

Article

Nitrogen Addition Decreased Respiration and Heterotrophic Respiration but Increased Autotrophic Respiration in a Cabbage (*Brassica pekinensis* Rupr) Experiment in the Northeast Plains

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Abstract: Farmland soil respiration (R_s) significantly impacts the global carbon (C) cycle. Although nitrogen (N) can promote crop growth and increase yields, its relationship with R_s and its constituents, including autotrophic respiration (R_a) and heterotrophic respiration (R_h), remains unclear. Therefore, a field study was carried out in a cabbage (*Brassica pekinensis* Rupr) system to probe the impact of N addition on R_s , R_a , and R_h . Five levels of N addition, including 0 kg N hm⁻²·yr⁻¹ (N0), 50 kg N hm⁻²·yr⁻¹ (N50), 100 kg N hm⁻²·yr⁻¹ (N100), 150 kg N hm⁻²·yr⁻¹ (N150), and 200 kg N hm⁻²·yr⁻¹ (N200), started in March 2022. The R_s (R_a and R_h) and soil samples were measured and collected twice a month. The findings revealed the following: (1) N fertilizer enhanced R_a while reducing R_s and R_h ; (2) soil temperature (ST), belowground net primary productivity (BNPP), soil inorganic N (SIN), and soil total C/total N (C/N) were the significant elements influencing R_a , and microbial biomass carbon (MBC), SIN, and microbial diversity (MD) were the primary factors influencing R_h ; (3) partial least squares-path models (PLS-PM) showed that ST and SIN directly impacted R_h , while ST and BNPP tangentially influenced R_a ; (4) 150 kg N hm⁻²·yr⁻¹ was the ideal N addition rate for the cabbage in the region. In summary, the reactions of R_a and R_h to N fertilizer in the Northeast Plains are distinct. To comprehend the underlying processes of R_s , R_a , and R_h , further long-term trials involving various amounts of N addition are required, particularly concerning worsening N deposition.

Keywords: N addition; soil respiration; soil temperature; autotrophic respiration; heterotrophic respiration; random forest; PLS-PM



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1. Introduction

Nitrogen (N) deposition in the atmosphere from human activities has increased significantly for over a century and is anticipated to worsen in the following decades [1]. The second-largest carbon (C) circulation in ecosystems [2] and the primary origin of atmospheric carbon dioxide (CO₂) emissions from terrestrial ecosystems are both significantly impacted by this worldwide rise in soil respiration (R_s) [3]. Currently, there is no consensus on how N addition affects R_s , as it can cause promotion [4,5], inhibition [6–8], or no alteration [9,10]. Forecasting atmospheric CO₂ absorption is difficult due to these contradictory and perplexing findings, raising uncertainty when evaluating C-climate feedback [11]. Although prior research has concentrated on forests and meadows [12–14], there are limited investigations on the reaction of farmland R_s on N fertilizer, despite its significant impact on global C cycling. N enhances crop yield, but its relationship with R_s remains unclear. Therefore, this research investigates how N-adding affects R_s , R_a , and R_h in a cabbage (*Brassica pekinensis* Rupr) system.

R_s is responsible for returning photosynthesis-sequestered C into the atmosphere [15]. However, predicting how R_s will respond to N addition is complicated because it consists of two elements: R_a (autotrophic respiration) and R_h (heterotrophic respiration), produced by the roots of plants, mycorrhizae, rhizosphere microorganisms, and the microbial degradation of vegetable matter and soil organic matter, respectively [11]. Determining the contribution of R_a and R_h to R_s is essential to understanding the balance of soil C storage, primarily influenced by the equilibrium of both C intake and R_s [16,17]. While it is broadly recognized that N deposition increases aboveground output, its effects on R_a and R_h are still being studied. This information gap hampers our knowledge of whether the agroecosystem acts as a C sink or source under increasing N deposition.

Furthermore, as the main processes of R_h and R_a are distinct, they may respond differently to environmental factors, emphasizing the importance of investigating their changes under varying conditions [17]. Understanding what controls R_s is critical for crop management because precise actions can enable the soil to take in atmospheric C [18]. N fertilizer has been shown to produce diverse effects on soil CO₂ effluences [19,20]. Whereas some studies found that N input increased R_s [21–23], they also suggested that the ecosystem-stimulating effects of N loading may cause C storage to decline. N fertilizer has been shown to suppress R_s by reducing organic C decomposition and increasing soil organic carbon (SOC).

Cabbage is a widely cultivated crop with significant nutritional and economic value. Optimizing N management in cabbage cultivation can enhance crop yield, improve quality, and reduce environmental impacts, such as nitrate leaching and greenhouse gas emissions. Specifically, different N levels have been found to affect yield, quality, and N utilization, indicating an optimal N application range for high productivity and quality [24]. Moreover, studies have demonstrated that reduced nitrogen with bio-organic fertilizer can enhance soil quality and increase the yield and quality of cabbage, pointing towards sustainable fertilization strategies that support both crop performance and environmental health [25]. Furthermore, some studies on the effects of N fertilizer on soil and plant respiration processes, while not exclusively focused on cabbage, offer insights applicable to its cultivation. For instance, research has shown that N fertilizer can significantly impact the total soil, root, and microbial respiration rates in plantations, suggesting a decrease in these rates with N fertilizer [26–28]. This information is critical for optimizing N application in cabbage systems to balance growth and soil microbial health.

