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Temperature sensitivity of soil respiration to nitrogen and phosphorous fertilization: Does soil initial fertility matter?

Qiqi Sun[a,b], Rui Wang[c], Ying Wang[a,b], Lanlan Du[d], Man Zhao[c], Xin Gao[d], Yaxian Hu[d], Shengli Guo[a,b,c,*]

[a] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling, Shannxi 712100, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] College of Resources and Environment, Northwest A&F University, Yangling, Shannxi 712100, PR China
[d] Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi 712100, PR China

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ABSTRACT

Temperature sensitivity of soil respiration (Q_{10}) is an important parameter when modeling the effects of global warming on terrestrial ecosystem carbon release. Widely applied chemical fertilizers can significantly affect soil productivity and carbon cycling in agroecosystems. However, little is known about how Q_{10} responds to chemical fertilization under different levels of initial soil fertility. On the Chinese Loess Plateau, changes in soil respiration rates and Q_{10} were investigated in soils of two fertility levels: low fertility (L) and high fertility (H). For each soil fertility level, there was one control plot and one chemical fertilized plot (+NP), which in total formed four treatments: L, L + NP, H and H + NP. All the treatments were replicated for three times on a continuous winter wheat cropping system. Respiration rates of surface soil in each treatment were in situ monitored from October 2010 through September 2015. Our results showed that after NP fertilization, soil respiration rates were increased by 46% in low fertility soil, yet only by 14% in high fertility soil ($P < 0.05$). The Q_{10} after NP fertilization was significantly decreased by 6.9% in low fertility soil, but was unchanged in the high fertility soil. The Q_{10} variation might be attributed to the different response of microbial respiration Q_{10} in the two soils. The decreased Q_{10} with NP fertilization in the low fertility soil was possibly due to N-induced increase of substrate quality for soil microbes and increased activities of both cellobiohydrolase and polyphenol oxidase. In the high fertility soil, the unchanged Q_{10} with NP fertilization may be the integrated result of less affected substrate quality and neutral response of polyphenol oxidase activity. Overall, our results suggested that the effects of NP fertilization on soil respiration and its temperature sensitivity varied with soil initial fertility levels, and therefore must be properly accounted for when estimating potential effects of local agricultural management to regional agroecosystems under future climate conditions.

1. Introduction

Temperature sensitivity of soil respiration in an ecosystem (Q_{10}: multiplier of soil respiration rate for a 10 °C increase in temperature) partially governs the amount of carbon released from soils to the atmosphere in response to global warming (Cox et al., 2000; Zhou et al., 2009). Large variations in the responses of soil respiration to temperature (Q_{10}) have been reported in different land uses, ecosystems or climatic conditions (Lloyd and Taylor, 1994; Davidson and Janssens, 2006; Zheng et al., 2009). The variation of the Q_{10} value in carbon cycle models may result in significant bias in the estimation of soil respiration (Townsend et al., 1997; Xu and Qi, 2001), which is regulated by multiple factors. For a certain soil, Q_{10} is closely related to the availability (Gershenson et al., 2009; Lützow and Kögelknabner, 2009) and quality (Davidson and Janssens, 2006; Wang et al., 2016) of respiration substrates, nutrient availability (Burton et al., 2002; Wang et al., 2017a; Zeng and Wang, 2015), the composition and size of soil microbial population (Coucheney et al., 2013; Ramirez et al., 2012) and activities of extracellular microbial enzymes (Stone et al., 2012; Wallenstein et al., 2009). All these factors are related to soil fertility (Lauber et al., 2008; Liu et al., 2010b), the effect of which on Q_{10} has been acknowledged but not well understood (Zheng et al., 2009; Uchida et al., 2010).

Chemical fertilization is a common field practice to sustain food production in agroecosystems (Liu, 1999; Fan and Zhang, 2000).

* Corresponding author at: Institute of Soil and Water Conservation, Xinong Road 26, Yangling, Shaanxi 712100, PR China.
E-mail address: slguo@ms.iswc.ac.cn (S. Guo).

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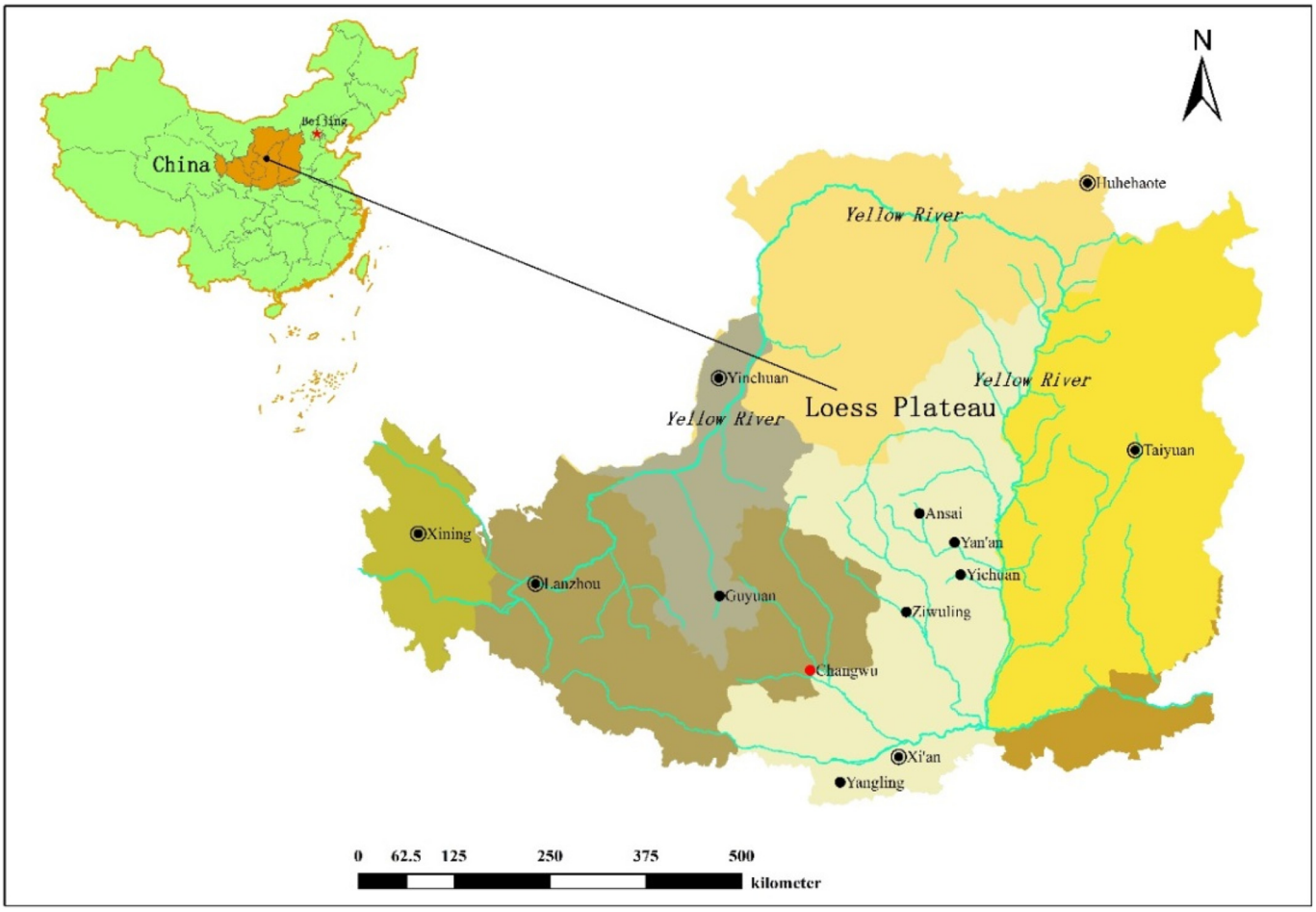

Fig. 1. Location of the experimental site in the Loess Plateau, China (cited from Wang et al., 2017a).

