

Enhancement of Carbon Sequestration in Soil in the Temperature Grasslands of Northern China by Addition of Nitrogen and Phosphorus

Nianpeng He^{1*}, Qiang Yu^{2,3}, Ruomeng Wang¹, Yunhai Zhang⁴, Yang Gao¹, Guirui Yu^{1*}

1 Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, CAS, Beijing, China,

2 State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, CAS, Shenyang, China, **3** Department of Biology, Graduate Degree Program in Ecology, Colorado State University, Fort Collins, Colorado, United States of America, **4** State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, CAS, Beijing, China

Abstract

Increased nitrogen (N) deposition is common worldwide. Questions of where, how, and if reactive N-input influences soil carbon (C) sequestration in terrestrial ecosystems are of great concern. To explore the potential for soil C sequestration in steppe region under N and phosphorus (P) addition, we conducted a field experiment between 2006 and 2012 in the temperate grasslands of northern China. The experiment examined 6 levels of N (0–56 g N m⁻² yr⁻¹), 6 levels of P (0–12.4 g P m⁻² yr⁻¹), and a control scenario. Our results showed that addition of both N and P enhanced soil total C storage in grasslands due to significant increases of C input from litter and roots. Compared with control plots, soil organic carbon (SOC) in the 0–100 cm soil layer varied quadratically, from 156.8 to 1352.9 g C m⁻² with N addition gradient ($R^2 = 0.99$, $P < 0.001$); and logarithmically, from 293.6 to 788.6 g C m⁻² with P addition gradient ($R^2 = 0.56$, $P = 0.087$). Soil inorganic carbon (SIC) decreased quadratically with N addition. The net C sequestration on grassland (including plant, roots, SIC, and SOC) increased linearly from -128.6 to 729.0 g C m⁻² under N addition ($R^2 = 0.72$, $P = 0.023$); and increased logarithmically, from 248.5 to 698 g C m⁻² under P addition ($R^2 = 0.82$, $P = 0.014$). Our study implies that N addition has complex effects on soil carbon dynamics, and future studies of soil C sequestration on grasslands should include evaluations of both SOC and SIC under various scenarios.

Citation: He N, Yu Q, Wang R, Zhang Y, Gao Y, et al. (2013) Enhancement of Carbon Sequestration in Soil in the Temperature Grasslands of Northern China by Addition of Nitrogen and Phosphorus. PLoS ONE 8(10): e77241. doi:10.1371/journal.pone.0077241

Editor: Xiujun Wang, University of Maryland, United States of America

Received April 24, 2013; **Accepted** August 30, 2013; **Published** October 10, 2013

Copyright: © 2013 He et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was partly funded by the Natural Science Foundation of China (31270519), by special fund for scientific research in the public interest (201209028), and by the strategic program of state eco-environmental investigation and evaluation in China (STS-N-02-03). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

* E-mail: heng@igsnrr.ac.cn (NH); yugr@igsnrr.ac.cn (GY)

Introduction

Human activity has doubled the atmospheric deposition of nitrogen (N) over the past century [1,2]. N deposition can influence the biogeochemical coupling of the carbon (C) and N cycles in soil by altering organic matter decomposition [3–5], belowground C allocation [6,7], and microbial communities and activity [8,9]. However, it remains unclear whether N limits soil C sequestration in terrestrial ecosystems, although the promotion of plant growth and ecosystem primary production are observed in many regions [3,6,7]. The effect of N addition on soil C sequestration is a concern because soil C sequestration in terrestrial ecosystems is an important approach to offset anthropogenic CO₂ emissions [10].

Increases in N deposition have been predicted to increase terrestrial C storage [11,12], but the results of field experiments were inconsistent. Zeng et al. (2010) reported that N addition

decreased the C storage of soil and aboveground content in ecosystems to some extent [13]. On the basis of a meta-analysis of 257 studies, Lu et al. (2011) found that N addition had no apparent effect on soil C storage in either the organic horizon or mineral soil in grasslands, despite substantial increases in C inputs from roots and litter [14].

Grasslands in northern China (approximately 150 million ha) have enormous capacity to sequester atmospheric CO₂ through good management of land use, especially via grazing-exclusion, mowing, and conversion from farmland to grassland [15–18]. Soil acidification or pH decrease was predicted in semi-arid regions with increasing N input, which might result in CO₂ emission from dissolution of soil inorganic carbon (SIC: carbonate minerals, such as calcium carbonate (CaCO₃) and dolomite (MgCO₃)), and from decomposition of soil organic C(SOC) by altering microbial communities and activity [19–21]. To date, few studies have evaluated the change of SIC and its

importance to soil C sequestration within N addition experiments, despite the abundance of SIC in semi-arid regions [22,23]. Inner Mongolian grassland, under semi-arid climate, is an important terrestrial ecosystem in northern China. Increasing N deposition may have a large influence on SOC dynamics and SIC storage, thus on the capacity of C sequestration in the Inner Mongolian grasslands.

In this paper, we used a seven-year field experiment, comprising a 6-level N addition gradient and 6-level P addition gradient to investigate the effects of N and P addition on soil C sequestration in Inner Mongolian grasslands. The main objectives were to: (1) explore the effect of N and P on soil C sequestration; and (2) quantify the role of SIC in soil C sequestration for under scenarios of increased N deposition.

Materials and Methods

Study site

This study was conducted in a typical steppe ecosystem at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS) of Chinese Academy of Sciences. The region had a semi-arid continental climate with mean annual precipitation of 345mm and mean annual temperature of 1.1°C over the period 1980 to 2010 [15]. The soil was classified as dark chestnut (calcic chernozem according to ISSS Working Group RB, 1998) or loamy sand in terms of texture. The experimental plot was located at 43°33'01"N, 116°40'20"E at an average elevation of 1200 m above sea level. The plot has been fenced off since 1999 to prevent grazing and trampling by large animals (e.g., sheep, cattle, and horses) [16].

Experimental design

Detailed information of the experimental design used in this research was reported in previous studies [24,25]. In brief, the field experiments for N (urea) and P (potassium phosphate) addition have been conducted in a *Leymus chinensis* grassland since 2006, where the predominant species were *L. chinensis*, *Stipa grandis*, *Cleistogenes squarrosa*, and *Agropyron michnoi*. N addition regimes were designated as control (CK), 0 (N1), 5.6 (N2), 11.2 (N3), 22.4 (N4), 39.2 (N5), or 56(N6) g N m⁻² yr⁻¹. Similarly, P addition regimes were designed as control (CK), 0 (P1), 1.55 (P2), 3.1(P3), 6.2 (P4), 9.3 (P5), or 12.4 (P6) g P m⁻² yr⁻¹. Other than in the control, 1.55 g P m⁻² was also added to each plot in the N addition experiment, and 2.8 g N m⁻² was added to each plot in the P addition experiment. Thus, there were seven levels for both N and P application with six replicates, giving a total of 84 experimental plots (6 m × 6 m) (Figure S1 in File S1). The fertilizer was thoroughly mixed with sand and then applied in late May, from 2006 to 2012. Our experimental design provided two unique series, one being a N-addition gradient without P limitation, and the other being a P-addition gradient without N limitation.

