# Effects of vegetation restoration in karst areas on soil nitrogen mineralisation (#100050)



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# Effects of vegetation restoration in karst areas on soil nitrogen mineralisation

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**Background.** Nitrogen mineralization is a crucial process in the ecosystem cycle, impacting ecosystem function and the nitrogen biogeochemical cycle. Therefore, studying the evolutionary traits of soil nitrogen mineralization in karst vegetation restoration is essential for understanding the significance of vegetation restoration in the terrestrial nitrogen cycle. **Methods.** This study analyzed soil profiles from different vegetation growth stages, including a 40-year-old woodland,20-year-old shrubland,15-year-old shrubland,5-year-old grassland, and nearby cropland. The aerobic incubation technique was utilized for 35 days to evaluate soil N mineralization features and their correlation with soil environmental factors. The investigation focused on variations in soil N mineralization rate, N nitrification rate, net nitrification rate, and $NH_4^+$-N and $NO_3^-$-N levels. **Results.** Nitrate nitrogen, the main form of inorganic nitrogen, displayed a 19.38% increase in the 0-40cm soil layer in the 20-year-old shrubland compared to the cultivated land. Soil $NH_4^+$-N levels exhibited fluctuations throughout the incubation period, with decreased levels on the 14th and elevated levels on the 21st. Soil $NO_3^-$-N and inorganic nitrogen levels showed a pattern of increase, decrease, and stabilization, reaching a peak on the 14th day of incubation. Soil nitrogen （NR） and nitrogen mineralization rate （NMR） decreased over time during vegetation restoration. Shrubland after 15 years, shrubland after 20 years, and woodland after 40 years displayed higher soil NR and NMR content. Specifically, shrubland after 15 years and shrubland after 20 years also demonstrated an increase in soil available nitrogen （AR） content. The Mantel test analysis revealed positive correlations between total nitrogen （TN）, total phosphorus （TP）, total potassium （TK）, silicon （Si）, AR, NR, and NMR. Furthermore, available phosphorus （AP） and NMR exhibited positive correlations with NR and NMR. Moreover, TN, TP, TK, and Si were positively associated with AR, NR, and NMR, while AP and nitrate nitrogen ($NO_3^-$-N) showed negative correlations with AR, NR, and

NMR. It is noteworthy that ammonium nitrogen ($NH_4^+$-N) had the most substantial impact on AR, bulk density BD had the most significant influence on NR, and ammonium nitrogen AN and soil organic carbon SOC had the most significant effect on NMR.

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## Abstract

**Background.** Nitrogen mineralisation plays a critical role in the ecosystem cycle, influencing ecosystem function and the nitrogen biogeochemical cycle. Therefore, it is essential to investigate the evolutionary characteristics of soil nitrogen mineralisation in karst vegetation restoration to comprehend the importance of vegetation restoration in the terrestrial nitrogen cycle.

**Methods.** This study analysed soil profiles from different vegetation growth stages, including a 40-year-old woodland, 20-year-old shrubland, 15-year-old shrubland, 5-year-old grassland, and nearby cropland. The aerobic incubation technique was utilised for 35 days to evaluate soil N mineralisation features and their correlation with soil environmental factors. The investigation focused on variations in soil N mineralisation rate, N nitrification rate, net nitrification rate, and $NH_4^+$-N and $NO_3^-$-N levels.

**Results.** Nitrate nitrogen, the main form of inorganic nitrogen, displayed a 19.38% increase in the 0-40 cm soil layer in the 20-year-old shrubland compared to the cultivated land. Soil $NH_4^+$-N levels exhibited fluctuations throughout the incubation period, with decreased levels on the 14th and elevated levels on the 21st. Soil $NO_3^-$-N and inorganic nitrogen levels showed a pattern of increase, decrease, and stabilisation, reaching a peak on the 14th day of incubation. Soil nitrogen (NR) and nitrogen mineralisation rate (NMR) decreased over time during vegetation restoration. Shrubland after 15 years, shrubland after 20 years, and woodland after 40 years displayed higher soil NR and NMR content. Specifically, shrubland after 15 years and shrubland after 20 years also demonstrated an increase in soil available nitrogen (AR) content. The Mantel test analysis revealed positive correlations between total nitrogen (TN), total phosphorus (TP), total potassium (TK), silicon (Si), AR, NR, and NMR. Furthermore, available phosphorus (AP) and NMR exhibited positive correlations with NR and NMR. Moreover, TN, TP, TK, and Si were positively associated with AR, NR, and NMR, while AP and nitrate nitrogen ($NO_3^-$-N) showed negative correlations with AR, NR, and NMR. It is noteworthy that ammonium nitrogen ($NH_4^+$-N) had the most substantial impact on AR, bulk density (BD) had the most significant influence on NR, and ammonium nitrogen (AN) and soil organic carbon (SOC) had the most significant effect on NMR.

