

Effects of Combined Biochar and Nitrogen Fertilizer Application on Spring Wheat Yield and Its C:N:P Ratio (Postprint)

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Abstract

Carbon (C), nitrogen (N), and phosphorus (P) ecological stoichiometry provides a novel perspective for studying material cycling and energy flow in crop-soil ecosystems; investigating the C, N, and P stoichiometric characteristics of wheat under biochar combined with different nitrogen fertilizer application rates can provide a theoretical basis for identifying regional nutrient limitations and rational fertilization. This study conducted a field plot experiment to measure wheat yield, CNP content, and their ecological stoichiometric ratios under treatments including application of $50 \text{ kg(N)} \cdot \text{hm}^{-2}$ nitrogen fertilizer, $100 \text{ kg(N)} \cdot \text{hm}^{-2}$ nitrogen fertilizer, biochar alone, biochar combined with $50 \text{ kg(N)} \cdot \text{hm}^{-2}$ nitrogen fertilizer, and biochar combined with $100 \text{ kg(N)} \cdot \text{hm}^{-2}$ nitrogen fertilizer. The results showed that, compared with the blank control (no nitrogen fertilizer and biochar) treatment, all other treatments significantly increased wheat straw and grain yields; except for the biochar alone treatment, other treatments increased N content in various aboveground organs of wheat to varying degrees; biochar combined with different nitrogen fertilizer rates significantly increased C and P contents in stems and grains. Stoichiometric ratio results indicated that, compared with the control treatment, biochar combined with $50 \text{ kg(N)} \cdot \text{hm}^{-2}$ nitrogen fertilizer significantly reduced leaf C/N and C/P, while biochar combined with $100 \text{ kg(N)} \cdot \text{hm}^{-2}$ nitrogen fertilizer significantly reduced stem C/N, C/P, N/P, as well as grain C/N and C/P. Leaf N/P ratios in the study area were mostly 18–23, suggesting that wheat growth may be limited by phosphorus. Biochar combined with nitrogen fertilizer significantly increased crop yield, enhanced wheat CNP nutrient contents, and reduced plant C/N, C/P, and N/P ratios. Overall, the treatment of biochar combined with $100 \text{ kg(N)} \cdot \text{hm}^{-2}$ nitrogen fertilizer showed the best overall performance.

Full Text

Preamble

Effect of Combined Application of Biochar and N-Fertilizer on Yield and C N P Ratio of Spring Wheat

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Abstract: Carbon (C), nitrogen (N), and phosphorus (P) are the three main elements in living organisms. The C N P stoichiometry of consumers and resources affects food web structure and function, influencing broad-scale processes such as global carbon cycles. Nutrient balance can regulate plant growth and stoichiometry in ecosystems. Wheat is a critical food crop, and its production impacts global food security. Combined biochar and N fertilizer application is a beneficial agronomic practice that affects grain yield and C N P allocation patterns. This study investigated wheat yield, C, N, and P contents, and their ratios under different treatments: 50 kg · hm⁻² N fertilizer, 100 kg · hm⁻² N fertilizer, biochar alone, biochar combined with 50 kg · hm⁻² N fertilizer, biochar combined with 100 kg · hm⁻² N fertilizer, and a control without N fertilizer or biochar. Results showed that all treatments significantly increased wheat straw and grain yield compared to the control. Except for biochar alone, other treatments increased N content in wheat organs. Biochar combined with different N fertilizer rates significantly increased C and P contents in stems and grains. Biochar with 50 kg · hm⁻² N fertilizer significantly decreased leaf C N and C P ratios, while biochar with 100 kg · hm⁻² N fertilizer significantly reduced stem C N, C P, and N P ratios, as well as grain C N and C P ratios. Leaf N P ratios in the study area ranged from 18 to 23, suggesting potential P limitation. Combined biochar and N fertilizer application significantly increased crop yield and CNP nutrient contents while decreasing C N, C P, and N P ratios. Overall, biochar combined with 100 kg · hm⁻² N fertilizer showed the best comprehensive performance.

