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Abstract

Planting cover crop has been suggested as a way of increasing soil organic carbon in agricultural land. Ryegrass (*Lolium multiflorum* L.), as a cover crop, could improve soil fertility and lower soil CO₂ emission. However, effects of soil water content and nitrogen on soil carbon mineralization after ryegrass incorporation are not fully understood. The present study was to investigate the effect of soil water content and nitrogen rate on soil carbon mineralization after ryegrass incorporated into upland red soil (Ferralsols). A laboratory experiment was established, including soil water contents [15% (W1), 30% (W2), 45% (W3)] and nitrogen rates [0 (N1), 60 mg/kg(N2), 120 mg/kg(N3)]. The results showed that the highest soil carbon mineralization accumulation was observed in W3N3. Nitrogen application inhibited carbon mineralization rate and accumulation in the late stage of ryegrass incorporation at W1, but increased carbon mineralization rate and accumulation at W2. With increasing soil water content, nitrogen application could improve soil carbon mineralization at the early stage of ryegrass incorporation. In conclusion, soil nitrogen and water content could regulate soil carbon mineralization. Considering to reduce the soil CO₂ emissions, rational nitrogen application should be taken seriously during cover crop (ryegrass) incorporated into the upland red soil.

Full Text

Preamble

Soil Carbon Mineralization as Affected by Water Content and Nitrogen Rate After Ryegrass Incorporation into Soil

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Abstract

Planting cover crops has been suggested as an effective way to increase soil organic carbon in agricultural land. Ryegrass (*Lolium multiflorum* L.), when used as a cover crop, can improve soil fertility and reduce soil CO₂ emissions. However, the effects of soil water content and nitrogen application on soil carbon mineralization following ryegrass incorporation are not fully understood. The present study investigated how soil water content and nitrogen rate influence soil carbon mineralization after ryegrass incorporation into upland red soil (Ferralsols). A laboratory experiment was conducted with three soil water contents [15% (W1), 30% (W2), 45% (W3)] and three nitrogen rates [0 (N1), 60 mg/kg (N2), 120 mg/kg (N3)]. The results showed that the highest cumulative soil carbon mineralization occurred in the W3N3 treatment. Nitrogen application inhibited carbon mineralization rate and accumulation during the late stage of ryegrass decomposition at W1, but increased both parameters at W2. With increasing soil water content, nitrogen application enhanced soil carbon mineralization during the early stage of ryegrass incorporation. In conclusion, soil nitrogen and water content can regulate soil carbon mineralization. To reduce soil CO₂ emissions, rational nitrogen application should be carefully considered when incorporating cover crops (ryegrass) into upland red soil.

Keywords: Ferralsols, carbon mineralization rate, accumulation, first-order kinetic equation

Introduction

Soil organic carbon plays a significant role in the global carbon cycle [?]. Soil carbon decomposition and sequestration are primarily affected by cropping systems [?], tillage methods, cover crops [?], organic manure, and chemical fertilizer [?] under field conditions. Soil carbon mineralization is limited by environmental factors such as field moisture capacity [?], temperature [?], and climate [?]. Soil water content influences soil oxygen availability, which can inhibit soil microbial activity and consequently affect soil carbon mineralization [?]. The effects of exogenous nitrogen input on soil carbon mineralization vary with environmental factors [?]. Most previous research has focused on forests [?], wetlands [?], grasslands [?], and croplands [?], but few studies have examined the effects of soil water content and nitrogen rate on soil carbon mineralization after cover crop incorporation into red soil.

Red soil is the third most important soil type worldwide, covering 13% of the

land area [?]. It is also a crucial land resource in southern China due to its great potential for agricultural production. However, several problems remain, including uneven spatiotemporal distribution of precipitation, serious soil and water loss, land degradation, and excessive fertilizer application [?]. These soils are highly leached and severely acidified, with low organic matter and nutrient deficiencies [?]. Nevertheless, the red soil region belongs to subtropical zones in China that have abundant natural resources—light, temperature, and water—for planting winter cover crops.