Field sampling

At the end of July 2012, we established one sampling quadrat (0.5 m × 1.0 m) in each plot. We first investigated aboveground biomass (AB) with all plant species combined.

Litter was subsequently collected. Root biomass was determined using a soil corer (diameter, 7 cm). The samples were collected separately from 5-points within each sampling quadrat, at 0–10 cm, 10–30 cm, 30–50 cm, and 50–100 cm in each plot. Similarly, soil samples were collected using a soil auger (4 cm in diameter) at 0–10 cm, 10–30 cm, 30–50 cm, and 50–100 cm. Bulk density of each soil layer was measured by the IMGERS, using the core method (volume 100 cm³) with 3 replicates.

Chemical analysis

Plant, litter, root, and soil samples were ground using a ball mill (M400, Retsch, Germany). The concentration of soil total carbon (STC) was measured by dry combustion using an elemental analyzer (VARIO MAX CN, Elementar, Hanau, Germany). The concentration of SIC was measured by manometric collection of CO₂ evolved during an HCl treatment process. SOC was calculated as the difference between STC and SIC. C concentrations in plant, root, and litter were measured using the elemental analyzer. Soil pH was determined via a pH meter using soil mixed with distilled water (ratio 1:2.5).

Calculations and statistical analysis

STC, SIC, and SOC (g C m⁻²) were calculated on an area basis to a soil depth of 100 cm, as described previously [15]:

$$\text{STC} = \sum D_i \times S \times B_i \times OM_i \times 10$$

where D_i, S, B_i, OM_i, and TN_i represent the thickness of the soil layer (cm), cross-sectional area (m²), bulk density (g cm⁻³), and total C concentration (g kg⁻¹), respectively; i = 1, 2, 3, and 4.

Moreover, the differences in STC and SOC storage between N or P treatments and CK plots was calculated, and was used as the capacity of soil C sequestration.

One-way analysis of variance (ANOVA) was used to determine the effects of N and P addition on the C storage in AB, litter, roots, and soil. Regression analyses were used to test the relationships between the storage of STC, SOC, and SIC and the addition intensities of N or P. Data were represented as mean ± 1 standard deviation (n = 6). All analyses were conducted using SPSS statistical software (ver. 13.0, SPSS, Chicago, IL, USA).

Results

Changes in plant and soil C storage

C stored in AB and litter significantly increased with increasing N addition ($F = 3.89, P = 0.04$ for AB; $F = 7.33, P < 0.001$ for litter) (Table 1), but no apparent effects were observed for P addition. In comparison with soil and roots, C storage in AB and litter was negligible (<1%). C storage in roots varied from 617.4 to 699.7 g C m⁻² in the 0–100-cm soil layer (Table 2), but did not increase significantly with addition of N and P (Table 1). C storage in grasslands (including AB, litter, root, SOC, and SIC in the 0–100-cm soil layer) varied from 16615 to 17448 g C m⁻² for the N-addition series, and from

Table 1. Changes of C storage in Inner Mongolian grasslands with N and P addition.

	C storage (g C m^{-2})				
	Aboveground biomass	Litter	Roots (0–100 cm)	SOC (0–100 cm)	SIC (0–100 cm)
N addition					
CK†	40.4±3.6 ^a	31.8±7.6 ^a	620.1±85.5 ^a	11770±1684 ^a	4339±111 ^a
N1	44.3±8.5 ^{ab}	31.5±7.4 ^a	635.7±65.0 ^a	11926±554 ^a	4355±706 ^a
N2	46.4±10.2 ^{ab}	33.9±7.2 ^a	639.8±84.6 ^a	12072±674 ^a	4108±202 ^{ab}
N3	44.2±7.7 ^{ab}	49.2±10.6 ^b	658.5±82.6 ^a	12254±1093 ^a	3725±460 ^b
N4	53.0±10.6 ^{bc}	46.2±6.6 ^{bc}	673.8±35.3 ^a	12620±569 ^a	3876±192 ^{ab}
N5	62.6±12.7 ^c	39.8±6.2 ^{ac}	698.5±96.8 ^a	12826±347 ^a	3778±404 ^a
N6	60.4±16.8 ^c	49.4±3.2 ^b	699.7±70.5 ^a	13122±637 ^a	3715±524 ^a
F-value	3.89 (0.04) (<0.001)	7.33	0.99 (0.444)	1.96(0.099)	2.67(0.031)
P addition					
CK	40.7±10.6 ^a	31.7±8.2 ^a	617.4±59.5 ^a	11882±808 ^a	4266±591 ^a
P1	45.2±5.6 ^a	33.7±6.0 ^a	647.6±46.4 ^a	12176±675 ^a	4221±675 ^a
P2	46.9±7.0 ^a	32.4±5.0 ^a	650.0±106.8 ^a	12294±926 ^a	4363±935 ^a
P3	48.4±11.6 ^a	33.9±2.2 ^a	662.6±68.8 ^a	12423±909 ^a	4305±251 ^a
P4	46.0±10.7 ^a	33.8±7.9 ^a	671.6±94.3 ^a	12671±634 ^a	4089±298 ^a
P5	45.8±9.9 ^a	33.3±7.2 ^a	671.9±48.9 ^a	12405±1519 ^a	4303±424 ^a
P6	46.7±6.5 ^a	35.2±4.9 ^a	679.6±102.6 ^a	12497±740 ^a	4350±550 ^a
F-value	0.424 (0.858)	0.207 (0.972)	0.43(0.853)	0.38(0.885)	0.97(0.463)

† CK, control; N1, 0 g N $\text{m}^{-2} \text{yr}^{-1}$; N2, 5.6 g N $\text{m}^{-2} \text{yr}^{-1}$; N3, 11.2 g N $\text{m}^{-2} \text{yr}^{-1}$; N4, 22.4 g N $\text{m}^{-2} \text{yr}^{-1}$; N5, 39.2 g N $\text{m}^{-2} \text{yr}^{-1}$; N6, 56 g N $\text{m}^{-2} \text{yr}^{-1}$; P1, 0 g P $\text{m}^{-2} \text{yr}^{-1}$; P2, 1.55 g P $\text{m}^{-2} \text{yr}^{-1}$; P3, 3.10 g P $\text{m}^{-2} \text{yr}^{-1}$; P4, 6.20 g P $\text{m}^{-2} \text{yr}^{-1}$; P5, 9.30 g P $\text{m}^{-2} \text{yr}^{-1}$; P6, 12.4 g P $\text{m}^{-2} \text{yr}^{-1}$.

‡ Data are presented as mean ± 1 SD (n = 6), and those designated with the same letters are not significantly different ($P < 0.05$).

doi: 10.1371/journal.pone.0077241.t001

16628 to 17538 g C m^{-2} for the P-addition series, but no significant differences were observed. However, C storage in grassland increased linearly with the intensity of N input ($R^2 = 0.82$, $P = 0.005$), and increased quadratically with P addition intensity ($R^2 = 0.86$, $P = 0.003$) (Figure 1).