Nevertheless, it remains obscure whether the correlations between soil biochemical parameters and R_s (R_a and R_h) vary at different levels of N addition. N fertilizer use is expected to continue rising with human-made N deposition rates. Therefore, it is essential to understand the connections between N fertilizer and R_s (R_a and R_h). Additionally, this research aims to provide a theoretical foundation for better ecosystem management techniques, offer parameter evaluation and model verification, and enhance predictions of farmland ecosystem responses to atmospheric N deposition. Although respiration does happen and its degree of impact on SOC build-up is also more or less well known, it still depends on the climates, soil type, and aboveground vegetation. This study had a specified study site, soil type, and aboveground vegetation, its study results are more targeted, and we further explained the relevant phenomena from a quantitative perspective. To achieve these objectives, we performed a field study based on the cabbage system in the Northeast China Plain. We measured and calculated R_s , R_h , R_a soil total C, total N, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial diversity (MD), microbial richness (MR), aboveground net primary productivity (ANPP), and belowground net primary productivity (BNPP). Specifically, this study aimed to (1) explore the implications of distinct N application levels on R_a and R_h , (2) identify the influencing factors and pathways between R_a and R_h , and (3) ascertain the ideal quantity of N addition for cabbage cultivation in this region.

2. Materials and Methods

2.1. Study Site Description

The research was conducted in Changchun City (43°88' N, 125°35' E), Jilin Province, Northeast China. The climate is a zone known as the temperate monsoon with an average yearly temperature of 6.9 °C and annual precipitation ranging from 582 to 635 mm. The accumulative annual temperature ranges from 2650 to 2860 °C. Based on the USDA soil taxonomy (Nachtergaele, 2001), the soil belongs to the Mollisols order, Ustolls suborder, Argiustolls great group, and Calcic Argiustolls subgroup. Before commencing the experiment of measuring R_s (23 October 2021), topsoil samples (0–20 cm) were collected and tested using Bao (1981) [29]. The results showed that the natural C content was 20 g·kg⁻¹, while the total N content was 3.6 g·kg⁻¹. The soil pH ranged from 6.3 to 7.4, and its sand, silt, and clay concentrations were 54.34%, 16.82%, and 29.12%, respectively.

2.2. Experimental Design

A field trial was carried out using a randomized block design consisting of five treatments: (1) 0 kg N hm⁻²·yr⁻¹ (N0), (2) 50 kg N hm⁻²·yr⁻¹ (N50), (3) 100 kg N hm⁻²·yr⁻¹ (N100), (4) 150 kg N hm⁻²·yr⁻¹ (N150), and (5) 200 kg N hm⁻²·yr⁻¹ (N200), with six replications for each treatment. This experiment involved planting cabbage on 15 August 2021 and 4 August 2022. A total of thirty plots were established on 9 December 2020 and 2021, with five treatment plots measuring 10 m × 10 m in every block, with a 10 m distance between each block. Ammonium nitrate (NH₄NO₃, N content over 33%) was used as the N addition and applied at the beginning of each growth season month (from August to October). The test variety was “Jijin”, a popular cabbage cultivar in Jilin Province. Cabbage was harvested in early October. The cabbage’s growth period, from the elongation to dough stage, was between late August and mid-September, with controlling weeds manually employed during the growing seasons. No chemical fertilizer or manure was used except for N fertilizer to limit variables. The specific dates of cabbage cultivation can be seen in Table 1.

Table 1. The specific dates of cabbage cultivation.

	2021	2022
Planting	15 August	4 August
N Fertilizing	25 August, 15 September, 5 October	15 August, 1 September, 1 October
Manually weeding	15 August to 11 October	4 August to 7 October
Harvesting	11 October	7 October

2.3. Measurement of R_s and R_h

2.3.1. Experimental Setup

On December 2020, one year preceding the commencement of the formal experiment, we initiated our experimental setup by inserting two distinct types of PVC collars into the soil of each plot designated for measurement. This preparatory step was crucial to minimize soil disturbance and acclimatize the site for accurate respiration assessments that followed.

2.3.2. Instrumentation and Measurement Conditions

Respiration rates were assessed using a soil respiration system (Model 3051T, Zhejiang Tuopuyunong Co., Ltd., Hangzhou, China), which includes a respiration chamber designed for seamless integration with the PVC collars, an industrial computer, and a display for real-time data visualization. This system calculates and displays CO₂ flux rates in μmol m⁻² s⁻¹. The measurements were meticulously scheduled on clear, sunny days between 9:00 and 14:00 to avoid diurnal variation impacts. ST at a 5 cm depth adjacent to each collar was monitored using a portable temperature probe, and SWC was measured at six proximate points with a handheld moisture probe (TDR 100, Spectrum Technologies,

Bellevue, WA, USA), ensuring that environmental variables that could influence respiration rates were accounted for.

2.3.3. *Rs* and *Rh* Measurement Protocol

Rs and *Rh* were measured in March 2022. For *Rs*, we employed surface PVC collars (5 cm in height) that were carefully placed 2–3 cm into the soil. These collars encapsulated both cabbage root systems and the associated soil microbial communities, thereby allowing for the measurement of total soil respiration (*Rs*), which includes contributions from both plant root and microbial respiration, as delineated in previous studies [30]. The measurements were strategically conducted during the cabbage growth cycle, from the onset of spring to the harvest period, bi-monthly. This timing was chosen to capture the dynamic respiration rates across different growth stages. To ensure the representativeness of our data, *Rs* measurements were obtained from five randomly selected, non-peripheral locations within each cabbage plot. These readings were then averaged to estimate the *Rs* rates effectively, mitigating potential edge effects.

To exclusively measure *Rh*, which represents microbial respiration in the absence of root activity, we utilized deeper PVC collars (40 cm in height) and inserted them 36–38 cm into the soil. This depth was informed by our preliminary field observations, which indicated that the majority of root biomass was confined to the top 30 cm of the soil profile. Thus, by removing all plant material from the vicinity of these deep collars, we ensured that the respiration measurements captured were predominantly microbial, enabling us to isolate *Rh* from *Rs*. The difference between *Rs* and *Rh* was subsequently used to calculate *Ra*, according to the methodologies outlined in prior research [31]. The collected data underwent rigorous analysis to parse out the contributions of microbial and root respiration to the total soil respiration rate.