## Introduction

Nitrogen plays a vital role in soils within terrestrial ecosystems, significantly affecting the growth of plants and their primary productivity(Delgado-Baquerizo et al., 2014). The availability of nitrogen in the soil often serves as a limiting factor for net primary production(Chapin et al., 2011), as plants predominantly absorb inorganic nitrogen(Li et al., 2013). Despite this, the majority of nitrogen present in the soil is in organic form. The ratio of organic to inorganic nitrogen, referred to as the soil's net nitrogen mineralisation rate (NMR), reflects the efficiency of soil nitrogen(Risch et al., 2020). The conversion of organic nitrogen to inorganic nitrogen, known as nitrogen mineralisation, directly influences nitrogen availability in the soil(Zhong and Makeschin, 2004), which is crucial for the growth of plants and ecosystem services(Booth et al., 2005). The mineralisation of organic nitrogen includes the processes of ammonification and nitrification, which are carried out by soil microorganisms(Maslov and Maslova, 2022). Several factors, including land use, soil characteristics, pH levels, temperature, moisture levels, type of vegetation, apoplastic quality, microbial populations, and human activities, can impact the mineralisation of nitrogen in the soil(Templer et al., 2005). Changes in nitrogen mineralisation have implications for nitrogen availability(Schlesinger and Bernhardt, 2013), primary productivity, ecosystem functioning, and long-term sustainability(Chen et al., 2009; Heitkamp et al., 2008). Thus, the characteristics of nitrogen mineralisation serve as critical indicators for evaluating soil quality.

Multiple studies have demonstrated that soil nitrogen (N) mineralisation is significantly affected by changes in land use and restoration techniques (Gurlevik and Karatepe, 2016; Li et al., 2018a; Wang et al., 2017). These effects stem mainly from differences in plant diversity and abundance, as well as variations in soil physical, chemical, and microbial properties under different restoration methods (Deng et al., 2014; Rhoades and Coleman, 1999). The impact of vegetation on N mineralisation is contingent upon the type of vegetation present, affecting both the quantity and quality of organic matter and the efficiency of N uptake by plants (Rahman et al., 2017; Unver et al., 2014). While much of the literature focuses on the consequences of altering land use(Contosta et al., 2011; Li et al., 2014), resulting in conflicting opinions on the direction and magnitude of N transformations following vegetation restoration (Li et al., 2014), several studies have reported conflicting findings, with some indicating an increase (Gurlevik and Karatepe, 2016; Wang et al., 2017), a decrease (Li et al., 2014; Yang et al., 2010), or no significant changes in N mineralisation rates (Zeng et al., 2009). Owen et al. (2003) observed higher soil mineralisation rates in forests compared to grasslands due to differences in carbon assimilation among plant functional groups and soil characteristics. Wei et al. (2017) found that root nitrogen content decreased alongside higher biomass despite increased functional group abundance, leading to a decline in the net soil nitrogen mineralisation rate. Seasonal variations in soil nitrogen availability and turnover are significant (Dujardin et al., 2012). Environmental factors such as moisture, temperature, and pH directly influence microbial activity (Unver et al., 2014; Ye et al., 2015). Dujardin et al. (2012) found that the peak in soil ammonium content occurs during summer due to increased microbial activity. Hu et al. (2015) determined that soil nitrogen transformations were similar in biocrust-covered soils and bare ground, attributed to decreased microbial abundance and activity in extremely low temperatures. However, there is a lack of research on soil nitrogen mineralisation in profiled soils in karst ecosystems. Ecosystem responses to these factors vary (Booth et al., 2005; Tapia-Torres et al., 2015; Zhou et al., 2009), highlighting the necessity for site-specific assessments of nitrogen transformation (Burke, 1989; Liu et al., 2017). Additionally, the impact of vegetation restoration on soil nitrogen mineralisation and the influence of soil environmental factors on this process remain poorly understood in most ecosystems. These knowledge gaps impede the precise prediction of nitrogen biogeochemical cycling.

