

THE EFFECT OF INCREASED NITROGEN LEVELS ON SOIL CO₂ EMISSION CAUSED BY MICROBIAL RESPIRATION IN THE RIPARIAN ZONE OF THE THREE GORGES RESERVOIR

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(Received 9th Sep 2020; accepted 30th Nov 2020)

Abstract. The increase of atmospheric nitrogen deposition has greatly affected soil CO₂ emission caused by microbial respiration, probably resulting in the acceleration of global warming. However, the effect of atmospheric nitrogen deposition on soil CO₂ emission is not still entirely clear, especially in riparian zone ecosystems. In this study, we studied the riparian zone of the Three Gorges Reservoir after a 36-d soil incubation with four nitrogen species, including NH₄Cl, NaNO₃, CO(NH₂)₂ and CO(NH₂)₂ (28%), as well as NH₄NO₃ (72%) with three nitrogen addition levels to the soil at 44.39, 88.77 and 133.16 μg N g⁻¹. Soil cumulative carbon respiration was promoted by 13.37%, 21.55% and 27.59% with the nitrogen addition of 44.39, 88.77 and 133.16 μg N g⁻¹ soil, respectively, increasing linearly with the increase of nitrogen addition levels. However, it was not changed with the nitrogen species. In conclusion, soil cumulative carbon respiration increased with the nitrogen addition levels regardless of the nitrogen species. Thus, induced by atmospheric nitrogen deposition, soil CO₂ emission from riparian zone should not be ignored in the twenty-first century.

Keywords: *atmospheric nitrogen deposition, soil cumulative carbon respiration, the Yangtze River, nitrogen species*

Introduction

Atmospheric nitrogen (N) deposition has increased more than 10-fold since the industrial revolution (Holland et al., 1999) and it will continuously do so in the coming decades (Galloway et al., 2008). The increase of atmospheric N deposition has greatly affected soil CO₂ emission caused by microbial respiration (He et al., 2018; Meyer et al., 2018), probably resulting in the acceleration of global warming. Therefore, assessing the effect of atmospheric N deposition increase on soil microbial respiration is an urgent issue.

A large number of studies have examined this scientific problem and found that atmospheric N deposition inhibits (Riggs and Hobbie, 2016; Li et al., 2017; Peng et al., 2020), promotes (Tu et al., 2013; Fang et al., 2017; Liang et al., 2018), or does not change (Peng et al., 2010; He et al., 2018; Zhao et al., 2020) soil microbial respiration in forest, grassland, pasture, cropland and wetland ecosystems. This dispute may be due to the difference of N addition species, N addition levels and soil properties. Firstly, inconsistent results were also discovered between the addition of ammonium and nitrate (Puri and Ashman, 1999; Min et al., 2011) and between that of inorganic and organic N (Ramirez et al., 2010; Du et al., 2014). Secondly, high level of ammonium addition relatively inhibits soil microbial respiration compared to that of nitrate (Min et al., 2011; Li et al., 2017) because the decline of soil acidity caused by ammonium addition is larger than that caused by nitrate addition. Finally, changed by N increase, soil properties such as pH

(Rousk et al., 2009; Chen et al., 2016; Li et al., 2017), dissolved organic carbon (DOC) (Wang et al., 2003; Chen et al., 2014; Eberwein et al., 2015) and Carbon (C):N ratio (Gong et al., 2019) are closely related to soil microbial respiration. Therefore, treated with different N species and levels, more soil ecosystems with different soil properties should be investigated for the accurate assessment of the effect of N increase on soil C loss by microbial respiration from the terrestrial ecosystem.

The riparian zone of the Three Gorges Reservoir (TGR) is the largest one in the world (Bao et al., 2015), locating in the third-largest N deposition area of the world. The atmospheric N flux in this area is even as high as 50 kg N ha⁻¹ a⁻¹ with the composition of 72% inorganic N and 28% organic N (Yuan et al., 2009; Zhang et al., 2020). The soil of the riparian zone is exposed to air when it comes out of water, which is conducive to the rapid propagation of aerobic microorganisms. Meanwhile, atmospheric N deposition will alleviate the N limitation of soil microbial metabolism, which may lead to a large amount of soil C respiration loss from the riparian zone (Chen et al., 2014; Kamble and Baath, 2016). Thus, the influence of N deposition on soil C loss by microbial respiration should be concerned in the riparian zone of the TGR.

In this study, we aimed to discuss the effect of atmospheric N deposition on soil CO₂ emission caused by microbial respiration based on a 36-d soil incubation executed by four N species with three N addition levels. We hypothesized that: (i) soil microbial respiration would be promoted by the increased N addition levels and the decreased soil C:N ratio; (ii) the N species had no significant effect on soil microbial respiration.

Materials and methods

Site description and soil sampling

The riparian zone of the TGR is located in the transection area of Chongqing and Hubei province, China. It is a north subtropical humid monsoonal climatic with an average annual temperature of 18.2°C and an average annual precipitation of 1053.15 mm (1981-2018, <https://power.larc.nasa.gov>). It has experienced continuous artificial drying-rewetting cycles since 2003. The area of its mainstream and tributaries is about 349 km² (He, 2011). A year is divided into the dry period and the flooding period, according to the fluctuation of the water level caused by the operation of the TGR and the dry period is about six months (Chen et al., 2019b). During the dry period, the soil of the riparian zone exposes to air, whereas during the flooding period, it is inundated by the water of the TGR.