Agroecosystems on Loess Plateau, characterized by low yet highly variable soil fertility (Wang et al., 2014), normally have low availability of natural N in soils, with the typical total N concentrations ranging from 0.042 to 0.077% (Zhu et al., 1983). Depletion of soil nutrients is a major constraint on sustainable food production in the loess region (Guo et al., 2012). To meet the local food requirement, arable soils in the Loess Plateau are normally heavily fertilized, especially with N and P fertilizers ($> 200\ kg\ N\ ha^{-1}$ each crop, $> 60\ kg\ P\ ha^{-1}$ each crop) (Guo et al., 2010; Ju et al., 2004), which results in even more variable soil fertility. In addition, climate change has resulted in increases in temperature and decreases in rainfall across the Loess Plateau since 1970 (Zhi et al., 2010; Sun et al., 2015). Therefore, how chemical fertilization affects the response of soil respiration to temperature changes at different fertility levels is of great relevance to management of agroecosystems, especially when confronting future climate change.

As one of the most effective ways regulating soil fertility (Liu et al., 2010a; Nest et al., 2014), chemical fertilization is playing increasingly important role in the carbon cycle of agroecosystems (Zhang et al., 2017). Chemical fertilization influences the magnitude of soil respiration and its components by altering soil physicochemical and biological properties and belowground carbon allocation (Chen et al., 2017; Ding et al., 2010). Specifically, through enhancing soil N availability, N fertilization could change microbial community (Allison et al., 2007; Ramirez et al., 2012; Xun et al., 2015) and soil enzyme activities (Allison and Vitousek, 2005; Stone et al., 2012; Waldrop et al., 2004), which is highly related to C turnover (Jiang et al., 2014). Furthermore, N and P fertilization generally exerts significant effect on photosynthetic rate and the belowground allocation of the photosynthesis especially in N-limited ecosystems (Wang et al., 2017a; Zeng and Wang, 2015). Moreover, increased nutrient (N in particular) inputs can change plant tissue N concentration (Burton et al., 2002; Burton et al., 2015; Lovelock et al., 2006) and then shift plant carbon supply to microbes (Högberg et al., 2003), further affecting microbial decomposition (Graham et al., 2012). The soil C:N:P ratio, highly related to substrate quality (Dignac et al., 2002; Leifeld and von Lutzow, 2014), drives key ecological processes (de Menezes et al., 2015; Dignac et al., 2002; Ma et al., 2013; Sardans et al., 2012) and may strongly affect the ecosystem C storage (Zeng and Wang, 2015). Previous studies have reported the effects of chemical fertilization on Q_{10}, yet the results varied with ecosystems (Zhou et al., 2014). For instance, N (and P) fertilization or N (and P) deposition reduced Q_{10} was more reported in forest (Mo et al., 2008; Sun et al., 2014), grassland ecosystems (Li et al., 2015; Zhang et al., 2014), and less in agroecosystems (Chen et al., 2017). In contrast, increased Q_{10} with increasing N (and P) inputs was reported in a Tibetan alpine meadow (Guo et al., 2017) and cold temperate forest (Liu et al., 2016). In addition, neutral responses were also reported in a young *Cunninghamia lanceolata* forest (Wang et al., 2017b) and a temperate grassland in Inner Mongolia, China (Li et al., 2015). However, at present, no consensus has been achieved with regards to the response of Q_{10} to chemical fertilization, as initial soil fertility is not always appropriately accounted for in these studies.

In this study, changes in surface soil respiration and its temperature sensitivity, soil and crop properties were investigated after applying NP fertilization for five years on soils of low and high fertility under a continuous winter wheat cropping system on the Loess Plateau. The aim of this study was to examine the responses of Q_{10} to NP fertilization in low and high fertility soils, and to further explore the driving factors for different responses. We proposed two hypotheses that 1) soil respiration

would increase but Q_{10} would decrease under NP fertilization; and 2) the increase in soil respiration and reduction in Q_{10} would vary with soil fertility under NP fertilization.

2. Materials and methods

2.1. Site description

This study was based on a long-term field experiment with continuous winter wheat (*Triticum aestivum* L., cv. 'Changwu 89 (1) 3–4') agroecosystems established in September 1984 at the Changwu State Key Agro-ecological Experimental Station (E107°40′, N35°12′, altitude of 1220 m), Changwu, Shannxi, China (Fig. 1, cited from Wang et al., 2017a). In particular to examine the responses of Q_{10} to NP fertilization in low and high fertility soils, the present study started from October 2010 to September 2015 with winter wheat (*Triticum aestivum* L.) crop. The study area is in a typical rain-fed farming region, and characterized by a semiarid continental monsoon climate. The annual mean precipitation is 542 mm (1956–2015), 60% of which occurs between July and September. The mean annual temperature was 9.6 °C (1956–2015), and the open pan evaporation is 1440 mm on average. The annual sunshine duration is 2230 h with a total radiation of 484 kJ cm^{-2}, and frost-free period is 171 days. All meteorological data were provided by Changwu State Key Agro-Ecological Experimental Station. Considering the growing cycle of winter wheat, the period from October to September of the following year was referred as a whole year.

As this study was based on a long-term field experiment, the soil was the same as used in Jiang et al., 2015a; Jiang et al., 2015b, Wang et al., 2017a and Wang et al., 2016. In brief, the soil in the study area is a uniform loam (Cumulic Haplustoll; USDA Soil Taxonomy System) originated from parent material of calcareous loess. Soil samples collected at 0–20 cm depth in 2010 are characterized by: bulk density of 1.3 g cm^{-3}, clay content (< 0.002 mm) of 24%, field water-holding capacity (WHC) of 22.4%, permanent wilting point of 9.0%, pH of 8.4 (1:1 soil:H_2O suspension), $CaCO_3$ of 10.5%, SOC of 6.5 g kg^{-1}, total soil N of 0.80 g kg^{-1}, and Olsen-P of 3.0 g kg^{-1}.

2.2. Experimental design and crop management

Two fertility levels were investigated in this study: one was amended with farmyard manure (75 t ha^{-1} yr^{-1}) since 1984 (as high fertility soil), the other was never fertilized as reference (as low fertility soil). Following local chemical-fertilization regime, it is more common to use N + P fertilization (Guo et al., 2010). Therefore, for each fertility level, there were two chemical fertilization sub-levels (no chemical fertilization, and 120 kg N ha^{-1} plus 26.2 kg P ha^{-1} recommended by the local agricultural extension services). Hence, there were in total four treatments and individually referred as L (low fertility), L + NP (low fertility with NP fertilization), H (high fertility), and H + NP (high fertility with NP fertilization).

The experimental plots were randomly laid out with three replicates per treatment. Each plot size was 10.3 m × 6.5 m, separated by 0.5-m buffer strips. Chemical N fertilizer (urea, 46.0% N) and triple super phosphate (P_2O_5, 46% P) were applied to the plots in mid-September of 2010, 2011, 2012, 2013 and 2014 by top-dressing and then incorporated into soils 5–7 days prior to sowing (Jiang et al., 2015a). All crops were harvested manually (the stubble height was about 5 cm), and all harvested biomass was removed from the plots at physiological maturity (late June) each year (Guo et al., 2012).