Keywords: CNP; ecological stoichiometry; nitrogen fertilizer; biochar; spring wheat

Introduction

Ecological stoichiometry examines the multivariate balance of chemical elements (C, N, P, O, S) in biological systems and their ecological interactions, primar-

ily using C N P ratios to link organismal traits and behaviors with ecosystem processes. This approach unifies ecological theories across molecular, cellular, organismal, population, ecosystem, and global scales. As a key indicator regulating organismal characteristics, C N P stoichiometry influences host-pathogen interactions, species symbiosis, community structure and dynamics, trophic interactions, and nutrient limitation in organisms, while also playing a crucial role in ecosystem nutrient cycling, supply-demand balance, and global biogeochemical cycles. The N P ratio particularly reflects both tissue properties and soil organic matter decomposition/mineralization, serving as an indicator of plant growth and development. Organismal C N P ratios relate to ecosystem structure and function, nutrient use efficiency, nutrient limitation, and growth rate, representing key metrics for determining community structure and function.

Combined biochar and N fertilizer application represents an effective agronomic practice for supplementing N and P nutrition, influencing plant behavior, growth, and physiological traits in croplands. This practice alters soil nutrient uptake and utilization in dryland farming, improving wheat growth and photosynthetic productivity. Studies have shown that biochar-fertilizer interactions significantly increase fertilizer apparent recovery and N use efficiency, promoting crop growth and yield. Research in northwestern arid regions demonstrated that high biochar rates with N fertilizer significantly improved wheat N and P uptake. These changes inevitably affect the distribution and variation of plant C N P stoichiometry. However, few studies have applied ecological stoichiometry theory to elucidate the mechanisms underlying biochar and N fertilizer effects on plant nutrient ratios. Investigating how fertilization practices influence crop C N P stoichiometry as an indicator of field nutrient status and its implications for nutrient limitation holds significant scientific and practical importance.

The rain-fed agricultural region of the Loess Plateau in central Gansu experiences concentrated precipitation from July to September, with severe drought and water scarcity. Traditional fertilization exacerbates soil water and nutrient loss, severely degrading the soil microbial environment. Enhancing regional farmland productivity and promoting sustainable use of cultivated land resources are urgent challenges. Biochar offers advantages including high stability, large specific surface area, and strong adsorption capacity for N and P nutrients, playing a major role in improving soil physicochemical properties, retaining nutrients, and regulating system nutrient balance. Based on these benefits, this study investigated plant C N P stoichiometry under combined biochar and N fertilizer application in Dingxi City, Li Jiabao Town, to explore regional nutrient limitations and provide theoretical support for understanding biogeochemical cycles and improving farmland productivity.

1. Materials and Methods

1.1 Study Area and Experimental Design

The study was conducted in Li Jiabao Town, Dingxi City, Gansu Province, located in the semi-arid hilly and gully region of the central Loess Plateau. The site has an elevation of approximately 2,000 m, with mean annual solar radiation of $594.7 \text{ kJ} \cdot \text{cm}^{-2}$, sunshine duration of 2,476.6 h, mean annual temperature of $6.4 \text{ }^{\circ}\text{C}$, $0 \text{ }^{\circ}\text{C}$ accumulated temperature of $2,933.5 \text{ }^{\circ}\text{C}$, $10 \text{ }^{\circ}\text{C}$ accumulated temperature of $2,239.1 \text{ }^{\circ}\text{C}$, frost-free period of 140 days, mean annual precipitation of 390.9 mm, annual evaporation of 1,531 mm, and aridity index of 2.53. Precipitation at 80% assurance is 365 mm with a coefficient of variation of 24.3%, characterizing a typical dryland farming region. The soil is typical loessial soil, soft and deep with uniform texture and good water storage capacity. A long-term field experiment was established in 2014 to study nutrient stoichiometry in the soil-plant system, with measurements taken in 2016. Pre-experiment soil properties (0–30 cm) were: pH 8.1, organic matter $16.0 \text{ g} \cdot \text{kg}^{-1}$, total N $1.55 \text{ g} \cdot \text{kg}^{-1}$, total P $0.82 \text{ g} \cdot \text{kg}^{-1}$, total K $14.4 \text{ g} \cdot \text{kg}^{-1}$, alkali-hydrolyzable N $51.1 \text{ mg} \cdot \text{kg}^{-1}$, and available P $21.2 \text{ mg} \cdot \text{kg}^{-1}$.