Intact soil was randomly collected from the riparian zone of the TGR within the Wanzhou section (N30°47.28'~30°50.10', E108°21.35'~108°23.41', *Fig. 1*) by polypropylene containers (13.5 cm×10.02 cm×6.8 cm) in June 2017. After the sampling, the containers were sealed tightly, stored at 4°C and immediately transported to a laboratory within 24 h. Soil physicochemical properties are listed in *Table 1*.

Experimental design and soil incubation

Nitrogen treatments consisted of four N species (NH₄Cl, NaNO₃, CO(NH₂)₂, CO(NH₂)₂ (28%) plus NH₄NO₃ (72%)) with three N levels (44.39, 88.77 and 133.16 µg N g⁻¹ soil). Meanwhile, a control without N treatment was set up. Three replicates were set up for both the control and the N treatments. In total, we incubated 39 jars ((4 × 3 + 1) × 3).

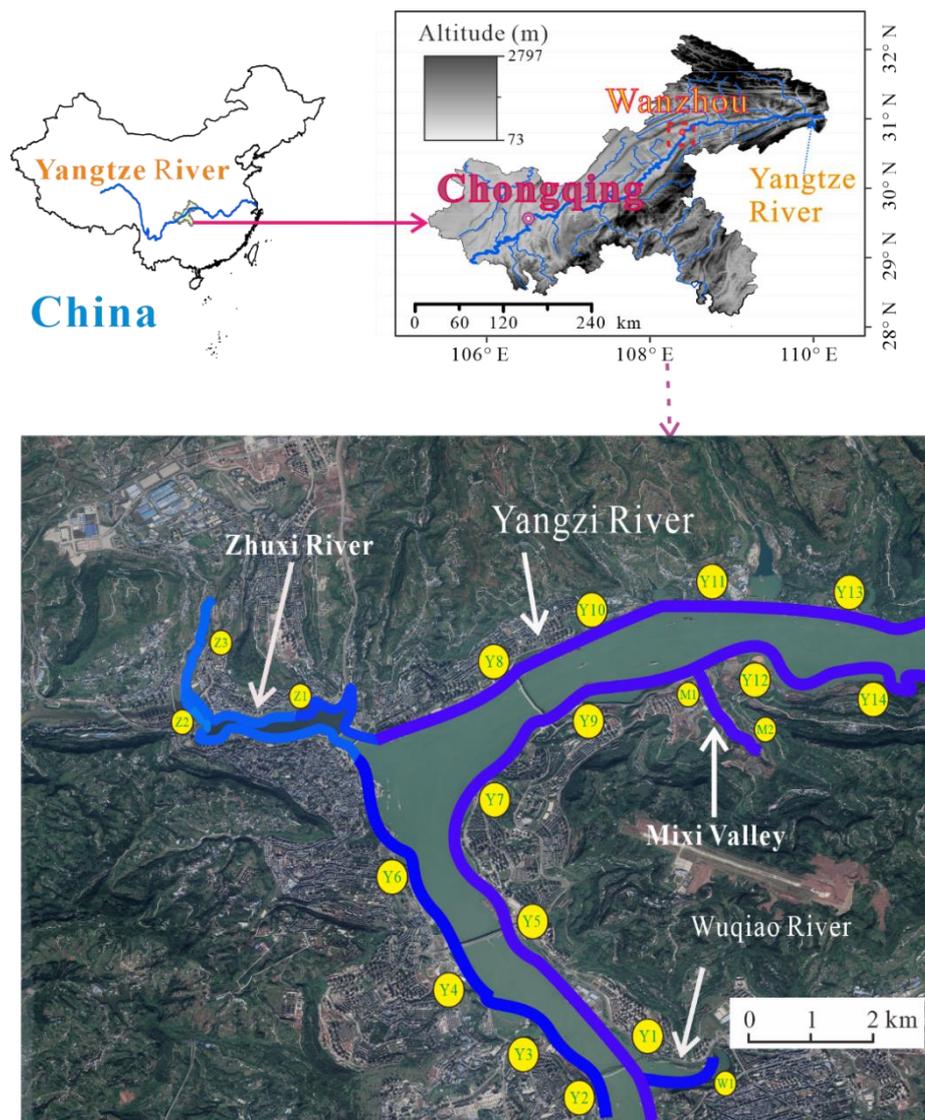


Figure 1. Soil sample sites

Table 1. Soil physicochemical properties in the riparian zone

Physicochemical properties	Values
pH	8.55 (0.03)
total C (mg g ⁻¹)	14.83 (0.24)
total N (mg g ⁻¹)	0.27 (0.01)
C/N ratio	54.36 (1.43)
DOC (mg kg ⁻¹)	61.38 (1.35)
Gravimetric water content (%)	40.00 (0.91)
Clay (%)	10.55 (5.25)
Silt (%)	23.40 (7.87)
Sand (%)	66.05 (15.23)

