# Soil microbial respiration responses of nitrogen addition:

# Evidence from a long-time semi-arid grassland soil incubation

Zhaomin Wang, Zhongmiao Liu, Binhui Guo, Zhengchao Qi, Decao Niu, Hua Fu<sup>\*</sup> State Key Laboratory of Grassland Agro-ecosystems; Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs; Engineering Research Center of Grassland Industry, Ministry of Education; College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, 730020, P. R. China

\* Corresponding author

Address: 768 West Jiayuguan Road, Chengguan District, Lanzhou, 730020, China. Phone: +86-13893621162. Fax: 0931-8912440. Email: fuhua@lzu.edu.cn

### Abstract:

Nitrogen is essential for the synthesis of key cellular compounds such as proteins and nucleic acids in all organisms, and it is one of the limiting elements in most terrestrial ecosystems. During past decades, terrestrial ecosystems nutrients availability have altered with nitrogen deposition increases rapidly so that under the soil microbial metabolism activities terrestrial ecosystem biogeochemical cycles are strongly affected. Therefore, maintaining the stability of soil carbon pools, especially microbial carbon pools has great importance for studying global carbon cycle and global climate change processes. Depending on whether soil microbial has already adapted to the environment nitrogen concentration, there exists different results, such as promotion, inhibition, and no impact. To date, how nitrogen will affect soil microbial respiration still has controversy. To determine the effects, we performed a 59 weeks incubation with the soil which has already treated with Urea for 9 years. The soil has been treated with four N addition levels in a semi-arid grassland where located in North-west part of China. We measured CO<sub>2</sub> effluxion under different treatments within the same temperature. Our results showed that during the first 8 weeks, soil microbial had strong responses about N addition and N9.2 showed greatest influence which means N addition has positive effect to soil microbial respiration. With the time passing, in the time of 9-59 weeks, N0 had highest soil microbial respiration rate while N2.3 was the lowest, this illustrated N2.3 had highest N use efficient (NUE), in order to meet soil microbial stoichiometry, microbial community became strong C-limitation under the N2.3

treatment. What's more, comparing with other studies which we shared same study area, we also found that the time of nitrogen application also had strong effect on soil microbial respiration. These results highlight the importance of microbial respiration and may also help us to have a better understand about how N deposition controls terrestrial C flows.

**Key Words:** Nitrogen deposition, Soil microbial respiration, CO<sub>2</sub> fluxes, semi-arid grassland, Loess Plateau

### 1. Introduction:

As a result of combustion of fossil fuels, application of fertilizers and development of animal husbandry, atmospheric nitrogen (N) deposition has been 3-5 times higher than the last century (Galloway et al., 2008). Increasing N which is one of the main limited nutrient elements for ecosystem will change the availability of nutrients in terrestrial ecosystem. As the consequence, soil physico-chemical properties will be strongly influenced (Vitousek et al., 1997) that expected to make soil microbial community change their living strategy with community structure and function group (Stuart and Elser, 2002) and finally impact terrestrial carbon biogeochemical cycles (Wei et al., 2019).Soil microorganisms as the major decomposers of terrestrial ecosystems in order to meet their own growth by acquiring nutrient resources (Liang et al., 2011),they release considerably amount of carbon into the soil carbon pool through respiration. The decomposition of nutrients by microorganisms not only drives the soil carbon flow but also mediates the balance of soil carbon pool (Stuart and Elser, 2002).

To get better understand about the effects of nitrogen addition on soil microbial respiration, Loess Plateau was chosen as it is sensitive for the N deposition with 0.2-2.2 g N m<sup>-2</sup> yr<sup>-1</sup> (Yuan et al. 2019) which belongs to nitrogen sensitive area. Prior works tested how soil microbial responses to N addition in the same experiment area showed different results, one reported that N addition positively influence the soil microbial respiration. Soil microbial activity is activated as N addition induces plant growth, leading to both primary productivity and litter are increased (Liu and Greaver, 2010; Wang et al., 2003) that brings environmental carbon substrates increased thus soil microbial respiration is promoted (Zhang et al., 2014). Meanwhile, other research showed there existed negative influence on soil microbial respiration with N deposition (Yuan et al. 2019). They uncovered this result mainly based on Ecological

Stoichiometry Theory (EST) due to the growth of microorganisms changed nitrogen and (or) phosphorus limitation to carbon limitation.

The objective of this study was to investigate how soil microbes cope with anthropogenic N deposition in a natural ecosystem and how microbial responses to this N input affect soil respiration with a long-time soil incubation.