Six treatments were established: CK (control, no N fertilizer or biochar), N50 ($50 \text{ kg(N)} \cdot \text{hm}^{-2}$ N fertilizer, no biochar), N100 ($100 \text{ kg(N)} \cdot \text{hm}^{-2}$ N fertilizer, no biochar), B (biochar $15 \text{ t} \cdot \text{hm}^{-2}$, no N fertilizer), BN50 ($50 \text{ kg(N)} \cdot \text{hm}^{-2}$ N fertilizer + biochar $15 \text{ t} \cdot \text{hm}^{-2}$), and BN100 ($100 \text{ kg(N)} \cdot \text{hm}^{-2}$ N fertilizer + biochar $15 \text{ t} \cdot \text{hm}^{-2}$). Each treatment had three replicates arranged in a randomized block design, with plot dimensions of $3 \text{ m} \times 6 \text{ m}$.

N fertilizer was applied annually before spring sowing starting in 2014, while biochar was applied once before spring sowing in 2014 at $15 \text{ t} \cdot \text{hm}^{-2}$. Wheat was sown in late March 2016 using a no-till seeder with cultivar ‘Dingxi 40’ at a seeding rate of $187.5 \text{ kg} \cdot \text{hm}^{-2}$, row spacing of 20 cm, and sowing depth of 7 cm. Harvest occurred in late July, followed by measurements. Biochar was produced from corn straw by Jinhefu Agricultural Technology Co., Ltd., with composition: C 53.28%, N 1.04%, P 0.26%, Ca 0.8%, K 0.51%, Mg 0.47%, and ash content 35.64%.

1.2 Sample Collection and Analysis

1.2.1 Sample Collection and Measurement At wheat maturity (July 26, 2016), samples were collected using an “S” pattern. Five $0.6 \text{ m} \times 0.5 \text{ m}$ quadrats were established per plot. Plants were cut at ground level, and fresh weight was recorded. Samples were separated into stems, leaves, and grains, washed with distilled water, air-dried, killed at $105 \text{ }^{\circ}\text{C}$ for 30 minutes, then oven-dried at $75 \text{ }^{\circ}\text{C}$ to constant weight. Fifty plants were randomly selected from each sample, ground, and sieved for C, N, and P analysis. Carbon content was determined using a C/N analyzer. For N and P, samples were digested with $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$, with total N measured by the Kjeldahl method and P by spectrophotometry. Mass ratios of C/N, C/P, and N/P were calculated for each organ and converted to

molar ratios for comparison with other studies. The calculations were performed as follows:

The mass ratios were first calculated for each organ, then converted to molar stoichiometric ratios. In the equations, RC:N, RC:P, and RN:P represent C/N, C/P, and N/P ratios, respectively, while wC, wN, and wP represent the relative atomic mass fractions of C, N, and P elements.

1.2.2 Data Processing and Analysis Data processing and graphing were performed using Microsoft Excel 2010. Variance analysis and significance testing were conducted using SPSS 22.0 with Duncan's and Pearson's methods.

2. Results

2.1 Effects of Combined Biochar and N Fertilizer Application on Spring Wheat Yield

All treatments significantly increased wheat biomass compared to the CK control. The BN100 treatment produced the highest biomass (54.60% increase), followed by BN50 (31.78% increase), while N50 showed the lowest increase (17.35%). Without biochar, both N50 and N100 treatments significantly increased straw yield by 23.08% and 33.07%, respectively, compared to CK. Under biochar amendment, BN100 significantly increased straw yield compared to biochar alone ($P < 0.05$). For grain yield, N100 without biochar differed significantly from N50 and CK ($P < 0.05$). All biochar-amended treatments showed significant differences ($P < 0.05$), and the interaction between biochar and N fertilizer significantly affected grain yield ($P < 0.05$).