Note: values represent means of three replicates with standard deviation in parenthesis

First, 200 g soil was placed in 470 mL jars as quickly as possible and stabilized at ambient temperature for three days to avoid soil disturbance. After the stabilization, the

jars were added with 50 mL N solution (corresponding to the above N treatments) every 10 days and incubated in a growth chamber (SW-96P, Sang woo scientific, Korea) at 20°C in the dark for 36 days. After four times of N solution addition, the N addition levels were 44.39, 88.77 and 133.16 µg N g⁻¹ soil corresponding to the atmospheric N deposition fluxes of 50, 100 and 150 kg N ha⁻¹ a⁻¹, respectively. The controls were added with four times of 50 mL deionized water. All samples were destructed for chemical analysis at the end of the incubation.

Soil respiration rate and cumulative C respiration

Soil respiration rate (R_s) was measured on the day of 1, 2, 4, 6, 8, 15, 22, 29 and 36. Before gas sampling, each jar was sealed airtight to accumulate CO₂ for 1 h. The headspace gas was extracted by a 10 mL syringe equipped with a three-way stopcock (Discofix^o, R C, B. Braun, Germany) through a septum in the middle of a lid at the start and the end of the CO₂ accumulative period. The collected gas was stored in a 5 ml syringe (GASTIGHT^o, R #1005 Hamilton, Shimadzu, Japan), and then was injected into a gas chromatography (GC7890, Agilent Technologies, USA) for analysing the CO₂ concentration. R_s was calculated according to the method of Robertson et al. (1999).

Soil cumulative C respiration (C_{um}) was calculated as Eq.1 (Lin et al., 2015):

$$C_{um(i+1)} = \frac{(R_{i+1} + R_i) \times (t_{i+1} - t_i) \times 24}{2} + C_{um(i)} \quad (\text{Eq.1})$$

where $C_{um(i+1)}$ is C_{um} on the $i+1$ sampling time (µg CO₂-C g⁻¹ soil, $i = 1$, $C_{um(i)} = 0$); R_{i+1} is R_s on the $i+1$ sampling time (µg CO₂-C g⁻¹ soil hr⁻¹), t is the sample time (d).

Soil physicochemical properties

Soil pH was determined in a soil: water (1:2.5 w/w) slurry (Orion 3 Star, Thermo Electron, USA). Total C and N content were detected by an element analyser (EA5000, Elementar, Germany). DOC was extracted with 10 ml deionized water (soil: water=1:10 w/w), then filtered through a 0.45µm filter (ALBET). Finally, it was determined by a total organic carbon analyser (TOC-LCPH/CPN, Shimadzu, Japan). Soil gravimetric water content was measured by drying 1 g soil in a furnace at 105°C for 24 h, and then was weighted. The distribution of soil particle size was analysed by hydrometer method (Ashworth et al., 2001).

Data analysis

The differences of R_s among the soil treatments were assessed by a repeated measure ANOVA after normality test (Shapiro-Wilk test). An exponential decay function was applied to explore the relationship between R_s and the incubation time, and a linear function was used to detect the relationship between C_{um} and the varieties of soil chemical properties. A two-way ANOVA was used to determine the statistical differences of C_{um} and the soil chemical properties after the incubation among the N addition species and levels at $P < 0.05$. The influence factors of soil respiration in terms of soil chemical properties were analysed by a principal component analysis (PCA) based on the standardization datum of soil pH, C: N ratio, DOC, total C and total N after the incubation. All statistical analyses were performed by IBM SPSS 17.0 for windows.

Results

Soil CO₂ emission

Soil respiration rate decreased rapidly during the initial 6 d and then tended to be stable, which was accorded with the trend of exponential decay for all the N treatments (Fig. 2). N addition significantly promoted R_s , especially during 1-4 days ($P < 0.05$). Compared to the control, C_{um} was promoted by 13.37% (i.e. 50 kg N ha⁻¹ a⁻¹), 21.55% (i.e. 100 kg N ha⁻¹ a⁻¹) and 27.59% (i.e. 150 kg N ha⁻¹ a⁻¹) (Fig. 3, $P < 0.05$), increasing linearly with the N addition levels for all the N species (Fig. 4, $P < 0.05$). C_{um} was significantly influenced by the increase of N levels ($P < 0.001$), but not changed with the N species (Table 2, $P = 0.129$).

