



Nitrogen addition decreased soil respiration and its components in a long-term fenced grassland on the Loess Plateau



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ARTICLE INFO

Keywords:

Soil respiration
Microbial respiration
Root respiration
N addition
Q₁₀ value

ABSTRACT

Knowledge of the impact of N enrichment on soil respiration components is critical for understanding carbon (C) cycling and its feedback processes with climate change. We conducted three N level addition experiments, control (CK, 0 g N m⁻²yr⁻¹), low nitrogen addition (LN, 10 g N m⁻²yr⁻¹), and high nitrogen addition (HN, 20 g N m⁻²yr⁻¹) to investigate the response of microbial and root respiration to N enrichment in a long-term fenced grassland on the Loess Plateau of China. Compared to the control, both the LN and HN treatments generally decreased soil respiration and its two components for both years. Under N addition, the decreased rates of root respiration and microbial respiration were significantly and positively correlated with the monthly root production and soil microbial biomass C, respectively. Nitrogen addition decreased the Q₁₀ values of root and microbial respiration, with the reduction more pronounced in root respiration. Overall, our results suggest that N enrichment may reduce the soil C loss through CO₂ emissions. With global warming, soil C loss will be less due to the lower Q₁₀ values of root and microbial respiration on the Loess Plateau of China under future scenarios of N deposition.

1. Introduction

Anthropogenic activities, such as fossil fuel burning and agricultural fertilization, have greatly increased deposition of reactive nitrogen (N) species to the biosphere (Galloway et al., 2004). Given the N limitation in most terrestrial ecosystems, the increase in soil N availability can change the primary productivity and plant litter decomposition, with consequent influence on ecosystem carbon (C) cycling (Vitousek et al., 1997). Soil respiration is the main pathway of C emission from the soil to the atmosphere in terrestrial ecosystems and represents an important component of global C cycle (Schlesinger and Andrews, 2000). Predictions based on global models indicate that even a small fluctuations of soil respiration can significantly amplify or mitigate atmosphere CO₂ concentration, causing positive or negative feedbacks to climate change (Raich and Schlesinger, 1992). Therefore, understanding the responses of soil respiration to N enrichment is of great significance in evaluating the terrestrial C balance and global C budget.

Grassland, storing 10–30% of global soil organic C (SOC) (Follett and Reed, 2010), can act as either a considerable C sink or source in response to global climate change through the balance between

photosynthesis and soil respiration (Parton et al., 1995). However, past investigations on the response of soil respiration to N addition have shown positive (Jia et al., 2012; Zhang et al., 2014), negative (Ren et al., 2016; Zhu et al., 2016) and neutral effects (Jiang et al., 2013) in different grassland ecosystems. One key reason for the divergence of previous studies in the effect of N addition on soil respiration is that soil respiration is composed of two different components (Ryan and Law, 2005). One of the components is root respiration, which refers to the CO₂ emission from plant roots, mycorrhizal fungi and other associated microorganisms (rhizosphere microorganisms) that depend on the contemporaneous. Another component is microbial respiration, which derives from the decomposition of plant litter and soil organic matter by soil microorganisms. Because the main controllers of the different components of soil respiration are variable (Han et al., 2016), the root and microbial respiration may respond differently to N addition in magnitudes or even directions. For example, N addition could either increase microbial respiration by plant litter accumulation or decrease it by limiting soil microbial biomass and enzyme activity (Ramirez et al., 2012; Yan et al., 2010; Zhang et al., 2014). Root respiration had been reported to increase resulting from the root biomass

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accumulation, or decrease due to the reduction of photosynthate allocation to belowground under N enrichment (Vallack et al., 2012; Zeng et al., 2010; Zhang et al., 2014). Great variations in the contribution of root respiration to total soil respiration in different ecosystems (ranging from 10 to 90%) may be an important factor in explaining the inconsistent results of the N addition on soil respiration (Hanson et al., 2000). Thus, partitioning and quantifying the individual root and microbial respiration components is essential for a better understanding of the underlying mechanisms driving the response of soil respiration to increased soil N availability. Furthermore, calculating C loss through heterotrophic respiration is essential for calculating net ecosystem production (Chen et al., 2011).

Soil temperature has been reported as the major abiotic factor that influences soil respiration (Thomas et al., 2011). The temperature sensitivity of soil respiration (Q_{10}), the relative change of soil respiration when temperature increases by 10 K, is considered as one crucial determinant of the climate-carbon cycle feedback in terrestrial ecosystems (Curjel yuste et al., 2004). Previous studies have shown that Q_{10} is largely dependent on the substrate quality and availability, and closely correlated with soil temperature and soil moisture (Luo et al., 2001). Therefore, N enrichment-induced changes of plant growth and soil microclimate may affect the Q_{10} values of soil respiration. However, there is little information about the effects of N fertilization on the Q_{10} of soil respiration in grassland ecosystems, and even less attention has been paid to microbial and root respiration.