Cover crops can scavenge nutrients, thereby decreasing nitrate leaching [?], alleviating soil erosion [?], and improving soil organic matter [?]. Ryegrass, as a forage grass or cover crop, exhibits high cold resistance, high biomass production, and rich nutritional value. Sowing ryegrass can mitigate nitrous oxide emissions [?] and remediate contaminated soil [?]. Ryegrass incorporation can improve crop yield and soil quality in paddy fields [?], and ryegrass residues can lower soil CO_2 emissions while increasing soil carbon stocks [?]. Soil carbon mineralization serves as an important indicator for measuring soil quality [?]. Planting ryegrass as a cover crop after sweet corn harvest in late October and incorporating it into soil in early April the following year—could this practice improve soil quality? Our previous results showed that applying chemical nitrogen fertilizer (60 mg/kg) could inhibit ryegrass decomposition and nitrogen release [?] as well as soil nitrogen mineralization [?] during the early stage of ryegrass incorporation, which might alleviate soil nitrogen loss. However, the effect of soil water content and nitrogen rate on soil carbon mineralization after ryegrass incorporation remains unclear.

The objectives of this laboratory experiment were to investigate the influence of soil water content and nitrogen rate on soil carbon mineralization and to optimize soil water and nitrogen management when incorporating ryegrass into upland red soil. We hypothesized that soil water content and nitrogen rate could affect ryegrass decomposition and thereby regulate soil carbon mineralization in upland red soil.

Materials and Methods

2.1 Ryegrass and Soil

Ryegrass (*Lolium perenne* L. cv. ‘Ganxuan No.1’) was planted after sweet corn harvest on October 15, 2013, and harvested before sweet corn sowing on April 1, 2014. The ryegrass was cut into small pieces and dried in an oven at 105°C for 30 minutes, then at 80°C until constant weight was achieved. The main nutritional components of the dried ryegrass were: total C 360.19 g/kg, total N 27.62 g/kg, C/N ratio 13.04, total P 21.08 g/kg, and total K 67.17 g/kg.

The experimental soil, classified as Ferralsols, was collected from the 0–20 cm layer at Jiangxi Agricultural University Sci-tech Park in Nanchang, China (28°45' N, 115°50' E). The soil was air-dried, visible plant roots were removed,

and it was passed through a 2-mm sieve for the incubation study. The basic chemical properties of the soil are shown in Table 1.

Table 1. Soil chemical characteristics

Soil type	Soil Organic C (g/kg)	Total N (g/kg)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
Original soil	4.85	—	—	—	—
Incubated soil	4.65	—	—	—	—

2.2 Soil Incubation

The experiment followed a completely randomized design with three replicates, conducted from May 11 to August 8, 2014. Three soil water contents [15% (W1), 30% (W2), 45% (W3)] and three nitrogen rates [0 (N1), 60 mg/kg (N2), 120 mg/kg (N3)] were tested. Dry ryegrass (2.5 g) was mixed into 100 g of soil to create the incubation soil. Each 250 ml jar was packed with 50 g of incubation soil. The jars received 7.5, 15, and 22.5 ml of deionized water for W1, W2, and W3, respectively, and 0, 14.15, and 28.30 mg of ammonium sulfate ($(\text{NH}_4)_2 \cdot \text{SO}_4$) for N1, N2, and N3, respectively.

2.3 Measurement of Soil Organic Carbon Mineralization

Potential carbon mineralization was measured using the soil CO_2 flux method described by [?]. A centrifuge tube (10 ml) containing 5 ml of 1 N NaOH was placed in each jar, and the jars were sealed airtight with wax, then incubated in the dark at 25°C. The tubes were collected on days 1, 3, 7, 13, 21, 31, 43, 57, 73, and 91. The amount of CO_2 -C produced during incubation was trapped in 1 M NaOH. For analysis, 1 ml of saturated BaCl_2 and phenolphthalein indicator were added to each tube, and the amount of CO_2 -C was determined by titration against 0.1 M HCl using an acid burette.

2.4 Parameter Simulation and Data Analysis

This experiment used a first-order kinetic model to assess the soil carbon mineralization process [?]:

$$C_t = C_0 \times (1 - e^{-kt})$$

The first-order kinetic model was simulated using Matlab 7.0, where C_t is the total organic carbon at time t , C_0 is the amount of potential soil carbon mineralization, k is the decay constant of soil carbon mineralization rate, and t is the incubation time.