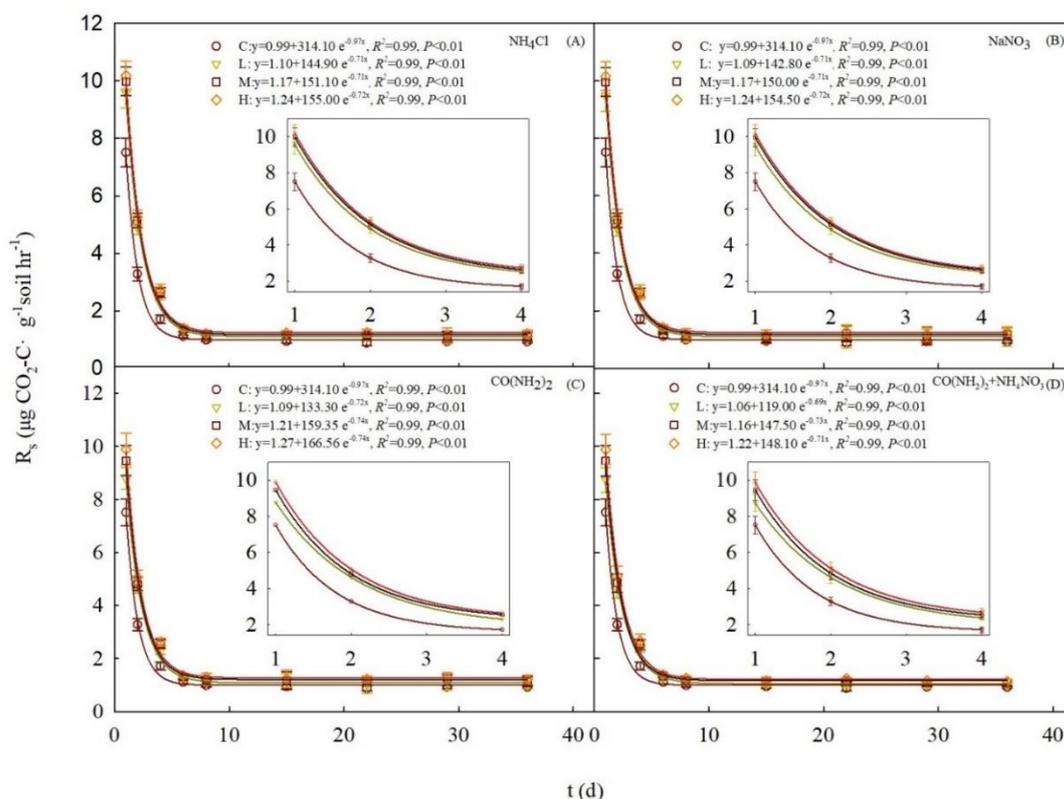


Figure 2. Soil respiration rate (R_s) during the 36-d incubation. C, L, M and H represent the N addition of 0, 44.39, 88.77 and 133.16 $\mu\text{g N g}^{-1}$ soil, respectively. “y” and “x” represent R_s and the incubation time, respectively. Bars represent the standard deviations of the mean ($n=3$)

The varieties of soil chemical properties

Compared to the control, soil pH significantly decreased under NH_4Cl (Table 3, $P < 0.05$), but was not significantly changed under NaNO_3 , $\text{CO}(\text{NH}_2)_2$ and $\text{CO}(\text{NH}_2)_2$ plus NH_4NO_3 ($P > 0.05$) at the end of the incubation. Soil DOC increased by 3.85%, while total C decreased by 0.59% across the N treatments, not changed by the N addition species and levels (Table 2, $P > 0.05$). Soil C: N ratio decreased and total N increased, with the N addition levels for all the N species (Tables 2 and 3, $P < 0.001$).