Table 2. C storage in roots within the different soil layers

	Carbon storage in roots (g C m^{-2})			
	0–10 cm	10–30 cm	30–50 cm	50–100 cm
N addition				
CK	232.6±81.4 ^{ab}	202.9±20.6 ^a	103.8±12.5 ^a	80.8±25.8 ^a
N1	234.4±45.3 ^{ab}	228.2±24.7 ^a	98.1±18.7 ^a	75.1±24.8 ^a
N2	202.0±25.5 ^{ab}	231.9±64.5 ^a	105.8±20.9 ^a	100.1±38.2 ^a
N3	244.2±54.5 ^{ab}	217.1±27.4 ^a	118.4±44.4 ^a	78.8±24.4 ^a
N4	272.2±23.6 ^{ab}	214.6±19.3 ^a	104.0±12.0 ^a	83.0±7.8 ^a
N5	269.3±43.5 ^a	205.3±31.2 ^a	123.7±22.1 ^a	100.3±29.6 ^a
N6	304.3±47.8 ^b	197.5±48.6 ^a	104.8±18.8 ^a	93.2±15.0 ^a
P addition				
CK	234.2±17.6 ^a	173.9±43.8 ^a	113.3±36.0 ^a	95.9±12.6 ^a
P1	235.4±34.3 ^a	198.4±30.8 ^a	110.7±13.6 ^a	103.1±12.5 ^a
P2	217.0±35.4 ^a	235.0±44.1 ^a	115.4±22.2 ^a	82.6±41.8 ^a
P3	258.8±26.7 ^a	218.4±59.4 ^a	103.3±19.5 ^a	82.1±17.4 ^a
P4	245.2±45.0 ^a	220.5±70.0 ^a	113.0±21.6 ^a	93.0±10.0 ^a
P5	246.5±56.4 ^a	198.4±28.6 ^a	132.6±11.0 ^a	94.4±19.9 ^a
P6	267.3±46.9 ^a	191.6±81.5 ^a	136.9±9.1 ^a	83.9±14.3 ^a

Data are presented as mean ± 1 SD (n = 6), and those designated with the same letters are not significantly different ($P < 0.05$).

doi: 10.1371/journal.pone.0077241.t002

Changes in SOC and SIC

SOC in the 0–100-cm soil layer ranged from 11770 to 13122 g C m^{-2} with N addition (Table 1, Table 3), and there was a strong, positive linear relationship with the intensity of N input within the 0–30 cm ($R^2 = 0.83$, $P = 0.003$) and 0–100 cm soil layers ($R^2 = 0.95$, $P < 0.001$) (Figure 1). Similarly, SOC increased quadratically with the intensity of P input in the 0–30cm ($R^2 = 0.92$, $P = 0.001$) and 0–100 cm ($R^2 = 0.74$, $P = 0.012$) soil layers (Figure 1).

In response to N addition, soil pH decreased from 7.0 to 6.0 in the 0–10-cm soil layer and from 7.5 to 7.2 in the 10–30-cm soil layer (Table S1 in File S1). SIC in the 0–30-cm soil layer differed significantly between N treatments ($F = 3.92$, $P < 0.05$), but there were no significant differences in the 0–100 cm soil layer (Figure 2). Regression analyses showed that SIC decreased quadratically in the 0–30 cm ($R^2 = 0.55$, $P = 0.05$) and 0–100-cm soil layers ($R^2 = 0.87$, $P = 0.002$) with increasing N addition (Table S2 in File S1). P addition had no apparent effect on SIC storage.

Effects of N and P addition on soil C sequestration

C sequestration by SOC content in the 0–100-cm soil layer ranged from 156 g C m^{-2} in N1 to 1352 g C m^{-2} in N6, and increased quadratically with the intensity of N input ($R^2 = 0.99$, $P < 0.001$; Figure 3). However, SIC storage decreased logarithmically with increasing N addition ($R^2 = 0.79$, $P = 0.018$), which was negatively correlated with soil pH (Figure 4). C sequestration by STC showed significant linear correlation with increasing N addition ($R^2 = 0.76$, $P = 0.023$). C sequestration by SOC and STC showed logarithmic response to increased P input ($R^2 = 0.56$, $P = 0.087$ for STC; $R^2 = 0.82$, P