2.4. Soil Sampling and Analysis

For soil analysis, a 6 cm diameter auger randomly assembled five soil cores per plot on the 15th and 30th of each month starting in March 2022. The five core samples were combined and filtered through a 2 mm mesh sieve. The resulting sample was then divided into two parts. One part was placed in a sterile plastic bag, sealed, and transported to the laboratory as soon as possible for MBC and MBN analysis at a temperature of 4 °C. The other part was air-dried to determine soil pH. Within one week, all samples were analyzed. Elemental analysis was conducted to measure the C and N levels in the soil. A continuous-flow auto-analyzer (Elementar vario EL cube, Frankfurt, Germany) was used to quantify the inorganic N ($\text{NH}_4^+\text{-N}$ add $\text{NO}_3^-\text{-N}$) in soil subsamples after removing it using a $2 \text{ mol}\cdot\text{L}^{-1}$ KCL solution [32]. For MBC and MBN extraction and quantification, the chloroform direct fumigation- K_2SO_4 method was utilized [33]. The number of observed species was determined to estimate bacterial and fungal richness [34]. Bacterial and fungal diversity was assessed using the Shannon–Wiener index ($H = -\sum P_i \cdot \ln P_i$) [35]. MD represented bacterial and fungal diversity, while MR represented bacterial and fungal richness.

2.5. ANPP and BNPP Estimations

A $1 \times 1 \text{ m}^2$ quadrat was used in each plot where there were no uncut plants to measure the aboveground vegetation. To estimate ANPP, live plants were taken in, sorted, and oven-dried at 70 °C. For the BNPP assessment, a root-in-growth approach was utilized. In the middle of each experimental plot, two 8 cm breadth and 40 cm deep holes were shoveled in the soil after each growing season. A 1 mm mesh sieve was used for filtering soil samples and filled into a nylon mesh bag with 1 mm pores. Root-free soil samples were utilized for filling the original holes. The root pieces still in the sieve after each following growing season were dried in the oven (Nanjing Ronghua Scientific Equipment Co., Ltd., Nanjing, China), weighed, and used to calculate BNPP.

2.6. Data Analysis

The statistical analysis was performed using R 3.4.2 (R Core Team, 2015), with a significance level of 0.05 unless otherwise stated. Firstly, we conducted a repeated-measures ANOVA to examine the impact of N on biotic and abiotic variables, *Rs*, and their constituents. Secondly, we utilized the random forest method to determine the relative importance of regulating biotic and abiotic factors on *Ra* and *Rh*. This powerful machine-learning technique is robust to multicollinearity and overfitting [36]. This study ran 100 times with evaluation based on the increased mean square error (lnMSE). The contribution of each factor was considered significant or not based on the resulting lnMSE. Finally, we employed a partial least squares-path model (PLS-PM) to gain a mechanistic understanding of how the soil and cabbage changes induced by N enrichment impacted *Rs* and its composition. PLS-PM is a data analysis method that uses latent variables to condense observed variables, assuming a linear relationship between them [37,38]. We quantified the direction and strength of the linear correlations between latent variables using path coefficients, while variability (R^2) was calculated to assess the goodness of fit. The GOF statistic assessed model accuracy, with a value exceeding 0.7 indicating an acceptable fit [39]. We used the “inner plot” function in the R package (pls pm) to create models with different structures.

3. Results and Discussion

3.1. N Fertilizer Effects on *Rs* and Its Constituent Parts (*Ra* and *Rh*)

The CO₂ fluxes exhibited uneven distribution during the N0, N50, N100, N150, and N200 treatments (Figure 1). With small amounts of N fertilizer application, the effect on *Rs* and its constituents was insignificant. However, the effect changed considerably with larger quantities of N fertilizer (beyond 150 kg N hm⁻²·yr⁻¹). Across all periods, N0 had the highest *Rs* values, while N200 had the lowest. The average *Rs* rate decreased by 6.68%, 0.93%, 5.25%, and 9.34% for N50, N100, N150, and N200 compared to N0. *Rh* showed a decline of 11.39%, 4.56%, 14.59%, and 16.42%, respectively. In contrast, *Ra* increased by 16.67%, 17.09%, 41.07%, and 25.73%, respectively. Although we acknowledge the variability in the CO₂ fluxes observed across different N treatment levels (N0, N50, N100, N150, and N200) and the increasing complexity in *Rs* responses with higher N additions, the uneven distribution of CO₂ fluxes and the variability observed in soil and microbial parameters across different nitrogen treatments emphasize the need for caution in interpreting PLS-PM results and the associated parameter uncertainty.