2.3. Measurements of soil respiration, soil temperature and soil moisture

The respiration rates of surface soil (R_s) in each fertility treatment were measured in situ using an automated closed soil CO_2 flux system equipped with a portable chamber (20 cm in diameter, Li-8100, Lincoln, NE, USA). The measurements were carried out normally every 15 days during the five-year observation period and continuously for three days after effective rainfall events, the same as Jiang et al., 2015a and Wang et al., 2016 did for long-term in situ monitoring experiment, but only once a month from December to February due to cold weather. Each measurement was conducted between 9:00 am and 11:00 am which could present mean value of the whole day (Iqbal et al., 2009) and all visible living organisms were removed prior to measurement by clipping vegetation and expelling insects without disturbance to soil. At least two measurements were taken for each plot, with a 90 s enclosure period and a 30 s delay between the measurements, and the average of the two measurements was taken as the daily soil respiration rate at this plot. However, if the variation between these two measurements was larger than 15%, one or more measurements were taken until the variation between two consecutive measurements was < 15%.

At the same time with the soil respiration measurement, soil temperature (three replicates) and soil moisture (four replicates) at 5-cm depth were also measured in different directions, each 10 cm away from the collar. The soil temperature was measured using a Li-Cor thermocouple probe, and the soil moisture was determined by a Theta Probe ML2X with an HH2 moisture meter (Delta-TDevices, Cambridge, England). Soil moisture was not acquired from December to February because of frost or snow cover. Soil water-filled pore space (WFPS) was calculated by the following equation (Ding et al., 2007): WFPS (%) = [volumetric water content/100 × (2.65 − soil bulk density)/2.65].

2.4. Soil sampling and chemical analysis

To obtain basic soil properties, soil samples were collected using a soil auger of 3 cm in diameter both during the growing and fallow season in the last experimental year (year5), and each sample consisted of six subsamples which randomly collected at top soil (0–20 cm). Each sample was passed through a 2.0-mm sieve and split into two halves: one half was stored at 4 °C for < 4 days before measuring enzyme activity, soil microbial biomass carbon (SMBC) and soil mineral N content (the sum of NO_3-N and NH_4-N), and the other half was air dried and then sieved through a 0.15 mm mesh for other analysis.

Soil organic matter (SOC) was determined using the K_2CrO_7-H_2SO_4 oxidation method (Sparks et al., 1996). The soil total nitrogen (STN) was measured following the Kjeldahl method (Grimshaw et al., 1989). The soil dissolved organic carbon (DOC) was determined using a total organic carbon analyzer (TOC-VCSH, Shimadzu, Japan) (Fujii et al., 2011). The soil nitrate (NO_3-N) and ammonium (NH_4-N) nitrogen were extracted with KCl and determined by colorimetry using a Bran & LuebbeIIAutoAnalyser (Fernandez-Escobar et al., 2009). The chloroform fumigation-extraction method was used to estimate soil microbial biomass carbon (SMBC) (Luo et al., 2015; Vance et al., 1987). Field moist soil samples (approximately 10 g oven-dry soil) were fumigated with ethanol-free chloroform for 24 h at 25 °C under dark condition and the control samples of equal weight were not fumigated. Both fumigated and un-fumigated samples were extracted with 40 ml 0.5 M K_2SO_4 solution (soil to solution ratio 1:4) and shaken at 300 rpm for 30 min, the extract was filtered through a 0.45 mm membrane and then measured in a Total Organic Carbon Analyzer (TOC-VCPH, Shimadzu, Japan). Biomass C was calculated as follows:

$$\mathrm{SMBC} = E_C / K_{EC} \quad (1)$$

where E_C = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and K_{EC} = 0.45 which is the scale factor to convert E_C to SMBC (Moore et al., 2000).

2.5. Microbial community and enzyme analysis

Soil DNA was extracted from 0.5 g soil using the FastDNA® Spin Kit for Soil (MP Biomedical, Cleveland, OH, USA) according to the mamufacturer's instructions. The purified DNA was diluted with 50 μL

sterilized water and checked for quality and quantity using a NanoDrop Spectrophotometer. Primers were tagged with unique barcodes for each replicate DNA sample. PCR reactions were carried out in a 30-μL mixture with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of each primer, and about 10 ng template DNA. The thermal cycling was as follows: 98 °C for 1 min; 30 cycles of 98 °C for 10 s, 50 °C for 30 s, and 72 °C for 1 min; and 72 °C for 5 min. Negative controls using sterilized water instead of soil DNA were included to avoid primer or sample DNA contamination. Each DNA sample was amplified in three technical replicates and then quantified with electrophoresis and mixed in one tube. All samples were pooled together with equal molar amounts from each sample and purified with the GeneJET gel extraction kit (Thermo Scientific). The purified library was generated using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) and mixed with the index codes. The library quality was assessed in the Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Then, the library was sequenced on an Illumina MiSeq platform by which 250 bp/300 bp paired-end reads were generated. All sequence reads were merged using FLASH (Magoč and Salzberg, 2011) and assigned to each sample according to their barcodes. Sequence analysis was performed by UPARSE software package using the UPARSE-OTU and UPARSE-OTUref algorithms (Edgar, 2013). Sequences with ≥97% similarity were clustered into operational taxonomic units (OTUs). The aligned 16S rRNA gene sequences were used for a chimera check using the Uchime algorithm (Edgar et al., 2011). Taxonomy was assigned using the Ribosomal Database Project classifier (Wang et al., 2007). The original sequence data are available at the European Nucleotide Archive (ENA) with accession number PRJEB11700 (Wang et al., 2017a).

The polyphenol oxidase and cellobiohydrolase are extracellular enzymes responsible for recalcitrant SOC decomposition (Jiang et al., 2014; Sinsabaugh et al., 2005; Zhang and Wang, 2012). The enhanced activities of polyphenol oxidase and cellobiohydrolase, in theory, indicate enhanced degradation of recalcitrant SOC and therefore decrease of Q_{10} according to the enzyme-kinetic theory (Bosatta and Agren, 1999). To acquire the activity of polyphenol oxidase, soil samples for each plot were assayed using 25 mM L-3,4-dihydroxyphenylalanine (DOPA) as substrate, following published protocols (German et al., 2011; Saiya-Cork et al., 2002; Steinweg et al., 2012). Sample suspensions were prepared by placing 1.0 g soil in a 150-ml Nalgene bottle. Phosphate buffer (90 ml, pH 6.5) was added to the bottle and the resulting suspension was homogenized using a blender for approximately 1 min. Polyphenol oxidase assays were conducted in clear 96-well microtiter plates (Ma et al., 2013). Sixteen replicate wells were used for enzyme activity assay, eight additional wells were used as negative substrate controls, and another eight wells served as negative sample controls. The assay wells received 200 μl aliquots of sample suspension and 50 μl of 25 mM DOPA substrate. The negative sample control wells contained 200 μl aliquots of sample suspension and 50 μl of phosphate buffer. The negative substrate control wells received 200 μl aliquots of phosphate buffer and 50 μl substrate. The plates were placed in an incubator at 25 °C in the dark for 22 h. Activity was measured spectrophotometrically at 460 nm absorbance using a plate reader (BioTek). The activities of polyphenol oxidase were expressed in unit of nmol $g^{-1} h^{-1}$.

The cellobiohydrolase was assayed as described by (Deforest, 2009) with slight modifications of the buffer concentrations due to the alkalinity of the soil in this experiment. Briefly, assays were conducted by homogenizing each fresh soil sample (equivalent weight to 1.0 g dry mass soil) in 125 ml of 50 mM Tris buffer (pH 8.2) in a 200 ml screw-cap Nalgene bottle, and then stirring the mixture vigorously to maintain a uniform suspension. The soil sample, Tris buffer, 10 μM references and 200 μM fluorometric substrates were distributed into a black 96-well plate in the order as described by Deforest (2009). Plates were incubated in the dark at 25 °C for 4 h until 10 μl 0.5 M NaOH was added to stop the reaction by bring the pH in the well to 10 and read using an automatic microplate reader (Deforest, 2009) at 365 nm excitation and 450 nm emission.