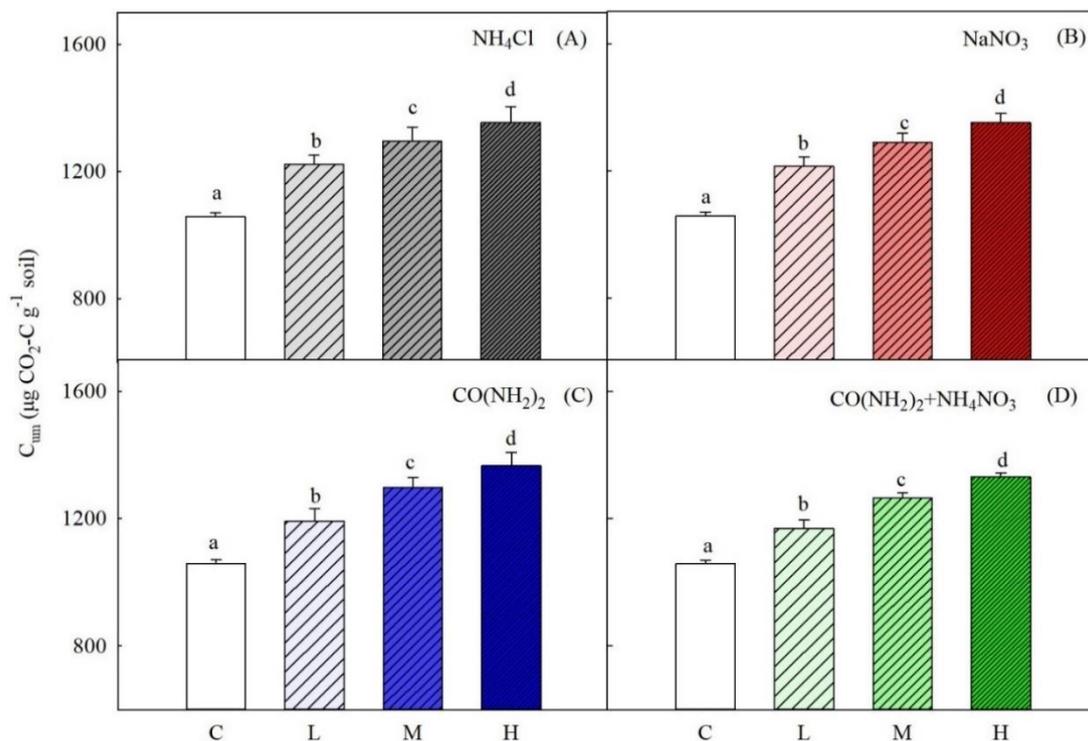


Figure 3. Cumulative soil C respiration (C_{um}) under the N treatments after the 36-d incubation. C, L, M and H represent the N addition of 0, 44.39, 88.77 and 133.16 $\mu\text{g N g}^{-1}$ soil, respectively. Different lowercases represent the significant differences among the N addition levels ($P < 0.05$); Bars represent the standard deviations of the mean ($n=3$)

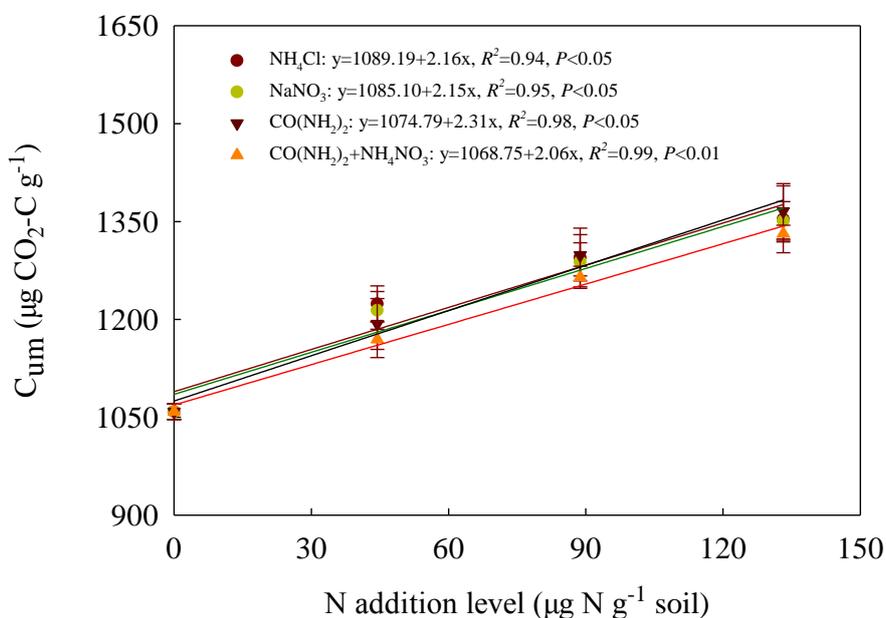


Figure 4. The relationship between Cumulative soil C respiration (C_{um}) and the N addition levels. “y” and “x” represent C_{um} and the N addition level, respectively. Bars represent the standard deviations of the mean ($n=3$)

Table 2. Results from two-way ANOVA (*P* values) to test the effects of N addition species and levels on Cumulative soil C respiration (*C_{um}*) and soil chemical properties

Response variable	Main effects		Interaction effects
	N species	N levels	N species*N levels
pH	0.001	0.523	0.245
Total C	0.300	0.990	1.000
Total N	<0.001	<0.001	0.003
C/N	1.000	<0.001	1.000
DOC	0.883	0.545	0.145
<i>C_{um}</i>	0.129	<0.001	0.925

Note: n=36

Table 3. Soil chemical properties at the end of the incubation

N species	Chemical properties	N levels (µg N g ⁻¹ soil)			
		C	L	M	H
NH ₄ Cl	pH	7.95 (0.08) a	7.75 (0.07) b	7.82 (0.01) b	7.78 (0.04) b
	Total C (mg g ⁻¹)	13.74 (0.29)	13.67 (0.19)	13.65 (0.24)	13.64 (0.22)
	Total N (mg kg ⁻¹)	270.33 (9.50) a	299.92 (9.10) b	329.51 (9.50) c	359.10 (9.50) d
	C/N	50.86 (1.07) a	45.61 (0.80) b	41.44 (0.60) c	37.99 (0.46) d
	DOC (mg kg ⁻¹)	57.19 (1.71)	59.91 (1.78)	60.03 (1.50)	57.46 (0.67)
NaNO ₃	pH	7.95 (0.08)	7.94 (0.04)	7.92 (0.06)	7.91 (0.08)
	Total C (mg g ⁻¹)	13.74 (0.29)	13.68 (0.19)	13.65 (0.20)	13.64 (0.25)
	Total N (mg kg ⁻¹)	270.33 (9.50) a	298.34 (10.02) b	331.28 (10.77) c	360.76 (10.21) d
	C/N	50.86 (1.07) a	45.60 (0.81) b	41.44 (0.58) c	38.00 (0.45) d
	DOC (mg kg ⁻¹)	57.19 (1.71)	58.85 (2.25)	60.54 (1.84)	58.76 (0.73)
CO(NH ₂) ₂	pH	7.95 (0.08)	7.91 (0.13)	7.88 (0.05)	7.86 (0.04)
	Total C (mg g ⁻¹)	13.74 (0.29)	13.68 (0.28)	13.66 (0.19)	13.66 (0.28)
	Total N (mg kg ⁻¹)	270.33 (9.50) a	300.42 (8.90) b	331.67 (9.48) c	361.44 (10.27) d
	C/N	50.86 (1.07) a	45.63 (0.76) b	41.46 (0.62) c	38.04 (0.49) d
	DOC (mg kg ⁻¹)	57.19 (1.71)	60.13 (1.68)	60.52 (2.15)	58.56 (0.73)
CO(NH ₂) ₂ plus NH ₄ NO ₃	pH	7.95 (0.08)	7.90 (0.12)	7.89 (0.07)	7.84 (0.08)
	Total C (mg g ⁻¹)	13.74 (0.29)	13.68 (0.20)	13.66 (0.21)	13.65 (0.20)
	Total N (mg kg ⁻¹)	270.33 (9.50) a	299.67 (9.17) b	328.77 (8.99) c	361.29 (9.13) d
	C/N	50.86 (1.07) a	45.62 (0.77) b	41.45 (0.57) c	38.41 (0.45) d
	DOC (mg kg ⁻¹)	57.19 (1.71)	60.39 (1.94)	58.23 (0.73)	59.27 (2.28)