The following calculations were performed: - Soil carbon mineralization rate ($\text{mg} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$) = Total soil carbon mineralization / days of incubation - Actual carbon mineralization rate (%) = Total soil carbon mineralization / Soil carbon content in incubated soil $\times 100\%$ - Potential carbon mineralization rate (%) = Total potential soil carbon mineralization / Soil carbon content in incubated soil $\times 100\%$ - Net carbon mineralization ($\text{mg} \cdot \text{g}^{-1}$) = Total soil carbon mineralization of nitrogen treatment – Total soil carbon mineralization without nitrogen treatment - Net carbon mineralization rate (%) = Net carbon mineralization / Soil carbon content in incubated soil $\times 100\%$

Statistical significance of differences among treatments was tested using ANOVA (SPSS 19.0, SPSS Inc., USA), with Duncan's test used to compare means. Differences at $p < 0.05$ were considered statistically significant.

Results

3.1 Dynamics of Soil Carbon Mineralization

Soil carbon mineralization was influenced by both nitrogen rate and soil water content. At W1, soil carbon mineralization under N2 and N3 was significantly reduced compared to N1 from day 7 to day 91, with decreases of 13.57% and 12.24% on day 91, respectively. In contrast, at W2 and W3, N3 significantly increased soil carbon mineralization compared to N1, with increases of 6.85% and 9.89% on day 91, respectively. No significant difference was observed between N2 and N3, and the highest soil carbon mineralization content was recorded in W3N3, reaching 4.14 mg/kg (Figure 1).

Different soil water contents exerted varying effects on soil carbon mineralization at the same nitrogen rate. Under N1, soil carbon mineralization in W2 was drastically reduced compared to W1 on days 1 and 3, while W3 significantly reduced mineralization on days 31 and 43 but increased it on day 13. Under N2 and N3, increasing soil water content (W2 and W3) significantly enhanced soil carbon mineralization during the middle and late periods (day 7-91) compared to W1, with no significant difference between W2 and W3.

Figure 1. Dynamics of soil carbon mineralization

Note: W1, W2, and W3 represent soil water contents of 15%, 30%, and 45%, respectively. N1, N2, and N3 represent nitrogen rates of 0, 60, and 120 mg/kg, respectively. Error bars indicate standard error of the mean ($n = 3$). Values with different lowercase letters within the same column indicate significant differences at $P < 0.05$.

Two-factor analysis of variance indicated that both nitrogen application and soil water content significantly affected soil carbon mineralization (Table 2). At the same water content (W1), increasing nitrogen fertilization (N2 and N3) significantly inhibited soil carbon mineralization amount and rate compared to N1, though no obvious difference existed between N2 and N3. Under W2 and W3,

increasing nitrogen fertilization (N3) significantly increased soil carbon mineralization amount and rate compared to N1, with N3 producing significantly higher amounts than N2. At the same nitrogen rate (N1), increasing soil water content (W2 and W3) did not significantly affect soil carbon mineralization amount and rate compared to W1. However, at N2 and N3, soil water content (W2 and W3) significantly increased soil carbon mineralization amount and rate compared to W1, with W3 showing obvious improvement over W2.

Net carbon mineralization amount and rate at N2 and N3 both showed negative values under W1. Under W2 and W3, net carbon mineralization amount and rate were significantly higher at N3 than at N2. At the same nitrogen rate (N1), no significant difference in net carbon mineralization amount and rate was observed among W1, W2, and W3.

Table 2. Effect of soil water content and nitrogen rate on accumulation and rate of soil carbon mineralization

Factors	Treatment	Accumulation	Mineralization	Net mineralization	Net mineralization
		(mg/g)	rate (%)	(mg/g)	rate (%)
Nitrogen rate	N1	3.75 \pm 0.02cd	21.50 \pm 0.12b	—	—
		0.51 \pm 0.06d	—		
		2.92 \pm 0.32d	N3 3.29 \pm 0.06e	18.86 \pm 0.33cd	—
		0.46 \pm 0.05d	—		
		2.63 \pm 0.31d	Soil water content W1 3.72 \pm 0.05d	18.62 \pm 0.33d	0.10 \pm 0.05bc
				0.55 \pm 0.31bc	W2 3.8
Nitrogen rate	W1N1	3.76 \pm 0.08cd	21.60 \pm 0.46b	—	—
×				W1N2 3.89 \pm 0.07bc	22.30 \pm 0.43b
Soil water content				—	—
				W1N3 4.14 \pm 0.02a	
Two-factor variance analysis (F value)	Nitrogen rate	4.769*	6.417**	20.271**	27.270**
Soil water content		115.147**	103.013**	24.139**	16.726**

