 10 mg C g⁻¹ soil). Means with different lowercase letters within different treatment types of same temperature are significantly different at $p < 0.05$.

The soil net N mineralization rate was significantly increased by N addition, especially in SW and ZW, but this reduced the net N mineralization rate in YW and BL (Figure 3). On average, it increased from -0.15 to -0.07 mg kg⁻¹ soil d⁻¹ in SW and from -0.11 to -0.06 mg kg⁻¹ soil d⁻¹ in ZW following N addition at 5 °C, whereas the soil net N mineralization rate significantly decreased from an average of 0.02 to -0.05 mg kg⁻¹ soil d⁻¹ in YW and from 0.03 to -0.02 mg kg⁻¹ soil d⁻¹ in BL under the same conditions (Figure 3). The addition of N had a significant effect on the Q_{10} value for soil net N mineralization rate in SW and ZW, while the difference in the Q_{10} value between the control and N addition was insignificant (Table 3).

3.3. Relationship of Soil CO₂ Flux to Net N Mineralization Rate

Soil CO₂ flux was negatively correlated with net N mineralization rate in the CK and N addition treatments in SW and ZW; however, after C addition, this negative correlation changed to a positive one. However, in YW and BL, a positive correlation between CO₂ flux and net N mineralization rate was found in the CK and C addition treatments, but this relationship was no longer apparent when N was added (Figure 4, Table 5).

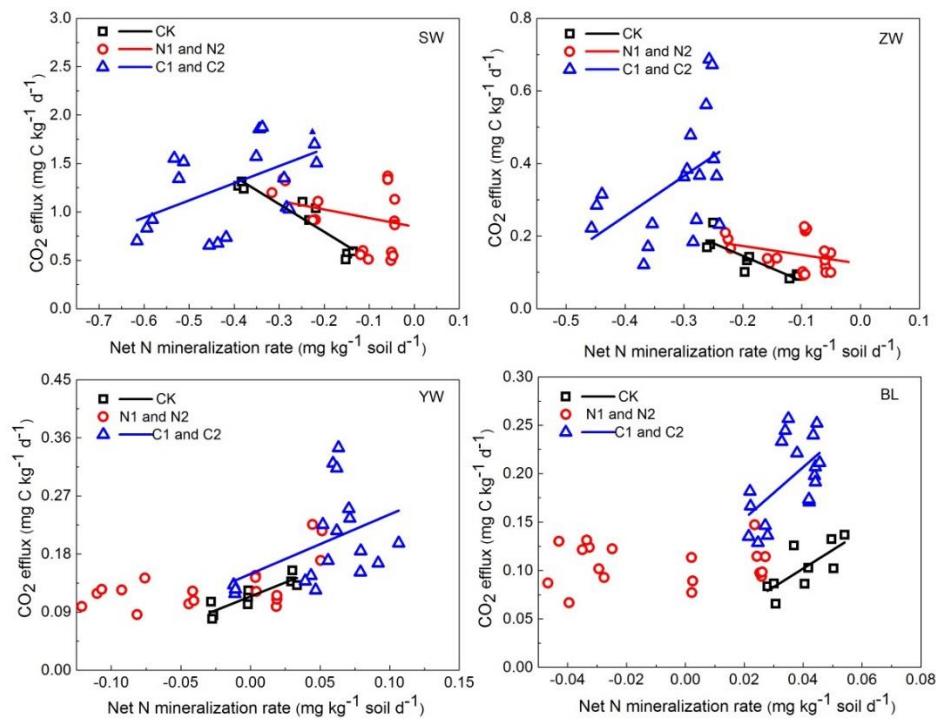


Figure 4. Relationships between CO₂ efflux and net N mineralization rate in four wetland soils with or without addition of NH₄NO₃ (N1 and N2) or glucose (C1 and C2) incubated at 5, 15, and 25 °C (CK: the control without C or N addition; N1: 0.005 mg N g⁻¹ soil; N2: 0.025 mg N g⁻¹ soil; C1: 2 mg C g⁻¹ soil; C2: 10 mg C g⁻¹ soil; SW: Sanjiang Plain wetland; ZW: Zoigê alpine wetland; YW: Yellow River estuary wetland; BL: Baiyangdian Lake).