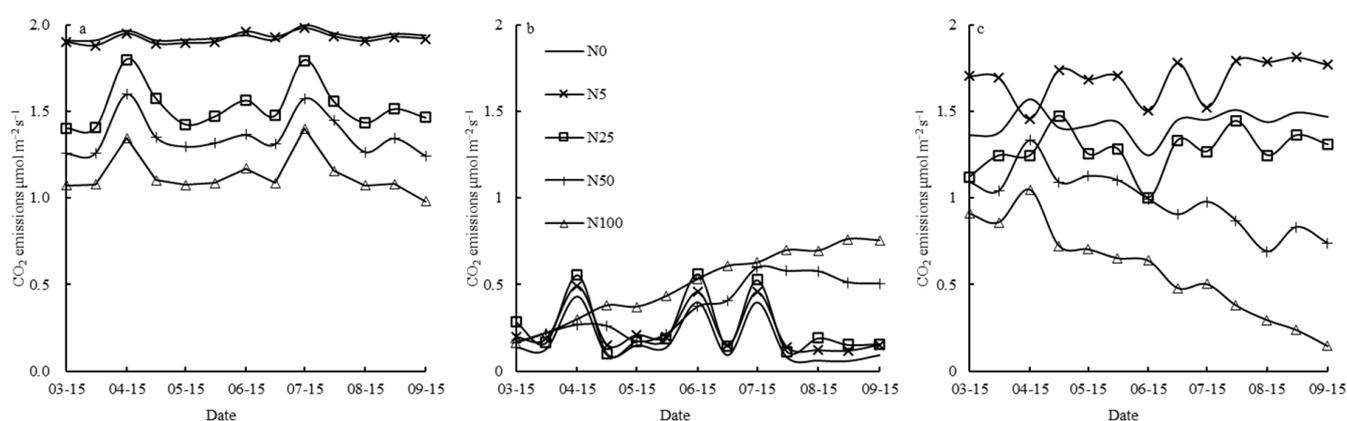


Figure 1. *Rs* under different N addition. Under various N treatments (N0 to N200), CO₂ fluxes displayed irregular patterns. Minimal N fertilization had negligible impacts on *Rs* and its components, but substantial changes occurred with higher N levels (>150 kg N hm⁻²·yr⁻¹). N0 consistently yielded the highest *Rs*, while N200 the lowest. Compared to N0, *Rs* decreased by 6.68–9.34% for N50–N200. *Rh* declined by 4.56–16.42%, but *Ra* rose by 16.67–41.07%. Note: (a–c) stand for *Rs*, *Ra*, and *Rh*, respectively.

Meta-analyses have shown that N fertilizer can prevent SOC decomposition and promote soil C storage, with estimates suggesting that European forests can sequester 50–230 kg ha⁻¹.yr⁻¹ of soil C in reaction to N fertilizer [40,41]. As more N becomes available, more of it will be lost to the environment. N reduces the effectiveness of adding more N to increase soil C content. Our study found that adding lower rates of N for shorter durations significantly increased SOC and MBC contents. However, adding higher rates for longer durations had the opposite effect, decreasing SOC and MBC contents.

3.2. Changes in Biotic and Abiotic Factors in Response to N Addition

With increasing N application, ST initially decreased before reaching a minimum under the N50 treatment, after which it increased. Similarly, SWC and SOC increased before decreasing, with maxima observed under the N150 treatment. Meanwhile, the pH and C/N values decreased while SIN continued to rise. Specifically, SOC content was 1.61%, 4.84%, 32.26%, and 22.58% upper in the N50, N100, N150, and N200 treatments, compared to the N0 treatment. Correspondingly, SIN was 1.22%, 2.44%, 34.63%, and 46.88% higher in the N50, N100, N150, and N200 treatments compared to N0. Figure 2 indicated that N addition at high levels reduced ST more than at low levels. This also illustrated the decrease in ST under N100 addition due to N enrichment stimulating ANPP. The shaded canopy and increased standing litter can reduce the amount of solar radiation that reaches the soil surface, decreasing ST and potentially affecting plant growth and nutrient cycling. Figure 2 also illustrates that C/N gradually declines with increasing N fertilizer. As a result, the capacity of the microorganisms that could break down SOC was decreased, allowing cabbage roots to absorb more N, which raised BNPP and Ra. Han et al. discovered that mycorrhizal respiration accounted for 38% of Ra, suggesting that the impact of BNPP on Ra may be overestimated due to the limitations of this approach [42].

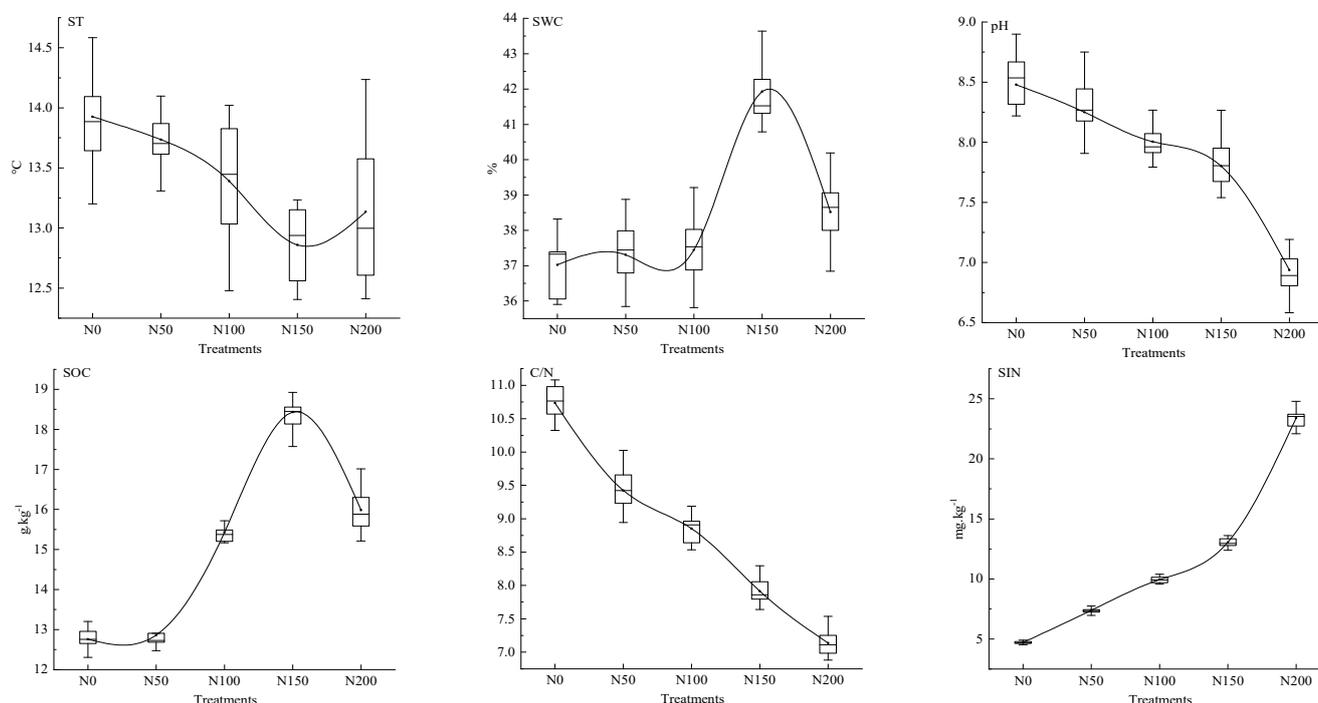


Figure 2. Effect of N fertilization on soil biotic and abiotic factors. As N application increased, ST decreased to a minimum under N50 then rose. Both SWC and SOC peaked under N150 before declining, pH and C/N decreased while SIN increased. Compared to N0, SOC was elevated by 1.61–32.26% across treatments, with SIN showing a 1.22–46.88% increase. Notes: ST stands for soil temperature, SWC for soil moisture content, SOC for soil organic matter carbon, C/N for soil total carbon/total nitrogen, and SIN for soil inorganic nitrogen.