2.6. Root biomass and photosynthetic rate measurements

To minimize root heterogeneity, six soil cores (0–20 cm) were taken in each plot (three cores from the rows, and another three cores between the rows) using a sharp iron tube (9 cm in diameter), and mixed well for the measurement of root biomass (Wang et al., 2016). Roots were separated from soils by soaking in water and gently washing over a 0.25 mm mesh. Wet roots were oven dried at 60 °C for 48 h to a constant weight.

The net photosynthetic rate was measured once for each phenological phase (Returning green, Elongating, Flowering, Filling and Ripening stages) during 9:00–11:00 am on sunny days using a Li-6400 Portable Photosynthesis system (LI-COR, Lincoln, Nebraska, USA) (Badier and Chauvel, 1997). The average of the whole year (i.e. October to September of the following year) was calculated as the annual photosynthetic rate.

2.7. Data analysis

An exponential function was used to represent the relationship between the soil respiration rate and soil temperature (Davidson et al., 1998):

$$R_s = \alpha e^{\beta T} \quad (2)$$

where R_s (μmol $m^{-2} s^{-1}$) is the measured soil respiration rate, T (°C) is the measured soil temperature at 5-cm depth, α is the intercept of soil respiration when the soil temperature is zero, and β is the temperature sensitivity of soil respiration.

The temperature sensitivity of soil respiration (Q_{10}) was calculated as following (Xu and Qi, 2001):

$$Q_{10} = e^{10\beta} \quad (3)$$

Data (mean ± SD, $n = 3$) were subjected to ANOVA, followed by a LSD test for post hoc comparisons of means. Differences in soil respiration, soil temperature and soil moisture among the treatments were analyzed using the general linear model (GLM) procedure in SAS 8.0 software (SAS Institute Inc., Cary, NC, USA). The Q_{10} values (mean ± SD, $n = 3$) between fertilized and unfertilized treatments were tested by the two independent samples *t*-test, at significance level of 0.05. Statistical significance was defined as $P \leq 0.05$. The statistical analyses were performed using SPSS 20.0 software for Windows (SPSS Inc., Chicago, USA). The figures were generated using Sigmaplot 12.5 software (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Responses of soil respiration to NP fertilization in low and high fertility soils

Soil respiration rate followed similar seasonal and annual patterns for all treatments, with the highest rates observed in the warm and wet summer and the lowest rates in winter (Fig. 3). However, there was a significant difference in the mean values of soil respiration (R_s) between the soils with and without NP fertilization (Table 1). In general, the R_s increased with NP fertilization regardless of soil fertility levels (Table 1, Fig. 3). However, such increase of R_s was evidently differentiated between two initial soil fertility levels. In the low fertility soil, the mean soil respiration after NP fertilization were 34.9% ($P < 0.05$), 45.4% ($P < 0.05$), 46.2% ($P < 0.05$), 53.7% ($P < 0.05$) and 50.7% ($P < 0.05$) greater than that without NP fertilization, respectively in the year1, year2, year3, year4 and year5 (Table 1). By contrast, in the high fertility soil, the mean soil respiration after NP fertilization were only 2.8% ($P > 0.05$), 22.7% ($P < 0.05$), 12.3% ($P > 0.05$), 22.1%

Table 1
Mean soil temperature, soil moisture and soil respiration rate (R_s) over the five years (n = 3).

Years	Treatments	Soil temperature (°C)	Soil moisture (%)	R_s (μmol $m^{-2} s^{-1}$)
Year1	L	15.2 ± 1.3 a	27.9 ± 1.2 a	1.29 ± 0.39 a
	L + NP	15.2 ± 1.1 a	27.0 ± 1.6 a	1.74 ± 0.47 b
	H	14.5 ± 1.2 a	26.1 ± 1.2 a	2.12 ± 0.52 b
	H + NP	14.6 ± 0.8 a	27.0 ± 1.8 a	2.18 ± 0.60 b
Year2	L	16.6 ± 1.1 a	30.2 ± 1.8 a	1.30 ± 0.33 a
	L + NP	16.3 ± 1.0 a	30.0 ± 1.8 a	1.89 ± 0.43 b
	H	16.3 ± 1.0 a	28.6 ± 1.2 a	1.94 ± 0.45 b
	H + NP	16.2 ± 1.0 a	28.1 ± 1.7 a	2.38 ± 0.57 c
Year3	L	16.1 ± 1.2 a	22.2 ± 1.5 a	1.17 ± 0.30 a
	L + NP	16.4 ± 1.2 a	21.2 ± 1.6 a	1.71 ± 0.40 b
	H	16.1 ± 1.1 a	19.6 ± 1.8 a	2.04 ± 0.44 bc
	H + NP	16.2 ± 1.0 a	19.7 ± 2.0 a	2.29 ± 0.55 c
Year4	L	15.2 ± 1.4 a	29.4 ± 3.2 a	1.49 ± 0.34 a
	L + NP	15.2 ± 1.3 a	29.8 ± 2.3 a	2.29 ± 0.55 b
	H	15.0 ± 1.3 a	28.2 ± 2.8 a	2.17 ± 0.54 b
	H + NP	14.7 ± 0.8 a	27.5 ± 2.2 a	2.65 ± 0.57 c
Year5	L	17.2 ± 1.7 a	32.7 ± 3.2 a	1.42 ± 0.37 a
	L + NP	17.5 ± 1.3 a	29.6 ± 2.4 a	2.14 ± 0.46 b
	H	16.9 ± 1.6 a	28.9 ± 4.4 a	2.23 ± 0.47 b
	H + NP	16.8 ± 1.1 a	28.3 ± 2.7 a	2.50 ± 0.33 b
Mean	L	16.0 ± 0.4 a	28.5 ± 1.8 a	1.33 ± 0.06 a
	L + NP	16.1 ± 0.4 a	27.5 ± 1.7 a	1.95 ± 0.10 b
	H	15.8 ± 0.4 a	26.3 ± 1.7 a	2.10 ± 0.12 b
	H + NP	15.7 ± 0.4 a	26.1 ± 1.6 a	2.40 ± 0.18 c

Note: The four treatments individually referred as L (low fertility), L + NP (low fertility with NP fertilization), H (high fertility), and H + NP (high fertility with NP fertilization). Different letters indicate significant differences among four treatments within each year at $P < 0.05$, and the values are means of three replicates ± SE.

($P < 0.05$) and 12.1% ($P > 0.05$) greater than that without NP fertilization, respectively in the five years. After averaging the five years, the annual mean R_s in the low fertility soil was significantly increased by 46% ($P < 0.05$) with NP fertilization (L + NP) compared to that without NP fertilization (L: 1.33 ± 0.12 μmol $m^{-2} s^{-1}$). However, the annual mean R_s in the high fertility soil was significantly increased only by 14% (H + NP vs. H, $P < 0.05$).

3.2. Responses of Q_{10} to NP fertilization in low and high fertility soils

For the low fertility soil, the temperature sensitivity of soil respiration (Q_{10}) generally decreased after NP fertilization, but the responses of Q_{10} to NP fertilization were not stable for each year, ranging from 0.09 to 0.18 (Table 2; Fig. 4a). In contrast, Q_{10} remained unchanged with NP fertilization in the high fertility soil. Specifically, the Q_{10} in the low fertility soils with NP fertilization was decreased by 0.0% ($P > 0.05$), 8.3% ($P < 0.05$), 7.1% ($P < 0.05$), 5.9% ($P > 0.05$) and 13.4% ($P < 0.05$) respectively in the five years. By contrast, the Q_{10} in the high fertility soil with NP fertilization showed numerical but not significant differences ($P > 0.05$) (Table 2; Fig. 4b). After averaging the five years, the annual mean Q_{10} in the low fertility soil was significantly reduced by 6.9% ($P < 0.05$) with NP fertilization (L + NP) relative to that without (L) (1.54 vs. 1.44); while the annual mean Q_{10} in the high fertility soil was non-significantly reduced by only 1.9% (H + NP vs. H, $P > 0.05$).