Table 5. Line equations between soil CO₂ efflux and net N mineralization rate under N or C addition incubated at 5, 15, and 25 °C in four wetland soils (CK: the control without C or N addition; N1: 0.005 mg N g⁻¹ soil; N2: 0.025 mg N g⁻¹ soil; C1: 2 mg C g⁻¹ soil; C2: 10 mg C g⁻¹ soil; SW: Sanjiang Plain wetland; ZW: Zoigê alpine wetland; YW: Yellow River estuary wetland; BL: Baiyangdian Lake).

| Wetland Type | N or C Addition | Line Equation | R ² | p |
|--------------|--------------------|---------------------|----------------|-------|
| SW | CK | $y = -2.86x + 0.22$ | 0.87 | <0.01 |
| | N1 and N2 addition | $y = -0.89x + 0.85$ | 0.08 | <0.05 |
| | C1 and C2 addition | $y = 1.77x + 2.00$ | 0.23 | <0.05 |
| ZW | CK | $y = -0.69x + 0.01$ | 0.72 | <0.01 |
| | N1 and N2 addition | $y = -0.24x + 0.12$ | 0.15 | <0.05 |
| | C1 and C2 addition | $y = 1.10x + 0.70$ | 0.24 | <0.05 |
| YW | CK | $y = 0.86x + 0.11$ | 0.23 | <0.01 |
| | C1 and C2 addition | $y = 0.92x + 0.15$ | 0.23 | <0.05 |
| BL | CK | $y = 1.91x + 0.03$ | 0.56 | <0.05 |
| | C1 and C2 addition | $y = 2.65x + 0.10$ | 0.30 | <0.05 |

3.4. The Response of Soil Labile C to Temperature, Labile C and N Addition

In postincubated soils, soil labile C in SW and ZW was 1272.17 and 1046.06 mg kg⁻¹, respectively, and was lower in YW (128.92 mg kg⁻¹) and BL (165.37 mg kg⁻¹) (Figure 5). Generally, labile C significantly decreased with increasing the temperature in the four wetland soils, and C and N addition did not influence the trend in labile C with temperature.