Figure 2 indicates that the soil C/N in this study is 7 to 11, and microbial reproduction and N fertilizer decomposition are quick. Excess N was released and applied to the soil for crop utilization, which is also helpful for *Ra* [43]. *Rh* is mostly made up of soil microbes decomposing leaf, branch, and root litter and mineralizing soil organic C [44]. When N inputs were high, we found that *Rh* reduced. The addition of N-affected MD and MR levels was significantly reduced, and the structure and function of the soil microbial community were improved. The changes in MD and MR could also influence *Rh* by affecting the C/N ratio. Our findings suggested an optimal addition rate of 150 kg N hm⁻².yr⁻¹ for SOC sequestration in the soil; maintaining soil as a carbon sink is low-level N addition, which can increase litter production above and below ground, thus promoting its decomposition. However, N addition had an improved impact on SOC. It later declined with increasing N supplement rates.

N addition significantly increased MBC and MBN while lowering MD and MR (Figure 3). MBC and MBN increased with higher levels of N addition, reaching their highest values at N150 before decreasing. Conversely, MD and MR decreased with increasing N addition. The microbial composition was strongly affected by N fertilization. N addition significantly increased soil SIN and the toxic impact on microorganisms, and decreased MD and MR in *Rh*. Wang et al. found that *Rh* changes significantly under N enrichment, and the decreased *Rh* in the N32 plot was attributed to changes in MBC [11]. The decline in MD and MR, consistent with reporting a decline in MD following N enrichment in grasslands, can be attributed to excess N [45]. In our study, *Rh* decreased more than *Ra* increased, resulting in a decline in *Rs*. Excess N can reduce BNPP in cabbage lodging, and the drop in MD may also be related to a decline in BNPP, further inhibiting *Rh*.

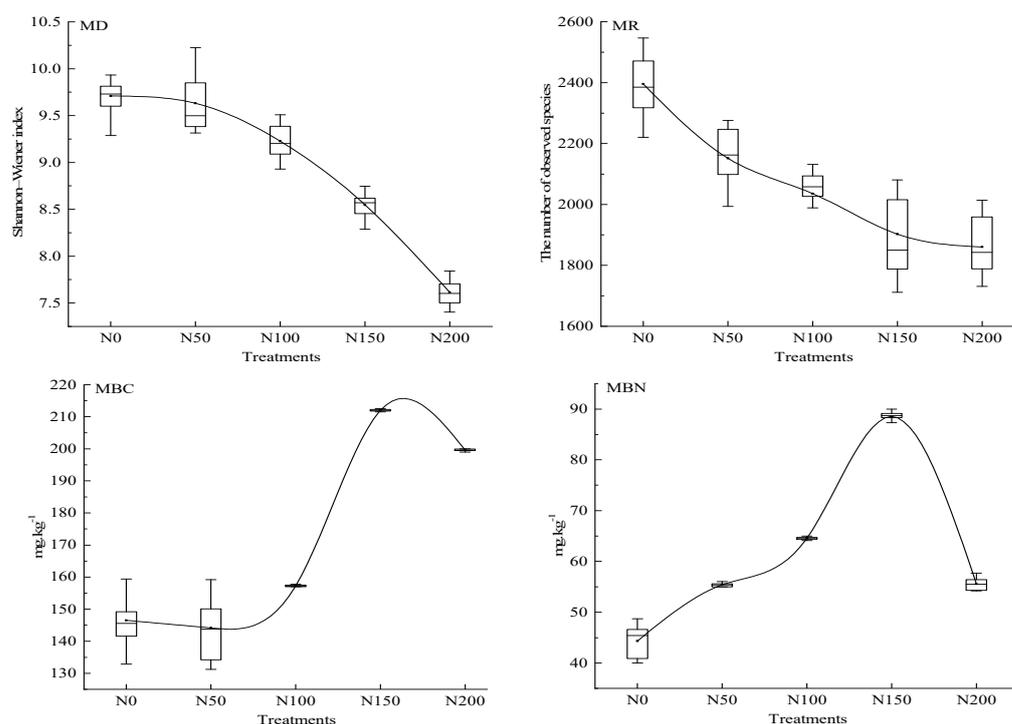


Figure 3. Effect of N addition on soil microbial activities. Nitrogen fertilization had a profound impact on soil microbial parameters, causing a notable surge in microbial biomass carbon (MBC) and nitrogen (MBN), which peaked at N150 before declining. Conversely, microbial diversity (MD) and richness (MR) diminished with escalating N levels. This altered the microbial composition significantly. Notes: MD stands for microbial diversity (bacterial diversity plus fungal diversity), MR stands for microbial richness (bacterial richness adds fungus richness), MBC for microbial biomass carbon, and MBN for microbial biomass nitrogen.

Figure 4 shows the trends of ANPP and BNPP. With constant N addition, BNPP and ANPP increased, peaking at N50 before declining. Compared to the N0 treatment, BNPP increased by 21.00%, 27.40%, 68.04%, and 38.81% in the N50, N100, N150, and N200 treatments, respectively.