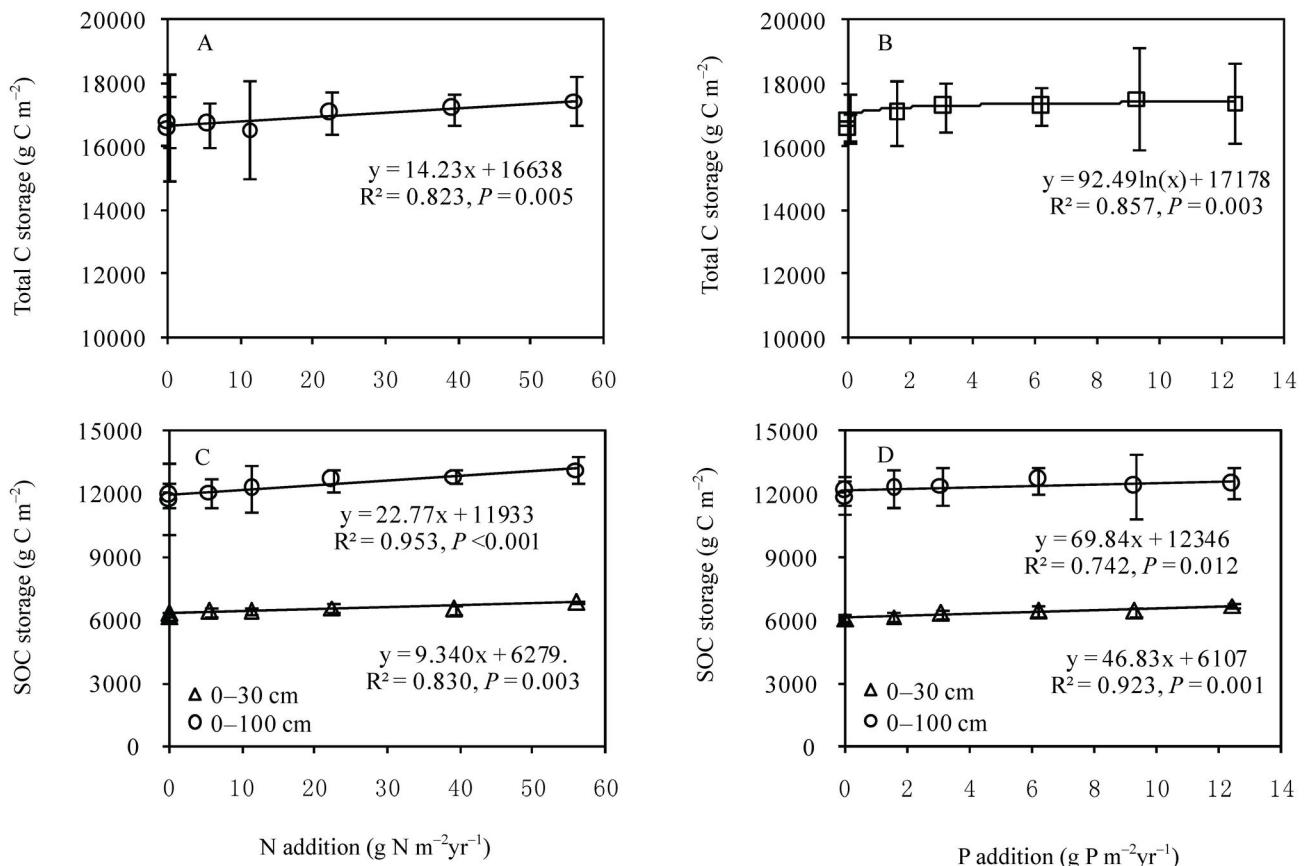


Figure 1. Changes in the carbon storage of Inner Mongolian grasslands with N addition (A and C) and P addition (B and D). Total C storage including C storage of ANPP, litter, roots, SOC, SIC (i.e. top 100-soil layer). Data are presented as mean \pm 1 SD ($n = 6$). See Table 1 for N and P addition and abbreviations.

doi: 10.1371/journal.pone.0077241.g001

= 0.014 for STC) (Figure 3). Moreover, the efficiency of C gain decreased with increasing N and P input (Figure 5).

Discussion

The addition of N enhances C storage in Inner Mongolian grasslands within aboveground biomass, litter, roots and soils. SOC storage in the 0–100 cm soil profile increased quadratically (from 156 to 1352 g C m⁻²) with the intensity of N input. The increase in C input by roots and aboveground biomass was the main reason for the increase in soil C storage (Table 1 and Table 2). Fornara and Tilman [26] found that the total ecosystem C storage increased significantly after 27 years of increasing N input, and suggesting N-induced increase in root mass and community transformation as the potential mechanisms. Roots make substantial C contributions to mineral soils [27], mainly through root turnover and decomposition but also through root exudation [14]. Compared with litter, roots are more important sources of new organic matter in Inner Mongolian grasslands [15]. Moreover, C gain efficiency decreased quadratically with increasing N and P addition in Inner Mongolian grasslands (Figure 5), similar to the

findings in the prairie grasslands in the USA [26] and forests in Sweden and Finland [28]. Therefore, it is important to further investigate the underlying mechanisms of decreasing C gain efficiency in the future.

The questions of where, how, and if N addition enhances soil C sequestration in terrestrial ecosystems remain controversial [14,29]. Through a 5-year N-addition experiment (20 g N m⁻²), Zeng et al. [13] found that the C storage of roots and SOC in Keerqin sandy grasslands decreased by 84.8 and 128.5 g C m⁻², respectively, although aboveground biomass and litter increased. In a 2-year N-addition experiment (17.5 g N m⁻²), Lu et al. [30] reported that the increase in C storage was 18.7, 8.7, and 377.9 g C m⁻² in aboveground biomass, litter, and the 0–40 cm soil layer, respectively, but decreased by 90 g C m⁻² in roots. Lu et al. [14] conducted a meta-analysis of 257 published studies and found that N addition had no significant effect on soil C storage in forests and grasslands, although N addition enhanced C inputs from vegetation to soil. Waldrop [31] found that soil C storage decreased significantly (20%) in a sugar maple-dominated ecosystem but increased significantly (10%) in an oak-dominated ecosystem under 3-year N addition. Based on our findings, we conclude that Inner Mongolian

Table 3. Changes in soil organic carbon (SOC) storage within the different soil layers.

	SOC storage (g C m ⁻²)			
	0–10 cm	10–30 cm	30–50 cm	50–100 cm
N addition				
CK	2611±101 a	3492±125 a	2370±180 a	3256±344 a
N1	2594±119 a	3563±235 a	2499±323 a	3270±564 a
N2	2627±20 ab	3761±212 a	2314±330 a	3370±296 a
N3	2715±128 b	3705±152 a	2337±305 a	3378±321 a
N4	2878±65 c	3709±233 a	2647±310 a	3350±46 a
N5	2852±83 c	3712±233 a	2653±166 a	3347±460 a
N6	2946±108 c	3997±238 b	2719±278 a	3397±904 a
P addition				
CK	2579±148 a	3486±463 a	2518±111 a	3300±327 a
P1	2596±61 a	3487±276 a	2568±343 a	3690±449 a
P2	2678±220 b	3518±313 a	2524±327 a	3692±334 a
P3	2833±111 b	3497±387 a	2620±464 a	3473±301 a
P4	2816±149 b	3631±282 a	2561±277 a	3663±352 a
P5	2788±80 b	3642±446 a	2452±284 a	3522±519 a
P6	2833±133 b	3695±528 a	2495±439 a	3475±290 a

Data are presented as mean ± 1 SD (n = 6), and those designated with the same letters are not significantly different ($P < 0.05$).

doi: 10.1371/journal.pone.0077241.t003

grasslands have an apparent potential to sequester atmospheric CO₂ in soil for scenarios of increasing N deposition.

P addition also enhanced soil C sequestration in semi-arid grasslands, but the increase showed a logarithmic relationship to the intensity of P input. This finding implied a lower saturation level for P in terms of C sequestration. C gain efficiency decreased quadratically with increasing P input, which also supported this assumption. The possible explanation for the observed logarithmic relationship is that P absorption proficiency of plant species decreased in response to P addition intensities[32]. Niu et al. [33] reported that P addition has no apparent effect on net ecosystem exchange in temperate steppe. Based on the increase and trend observed for SOC, we suggest that N addition will have a greater effect than P addition in Inner Mongolian grasslands. Moreover, stoichiometry can provide a new approach for discussing the constraining effect of N and P on C sequestration [34], because the N:P ratio of soil organic matter (SOM) in Inner Mongolian grassland is constrained within a rather narrow range, with the C:N:P ratio being 98:6:1 for the experimental plot [24,35].

A decrease in soil pH is commonly observed with increasing reactive N input (N deposition or N addition experiments) [19–21]. Most studies have emphasized the regulatory effect of soil pH on SOM turnover due to the alternation of microbial communities and activity [9,36], but have ignored the effect of soil acidification on SIC. In this study, the soil pH decreased by

0.4–1.0 units in the surface soil layer and was significantly correlated with the intensity of N input ($P < 0.001$). However, there were no apparent changes in pH within deeper soil profile during this 7-year N-addition experiment. However, a longer field experiment may be required to observe effects of N addition on pH in deeper soil layers. Soil pH after N addition depends on the balance between acid and non-acid cations on colloid surfaces and the balance between hydrogen (H⁺) and hydroxide (OH⁻) ions in soil solution. Theoretically, if all of the ammonium ions from ammonium sulfate are nitrified then 1 mol ammonium sulfate produces 4 mol acid (+). Thus, the application of 24 kg N ha⁻¹ as ammonium sulfate requires nearly 165 kg CaCO₃ ha⁻¹ to neutralize it [37]. Along with increasing reactive N input (N fertilizer or increasing atmospheric N deposition), croplands and grasslands in China showed significant acidification from the 1980s to the 2000s [21,38].