The Southwest Karst region is acknowledged as one of the world's three continuous karst distribution zones (Sheng et al., 2018). Throughout the latter half of the 20th century, substantial carbonate development occurred in this area, leading to shallow soil stratum, intricate karst ecosystems, high human population density, and frequent human activities. These elements contributed to significant vegetation destruction and ecosystem degradation (Wang et al., 2004). To combat these challenges, numerous vegetation restoration projects were initiated by the Chinese government in the region (Basile-Doelsch et al., 2020; Chen et al., 2018; Wang et al., 2018). Despite the increased vegetation cover resulting from these initiatives, the impacts (Li et al., 2019) and the underlying mechanisms of long-term restorations on soil inorganic nitrogen accumulation (Li et al., 2019; Liu et al., 2024)and nitrogen mineralisation remain uncertain. This investigation explores the condition of soil nutrients in the restoration area, focusing on soils derived from calcareous materials in the karst region. The study assesses the nitrogen mineralisation characteristics of different vegetation restoration soils and investigates the soil environmental factors that influence nitrogen mineralisation. Through these comparative analyses, our goal is to establish a theoretical basis for evaluating the impacts of vegetation restoration on nitrogen mineralisation in karst regions.

# Materials & Methods

## Study area

Pingba District (26°15′–26°37′40″N, 105°59′20″–106°33″43″E), Anshun City, Guizhou Province, falls in a subtropical humid monsoon climate zone and has an elevation and average annual temperature of 963–1,645.6 meters and 13.3 °C, respectively. The study area has complex topographic conditions, characterised by typical karst landscapes and diverse types of restored vegetation. The parent rock of the study area is dominated by limestone, whereas the soil is mainly calcareous. The maturity of restored vegetation was determined through a combined approach of examining Google Earth images, fieldwork, and visits to local villages (See Figure 1 for details).

**Figure 1 Basic information about the sample plots**

## Selection of sample plots and sampling

The present study identified different types of restored vegetation sample plots with common environmental conditions, focusing on vegetation restoration type, topography, and soil type. These selected restored vegetation types were grassland, shrub grassland, shrub, and woodland with restoration maturities of 5 a, 15 a, 20 a, and 40 a, respectively. In contrast, adjacent cultivated land was used as a control (CK). The dominant vegetation type in grassland was Leucaena [*Imperata cylindrical (L.) Beauv*].; that in shrubs included pyracantha (*Pyracantha fortuneana*), artemisia (Artemisia annua), wild berry (*Rubus et al.*), and wild peppercorn (*Zanthoxylum simulans*); that in woodland included Park and Rowan (*CatalpabungeiC.A.Mey, Celtis sinensis Pers*); that in arable land was maise (*Zea mays*). Table 1 provides more details on the sample plots. After collecting soil samples, visible impurities, such as roots, gravel, plants, and animal debris, were removed, and the samples were filtered through a sieve. Sample points were categorised into 0–5 cm, 5–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm soil stratum. The reliability of the experimental data was ensured through triplicate sampling at each sample point.

**Table 1 Basic Information of Sample Sites**

## Sample analysis and methods

pH was determined through the potentiometric method (water: soil = 2.5:1); total phosphorus (TP) and total potassium (TK) through NaOH dissolution; alkali dissolved N (AN) through the alkali diffusion method; immediate phosphorus (AP) by the 0. 5 mol•$L^{-1}NaHCO_3$ method; primary potassium (AK) by ammonium acetate leaching flame photometry; water content (SMC) through dehydration; and soil bulk-density (BD) and total portfolio porosity (STP) by the ring knife method; soil mechanical composition by the hydrometer method. The classification of soil particles was according to the international system (Ge et al., 2019) [sand (Sa) 2–0.02 mm, powder (Si) 0.02–0.002 mm, and clay (Cl) < 0.002 mm]. A more detailed methodology explanation is provided (Sparks et al., 1996).