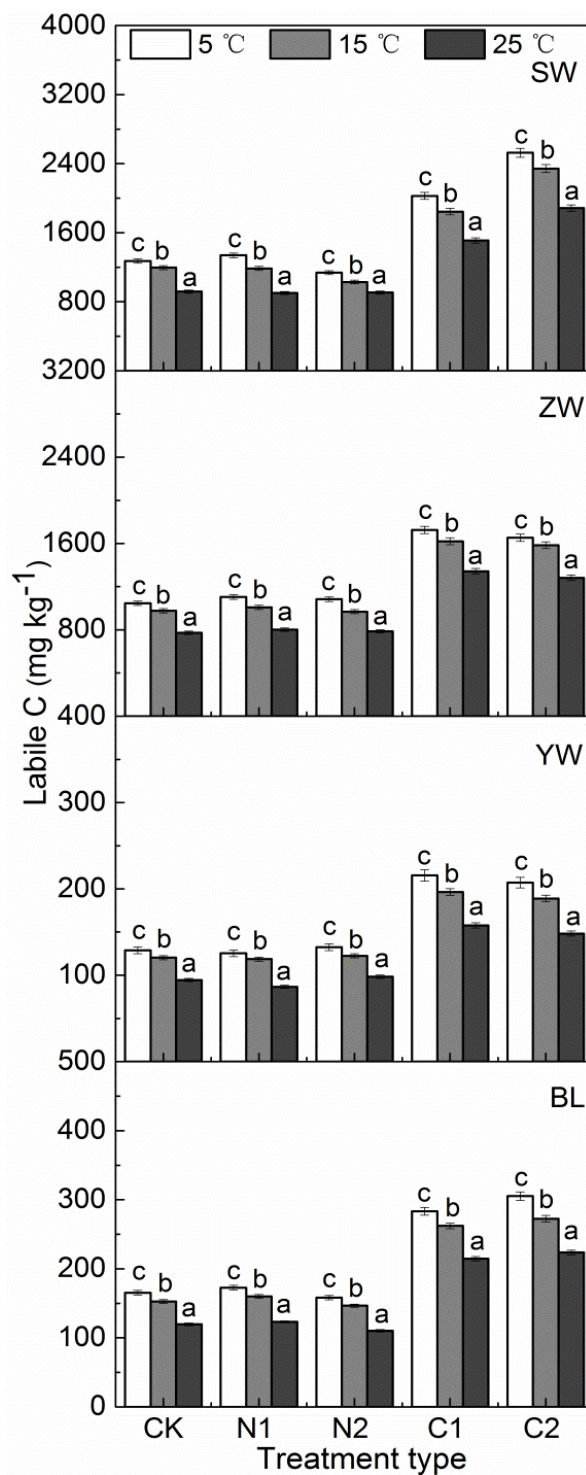


Figure 5. Labile C under N or C treatments from wetland soils (SW: Sanjiang Plain wetland; ZW: Zoigê alpine wetland; YW: Yellow River estuary wetland; BL: Baiyangdian Lake), Vertical bars represent \pm SE. CK: The control without C or N addition; N1: $0.005 \text{ mg N g}^{-1}$ soil; N2: $0.025 \text{ mg N g}^{-1}$ soil; C1: 2 mg C g^{-1} soil; C2: 10 mg C g^{-1} soil. Means with different lowercase letters at different temperatures (i.e., 5, 15 and 25 °C) of the same treatment type were considered significantly different at $p < 0.05$.

4. Discussion

4.1. Positive Response of Soil C Mineralization to Temperature and C Addition

Our findings showed that soil C mineralization was influenced by the incubation temperature, labile C addition, and wetland type, but not the addition of inorganic N. Higher temperatures increased the soil C mineralization rate, especially in SW and ZW, showing that low temperature was a limiting factor in this process at our study sites. It was shown that soil labile C significantly decreased with the increasing of temperature, suggesting that the labile C pool is an important source of CO₂ in soil. The Q10 value of soil CO₂ flux increased up to a maximum with labile C addition (C2 addition) in the four wetland soils (Table 3), indicating that the labile external C supply increased the sensitivity of SOM decomposition to temperature [11]. Thus, if global warming increases, the biomass of leaves and roots, the supply of labile C through leaves and roots turnover will enhance and drive the growth and activity of soil microorganisms and the regulation of CO₂ production.

4.2. Neutral Impact of N Addition on Soil C Mineralization

A neutral effect of N addition on C mineralization rates was seen in the four wetland soils, i.e., similar to that in other studies carried out in wetland ecosystems [15,31,32]. This phenomenon was probably due to the microbial community structure and function, which had a certain tolerance to N in wetland soils; thus, there was a particular N addition threshold level for increased N mineralization [16]. In addition, the short incubation time used (28 days) and the small amount of N added might have precluded the detection of statistically significant changes in the C mineralization process. If the research had been carried out over a longer period, the increased N deposition may have affected soil C processes by enhancing plant biomass and changing plant community composition, thereby providing more organic matter to soil microbes to increase soil C mineralization [14]. Hence, further research is needed to verify how increasing N deposition can regulate soil C over longer periods.

4.3. Soil Net N Mineralization Rate Responses to Temperature and the Addition of N in Four Wetland Soils

The effect of increasing temperature on N mineralization was soil-specific and enhanced the net N mineralization rate in YW and BL, but decreased this it in SW and ZW. The response of soil N mineralization rate to the increasing of temperature depended on the wetland type and soil nutrient availability. The soil ratio of C: N in SW and ZW was 18.89 and 21.68, and 6.60 and 6.19 in YW and BL, respectively. The lower soil C:N ratio in YW and BL suggested that microbes associated with N mineralization were likely limited by C availability, rather than N availability. In addition, the microbial biomass C:N ratio in SW and ZW was 5.54 and 5.61, respectively. Thus, the lower soil microbial biomass C:N ratio and N limitation allowed soil microbes from wetland soils to immobilize all available N to meet their N needs [30], resulting in a negative mineralization rate. Soil net N mineralization rates were significantly decreased by increasing temperature in SW and ZW; this may be partly attributable to the limited N status and higher N immobilization in those soils. Furthermore, the response of net N mineralization to N addition also showed regional differences, which reduced net N mineralization after the addition of N in YW and BL, but increased in SW and ZW. Adding inorganic N could alleviate N-limitation, improve microbial turnover efficiency, and promote a positive excitation effect of N mineralization in SW and ZW soils, which ought to increase the overall N mineralization rate [16,17,33]. In YW and BL, labile C was only 11% of that in SW and ZW. Microbial N mineralization is the process by which some microorganisms obtain N by using labile C to decompose recalcitrant SOM [34], whereas in short-term incubation experiments, the greater N availability after N addition, and the lack of labile C, led to recalcitrant C mining for N suppression, thereby reducing the net N mineralization rate [35].