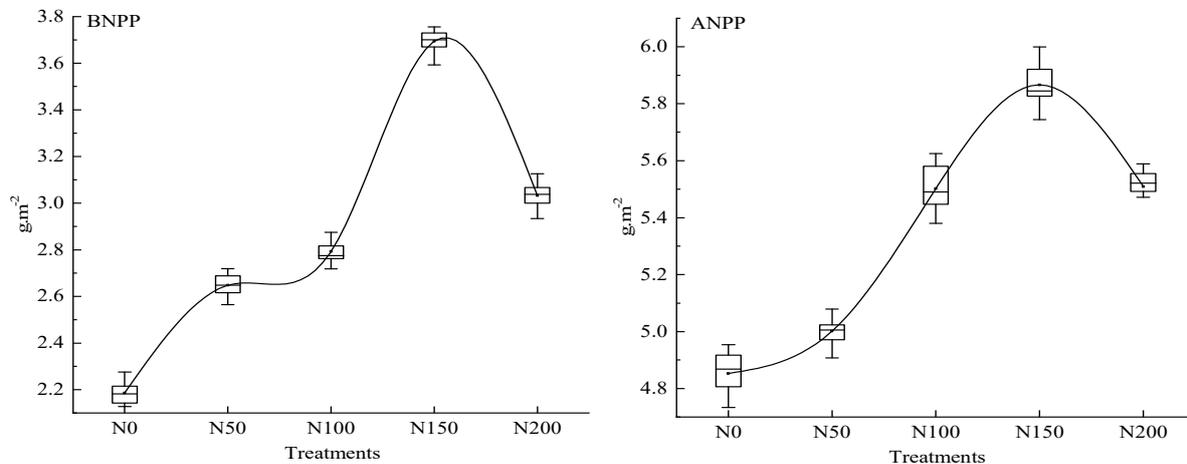


Figure 4. Effects of N fertilizer on ANPP and BNPP. Under sustained nitrogen fertilization, both aboveground (ANPP) and belowground (BNPP) net primary productivity exhibited an initial rise, peaking at the N50 level before showing a decline. Specifically, in comparison to the control (N0), BNPP experienced notable increases of 21.00%, 27.40%, 68.04%, and 38.81% across the N50, N100, N150, and N200 treatments, respectively. Notes: The ANPP and BNPP stand for aboveground net primary productivity and belowground net primary productivity, respectively.

The combined analysis of several N addition experiments revealed that increasing soil N availability markedly decreased MD and MR [46–48]. The reasons can be divided into the following aspects. Firstly, adding more N reduced the energy needed to assimilate it into the soil. Secondly, it also lowered the pH value of the soil and enriched dangerous metal ions, which inhibited the growth and activity of microorganisms. In addition to drastically reducing soil bacteria and fungi biomass, N altered the MD and MR makeup and lowered *Rh*.

3.3. Pearson Correlation Analysis for *Ra* and *Rh*

The Pearson correlation revealed significant negative associations between ST and C/N with *Ra*. At the same time, BNPP, SIN, ANPP, and SOC showed positive correlations with *Ra* (Figure 5a). The decrease in ST, in turn, contributed to the increase in *Ra*. This result is also consistent with a survey conducted by Yang et al. [17], who found that N was 12.5 °C in N0, decreased to 11.5 °C in the N16 plots, and 11.4 °C in the N32 properties. Figure 5a also indicated a strong positive association between *Ra* and BNPP. *Ra* rising in line with the increase in BNPP was not unexpected. The cause of the incident was that BNPP was linked to root growth, an essential source of C for *Ra* [49,50]. At the same time, a global synthesis determined that BNPP was the primary factor influencing *Ra* and independently explained 54% of the variation in *Ra* [51]. Figure 5a also shows a significant positive correlation between *Ra* and SIN. The main reason was the N application significantly increasing SIN content in this cabbage field. Using SIN as fertilizer can encourage BNPP and increase *Ra* [52]. The C/N ratio is crucial to how well bacteria can break down SOC [53]. The results in Figure 5b indicated positive correlations between MD, MR, SIN, and SOC with *Rh*, while MBC and MBN were significantly negatively associated with *Rh* (Figure 5b).

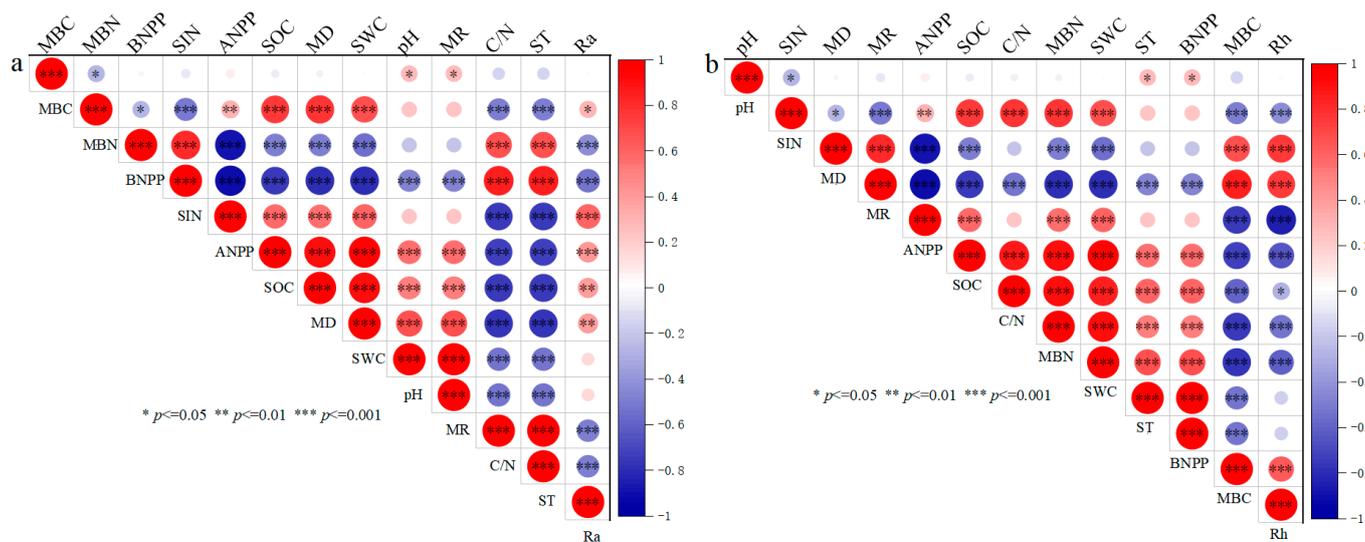


Figure 5. Pearson correlation analysis for *Ra* and *Rh*. (a) Represents the correlations among *Ra* and MBC, MBN, BNPP, SIN, ANPP, SOC, MD, SWC, pH, MR, C/N, and ST. (b) Represents the correlations among *Rh* and pH, SIN, MD, MR, ANPP, SOC, C/N, MBN, SWC, ST, BNPP, and MBC.