The soil's ammonium nitrogen content was obtained using a 2 mol·$L^{-1}$ KCl solution and the indophenol blue colourimetric technique. On the other hand, the nitrate nitrogen levels were determined through the dual-wavelength ultraviolet spectrophotometric approach along with a correction factor.

## Determination of mineralisable nitrogen

Soil organic nitrogen mineralization was evaluated by conducting aerobic incubation. Initially, 60 grams of soil (sieved through a 2 mm mesh) were placed in 250 mL of PE for the incubation process. The soil moisture was set at 30% of its maximum capacity and kept constant at 25°C for a period of 7 days to activate soil microorganisms. Subsequent to the pre-cultivation stage, samples were sealed with black cling film, aerated through punctures, and kept in darkness at 25°C for a total of up to 35 days. Aeration was performed every 3 days for a duration of 30 minutes, with moisture levels monitored through regular weighing. Destructive sampling was conducted on days 7, 14, 21, 28, and 35 following the incubation period. On each occasion, 10 grams of soil were combined with 35 ml of 2 mol·$L^{-1}$ KCl solution (in a 5:1 ratio), shaken for 1 hour, and filtered into plastic containers for subsequent analysis. Ammonium nitrogen content was assessed by leaching with KCl and colorimetric analysis utilizing indophenol blue, while nitrate nitrogen levels were determined using a dual wavelength UV spectrophotometric method with a correction factor.

## Statistical methods

The impact of vegetation restoration on soil inorganic nitrogen components was analysed using a one-way analysis of variance (ANOVA). The Mantel test was employed to determine the significance of different factors on nitrogen mineralisation. The data analysis was performed in SPSS 26.0 for ANOVA, and the Mantel tests, along with visualisation, were completed using the dplyr, ggcor, and ggplot2 packages in R v4.2.2.

## Calculation of Indicators

The formula for calculating the indicator of soil N mineralisation characteristics is as follows:

| |
|---|
| Net ammonification amount (mg/kg) = $NH_4^+$-N content after culture-$NH_4^+$-N content before culture |
| Net nitrification(mg/kg) = $NO_3^-$-N content after culture - $NO_3^-$-N content before culture |
| Soil mineral nitrogen content (mg/kg) = $NH_4^+$-N + $NO_3^-$-N |
| Net mineralisation (mg/kg) = soil mineral N content after incubation-soil mineral N content before incubation |
| Net nitrogen mineralisation rate (mg/kg· d) = net mineralisation (mg/kg)/ culture |

| |
|---|
| days. |
| Net ammonification rate (mg/kg· d) = net ammonification amount (mg/kg) / culture days |
| Net nitrification rate (mg/kg · d) = net nitrification amount (mg/kg) / culture days |

## Results and analysis

### Effects of vegetation restoration on inorganic nitrogen

**Fig. 2 Effects of vegetation restoration on inorganic nitrogen**

The range of soil ammonium nitrogen levels varied from 0.19 to 1.28 mg/kg across different vegetation restoration sites, with the highest concentration observed in shrub soil following two decades of growth. More specifically, the soil's ammonium nitrogen values were documented as 0.84 to 1.28 mg/kg for 40-year-old woodlands, 0.19 to 0.48 mg/kg for 20-year-old shrubs, 0.17 to 0.76 mg/kg for 15-year-old shrub grasslands, 0.15 to 0.36 mg/kg for 5-year-old grasslands, and 0.19 to 0.28 mg/kg for cultivated areas.

The top 5 cm of soil, the ammonium nitrogen content varies from 0.26 to 1.23 mg/kg. Over 40 years, the woodland area showed a significant increase in soil ammonium nitrogen compared to cultivated land, reaching 1.23 mg/kg, a 3.31-fold rise from the cultivated area. The sequence of soil ammonium nitrogen levels is as follows: 40-year-old woodland > 20-year-old shrub > 5-year-old grassland > cultivated land > 15-year-old shrub grassland. This pattern remains consistent in the 5-10 cm soil depth. In the 10-20 cm stratum, the order shifts to 40-year-old woodland > 15-year-old shrubland > cultivated land > 20-year-old shrub > 5-year-old grassland. As we go deeper into the soil, in the 20-30 cm and 30-40 cm stratums, the sequence changes to 40-year-old woodland > 15-year-old shrubland > 20-year-old shrub > 5-year-old grassland > cultivated land, with respective increases compared to cultivated land of 4.89, 2.03, 0.58, and 0.41 times.