Typically, net N release in mineral form (NO₃⁻ and NH₄⁺) only occurs after the N demand of soil microbes has been satisfied [36]. Soil organic matter is primarily decomposed by microbial decomposers, and their C/N ratio is lower than that of most litter types [36]. This leads to higher

N demand. Although decomposers need a considerable fraction of assimilated C, inorganic N is required, at least in the early stages of decomposition. Globally, the initial nitrogen concentration of litter is a dominant driver of net N immobilization and release during long-term litter decomposition. Their results also showed that N could be released early in the decomposition process of high-quality litter in an environment with a low decomposition rate [37]. The C:N ratio was 13:34 in the senesced plant materials, 7:22 in the soil, and 4:6 in the soil microbial biomass for the wetland studied. The C:N ratio of soil microbial biomass is low, which makes soil microorganisms immobilize all available N in the short-term cultivation process to meet their high nitrogen demands.

4.4. Correlation Between Soil CO₂ Efflux and Net N Mineralization Responses to Soil Type, and Addition of N or C in Four Wetland Soils

Based on previous studies, there are three relationships between soil CO₂ emissions and net N mineralization rate. Positive correlations were observed in soils limited by C availability [23,38] and negative correlations in an N-limited soil (Bosatta and Berendse, 1984; Schimel, 1986). No correlations were observed in soils with a large variability in soil C:N ratios [38,39]. No correlation between respiration and net N mineralization was detected in soils with a large variability in soil C:N ratios [20,40].

We analyzed the correlation between soil CO₂ efflux and net N mineralization rate in response to N or C addition. A negative relationship between soil CO₂ efflux and net N mineralization rate was found in the control in SW and ZW soils. This may be attributed to the fact that these soils had a higher C:N ratio and were more limited by N. N mineralization is characterized by microbe immobilization, as microbes need additional available N to metabolize organic matter with high C content [41]. Higher N immobilization is often accompanied by high rates of C mineralization, as C substrates probably promote N retention by microbes [42]. The decomposition of labile C and recalcitrant C was susceptible to increasing temperature, and produced more CO₂ efflux in SW and ZW soils [43,44]. However, N addition did not shift the correlation between soil CO₂ flux and net N mineralization rate in SW and ZW, which suggested that soil microbes from those wetlands immobilized nearly all the added N to meet their N needs. C addition shifted the relationship between soil CO₂ flux and net N mineralization rate in SW and ZW, which was consistent with the theory that available N (ammonium and nitrate) does not appear in the definition of the critical C:N ratio [45]. The addition of C increased the pool of labile C, which, in turn, altered the C:N critical ratio and microbial turnover efficiency [46]. Our results showed that positive correlations between soil CO₂ flux and net N mineralization rate were observed in YW and BL with lower C availability, and in the four wetland soils with labile C addition, unlike those in other studies of wetland [30] or forest ecosystems [21,47]. However, the soil CO₂ flux and net N mineralization rate seemed to be unrelated to N addition in YW and BL; this may be partly attributable to the fact that a lower temperature sensitivity (Q_{10}) and N addition had neutral effects on CO₂ efflux in those soils.

The response of soil mineralization rate to both temperature increases and the addition of N or C had high spatial variability among the studied wetland ecosystems, differing due to climate environment and soil substrate availability. The average Q_{10} values of soil mineralization rate in soils from SW and ZW were significantly higher than those from YW and BL. SW and ZW are cold and humid environments, which hold a lot of C in the soil. Due to the fragility of ecosystems in high latitudes and altitudes, they are highly sensitive to climate change. For instance, in the past 40 years, the temperature of the Tibetan Plateau has increased by 0.016 °C year⁻¹, higher than the national average, i.e., 0.004 °C year⁻¹ [30]. These regions showed a faster warming rate and higher temperature sensitivity to carbon mineralization.