Factors	Treatment (mg/g)	Accumulation rate (%)	Mineralization rate (%)	Net mineralization (mg/g)	Net mineralization rate (%)
Nitrogen rate	—	—	—	—	—
× Soil water content	—	—	—	—	—

*Note: Values with different lowercase letters within the same column indicate significant differences at $p < 0.05$. * indicates significant differences at $p < 0.05$, ** indicates extremely significant differences at $p < 0.01$.*

3.2 Dynamics of Soil Carbon Mineralization Rate

All treatments exhibited maximum soil carbon mineralization rates on day 1 of incubation, which then dropped rapidly until day 91 (Figure 2). At W2, soil carbon mineralization rates of N2 and N3 were significantly higher than that of N1. At W3, the mineralization rate of N2 decreased by 7.26% compared to N1, while that of N3 increased by 5.73%. Under N1, the mineralization rate of W2 was significantly decreased compared to W1, but the rate of W3 was obviously higher than that of W2. Under N2 and N3, no significant difference in soil carbon mineralization rate was observed among W1, W2, and W3. However, the mineralization rate of W2 was significantly higher than that of W3 at N2. The analysis results in Table 3 show that both nitrogen rate and soil water content significantly influenced soil carbon mineralization rate.

Figure 2. Dynamics of soil carbon mineralization rate

Note: W1, W2, and W3 represent soil water contents of 15%, 30%, and 45%, respectively. N1, N2, and N3 represent nitrogen rates of 0, 60, and 120 mg/kg, respectively. Error bars indicate standard error of the mean ($n = 3$). Values with different lowercase letters within the same column indicate significant differences at $P < 0.05$.

Table 3. Two-way analysis of variance of soil carbon mineralization rate

Incubated days	Nitrogen rate	Soil water content	Nitrogen rate × Soil water content
1	12.93**	36.026**	14.511**
3	8.956**	111.088**	7.689**
7	8.049**	71.872**	7.313**
13	4.704*	57.272**	16.902**
21	7.791**	44.554**	22.200**

Incubated days	Nitrogen rate	Soil water content	Nitrogen rate × Soil water content
31	5.257*	99.258**	26.136**
43	5.099*	63.008**	33.159**
57	8.431**	92.652**	28.477**

Note: * indicates significant differences at $p < 0.05$, ** indicates extremely significant differences at $p < 0.01$.

3.3 Kinetic Parameters of Soil Carbon Mineralization

The carbon mineralization curves fit well with the first-order kinetic model ($R^2 = 0.97\text{--}0.99$) (Table 4). Across all treatments, the highest potential mineralization amount was observed in W2N3, while the lowest was recorded in W1N2. At the same nitrogen rate (N1, N2, N3), W2 significantly increased the potential mineralization amount C_0 compared to W1. However, no obvious difference was found between W2 and W3 at N2 and N3. Appropriate increases in soil water content and nitrogen rate could enhance the potential mineralizable carbon in red soil during ryegrass incorporation.

Both nitrogen rate and soil water content significantly impacted the K value. The K value of W3N3 was significantly higher than all other treatments. The K values of each treatment at W2 were significantly lower than those at W1 and W3. The maximum potential mineralization rate was obtained from W2N3, and the minimum from W1N2.