Note: values represent means of three replicates with standard deviation in parenthesis. Means within each row followed by different lowercases represent a significant difference among the N levels (*P*<0.05)

The relationship between *C_{um}* and the varieties of soil chemical properties

Soil cumulative C respiration was negatively and linearly related to the varieties of soil C: N ratio and total C (Fig. 5A and E, *P*<0.01), while positively and linearly related to the varieties of soil total N (Fig. 5B, *P*<0.01). No significant correlations were observed between *C_{um}* and the varieties of soil pH and DOC (Fig. 5C-D, *P*>0.05).

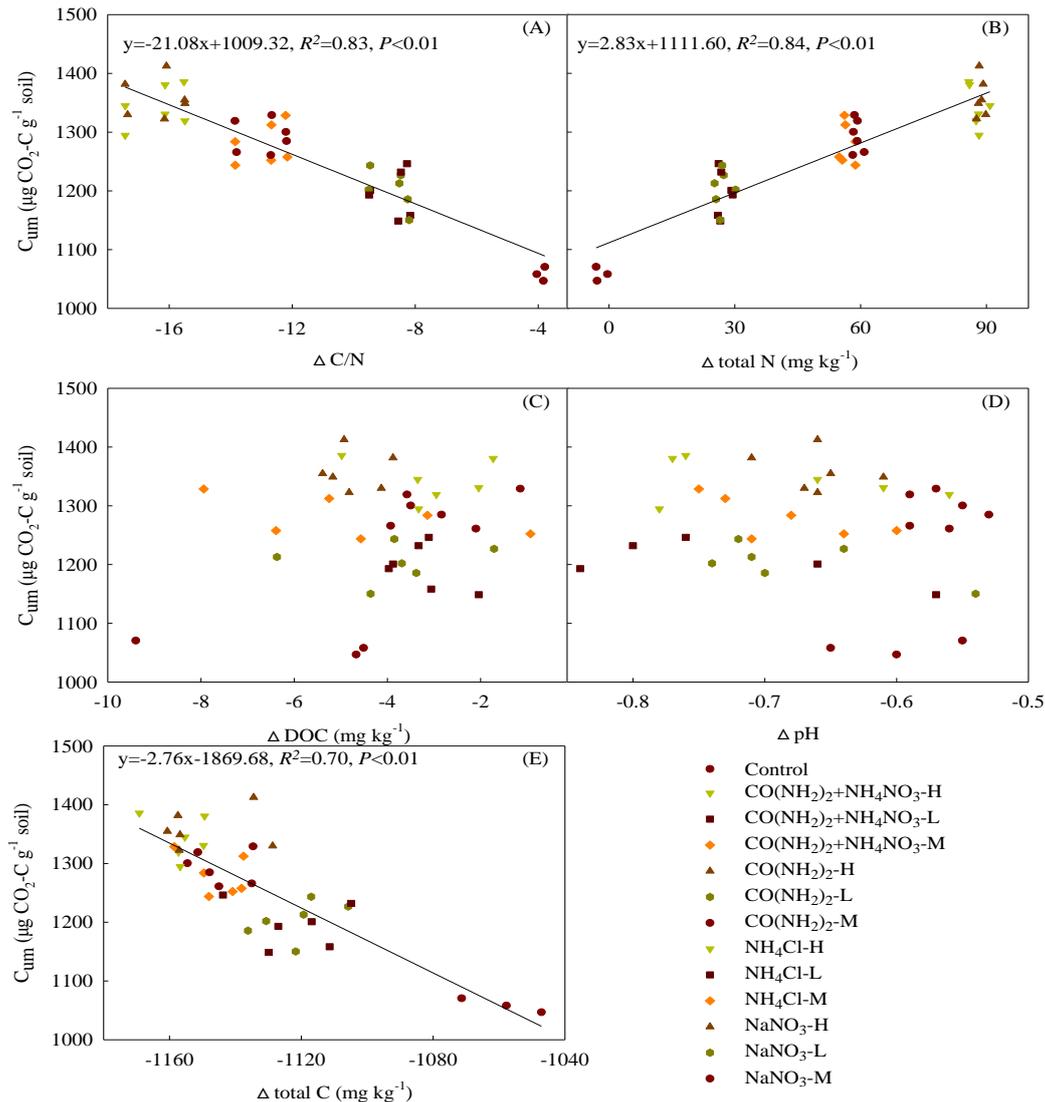


Figure 5. The relationships between Cumulative soil C respiration (C_{um}) and the varieties of the chemical properties. L, M and H represent the N addition of 44.39, 88.77 and 133.16 $\mu\text{g N g}^{-1}$ soil, respectively. “ Δ ” represents the varieties of the soil chemical properties before and after the incubation. “y” and “x” represent the variables in horizontal and vertical coordinates

Discussion

Our results indicated that C_{um} increased with the N addition levels by alleviating soil microbial N limitation regardless of the N species (Fig. 6), which would result in a large amount of soil CO₂ emission from riparian zone in the future. Thus, induced by atmospheric N deposition, soil CO₂ emission should be concerned in riparian zone.

The effect of N addition levels on soil C/N ratio and CO₂ emission