Soil acidification could induce substantial CO₂ emission from dissolution of carbonates (such as calcium carbonate (CaCO₃) and dolomite (MgCO₃)) in semi-arid Inner Mongolian grasslands because of the high SIC content [22,23]. Our results showed that SIC decreased from 230.8 to 623.9 g C m⁻² in the 0–100-cm soil layer in Inner Mongolian grasslands, and that the decreases in SIC could offset 46–76% of the increases in SOC associated with N addition. Yang et al. [23] estimated that, with a decrease 0.63 in the soil pH, SIC storage in the top 10 cm decreased by an average of 26.8 g C m⁻² yr⁻¹ in northern China. However, a few studies suggest that carbonates may be dissolved in surface soil (through the release of CO₂ from decomposition of soil organic matter or by the input of hydrogen ions from acid deposition) but re-precipitated in deep soil layers [23,38,39]. The relationship between soil acidification and SIC storage could be very important for the carbon cycle in semi-arid grasslands in northern China[22]. Therefore, future studies should also include assessment of SIC dynamics in order to accurately evaluate soil C sequestration under N deposition scenarios in semi-arid regions.

Conclusion

In the semi-arid Inner Mongolian grasslands, soil C sequestration increased quadratically with increasing N input due to the significant increase in litter and root C input; but C gain efficiency decreased with increasing N input. Soil C sequestration increased logarithmically with the intensity of P input and showed a lower saturation level for P than for N. Soil pH apparently decreased with N addition, which resulted in a loss of SIC of 230.8 to 623.9 g C m⁻² in the 0–100-cm soil layer. Our findings demonstrated that loss of SIC could partially offset the increase in SOC associated with N addition. Therefore, future evaluation of soil C sequestration for scenarios of increasing N deposition should include changes in SIC storage, especially in semi-arid regions.

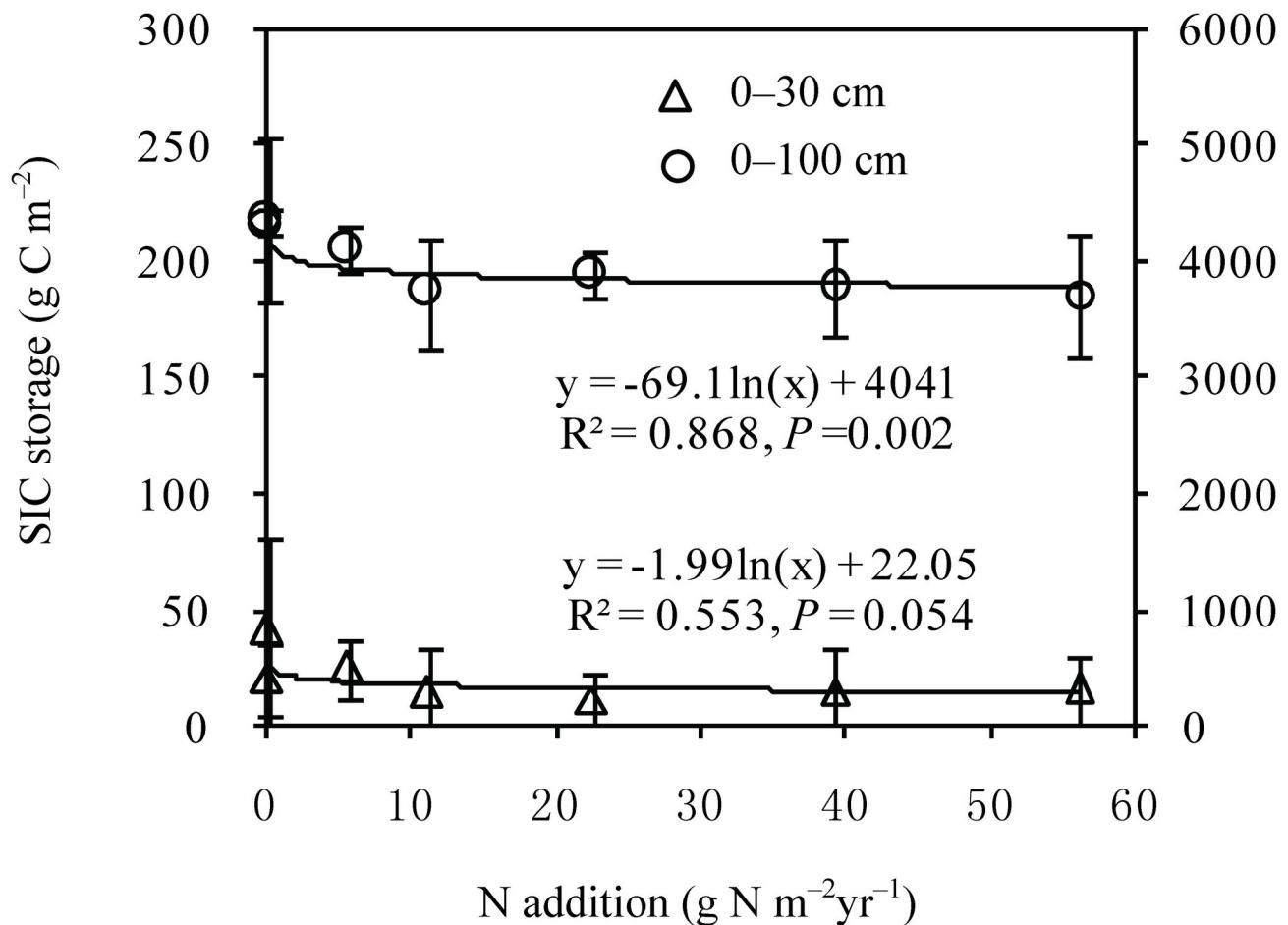


Figure 2. Changes in SIC within 0–30 cm and 0–100 cm soil layers with N addition.