3.3. Soil physicochemical properties in low and high fertility soils

Soil temperature, ranging from −3.9 °C to 31.9 °C, showed similar seasonal and annual variations for all treatments, which agreed well with the air temperature (Fig. 2a, b). Soil moisture, ranging from 19.6% to 32.7%, fluctuated in response to the precipitation within each season (Fig. 2c). The mean soil temperature showed no significant difference with or without NP fertilization (Fig. 2b, Table 1), neither did the mean soil moisture (Fig. 2c, Table 1).

Initially, the SOC (9.4 g kg^{-1}), DOC (60.1 mg kg^{-1}) and SMBC (254.2 mg kg^{-1}) in the high fertility soil (H) were 42.4% ($P < 0.05$), 143.3% ($P < 0.05$) and 68.6% ($P < 0.05$) greater than that in the low fertility soil (L), respectively. After NP fertilization, the SOC, DOC and SMBC in general showed increasing trends for low fertility soils, but remained unchanged for high fertility soil (Table 3). Specifically, in low fertility soil with NP fertilization (L + NP), the SOC, DOC and SMBC were significantly increased by 15.2% ($P < 0.05$), 37.7% ($P < 0.05$) and 41.8% ($P < 0.05$), respectively. In contrast, all of them were unchanged ($P > 0.05$) in high fertility soil with NP fertilization (H + NP). Without NP fertilization, the soil mineral N in the high fertility soil (H: 20.5 mg kg^{-1}) was 61.4% greater ($P < 0.05$) than that in the low fertility soil (L: 12.7 mg kg^{-1}). After NP fertilization, soil mineral N content was increased by 19.7% ($P < 0.05$) and 30.2% ($P < 0.05$) for low and high fertility soil, respectively (Table 3).

3.4. Changes in crop properties in low and high fertility soils

After NP fertilization, the root biomass showed significantly increasing trends for both low and high fertility soils (Table 4). The mean root biomass was lowest in the low fertility soil (L: 3.94 t ha^{-1}), and significantly increased by 15.0% with NP fertilization ($P < 0.05$). However, for the high fertility soils, mean root biomass with NP fertilization (H + NP) was significantly increased by 34.7% ($P < 0.05$) compared with that without NP fertilization (H: 4.76 t ha^{-1}).

The crop yields with NP fertilization were also significantly increased for both low and high fertility soils with varying degrees (Table 4). The mean crop yield was lowest in the low fertility soil (L: 1.87 t ha^{-1}), and significantly increased by 161.5% under NP fertilization (L + NP) ($P < 0.05$). For the high fertility soils, however, mean crop yield with NP fertilization (H + NP) was non-significantly increased only by 7.9% ($P > 0.05$) compared with that without (H: 5.06 t ha^{-1}).

Between the two control treatments without NP fertilization, the mean photosynthetic rate of the high fertility soil (H) was 19.6% greater than that of the low fertility soil (L) (20.1 vs. 16.8 μmol CO_2 $m^{-2} s^{-1}$, $P < 0.05$). With NP fertilization, the photosynthetic rate increased, but numerically different between soil fertility levels ($P < 0.05$, Table 4): in specific of the low fertility soil, mean photosynthetic rate with NP fertilization (L + NP) increased by 20.2% compared with that without (L) ($P < 0.05$), while applying NP fertilizers in the high fertility soil did only 8.0% (H + NP vs. H: $P > 0.05$).

3.5. Changes in microbial community and enzyme activities in low and high fertility soils

When no NP fertilization was applied, the polyphenol oxidase activity in high fertility soil (H) was only 18.2% of that in low fertility soil (L) ($P > 0.05$). After NP fertilization, the polyphenol oxidase activities were stimulated to different degrees (Table 4). For low fertility soils, the polyphenol oxidase activity was 3 times greater ($P < 0.05$) with NP fertilization (L + NP: 0.034 nmol $g^{-1} h^{-1}$) than without (L: 0.011 nmol $g^{-1} h^{-1}$). For high fertility soil, it remained unchanged with NP fertilization ($P > 0.05$).

The cellobiohydrolase activity was initially greater in high fertility soil (H: 38.0 nmol $g^{-1} h^{-1}$) than in low fertility soil (L: 25.5 nmol $g^{-1} h^{-1}$) (by 48.8%, $P < 0.05$), both being significantly stimulated to different degrees with NP fertilization (Table 4). In the low fertility soil, the cellobiohydrolase activity was increased by 26.9% with NP fertilization (L + NP vs. L: $P < 0.05$). By contrast, it was increased by only 18.0% due to NP fertilization in the high fertility soil (H + NP vs. H: $P < 0.05$).

In the low fertility soil, the relative abundance of Actinobacteria increased by 32.9% due to NP fertilization ($P < 0.05$). However, in the high fertility soil, it was unchanged with NP fertilization ($P > 0.05$). In

Table 2
The Q_{10} and relationship between soil respiration and soil temperature over the five years (n = 3).

Years	Treatment	Equations	α	β	N	r^2	P	Q_{10}
Year1	L	$F = 0.716e^{0.039T}$	0.716 ± 0.201a	0.039 ± 0.014a	17	0.63	< 0.01	1.48
	L + NP	$F = 0.919e^{0.229T}$	0.919 ± 0.229b	0.039 ± 0.012a	18	0.78	< 0.01	1.48
	H	$F = 0.926e^{0.051T}$	0.926 ± 0.175b	0.051 ± 0.010b	17	0.73	< 0.01	1.67
	H + NP	$F = 0.919e^{0.053T}$	0.919 ± 0.194b	0.053 ± 0.011b	18	0.73	< 0.01	1.70
Year2	L	$F = 0.605e^{0.045T}$	0.605 ± 0.148a	0.045 ± 0.012a	25	0.69	< 0.01	1.57
	L + NP	$F = 1.003e^{0.037T}$	1.003 ± 0.219c	0.037 ± 0.011a	26	0.82	< 0.01	1.45
	H	$F = 0.793e^{0.053T}$	0.793 ± 0.142b	0.053 ± 0.009a	26	0.78	< 0.01	1.69
	H + NP	$F = 1.030e^{0.049T}$	1.030 ± 0.191c	0.049 ± 0.009a	26	0.72	< 0.01	1.63
Year3	L	$F = 0.620e^{0.041T}$	0.620 ± 0.129a	0.041 ± 0.010a	19	0.70	< 0.01	1.50
	L + NP	$F = 0.987e^{0.034T}$	0.987 ± 0.194b	0.034 ± 0.009a	19	0.68	< 0.01	1.40
	H	$F = 0.997e^{0.043T}$	0.997 ± 0.157b	0.043 ± 0.007a	18	0.72	< 0.01	1.53
	H + NP	$F = 1.037e^{0.044T}$	1.037 ± 0.178b	0.044 ± 0.008a	18	0.72	< 0.01	1.55
Year4	L	$F = 0.783e^{0.049T}$	0.783 ± 0.131a	0.048 ± 0.010a	21	0.69	< 0.01	1.62
	L + NP	$F = 1.299e^{0.043T}$	1.299 ± 0.300b	0.043 ± 0.014a	21	0.78	< 0.01	1.53
	H	$F = 1.117e^{0.051T}$	1.117 ± 0.235b	0.051 ± 0.012a	21	0.72	< 0.01	1.67
	H + NP	$F = 1.421e^{0.047T}$	1.421 ± 0.287c	0.047 ± 0.012a	21	0.87	< 0.01	1.60
Year5	L	$F = 0.626e^{0.042T}$	0.626 ± 0.203a	0.042 ± 0.016b	18	0.85	< 0.01	1.52
	L + NP	$F = 1.095e^{0.029T}$	1.095 ± 0.326b	0.029 ± 0.014a	16	0.67	< 0.05	1.34
	H	$F = 1.356e^{0.029T}$	1.356 ± 0.370c	0.029 ± 0.014a	18	0.66	< 0.05	1.34
	H + NP	$F = 1.623e^{0.024T}$	1.623 ± 0.302d	0.024 ± 0.010a	18	0.73	< 0.05	1.27

Note: The four treatments individually referred as L (low fertility), L + NP (low fertility with NP fertilization), H (high fertility), and H + NP (high fertility with NP fertilization). N is the number of statistical variables; r^2 is the determinant coefficient; P is the significance level. Different letters indicate significant differences among four treatments within each year at $P < 0.05$, and values are means of three replicates ± SE.

the low fertility soil, the relative abundance of Ascomycota was significantly increased by 0.39% ($P < 0.05$) due to NP fertilization. However, it was unchanged with NP fertilization in the high fertility soil ($P > 0.05$) (Table 5).