5. Conclusions

In conclusion, soil CO₂ flux was not regulated by N addition, but by elevated temperature, wetland type, and labile C addition, especially with high C doses. The temperature sensitivity of soil CO₂ flux (Q_{10} values) in wetland soils under labile C addition was significantly higher than that under

control and N addition, showing that labile C content was the most important factor affecting SOM mineralization. Soil C mineralization response to elevated temperature was mediated by wetland type. Q_{10} values in SW and ZW were significantly higher than those in YW and BL. The SOC was unstable and decomposed more readily in soil from wetlands SW and ZW. In contrast to the lack of response to C addition, net N mineralization was inconsistently affected by elevated temperature and N addition, and mediated by wetland type. Furthermore, the correlation between soil CO_2 flux and net N mineralization rate varied among different wetland soils, which was intensively regulated by soil labile C content and the C:N ratio. In conclusion, wetland type and labile C addition were shown to control the temperature sensitivity of soil C and N mineralization more than N addition in wetland soils in China.

Author Contributions: Conceptualization, C.W.; methodology, C.W.; software, Y.Z., Y.L.; validation, C.W. and Z.Z.; formal analysis, Y.Z.; investigation, Y.Z., Y.L.; resources, C.W.; data curation, Y.Z. and Y.L.; writing—original draft preparation, Y.Z.; writing—review and editing, C.W. and Y.Z.; visualization, Y.Z. and Y.L.; supervision, C.W.; project administration, C.W.; funding acquisition, C.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China, grant number 41373069 and 41971024.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

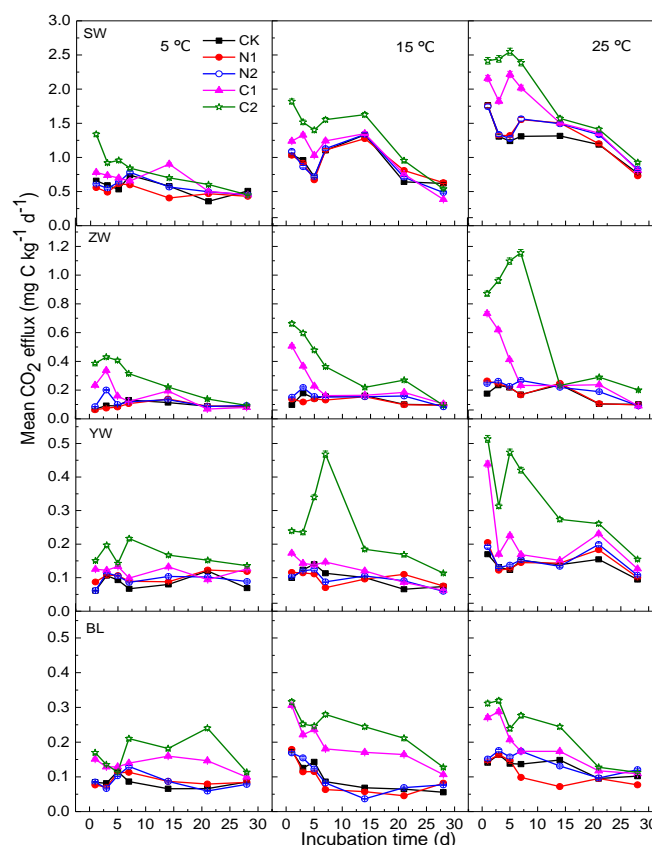


Figure A1. Mean CO_2 efflux under different temperature, N or C treatments from wetland soils in China (SW: Sanjiang Plain wetland; ZW: Zoigê alpine wetland; YW: Yellow River estuary wetland; BL: Baiyangdian Lake), Vertical bars represent \pm SE. CK: The control without C or N addition; N1: $0.005 \text{ mg N g}^{-1}$ soil; N2: $0.025 \text{ mg N g}^{-1}$ soil; C1: 2 mg C g^{-1} soil; C2: 10 mg C g^{-1} soil.

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