doi: 10.1371/journal.pone.0077241.g002

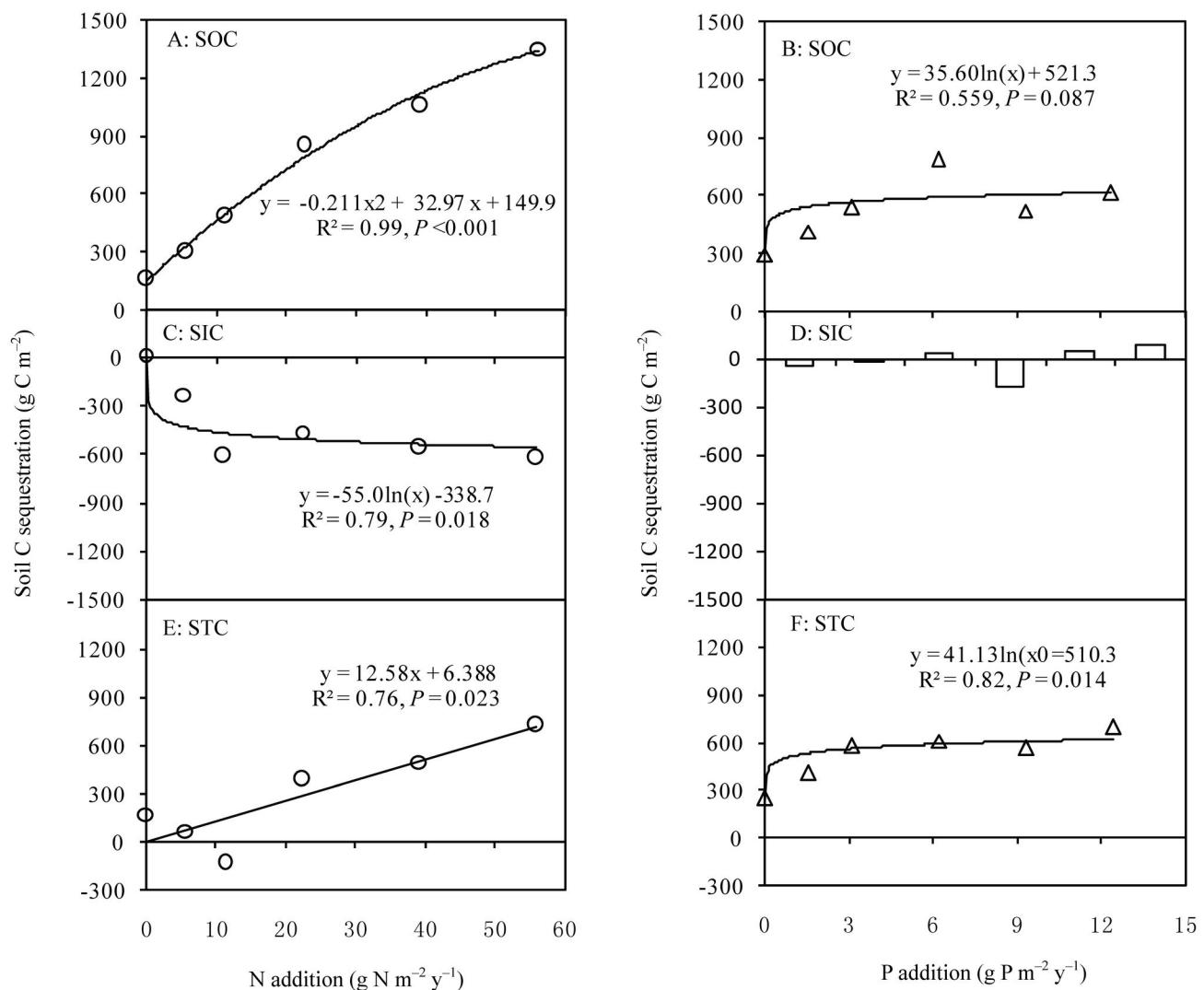
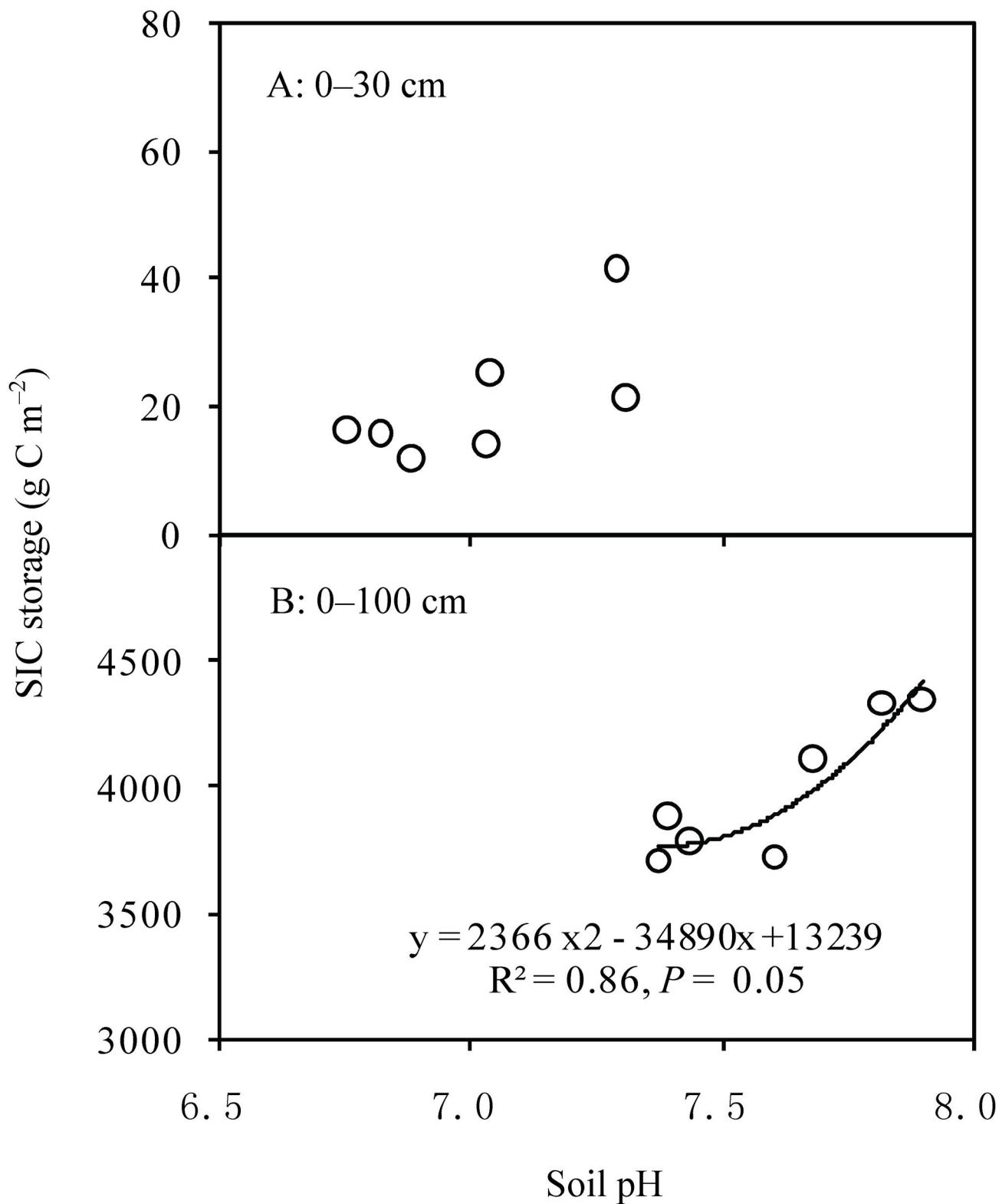