4. Discussion

4.1. Positive response of soil respiration regardless of initial soil fertility

The significantly increased mean soil respiration regardless of initial soil fertility levels (Table 1), clearly illustrates the stimulating effects of NP fertilization on soil respiration. Such effects were associated with increased input of plant carbon, photosynthesis, root biomass and crop yield increased with NP fertilization regardless of initial soil fertility levels (Table 4). We thus infer an increase in root respiration may also have occurred (Table 4). NP fertilization can stimulate root respiration by increasing soil nutrient (i.e., N and P) availability (Chen et al., 2017; Sun et al., 2014), which led to increased root N concentration (Burton et al., 2002) and belowground allocation of photosynthesis (i.e., root biomass), consequently stimulating root respiration (Sun et al., 2014;

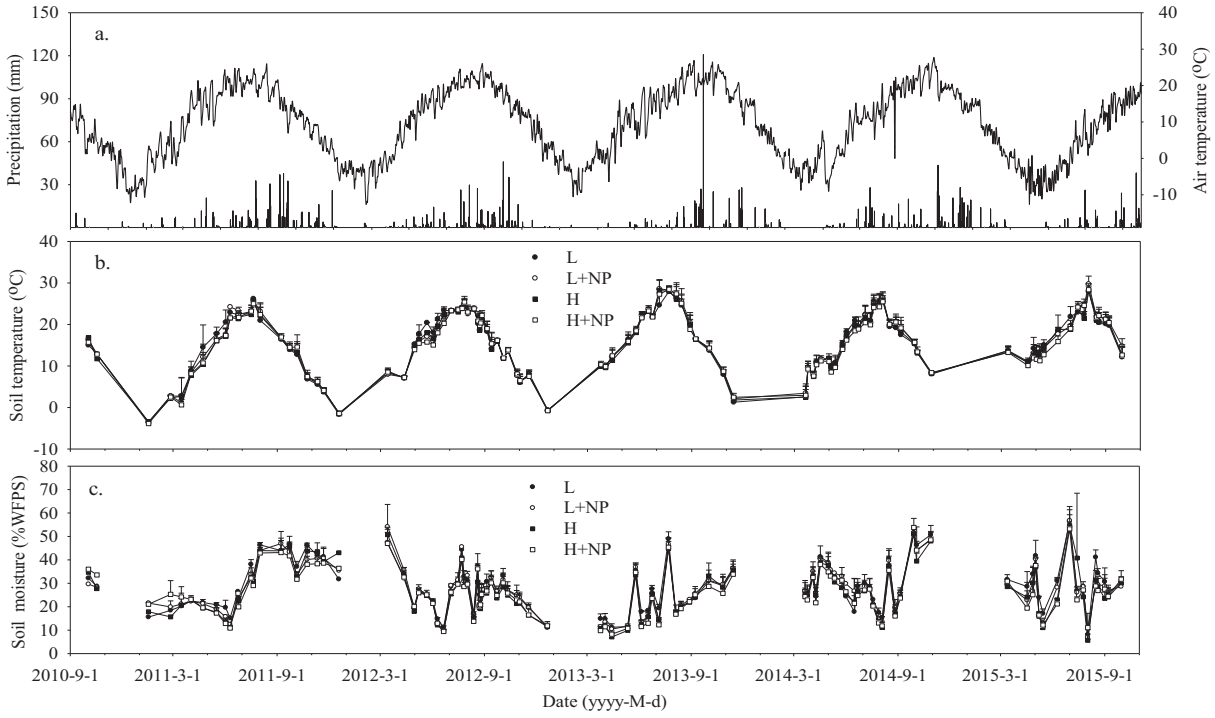

Fig. 2. Variations of precipitation (mm) and air temperature (°C) (a.), soil temperature (°C) (b.), and soil moisture (% WFPS) (c.) over the five-year observation period (± SE, n = 3). The four treatments individually referred as L (low fertility), L + NP (low fertility with NP fertilization), H (high fertility), and H + NP (high fertility with NP fertilization).

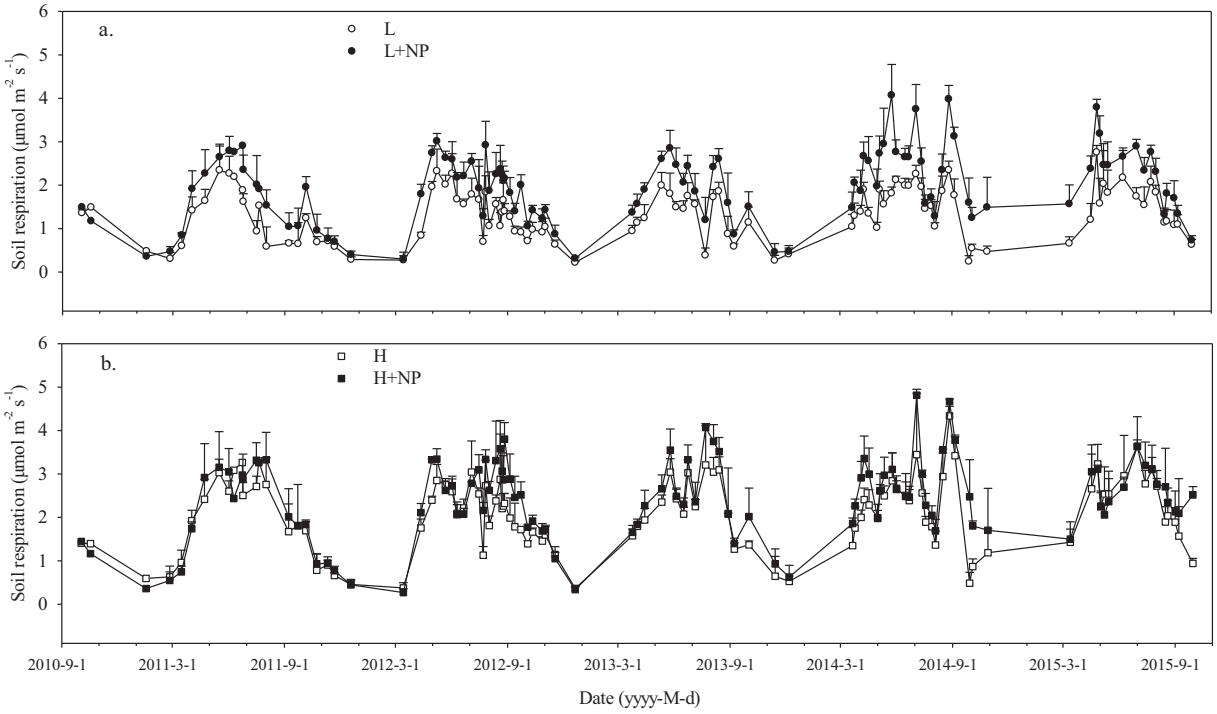

Fig. 3. Dynamics of soil respiration rates under low (a.) and high (b.) fertility levels with and without NP fertilization respectively, i.e., L + NP vs. L and H + NP vs. H over the study years (± SE, n = 3). The four treatments individually referred as L (low fertility), L + NP (low fertility with NP fertilization), H (high fertility), and H + NP (high fertility with NP fertilization).