Figure 5a shows that N fertilizer also promotes the content of SIN, which as a fertilizer, can promote BNPP, which in turn enables *Ra* [54]. N addition has been discovered to impact C/N significantly, and one potential reason was as follows. Soil microorganisms need 5 parts of C and 1 part of N for every aspect of dissolved SOC and 20 parts of C as an energy source. The ideal C/N ratio in the soil is 25:1. This ratio is better for developing soil microbes [55]. When the C/N ratio exceeds 25:1, microorganisms cannot reproduce in significant quantities, consuming all of the N generated by SOC for their growth. When the C/N balance of the soil is less than 25:1, microorganisms proliferate fast, N breaks down quickly, and excess N is produced, which is transferred to the ground for crop use. The Pearson correlation analysis provided valuable insights into the relationships between N-induced changes in soil parameters and microbial activities, while highlighting the influence of N fertilization on soil microbial dynamics. However, caution must be exercised in interpreting these correlations, considering potential confounding factors and the limitations of the dataset.

3.4. Controlling Factors and Pathways for the *Ra* and *Rh*

According to a random forest analysis, ST, BNPP, SIN, and C/N were the most critical factors that affected *Ra* (Figure 6a). Figure 6b indicated that the most effective predictors of *Rh*, however, were MBC, SIN, and MD (all $p < 0.01$). The study can account for 56% and 80%, respectively, of the variation in *Ra* and *Rh*. Figure 6 indicated that ST, BNPP, SIN, and C/N predominantly controlled the *Ra* variation. The primary factors affecting soil *Rh* were MBC, SIN, and MD. Soil MBC reflects the amounts of microbes gaged in *Rh*, which is adversely affected by excessive N and corroborates global evidence of a decline in MD and MR [50,56,57]. A recent meta-analysis showed that the global soil microbial biomass carbon (MBC) decreased by an average of 11%. This decline was positively correlated with increasing rates of N addition to the soil [58]. Excessive N also has a specific impact on MD and MR; reducing the formation of refractory compounds increases the availability of C to soil microorganisms, for instance, alkyl, lignin, and aromatic C, ultimately inhibiting *Rh* [59,60].

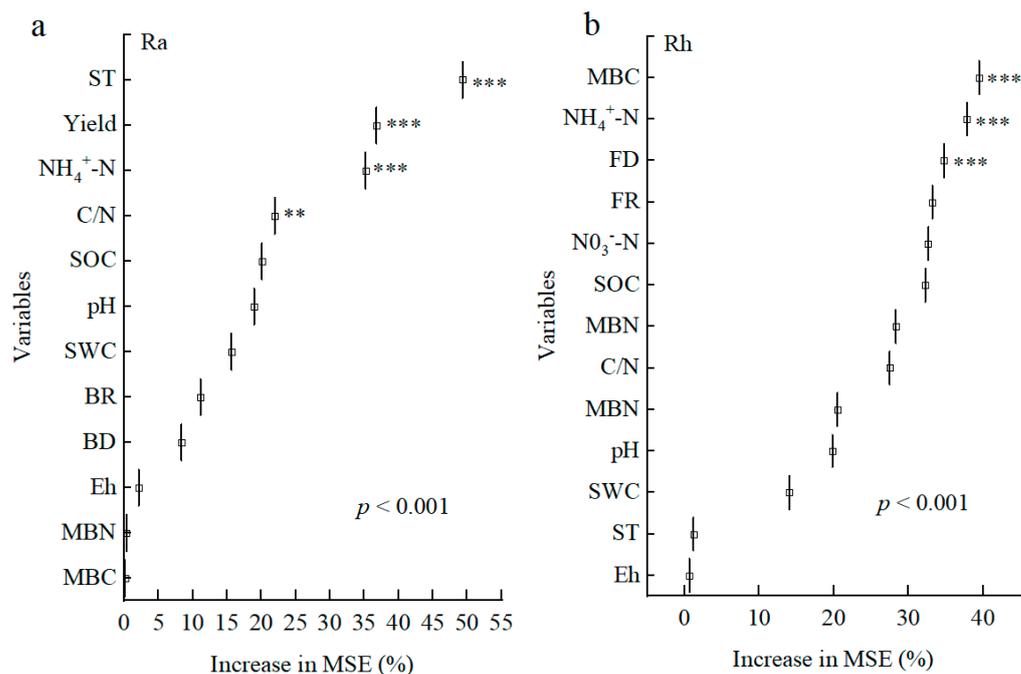


Figure 6. Box plots of the relative contributions of driving factors on Ra and Rh . The most significant factors influencing Ra were ST, BNPP, SIN, and C/N, whereas Rh was most accurately predicted by MBC, SIN, and MD, all with p -values less than 0.01. This study explained 56% and 80% of the variance in Ra and Rh , respectively. Note: (a,b) represent Ra and Rh , respectively. The importance of predictor variables is assessed based on the percentage increased mean square error (lnMSE) from 100 runs of the random forest model, with $** p < 0.01$, $*** p < 0.001$.