The grassland on the Loess Plateau is an important component of China's grasslands, with a great significance in the global C cycle. Since the 1950s, to solve the problem of soil erosion, the Chinese government initiated a long-term vegetation restoration project (Fu et al., 2002). After decades of fencing, the vegetation coverage and soil nutrients have greatly improved (Qiu et al., 2013; Jing et al., 2014). In the context of increasing deposition of atmospheric N on the Loess Plateau (Wei et al., 2011), how the N enrichment influences soil respiration, especially root and microbial respiration separately in these long-term fenced grasslands is still unclear. Given the abovementioned context, we performed a field experiment to test the effects N addition on components of soil respiration in the long-term fenced grassland on the Loess Plateau of China. We hypothesized that (1) N addition would increase soil respiration through both microbial and root respiration because grassland is always N limited; (2) Root respiration may be more sensitive to N addition than microbial respiration considering the more directly control of plant growth on root respiration; (3) N addition would alter the temperature sensitivity of soil respiration and its components.

2. Materials and methods

2.1. Study site

The study was performed in a national nature reserve of restored grasslands (since 1982) on the Loess Plateau in Ningxia Hui Autonomous Region (106°21'–106°27'E, 36°10'–36°17'N), China. The site covers a total area of 6660 ha, with an elevation ranging from 1800 to 2100 m. The mean annual temperature is 6.9 °C, with the maximum and minimum temperatures occur in July (24 °C) and January (−14 °C), respectively. The average rainfall in 26 years is 455 mm, and the rainfall from July to September accounts for 65–85% of the annual precipitation. The vegetation community consists of 297 plant species and is dominated by *Stipa* plants (*S. bungeana*, *S. grandis*, *S. przewalskyi*), and main forbs include *Artemisia sacrorum* and *Thymus mongolicus*. The soil types in this area are mainly Loessial soil and mountain gray-cinnamon soil.

2.2. Experimental design

The experiment was designed as a randomized block with five

replicate blocks separated by 2 m walkways. In each block, we established three 3 m × 4 m plots (3 N treatment × 5 replicates = 15 plots). In these three plots, we established one of three N-addition treatments: control (CK, 0 g N m^{−2}yr^{−1}), low N addition (LN, 10 g N m^{−2}yr^{−1}), and high N addition (HN, 20 g N m^{−2}yr^{−1}). Nitrogen was applied in April of each year. Nitrogen was manually added to the grassland surface in the form of dry urea (CO(NH₂)₂). Soil respiration (SR) was separated into microbial respiration (MR) and root respiration (RR) in the field by using the trenching method. In September 2013, trenches (0.1 m wide and 0.5 m deep) were excavated in each plot and lined with nylon mesh (0.038 mm mesh size) to set up root-free small plots (0.3 m × 0.3 m). The trench was then refilled with soil according to its original soil profile. The meshes with smaller pore sizes than fine root diameter can inhibit root growth into the plots but permit the penetration of water, bacteria, organic matter, and materials (Moyano et al., 2007). The root-free plots were then kept free of seedlings and herbaceous vegetation by periodic manual removal during the study period. After eight months of equilibration of excavated trenches, we measured CO₂ efflux in the root-free plots as microbial respiration only, while the CO₂ from the whole-soil plots with intact vegetation was composed of both microbial and root respiration. Root respiration was then determined by the difference of CO₂ efflux between the whole-soil and root-free plots.

2.3. Measurement protocols

2.3.1. Soil temperature and moisture

Soil temperature and moisture near each collar were measured at the time of measuring soil respiration. Soil temperature at a depth of 5 cm was determined using a thermocouple probe connected to the LI-6400 adjacent to each PVC collar. Soil moisture (volumetric soil content) was determined at a depth of 0–10 cm using a TRIME TDR probe (IMKO, Ettlingen, Germany) near the same sites after soil temperature measurements.

2.3.2. Soil CO₂ efflux

In June 2014, PVC collars (11 cm in diameter and 5 cm in height) were permanently installed 2–3 cm into the soil for the measurement of soil respiration (one for each root-free plot and two for each whole-soil plot). Soil respiration was measured once or twice a week from 18 June to 17 October in 2014 and from 24 April to 15 October in 2015 using an LI-6400 portable photosynthesis system attached to a soil CO₂ efflux chamber (800 cm³ in total volume; LI-COR 6400-09 TC, LI-COR Inc., Lincoln, NE, USA). All measurements were conducted between 09:00 h and 11:00 h (local time).

However, we observed the soil temperature and moisture in root-free plots were significantly higher than that in whole soil plots. The actual root respiration would be underestimated if it is directly calculated from the difference of measured CO₂ flux between the whole-soil plot and root-free plot. To eliminate this error, we corrected the measured microbial respiration by using a bivariate linear equation (1) simulating the relationship between microbial respiration, soil temperature and soil moisture in root-free plots:

$$\text{LN}(\text{MR}_{\text{measured}}) = a \times T + b \times W + c \quad (1)$$

where LN(MR_{measured}) is the natural logarithm of measured microbial respiration, T and W are the soil temperature (°C) and volumetric soil water content (%) measured in the root-free plot, respectively. The symbols a, b and c are coefficients relevant to soil temperature and moisture. We solved for the three coefficients and established a function according to Eq. (1) for each treatment (Table 1). However, because of severe soil drought during July–August in 2015, the relationships of microbial respiration with soil temperature and moisture during this period are very different from other measuring periods. To ensure accurate calibration, we separated the measured data from July to August

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