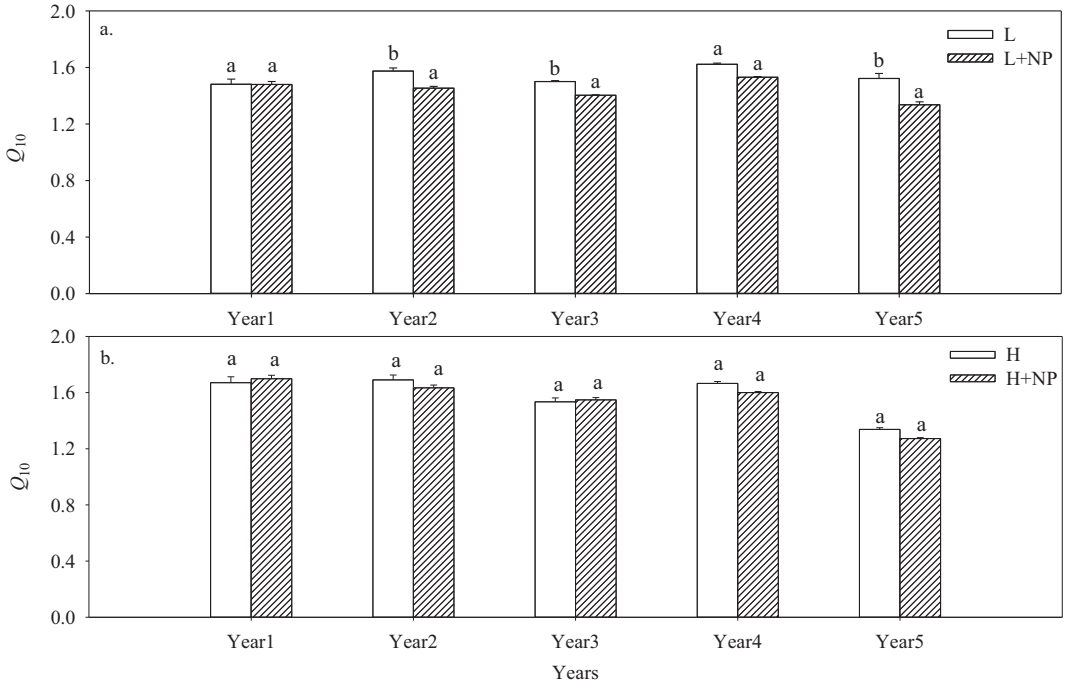

Fig. 4. The average Q_{10} values under low (a.) and high (b.) fertility levels with and without NP fertilization respectively, i.e., L + NP vs. L and H + NP vs. H over the study years (± SE, n = 3). The four treatments individually referred as L (low fertility), L + NP (low fertility with NP fertilization), H (high fertility), and H + NP (high fertility with NP fertilization). Different letters indicate significant difference between treatments within each year at $P < 0.05$.

Yan et al., 2010). In the low fertility soil, NP fertilization likely also stimulated microbial respiration through increased microbial biomass, soil C availability and enzyme activities (Tables 3 and 4) (Tu et al., 2013; Zhang et al., 2014), as well possible priming effects on SOM decomposition (Graham et al., 2012). In contrast, neutral responses of microbial biomass and soil enzyme activity in the high fertility soil (Tables 3 and 4) implied that it was primarily root respiration driving variation with NP fertilization. The increased soil respiration contradicted the result of Ramirez et al., 2010, which reported a negative effect of nitrogen fertilization on soil microbial respiration. The difference may be because the calcareous soils in this study responded differently to chemical fertilization from the acid soils in their study.

Table 3
Effect of NP fertilization on soil C, N contents in low and high fertility soils (n = 3).

Treatments	SOC (g kg^{-1})	DOC (mg kg^{-1})	SMBC (mg kg^{-1})	STN (g kg^{-1})	Soil mineral N (mg kg^{-1})
L	6.6 ± 0.2 a	24.7 ± 1.7 a	150.8 ± 4.37 a	0.82 ± 0.02 a	12.7 ± 1.4 a
L + NP	7.6 ± 0.4 b	34.0 ± 1.8 b	213.8 ± 5.67 b	0.94 ± 0.05 a	15.2 ± 1.8 b
Increase/%	15.2	37.7	41.8	14.6	19.7
H	9.4 ± 0.2 c	60.1 ± 6.8 c	254.2 ± 7.53 c	1.04 ± 0.06 b	20.5 ± 7.1 c
H + NP	10.5 ± 0.4 c	65.7 ± 6.1 c	231.2 ± 8.13 c	1.17 ± 0.07 b	26.7 ± 9.7 d
Increase/%	11.7	9.3	−9.0	12.5	30.2

Note: SOC: soil organic carbon; STN: soil total nitrogen; DOC: soil dissolved organic carbon; SMBC: soil microbial biomass carbon; Soil mineral N: the sum of nitrate (NO_3-N) and ammonium (NH_4-N) nitrogen. The four treatments individually referred as L (low fertility), L + NP (low fertility with NP fertilization), H (high fertility), and H + NP (high fertility with NP fertilization). Different letters indicate significant differences at $P < 0.05$, and values are means of three replicates ± SE.

Table 4
Effect of NP fertilization on crop properties and enzyme activities in low and high fertility soils (n = 3).

Treatments	Root biomass (t ha^{-1})	Crop yield (t ha^{-1})	Photosynthesis (μmol CO_2 m^{-2} s^{-1})	Cellobiohydrolase activity (nmol g^{-1} h^{-1})	Polyphenol oxidase activity (nmol g^{-1} h^{-1})
L	3.94 ± 0.11 a	1.87 ± 0.39 a	16.8 ± 0.7 a	25.54 ± 1.25 a	0.011 ± 0.0044 a
L + NP	4.53 ± 0.20 b	4.89 ± 0.97 b	20.2 ± 1.1 b	32.40 ± 1.25 b	0.034 ± 0.0082 b
Increase/%	15.0	161.5	20.2	26.9	209
H	4.76 ± 0.32 b	5.06 ± 0.97 c	20.1 ± 1.1 b	38.01 ± 0.62 b	0.002 ± 0.0005 a
H + NP	6.41 ± 0.55 c	5.46 ± 1.09 c	21.7 ± 1.6 b	44.86 ± 0.00 c	0.012 ± 0.0046 a
Increase/%	34.7	7.9	8.4	18.0	500

Note: The four treatments individually referred as L (low fertility), L + NP (low fertility with NP fertilization), H (high fertility), and H + NP (high fertility with NP fertilization). Different letters indicate significant difference at $P < 0.05$, values are means of three replicates ± SE.

Table 5
Effect of NP fertilization on abundant community in low and high fertility soils (n = 3).