Soil C: N ratio decreased with the N addition levels for all the N species (Table 2 and Table 3, $P < 0.001$). Soil total N increased with the N addition levels ($P < 0.001$), which is the reason for the results. Gong et al. (2019) discovered the same trend in a field study of temperate grassland.

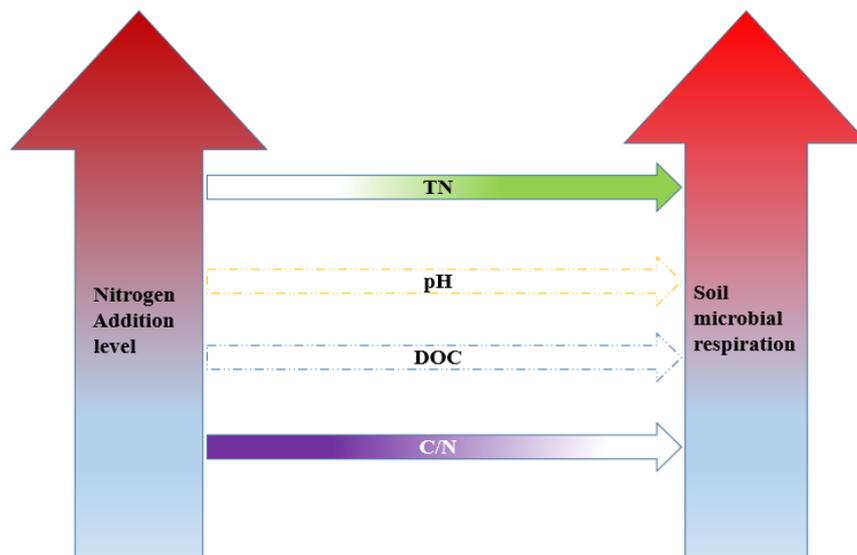


Figure 6. The response of soil microbial respiration to the N addition levels in terms of the varieties of soil chemical properties. The colours of the arrows changing from light to dark and from dark to light represent the increase and decrease of soil chemical properties, respectively. Dash and solid lines represent insignificant and significant relationships, respectively

As we hypothesized, C_{um} increased with the N addition levels for all the N species (Fig. 3, $P < 0.05$). The alleviation of soil microbial nutrition limitation is the reason for the results, supported by the results of PCA (PC1 represented by soil C: N ratio and total N, Fig. 7) and the negatively linear relationship between C_{um} and the variety of soil C: N ratio (Fig. 5A, $P < 0.01$). N addition increases soil microbial respiration in forest, pasture, grassland, wetland, cropland and bamboo ecosystems, which is consistent with our results; Meanwhile, inconsistent results of inhibiting and no effect were also observed (Table 4). The differences of soil pH and C and N availability (Leifeld et al., 2008; Eberwein et al., 2015) might be the reasons for the inconformity.

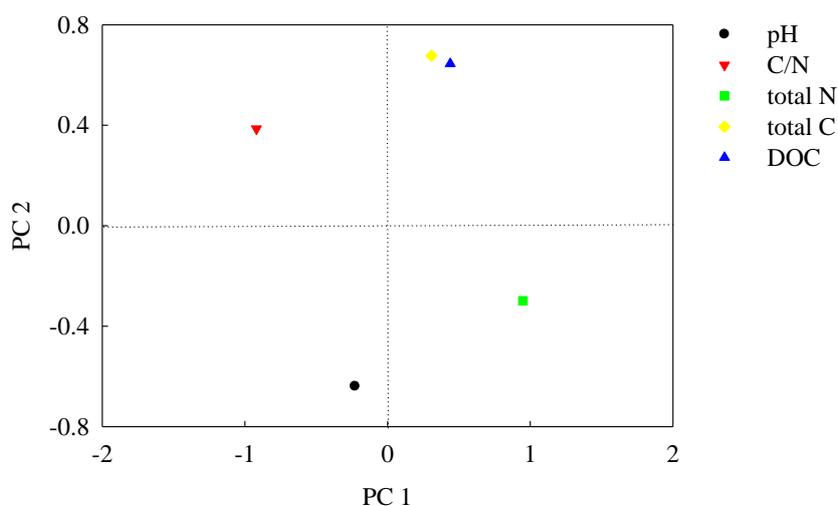


Figure 7. The principal component analysis (PCA) of the soil chemical properties after the incubation. The first two principal components (PCs) account for 41 and 30% of the variances in the soil chemical properties