## 2. Materials and Methods:

### 2.1 Field description

This study was conducted in the grassland which located inside the Semiarid Climate and Environment Observatory of Lanzhou University (SACOL) in Lanzhou, Gansu Provience, China ( $35^{\circ} 57'$  N,  $104^{\circ} 09'$  E; 1966 m a.s.l). This area has a history of farming for more than 30 years and the distance away from Lanzhou city is nearly 40km (Zhang, et al, 2014). The annual mean temperature is  $6.7^{\circ}$ C, and the average precipitation is about 382 mm per year. According to the classification, soil in this region is classified as Sierozem (Li, et al, 2010). This area is mainly dominated by *Stipa bungeana* and *Leymus secalinus*, the period of the plant growth in SACOL is from the beginning of April to the end of October.

## 2.2 Experimental design and soil sampling

All samples were collected in 15<sup>th</sup> of October, 2018. We used Urea  $[CO(NH_2)_2)]$ as the additional nitrogen resource, there existed 4 different nitrogen addition treatments, N0 (without addition), N2.3 (2.3g N m<sup>2</sup> yr<sup>-1</sup>), N4.6 (4.6g N m<sup>2</sup> yr<sup>-1</sup>) and N9.2 (9.2g N m<sup>2</sup> yr<sup>-1</sup>), and every treatment contains five replicates. Each plot was in the size of 4×5m, and all plots were separated. We applied Urea twice per year and each time we fertilized half amount of the N fertilizer. We conducted this treatment followed the different concentrations in the middle of April and May 2018, respectively.

We sampled soil inside each site with  $1 \times 1$ m plot which were randomly established. Three composite of 0-10cm top layer soil samples were collected with 3 soil cores (7.5 cm inner diameters). Then 3 soil cores were well mixed. Composite soil samples were stored at  $-4^{\circ}$ C and transferred to laboratory in Lanzhou University within 24hours. Prior to analysis, all soil samples were passed though 2mm sieve, roots and plant detritus were removed by hand carefully.

## 2.3 Microbial respiration assay

Soil microbial respiration was measured as CO<sub>2</sub> evolution of fresh soil samples at 60%

of water holding capacity. We incubated samples in sealed brown glass jars with the presence of a CO<sub>2</sub> trap (0.5 M NaOH) at 25°C. The CO<sub>2</sub> efflux trapped in the NaOH solution for each soil sample was measured by titrating the residual OH<sup>-</sup> with 0.05 M HCl (Wei et al., 2013). We measured the CO<sub>2</sub> evolution for 413 days, during the first week, we measured every 2 days, then measured each weeks from 2 to 13 weeks, finally we take 2 weeks as a testing period till the incubation time reaches 413 days. All date was analysis using Microsoft Excel 2013, and graphs were showed by using Origin 2018.

### 3. Results and Discussion

After 59 weeks incubation under  $25^{\circ}$ C, we found that soil microbial respiration shows different responses under different nitrogen concentrations. During the first 56 days, N 9.2 shows it has the highest influence for the soil microbial respiration, other treatments showed fluctuation with the soil microbial respiration rate (Fig 1a), this results indicated the higher N concentration has higher soil microbial respiration rate, and during these incubation time, soil microbial has strong self-adjustment with the N input. After 8 weeks, N0 had the highest CO<sub>2</sub> effluxion while the N2.3 was the lowest one. This might because of when nitrogen input may make the resource C:N imbalance (Yuan et al. 2019), in order to cope with the increasing nitrogen, soil microbial community will release more soil C-acquiring enzymes to balance the stoichiometry themselves. What's more, N2.3 is more effective for soil microbial growth (Zhou et al., 2012). So, under N 2.3 soil microbial is easier to become C-limitation. And combine researches conducted with Zhang (Zhang et al., 2014) and Yuan (Yuan, et al., 2019), although they shared the same study area with opposite results, the main factor make this different is the different time scale, the previous was investigated the soil microbial respiration under 5 years, the later one was mainly focus the soil microbial respiration for 8 years. There is an evidence that during the first 5 years soil microbial respiration will have significant difference (Treseder 2008).



Figure 1 Relationships between Soil Respiration Rate and Incubation time (a), Total CO<sub>2</sub> Effluxion correlated with the Incubation time (b). Black square, red dot, blue triangle and green triangle represent N0, N2.3, N4.6 and N9.2, respectively.

More deeply investments are need to unveil to strategies for the reasons about nitrogen deposition effect soil microbial respiration. Overall, our research gives a better understanding of how soil microbial deal with the nutrients input especially nitrogen. This finding will help to improve the predictions of soil microbial feedback to climate change.

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