2.2 Effects of Combined Biochar and N Fertilizer Application on C, N, and P Contents

Mean C content in stems was $381.17 \text{ g} \cdot \text{kg}^{-1}$, while leaves and grains averaged $353.30 \text{ g} \cdot \text{kg}^{-1}$ and $363.83 \text{ g} \cdot \text{kg}^{-1}$, respectively. Without biochar, leaf C content showed no significant differences among treatments. Biochar amendment in BN50 and BN100 increased C content in leaves, stems, and grains. BN50 increased C content by 3.5%, 2.49%, and 1.9% in leaves, stems, and grains compared to CK, while BN100 increased them by 4.13%, 2.23%, and 2.34%, respectively, though differences between BN50 and BN100 were not significant ($P > 0.05$). Biochar alone did not significantly increase C content in any organ ($P > 0.05$).

Mean N content was $22.59 \text{ g} \cdot \text{kg}^{-1}$ in grains, $8.9 \text{ g} \cdot \text{kg}^{-1}$ in leaves, and $6.05 \text{ g} \cdot \text{kg}^{-1}$ in stems. Except for biochar alone, all fertilization treatments increased grain N content, with BN100 showing the highest values, followed by BN50. Biochar alone produced the lowest N content. Leaf N content was highest in BN100, followed by N100, with CK showing the lowest. Stem N content was highest in BN100, followed by BN50, with CK lowest. Overall, all treatments

except biochar alone increased aboveground N content. The ranking for grain N content was BN100, BN50 > N100 > N50 > CK, B; for leaf N: BN100, N100 > BN50 > N50 > CK, B; and for stem N: BN100 > BN50 > N100 > N50 > CK, B.

Mean P content was $2.29 \text{ g} \cdot \text{kg}^{-1}$ in grains, $1.04 \text{ g} \cdot \text{kg}^{-1}$ in leaves, and $0.64 \text{ g} \cdot \text{kg}^{-1}$ in stems. Without biochar, P content in aboveground organs showed no significant differences. Under biochar amendment, P content increased with N fertilizer rate. BN100 significantly increased grain P content by 8.33% compared to biochar alone, while stem and leaf P content showed no significant differences among treatments. At equivalent N rates, biochar significantly increased stem and grain P content, with BN100 showing the largest increases of 33.93% and 9.78%, respectively. BN50 increased stem and grain P content by 32.73% and 10.10% compared to N50. Biochar alone did not increase leaf and grain P content except for stem P.

2.3 Ecological Stoichiometric Characteristics

2.3.1 C:N Ratio in Aboveground Organs C N ratios ranged from 44.07–49.06 in leaves, 71.50–75.72 in stems, and 19.01–19.91 in grains, with coefficients of variation of 4.26%, 2.12%, and 1.91%, respectively. Stem C N was significantly higher than leaf and grain C N, which also differed significantly from each other. With increasing N fertilizer rates, C N ratios in aboveground organs decreased regardless of biochar application. At equivalent N rates, only BN50 decreased leaf C N, while other treatments showed no significant differences in organ C N ($P > 0.05$). Overall, the lowest C N values occurred in BN100, decreasing by 4.43, 4.06, and 0.89 units in leaves, stems, and grains compared to CK.

2.3.2 C:P Ratio in Aboveground Organs C P ratios ranged from 830.01–940.55 in leaves, 1,347.83–2,055.37 in stems, and 385.51–428.36 in grains, with coefficients of variation of 5.39%, 15.31%, and 3.47%, respectively. Except for N100, which had high stem C P, stem C P was significantly higher than leaf and grain C P, which differed significantly. Without biochar, leaf, stem, and grain C P increased with N fertilizer. Under biochar amendment, C P remained relatively stable. At equivalent N rates, BN100 significantly decreased stem and grain C P compared to N100 ($P < 0.05$), while BN50 significantly decreased grain C P compared to N50. The highest C P values occurred in N100 for leaves and stems (increasing by 42.95 and 301.77 units vs. CK) and in N50 for grains (increasing by 12.09 units vs. CK).