This investigation revealed N response patterns and the parameters influencing Ra and Rh in a cabbage field. Figure 6 demonstrates that ST, BNPP, SIN, and C/N were the most dominant drivers of Ra , while the three most important drivers of Rh were MBC, SIN, and MD. One of the significant essential elements limiting plant growth is temperature [61]. Ra largely depends on associated rhizosphere respiration [62]. Moreover, Ra can be impacted by slight temperature changes that immediately modify the supply of plant photosynthates [11]. The reduction in ST with increased N enrichment rates can be due to a decrease in the amount of photosynthetic C delivered to the roots [63], which would have an impact on Ra . Figures 5b and 6 indicate that the lack of a significant response of Rh to N fertilizer can be ascribed to the corrective effects of the enhanced MBC on the Rh . The enhanced MBC with N treatment was consistent with earlier investigations [64] because MBC and MBN restrictions have been relieved due to an improved aboveground C substrate supply. According to the N mining theory [65], when N is sufficient to suit the needs of soil microorganisms, labile C should be preferentially degraded over recalcitrant C, which reduces soil labile C, which suppresses microbial activity.

In PLS-PM analysis (Figure 7), ST and BNPP were identified as direct controllers of Ra , while ST and MBC drove Rh . SIN had an indirect effect on Ra , and its direct effect on Rh was weak ($p < 0.1$). Overall, the dominant drivers controlling Ra have decreased ST and increased BNPP, considering the combined effects of various drivers (Figure 7). BNPP and SIN directly exerted a positive effect on Ra . In contrast, the differences in Rh were primarily explained by MBC, SIN, and MD (Figure 7).

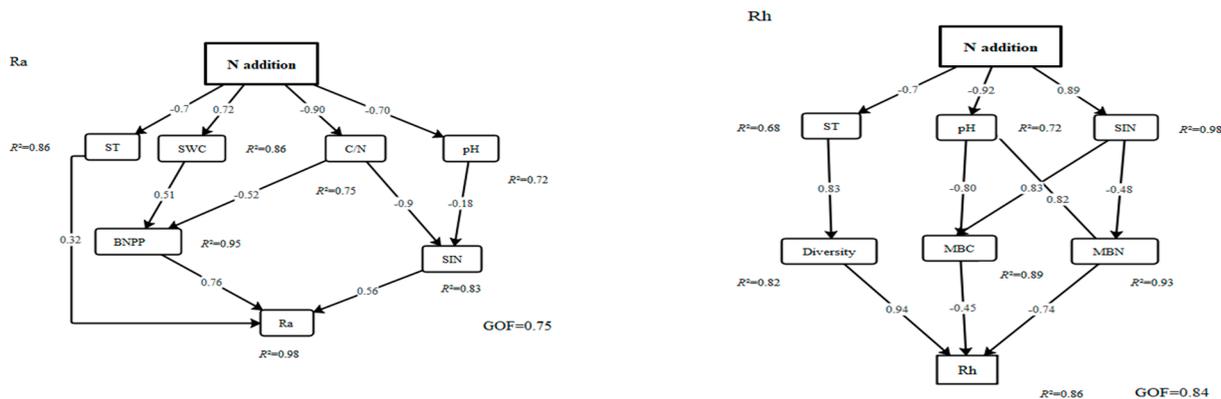


Figure 7. PLS-PM for the effect of N addition on the soil-microbial-biomass system. ST and BNPP are the primary direct regulators of *Ra*, while ST and MBC are the main drivers of *Rh*. SIN indirectly influenced *Ra*, with a weak direct impact on *Rh* ($p < 0.1$). Changes in *Ra* are predominantly governed by decreased ST and elevated BNPP, mediated by various factors. Both BNPP and SIN positively influenced *Ra*, whereas *Rh* variations were primarily attributed to MBC, SIN, and MD. Note: The letters a and b represent *Ra* and *Rh*, respectively.

The high rates of N fertilization in earlier studies may overestimate the impact of N addition on *Rs* due to the potential decline or stabilization of background N deposition rates over several decades. Consequently, the impact of N addition on *Rs* is determined by adding the experimental N addition rate to the background N deposition rate [66]. Therefore, accounting for early environmental conditions is critical to accurately predicting the effect of N fertilization on soil processes, especially in light of how crucial *Rs* is to maintaining the net ecosystem C balance.

4. Conclusions

The investigation uncovered the impact of nitrogen fertilization on the nitrogen cycling dynamics within cabbage cultivation sites in the designated experimental area. The augmentation of nitrogen was found to have modulated several soil and microbial parameters, specifically leading to a reduction in ST, pH, C/N, microbial diversity (MD), and MR. Conversely, it resulted in an elevation of SOC, SIN, MBC, MBN, ANPP, and BNPP. These observed alterations appeared to be facilitated by the intricate interactions between soil microorganisms, soil properties, and plant biomass.

Furthermore, the application of nitrogen was associated with a decline in *Rs* and *Rh*, but an increase in *Ra* in the cabbage cultivation experiment. A detailed analysis revealed that ST, BNPP, SIN, and C/N emerged as significant factors influencing *Ra*, while MBC, SIN, and MD primarily governed *Rh*. Notably, ST and SIN exhibited a direct influence on *Rh*, whereas ST and BNPP indirectly influenced *Ra*.

Our findings provide valuable insights into how nitrogen enrichment shapes *Ra* and *Rh* dynamics in cabbage fields. Based on our observations, we propose that an optimal nitrogen application rate of $150 \text{ kg N hm}^{-2} \cdot \text{yr}^{-1}$ would be beneficial in this experimental region, balancing plant growth requirements with soil health and microbial activity. Another thing that should be mentioned is that while the PLS-PM approach offers a comprehensive framework for analyzing complex relationships in nitrogen-enriched cabbage fields, its application should be accompanied by a critical assessment of data limitations and model validity. Future research efforts should aim to address these challenges by incorporating robust experimental designs, increasing sample sizes, and integrating complementary analytical techniques to enhance the reliability of findings and facilitate more informed management decisions in agricultural systems.

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