Figure 3. Soil C sequestration of grasslands in the 0–100 cm soil layer with N addition and P addition. STC, soil total carbon; SOC, soil organic carbon; SIC, soil inorganic carbon.

doi:10.1371/journal.pone.0077241.g003

**Figure 4. Relationship between SIC and soil pH in response to N addition.**

doi:10.1371/journal.pone.0077241.g004

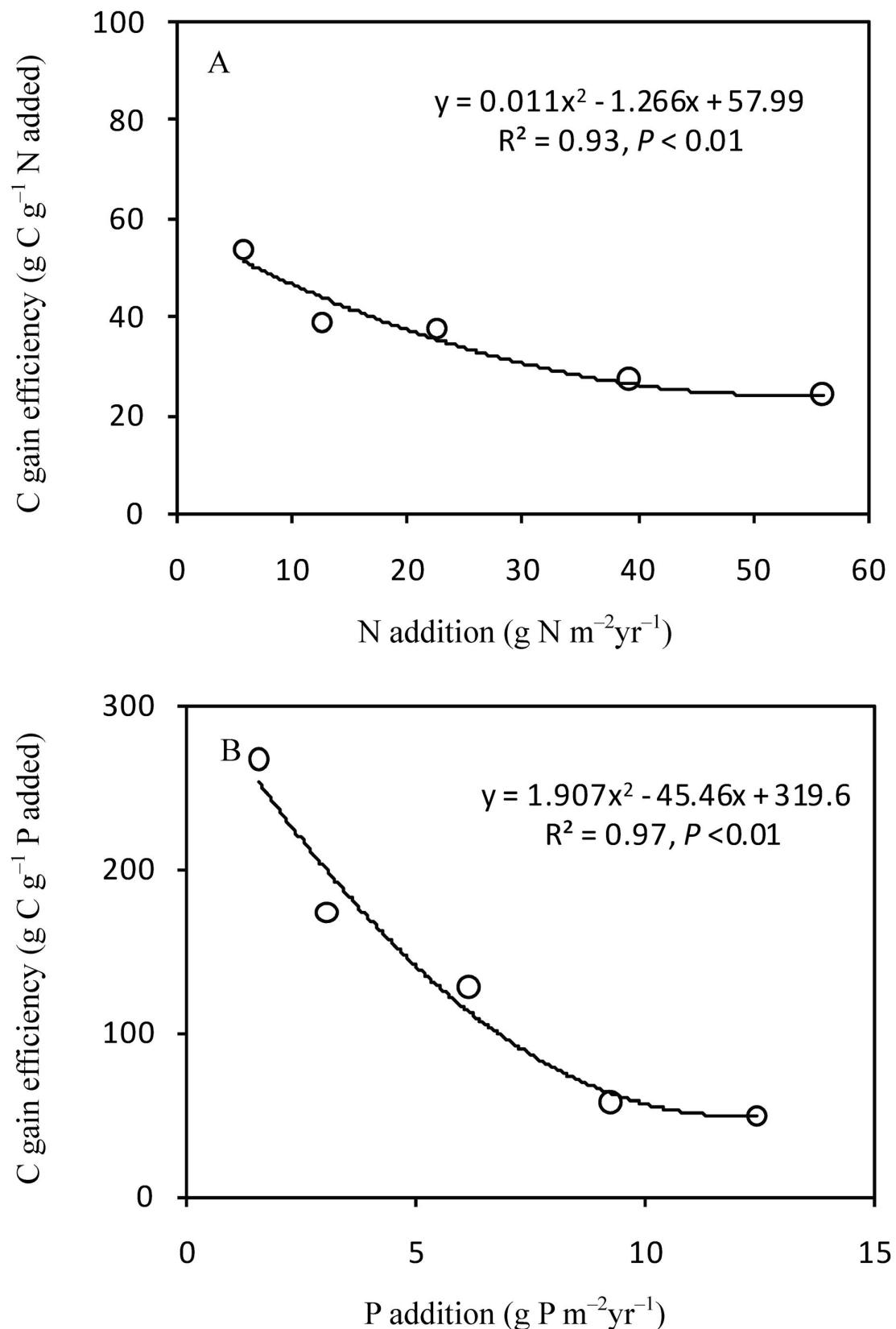


Figure 5. C gain efficiency of SOC under N addition (A) and P addition (B).

doi: 10.1371/journal.pone.0077241.g005

Supporting Information

File S1. Figure S1, Table S1 and Table S2. (DOCX)