2.3.3 N:P Ratio in Aboveground Organs N P ratios ranged from 17.10–21.68 in leaves, 18.72–28.41 in stems, and 20.28–22.33 in grains, with coefficients of variation of 7.19%, 14.90%, and 3.99%, respectively. Without biochar, stem N P was significantly higher than leaf N P in CK and N50, while N100 showed no significant differences among organs. Under biochar amendment, grain N P

was significantly higher than leaf N P in B and BN50, while BN100 showed no significant differences ($P > 0.05$). Overall, without biochar, stem N P was highest, followed by grain and leaf. With biochar, grain N P was highest, followed by stem and leaf, showing greater fluctuation and sensitivity.

Without biochar, leaf, stem, and grain N P increased with N fertilizer. Under biochar amendment, leaf N P decreased while stem and grain N P fluctuated. At equivalent N rates, only BN100 significantly decreased stem and grain N P ($P < 0.05$). The highest N P values occurred in N100, increasing by 3.15, 5.2, and 2.05 units in leaves, stems, and grains compared to CK.

3. Discussion

3.1 Wheat Yield Response to Combined Biochar and N Fertilizer Application

Previous two-year studies on biochar and N fertilizer co-application showed significant wheat yield increases in the second year, with grain yield increasing by 8.19% compared to N fertilizer alone. Biochar application without N also significantly increased both grain and straw yields. Other research found that N fertilizer combined with high biochar rates ($100 \text{ t} \cdot \text{hm}^{-2}$) increased radish yield by 95–266%. Our study demonstrated that biochar significantly increased grain yield regardless of N application, with the combination of $100 \text{ kg(N)} \cdot \text{hm}^{-2}$ and biochar showing a 32.99% increase over the control. This aligns with previous findings, primarily because biochar's high stability and adsorption capacity provide slow-release fertilizer properties, creating complementary synergies. The combined application showed more significant effects than biochar alone, likely due to biochar's low inherent nutrient content and its ability to improve soil porosity, water retention, pH, reduce active aluminum, enhance nutrient availability (K, P, Mg, Ca), increase cation exchange capacity, and interact with crop type. Specific mechanisms require further investigation.

3.2 Plant C, N, and P Content Response to Combined Biochar and N Fertilizer Application

Our study showed that aboveground C content increased gradually with N rate regardless of biochar application. However, leaf C content across treatments was substantially lower than the global average of $464 \text{ g} \cdot \text{kg}^{-1}$ for 492 terrestrial plant species, indicating low organic matter and carbon storage in this region. Non-biochar treatments showed a trend of higher C content than biochar treatments, though differences were not significant. Under biochar with $100 \text{ kg(N)} \cdot \text{hm}^{-2}$, grain C content reached $370.44 \text{ g} \cdot \text{kg}^{-1}$, a 2.34% increase over CK, likely due to biochar's high C content. Biochar and N fertilizer application increased plant and soil organic matter through enhanced straw production and litter decomposition, improving root growth and photosynthetic function, thereby increasing wheat C content. Biochar alone did not increase organ C content because plants primarily acquire C through photosynthesis, resulting in relatively

small changes.

Mean aboveground N content was $10.51 \text{ g} \cdot \text{kg}^{-1}$, substantially lower than the national average of $18.6 \text{ g} \cdot \text{kg}^{-1}$ for 753 terrestrial plant species, likely due to low soil N content in this region. As grain yield increased with N and biochar application, N redistribution to grains occurred, causing relatively lower N content in stems and leaves through dilution effects. P supplementation in this study significantly increased N and P contents under biochar amendment, consistent with previous research. Biochar's high N, P, K, and Mg content compensated for insufficient N supply when applied alone, significantly improving fertilizer apparent recovery and N use efficiency. This decreased soil C/N, increased N availability, and enhanced N content in leaves, stems, and grains. Similar to N, combined biochar and N fertilizer improved P uptake, likely by increasing soil P content, micronutrient availability, and available nutrient concentrations. In nutrient-poor environments, plants adapt by increasing nutrient resorption efficiency, while in nutrient-rich conditions, they enhance nutrient uptake capacity. Thus, wheat may have high P translocation rates and notable nutrient resorption during senescence.