Taxonomy	L	L + NP	H	H + NP
Bacterial 16S				
Acidobacteria	12.50 ± 1.10 a	18.25 ± 0.81 b	18.06 ± 0.85 b	17.60 ± 0.25 b
Actinobacteria	19.67 ± 0.43 a	26.14 ± 0.46 b	22.45 ± 0.69 ab	24.50 ± 1.80 b
Bacteroidetes	2.14 ± 0.48 a	2.73 ± 0.01 a	4.14 ± 0.08 ab	4.41 ± 0.39 b
Chloroflexi	4.82 ± 0.56 a	7.43 ± 0.15 b	7.50 ± 0.18 b	6.69 ± 0.06 b
Planctomycetes	3.01 ± 0.09 a	3.74 ± 0.20 ab	6.04 ± 0.53 b	3.58 ± 0.18 a
Verrucomicrobia	1.38 ± 0.11 a	1.71 ± 0.04 a	2.33 ± 0.15 b	1.71 ± 0.08 a
WS3	0.55 ± 0.01 b	0.39 ± 0.02 ab	0.31 ± 0.02 a	0.27 ± 0.01 a
Fungal ITS				
Ascomycota	88.09 ± 1.95 a	88.44 ± 2.19 b	92.81 ± 0.51 b	92.11 ± 0.09 b
Basidiomycota	7.41 ± 1.37 b	4.50 ± 0.48 a	3.34 ± 0.05 a	2.91 ± 0.12 a
Zygomycota	0.35 ± 0.05 a	0.42 ± 0.02 a	0.66 ± 0.37 a	0.40 ± 0.03 a
Glomeromycota	0.22 ± 0.05 a	0.28 ± 0.05 a	0.18 ± 0.02 a	0.14 ± 0.02 a
Chytridiomycota	0.60 ± 0.07 a	0.93 ± 0.55 a	0.13 ± 0.07 a	0.20 ± 0.10 a

Note: The four treatments individually referred as L (low fertility), L + NP (low fertility with NP fertilization), H (high fertility), and H + NP (high fertility with NP fertilization). Different letters indicate significant difference at $P < 0.05$, values are means of three replicates ± SE.

Alternatively, the addition of N and P together may induce different responses than N alone. This calls for further investigations on a wider range of soils to detect the responses of soil respiration to chemical fertilization under diffferent intial fertility levels.

4.2. Divergent responses of Q_{10} in initially low and high fertility soils

A notable finding from our data is the contrasting effects of NP fertilization on the Q_{10} in low and high fertility soils. Although soil respiration increased regardless of initial soil fertility, Q_{10} with NP fertilization decreased from 1.54 to 1.44 in the initially low fertility soil, but remained unchanged in the initially high fertility soil (Fig. 4). The mechanism of the effect of N and P fertilization on Q_{10} of soil respiration was complicated partly because the various respiration components (i.e., root respiration and microbial respiration) have different patterns of temperature responses (Boone et al., 1998; Uchida et al., 2010; Vicca et al., 2010). No systematic differences were found in Q_{10} among roots with different root biomass or root N concentration in previous studies (Atkinson et al., 2007; Tu et al., 2013; Zhang et al., 2014). It is plausible that microbial response to either soil nutrient availability or increased plant litter quality drove the observed decrease in Q_{10}. In specific for the soil initially low in fertility, NP fertilization increased labile C availability through enhanced plant C inputs to soil (Table 3, Table 4), which would require lower activation energy to be decomposed (Bosatta and Agren, 1999; Vanhala et al., 2007). Increased soil N availability in turn may also have reduced C:N ratios in plant C inputs to soil (e.g., residues). The additional P application may compel plants to increase N uptake rates to balance the N:P ratios, which would further reduce C:N ratios in residues (Zeng and Wang, 2015). This change in the quality of plant litter may have reduced the activation energy of decomposition in the soil with low initial fertility (Bosatta and Agren, 1999; Leifeld and von Lutzow, 2014; Jiang et al., 2015b). However, for the soil initially high in fertility where labile C and soil mineral N content was already abundant (Table 3), the soils may have already been approaching N and P saturation (Khan et al., 2007). The NP fertilization therefore contributed little to reducing substrate C:N ratios or soil microbial activation energy, with a resulting neutral response of Q_{10} to NP fertilization. Nevertheless, given the complexity of

soil respiration in situ and the limited soil type investigated in this experiment, great cautions should be paid when extrapolating the findings in this study.

Apart from the divergent patterns discussed above, there were also inter-annual variations of Q_{10} values that cannot be ignored. In particular, these variations were more correspondent to the variations of natural precipitation (Fig. 2a) and soil moisture content (Fig. 2c, Table 1). This implies that, in the rain-fed region as this study area where precipitation is highly variable across years, soil water availability may also limit the effects of NP fertilization on soil respiration Q_{10}. This further highlights the relevance of soil respiration under different temperature and precipitation patterns under current and future climate conditions.

4.3. Possible role of microbial community in soil respiration and Q_{10}

Varying changes in microbial communities in the two soils (Table 5) were another reason causing their different responses of microbial respiration Q_{10} to NP fertilization. Previous studies reported that the relative abundances of Actinobacteria are considered responsible for the activities of cellobiohydrolase (de Menezes et al., 2015), and that of Ascomycota may be associated with the activities of polyphenol oxidase (Fog, 2010). In specific for the soil at initially low fertility, the recalcitrant C may be not decomposed due to the preferential use of plant C by microbes under nutrient limitation (Graham et al., 2012; Uchida et al., 2010). After applied NP fertilization, more abundantly available soil mineral N enhanced the activities of both cellobiohydrolase and polyphenol oxidase (26.8% and 209%, Table 4). This may ease the degradation of both hemicellulose, cellulose (Stone et al., 2012) and lignin and phenolic compounds (Jiang et al., 2014; Sinsabaugh et al., 2005; Zhang and Wang, 2012) with lower energy cost. In addition, the decomposition and mineralization of substrate with lower C:N ratios in low fertility soils could also be better stimulated by polyphenol oxidase activities (Zhang et al., 2009). These processes may jointly contribute to the significant decrease of microbial respiration Q_{10}. Nevertheless, in the soil with initially high fertility, NP fertilization significantly increased cellobiohydrolase activity, but the polyphenol oxidase activity was not affected (Table 4). Although both enzymatic activities matter, the polyphenol oxidase was more likely the driver of recalcitrant C pool stability (Carreiro et al., 2000; Jiang et al., 2014), and consequently the microbial respiration Q_{10}. The unchanged polyphenol oxidase activity (Table 4) indicates that the breakdown of recalcitrant C and thus the microbial respiration Q_{10}, was not affected by NP fertilization in high fertility soil, which was likely due to the preferential use of nutrient-rich SOC by microbes when soil is rich in decomposable SOC (Ding et al., 2010). However, given the difficulty to link soil respiration or Q_{10} to specific genes or enzymes, future research should employ a combination of various approaches, including quantification of functional gene activity and determination of the chemical structure of SOC to identify the variations in Q_{10} driven by soil microbial community.

5. Conclusions

This paper investigated the responses of soil respiration and Q_{10} to NP fertilization in two soils with different initial fertility levels. Our five-year results from the winter wheat-cropping agroecosystem of the semiarid Loess Plateau supported the two hypotheses raised in the study, indicating that the effects of NP fertilization to soil respiration and Q_{10} differed with soil initial fertility. In particular, NP fertilization mainly affected the Q_{10} of microbial respiration, which was likely due to the insensitivity of root respiration Q_{10} to root N concentration. The significantly decrease of microbial respiration Q_{10} in low fertility soil was mostly because of the increased substrate quality for microbes induced by more abundant soil mineral N content and increased activities of both cellobiohydrolase and polyphenol oxidase. However, the microbial respiration Q_{10} in high fertility soil remained unchanged, possibly because of the less effect of NP fertilization on substrate C:N ratio and neutral response of polyphenol oxidase activity.

Our results showed that the NP fertilization effect on Q_{10} varied with soil fertility, casting new lights on current understanding of the potential impacts of NP fertilization onto regional agroecosystem and the associated feedbacks in the terrestrial agroecosystem. This points out that the potential impacts of soil initial fertility on soil respiration and Q_{10} responses should be properly accounted for when simulating the NP fertilization effects to regional agroecosystem carbon balances.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.geoderma.2018.04.001. These data include the Google map of the most important areas described in this article.

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