References

1. Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen cycle. *Nature* 451: 293–298. doi:10.1038/nature06592. PubMed: 18202647.
2. Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW et al. (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* 70: 153–226. doi:10.1007/s10533-004-0370-0.
3. Neff JC, Townsend AR, Gleixner G, Lehman SJ, Turnbull J et al. (2002) Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature* 419: 915–917. doi:10.1038/nature01136. PubMed: 12410307.
4. Hagedorn F, Spinnler D, Siegwolf R (2003) Increased N deposition retards mineralization of old soil organic matter. *Soil Biol Biochem* 35: 1683–1692. doi:10.1016/j.soilbio.2003.08.015.
5. Hagedorn F, Kammer A, Schmidt MWI, Goodale CL (2012) Nitrogen addition alters mineralization dynamics of 13C-depleted leaf and twig litter and reduces leaching of older DOC from mineral soil. *Glob Change Biol* 18: 1412–1427. doi:10.1111/j.1365-2486.2011.02603.x.
6. Gamboa AM, Hidalgo C, De Leon F, Etchevers JD, Gallardo JF et al. (2010) Nutrient addition differentially affects soil carbon sequestration in secondary tropical dry forests: early- versus late-succession stages. *Restor Ecol* 18: 252–260. doi:10.1111/j.1526-100X.2008.00432.x.
7. Yang YH, Luo YQ, Lu M, Schadel C, Han WX (2011) Terrestrial C:N stoichiometry in response to elevated CO₂ and N addition: a synthesis of two meta-analyses. *Plant Soil* 343: 393–400. doi:10.1007/s11104-011-0736-8.
8. Grandy AS, Robertson GP (2007) Land-use intensity effects on soil organic carbon accumulation rates and mechanisms. *Ecosystems* 10: 58–73.
9. Keeler BL, Hobbie SE, Kellogg LE (2009) Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: Implications for litter and soil organic matter decomposition. *Ecosystems* 12: 1–15. doi:10.1007/s11252-008-0079-2.
10. Liu LL, Greaver TL (2010) A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecol Lett* 13: 819–828. doi:10.1111/j.1461-0248.2010.01482.x. PubMed: 20482580.
11. Johnston CA, Groffman P, Breshears DD, Cardon ZG, Currie W et al. (2004) Carbon cycling in soil. *Front Ecol Environ* 2: 522–528. doi:10.1890/1540-9295(2004)002[0522:CCIS]2.0.CO;2.
12. Reay DS, Dentener F, Smith P, Grace J, Feely RA (2008) Global nitrogen deposition and carbon sinks. *Nat Geosci* 1: 430–437. doi:10.1038/ngeo230.
13. Zeng DH, Li LJ, Fahey TJ, Yu ZY, Fan ZP et al. (2010) Effects of nitrogen addition on vegetation and ecosystem carbon in a semi-arid grassland. *Biogeochemistry* 98: 185–193. doi:10.1007/s10533-009-9385-x.
14. Lu M, Zhou XH, Luo YQ, Yang YH, Fang CM et al. (2011) Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis. *Agric Ecosyst Environ* 140: 234–244. doi:10.1016/j.agee.2010.12.010.
15. He NP, Yu Q, Wu L, Wang YS, Han XG (2008) Carbon and nitrogen store and storage potential as affected by land-use in a *Leymus chinensis* grassland of northern China. *Soil Biol Biochem* 40: 2952–2959. doi:10.1016/j.soilbio.2008.08.018.
16. He NP, Zhang YH, Dai JZ, Han XG, Baoyin TGT et al. (2012) Land-use impact on soil carbon and nitrogen sequestration in typical steppe ecosystems, Inner Mongolia. *J Geogr Sciences* 22: 859–873. doi:10.1007/s11442-012-0968-4.
17. He NP, Zhang YH, Yu Q, Chen QS, Pan QM et al. (2011) Grazing intensity impacts soil carbon and nitrogen storage of continental steppe. *Ecosphere* 2: (1):art8 doi:10.1890/ES110-00017.00011.
18. Wang SP, Wilkes A, Zhang ZC, Chang XF, Lang R et al. (2011) Management and land use change effects on soil carbon in northern China's grasslands: a synthesis. *Agric Ecosyst Environ* 142: 329–340. doi:10.1016/j.agee.2011.06.002.
19. Kemmitt SJ, Wright D, Goulding KWT, Jones DL (2006) pH regulation of carbon and nitrogen dynamics in two agricultural soils. *Soil Biol Biochem* 38: 898–911. doi:10.1016/j.soilbio.2005.08.006.
20. Noble AD, Suzuki S, Soda W, Ruaysoongnern S, Berthelsen S (2008) Soil acidification and carbon storage in fertilized pastures of Northeast Thailand. *Geoderma* 144: 248–255. doi:10.1016/j.geoderma.2007.11.019.
21. Yang YH, Ji CJ, Ma WH, Wang SF, Wang SP et al. (2012) Significant soil acidification across northern China's grassland during 1980s–2000s. *Glob Change Biol* 18: 2293–2300.
22. Mi N, Wang SQ, Liu JY, Yu GR, Zhang WJ et al. (2008) Soil inorganic carbon storage pattern in China. *Glob Change Biol* 14: 2380–2387. doi:10.1111/j.1365-2486.2008.01642.x.
23. Yang YH, Fang JY, Ji CJ, Ma WH, Mohammat A et al. (2012) Widespread decrease in topsoil inorganic carbon stocks across China's grasslands during 1980s–2000s. *Glob Change Biol* 18: 3672–3680. doi:10.1111/gcb.12025.
24. Yu Q, Chen QS, Elser JJ, He NP, Wu HH et al. (2010) Linking stoichiometric homeostasis with ecosystem structure, functioning and stability. *Ecol Lett* 13: 1390–1399. doi:10.1111/j.1461-0248.2010.01532.x. PubMed: 20849443.
25. Yu QA, Elser JJ, He NP, Wu HH, Chen QS et al. (2011) Stoichiometric homeostasis of vascular plants in the Inner Mongolia grassland. *Oecologia* 166: 1–10. doi:10.1007/s00442-010-1902-z. PubMed: 21221646.
26. Fornara DA, Tilman D (2012) Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition. *Ecology* 93: 2030–2036. doi:10.1890/12-0292.1. PubMed: 23094375.
27. Guo LB, Halliday MJ, Siakimotu SJM, Gifford RM (2005) Fine root production and litter input: Its effects on soil carbon. *Plant Soil* 272: 1–10. doi:10.1007/s11104-004-3611-z.
28. Hyvonen R, Persson T, Andersson S, Olsson B, Agren GI et al. (2008) Impact of long-term nitrogen addition on carbon stocks in trees and soils in northern Europe. *Biogeochemistry* 89: 121–137. doi:10.1007/s10533-007-9121-3.
29. Ammann C, Spirig C, Leifeld J, Neftel A (2009) Assessment of the nitrogen and carbon budget of two managed temperate grassland fields. *Agric Ecosyst Environ* 133: 150–162. doi:10.1016/j.agee.2009.05.006.
30. Lu FM, Lu XT, Liu W, Han X, Zhang GM et al. (2011) Carbon and nitrogen storage in plant and soil as related to nitrogen and water amendment in a temperate steppe of northern China. *Biol Fertil Soils* 47: 187–196. doi:10.1007/s00374-010-0522-4.
31. Waldrop MP, Zak DR, Sinsabaugh RL, Gallo M, Lauber C (2004) Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecol Appl* 14: 1172–1177. doi:10.1890/03-5120.
32. Li LJ, Zeng DH, Mao R, Yu ZY (2012) Nitrogen and phosphorus resorption of *Artemisia Scoparia*, *Chenopodium acuminatum*, *Cannabis sativa*, and *Phragmites communis* under nitrogen and phosphorus additions in a semiarid grassland, China. *Plant Soil Environ* 58: 446–451.
33. Niu SL, Wu MY, Han Y, Xia JY, Zhang Z et al. (2010) Nitrogen effects on net ecosystem carbon exchange in a temperate steppe. *Glob Change Biol* 16: 144–155. doi:10.1111/j.1365-2486.2009.01894.x.
34. Taylor PG, Townsend AR (2010) Stoichiometric control of organic carbon-nitrate relationships from soils to the sea. *Nature* 464: 1178–1181. doi:10.1038/nature08985. PubMed: 20414306.
35. Hessen DO, Agren GI, Anderson TR, Elser JJ, de Rueter PC (2004) Carbon sequestration in ecosystems: The role of stoichiometry. *Ecology* 85: 1179–1192. doi:10.1890/02-0251.
36. Grandy A, Sinsabaugh R, Neff J, Stursova M, Zak D (2008) Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. *Biogeochemistry* 91: 37–49. doi:10.1007/s10533-008-9257-9.
37. Goulding KWT, Blake L (1998) Land use, liming and the mobilization of potentially toxic metals. *Agric Ecosyst Environ* 67: 135–144. doi:10.1016/S0167-8809(97)00111-4.

38. Guo JH, Liu XJ, Zhang Y, Shen JL, Han WX et al. (2010) Significant acidification in major Chinese croplands. *Science*, 327: 1008-1010. doi: 10.1126/science.1182570. PubMed: 20150447.
39. Marion GM, Schlesinger WH, Fonteyn PJ (1985) CALDEP: a regional model for soil CaCO_3 (caliche) deposition in south western deserts. *Soil Sci* 139: 468-481. doi:10.1097/00010694-198505000-00014.