Table 4. Kinetic parameters of soil carbon mineralization

Fitting parameters	Treatment	Potential mineralization amount C_0 (mg/g)	Mineralization rate constant K (d^{-1})	Potential mineralization rate (%)
Nitrogen rate	N1	3.89 ^{±0.17bcd} 0.0271 ^{±0.0026b} 22.33 ^{±0.96cde} N2 3.38 ^{±0.06e} 0.0267 ^{±0.0004b} 19.39 ^{±0.33}		
Nitrogen rate × Soil water content	W1N1	3.97 ^{±0.08bc} 0.0246 ^{±0.0002b} 22.76 ^{±0.46bcd} W1N2 3.93 ^{±0.10bc} 0.0277 ^{±0.0012b} 22.58 ^{±0.0}		
Two-factor variance analysis (F value)	Nitrogen rate	34.648**	4.82*	32.775**

Fitting parameters	Potential mineralization amount C_0 (mg/g)	Mineralization rate constant K (d $^{-1}$)	Potential mineralization rate (%)
Soil water content	108.878**	3.246*	11.673**
Nitrogen rate		—	3.313*
Soil water content			

Note: Values with different lowercase letters within the same column indicate significant differences at $p < 0.05$. * indicates significant differences at $p < 0.05$, ** indicates extremely significant differences at $p < 0.01$.

Discussion

The present work demonstrated that exogenous nitrogen addition inhibited soil carbon mineralization under low soil water content (15%). Under such conditions, soil microorganisms would prioritize aerobic respiration, decomposing small molecular substances, enhancing microbial activity, and accelerating mineralization efficiency [?]. Low soil water content might inhibit soil enzyme activity [?] and microbial activity [?], while excessive mineral nitrogen could increase the aromaticity and complexity of dissolved organic matter (DOM) molecules [?], which might mitigate the mineralization of soil organic matter.

Increasing soil water content (30% and 45%) combined with nitrogen fertilizer application improved soil carbon mineralization in our results. Similar findings have been reported in wheat-maize cropping systems [?], floodplain wetlands [?], and the Wuyi mountains [?]. Increasing nitrogen fertilization could enhance soil carbon mineralization during ryegrass incorporation at soil water contents of 30% and 45%. Reasonable soil moisture can increase microbial activity [?] and improve rates of aerobic heterotrophic respiration [?], thereby increasing soil carbon mineralizability. Nitrogen fertilizer can increase soil carbon mineralization compared to treatments without nitrogen fertilizer [?]. The initial nitrogen concentration of residues exerts a strong influence on carbon mineralization. High nitrogen content supplies available nitrogen to soil microorganisms in the short term and stimulates microbial activity [?]. Residues with high nitrogen

concentrations and low C/N ratios can accelerate initial carbon mineralization [?], which benefits soil organic carbon mineralization.

High soil carbon mineralization rates were observed during the early stage (the first 7 days) after ryegrass incorporation. This likely occurred because sufficient carbon and nitrogen resources in the soil and rapidly degrading ryegrass provided fundamental materials for soil microorganism reproduction during the initial period. In our study, nitrogen application inhibited the rate of soil carbon mineralization at low soil water content. Soil enzyme activity is a key factor in soil organic matter decomposition and may be limited by low soil water content [?]. As soil water content increased, adding nitrogen fertilizer significantly improved the soil carbon mineralization rate, consistent with Li et al. [?]. Previous studies found that increasing water content could improve soil carbon mineralization rate at 45–75% of upland field capacity [?]. Reasonable soil water content might promote soil microbial and enzymatic activities [?], while nitrogen fertilizer supplies more available nitrogen [?], both leading to higher rates of soil carbon mineralization.

In our incubation experiment, the first-order kinetic equations provided a good fit for SOC mineralization processes, similar to previous reports [?]. Soil water content significantly influenced potential carbon mineralization. Potential mineralizable C (C_0), determined from the equation, measures active or labile and easily decomposable SOC. These parameters can be used to determine the effects of agricultural practices and tillage on carbon sequestration and short-term nutrient turnover or fertility [?].

Conclusions

Nitrogen application significantly inhibited soil carbon mineralization during the late stage of ryegrass incorporation at 15% soil water content, but increased carbon mineralization at 30% soil water content. Without nitrogen application, increasing soil water content significantly inhibited soil carbon mineralization during the early stage of ryegrass incorporation. However, soil water content increased soil carbon mineralization when nitrogen was applied. In conclusion, nitrogen fertilizer application and soil water content significantly affected soil carbon mineralization after ryegrass incorporation into red soil.

To improve soil carbon sequestration and reduce greenhouse gas emissions, rational nitrogen application should be carefully considered when incorporating cover crops (ryegrass) into upland red soil.

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Note: Figure translations are in progress. See original paper for figures.

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