3.3 Ecological Stoichiometric Ratio Response to Combined Biochar and N Fertilizer Application

The N/P ratio effectively reflects N and P limitation, serving as an important indicator of soil nutrient supply and plant productivity constraints. C/N and C/P ratios indicate nutrient use efficiency. Different management practices trigger various physiological and biochemical responses that regulate C, N, and P metabolism and cycling, resulting in specific elemental stoichiometric characteristics. Our study showed that increasing N rates and biochar supplementation significantly decreased C/N but relatively increased C/P, indicating higher N use efficiency under N deficiency and strong C assimilation capacity—likely an adaptation strategy to nutrient-poor conditions.

Regarding N/P ratios, previous research on the Loess Plateau showed that increasing N rates significantly increased stem and grain N content while decreasing P content, leading to significantly higher N/P. Our results differ, possibly due to P supplementation creating nutrient interactions that increased plant P content and thus N/P. Under combined biochar and varying N rates, plant N/P remained relatively constant, and biochar with N fertilizer significantly reduced wheat N/P compared to N fertilizer alone. This may occur because biochar's organic macromolecules and porous structure facilitate macroaggregate formation, strongly adsorbing various nutrient forms such as $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ and available P, thereby influencing N/P distribution and metabolism among aboveground organs.

3.4 Plant N P Ratio and Nutrient Limitation

The ecological mechanism of high N P remains debated. Due to variation in soil nutrient availability, species, and plant age across regions, ecosystems, and vegetation types, N P shows high variability, making critical thresholds for N or P limitation inconsistent. Some researchers suggest N P <13 indicates N limitation while N P >16 indicates P limitation, with intermediate values suggesting co-limitation. Other studies propose N P >16 indicates P limitation in wetland ecosystems, while N P <14 indicates N limitation. Alternative criteria suggest P limitation occurs when N P >14 with leaf P content <1.0 g · kg⁻¹, and N limitation when N P <10 with leaf N content <20.0 g · kg⁻¹; N P between 10–14 may indicate co-limitation or adequate supply depending on absolute nutrient concentrations.

In our study area, wheat leaf N content was below 10 g · kg⁻¹, P content was approximately 1.0 g · kg⁻¹, and N P ranged from 18–23. N addition experiments suggest that N fertilizer intensifies P limitation on plant growth, indicating that dryland wheat may be primarily P-limited. From a global perspective, climate change intensifies P limitation in tropical regions, while high-altitude or high-latitude areas show a trend shifting from N to P limitation. Analysis of China's second national soil survey showed that loessial soil in the Loess Plateau has lower available P content than the national average (1.9 g · m⁻² vs. 3.4 g · m⁻²). Research on 126 plant species across seven Loess Plateau regions found mean leaf N P of 14.9, with N P increasing with latitude and solar radiation but decreasing with precipitation and temperature. Our N P ratios were significantly higher than those reported for grasses in that study. Since our study area is in the western Loess Plateau with stronger solar radiation and less precipitation, the higher C N P ratios support the conclusion of P limitation, consistent with critical threshold hypotheses.

Thus, wheat populations in this study appear more P-limited than N-limited. Aboveground organ C, N, and P contents varied substantially with biochar and N fertilizer treatments. The 100 kg(N) · hm⁻² + biochar treatment produced the highest photosynthetic yield with the lowest stem and grain C N and C P ratios, while 50 kg(N) · hm⁻² + biochar yielded the lowest N P. However, combined biochar and N fertilizer intensified P limitation, potentially shifting the system from N to P constraint. While this study explored biochar and N fertilizer effects on crop stoichiometry, these impacts are long-term and influenced by temperature, precipitation, and soil properties. Multi-year observations are needed to fully understand ecosystem responses. To prevent nutrient depletion and promote sustainable development in dryland agriculture, we recommend applying varying P fertilizer rates combined with appropriate biochar amendments.

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