Table 4. The response of soil respiration to N addition in different soil ecosystems

Ecosystems	N species	N levels (kg N ha ⁻¹ yr ⁻¹)	Response of soil respiration to N addition	References
Forest	NH ₄ NO ₃	100	-	(Zheng et al., 2018)
	NH ₄ NO ₃	100	-	(Li et al., 2017)
	NH ₄ NO ₃	50,150	-	(Peng et al., 2020)
	NH ₄ NO ₃	50	+	(Sun et al., 2019)
	NH ₄ NO ₃	50	+	(Chen et al., 2019a)
	CO(NH ₂) ₂	50, 100	ns	(Zhao et al., 2018)
	NH ₄ NO ₃	50,150,300	-	(Yan et al., 2020)
Pasture	NH ₄ NO ₃	44.20	+	(Fang et al., 2017)
	CO(NH ₂) ₂	100	+	(Wang et al., 2020)
	NH ₄ NO ₃	<8, > 8	ns, -	(Wang et al., 2019b)
Grassland	CO(NH ₂) ₂	100	-	(Riggs and Hobbie, 2016)
	CO(NH ₂) ₂	100, 200, 300, 400, 500	-	(Zeng et al., 2018)
	CO(NH ₂) ₂	100	-	(Riggs et al., 2015)
	CO(NH ₂) ₂	23, 92	+	(Zhang et al., 2014)
	CO(NH ₂) ₂	100	ns	(Zhao et al., 2020)
	NH ₄ NO ₃	50, 100	ns	(He et al., 2018)
	NH ₄ NO ₃	44.2	ns	(Fang et al., 2018)
	CO(NH ₂) ₂	100, 200	-	(Wei et al., 2018)
Wetland	NH ₄ NO ₃	0.10, 0.20, 0.50 mg N g ⁻¹ soil	-	(Tao et al., 2013)
	NH ₄ NO ₃	30, 60	-	(Min et al., 2011)
	NH ₄ NO ₃	40, 80	+	(Wang et al., 2019a)
Bamboo	NH ₄ NO ₃	50,100	+	(Tu et al., 2013)
Cropland	CO(NH ₂) ₂	120, 180, 240	+	(Liang et al., 2018)
Riparian zone	NH ₄ Cl, NaNO ₃ , CO(NH ₂) ₂ , CO(NH ₂) ₂ (28%) plus NH ₄ NO ₃ (72%)	50, 100, 150	+	This study

Note: “+”, “-” and “ns” represent promoting, inhibiting and no significant impact of N addition on soil microbial respiration

The effect of N species on soil CO₂ emission

Again, as we hypothesized, the N species had no effect on C_{um} (Fig. 3 and Table 2, P>0.05), which is supported by the results of Ramirez et al. (2010). However, Du et al. (2014) discovered that inorganic and organic N addition inhibits and promotes soil microbial respiration, respectively, and Wang et al. (2018) found that the mixture addition of inorganic and organic N has higher inhibition on soil microbial respiration than single organic or inorganic N addition, which are inconsistent with our results. This inconformity should be due to the difference of soil microbial activity caused by different N addition species and soil properties (Geisseler and Scow, 2014), and needs a further study.

The results suggested that soil CO₂ emission from riparian zone caused by atmospheric N deposition should be considered when we establish an estimation model of soil CO₂ emission from the terrestrial ecosystem. In addition, for improving the ecological effect of the TGR, land management measures should be taking to reduce exogenous N input to the riparian zone during the dry period.

Conclusion

This study discussed the effect of nitrogen increase on soil carbon dioxide emission caused by microbial respiration in the riparian zone of the Three Gorges Reservoir based on a 36-d soil incubation. The increase of the nitrogen addition levels decreased soil carbon: nitrogen ratio and promoted soil microbial respiration regardless of the nitrogen species. The findings suggest that, induced by atmospheric nitrogen deposition, soil carbon dioxide emission should be concerned in riparian zone. However, a long-term soil incubation should be launched for the better understanding of the effect of exogenous nitrogen input on soil microbial carbon dioxide emission. Meanwhile, this study only discussed the effect of nitrogen increase on soil microbial respiration in terms of soil chemical properties; the deep microbial mechanism should be discussed in the future.

Acknowledgments. This work was supported by the National Natural Science Foundation of China (31770529); Program of Chongqing Science and Technology Commission (cstc2018jcyjAX0813); the Science and Technology Research Program of Chongqing Municipal Education Commission (KJ1710260, KJQN201801233, KJZD-K201801201); the Chongqing Municipal Key Laboratory of Institutions of Higher Education (WEPKL2016LL-03).

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