The nitrogen content of nitrate in the soil ranged from 5.58 to 501.56 mg/kg in various scenarios of vegetation restoration. In particular, the highest soil nitrate nitrogen content in the shrub was observed after 20 years. The levels of soil nitrate nitrogen in a woodland of 40a, shrub of 20a, shrub grassland of 15a, grassland of 5a, and cultivated land varied from 65.9-267.54, 120.02-501.56, 82.88-187.32, 5.58-223.3, and 71.56-413.96 mg/kg, respectively.

The 0-5 cm soil stratum, the soil nitrate-nitrogen content ranged from 187.32 to 501.56 mg/kg. The nitrate nitrogen content of shrub soil after 20 years was significantly higher than that of cultivated land, reaching 501.56 mg/kg, representing a 21.16% increase compared to cultivated land. The ranking of nitrate nitrogen performance is as follows: shrubs after 20 years > cultivated land > woodland after 40 years > grassland after five years > shrubland after 15 years. In the 5-

10 cm soil stratum, the order of nitrate nitrogen content is cultivated land > shrubs after 20 years > woodland after 40 years > shrubland after 15 years > grassland after five years. In the 10-20 cm soil stratum, the nitrate nitrogen performance is similar to that of the 0-5 cm stratum. In the 20-30 cm soil stratum, the nitrate nitrogen performance is shrubs after 20 years > cultivated land > woodland after 40 years > shrub grassland after 15 years > grassland after five years. Finally, in the 30-40 cm soil stratum, the ranking of nitrate nitrogen performance is shrubs after 20 years > shrub grassland after 15 years > cultivated land > woodland after 40 years > grassland after five years. Overall, shrub growth in the 0-40 cm soil stratum increased by 19.38% over 20 years compared to cultivated land.

## Effects of vegetation restoration on nitrogen mineralisation

### Variation characteristics of soil ammonium nitrogen

**Fig. 3 Variation characteristics of soil ammonium nitrogen**

The nitrogen content of $NH_4^+$-N in the soil varies from 0.09 to 4.19 mg/kg depending on the stage of vegetation restoration. Specifically, concentrations range from 0.25 to 4.19 mg/kg in a 40-year-old forest, 0.19 to 2.54 mg/kg in a 20-year-old shrub area, 0.15 to 2.18 mg/kg in a 15-year-old grassland, 0.10 to 1.13 mg/kg in a 5-year-old field, and 0.09 to 1.17 mg/kg in farmland. The fluctuations of $NH_4^+$-N levels in the soil follow a cyclic pattern of increase, decrease, increase, decrease, and stabilisation over time. After seven days of cultivation, notable differences were observed between various vegetation types and cultivated land. By the 14th day, the overall content decreased, only to rise again by the 21st day. Furthermore, $NH_4^+$-N concentrations are higher in the 0-10cm topsoil stratum compared to the deeper stratum (10-20cm, 20-30cm, 30-40cm).

In the 0-5cm soil stratum, the concentration of soil $NH_4^+$-N displayed the lowest trend on the 14th day, with no statistically significant difference; it exhibited the highest trend on the 21st day. The ranking of soil $NH_4^+$-N was as follows: woodland (40a) > shrub (20a) > shrub grassland (15a) > Grassland (5a) > cultivated land. Specifically, woodland (40a) and shrub (20a) increased by 3.39 times and 1.66 times, respectively, compared to cultivated land. Shifting to the 5-10cm soil stratum after 21 days, the sequence of soil $NH_4^+$-N was shrub (20a) > woodland (40a) > shrub grassland (15a) > cultivated land > Grassland (5a). Notably, shrub (20a) and woodland (40a) increased by 3.47 times and 1.15 times compared to cultivated land. After 21 days in the 10-20cm soil stratum, the order of soil $NH_4^+$-N was shrub (20a) > woodland (40a) > shrub grassland (15a) > Grassland (5a) > cultivated land. In this case, shrub (20a) and woodland (40a) increased by 2.22 times and 1.95 times compared to cultivated land. This trend persisted in the 20-30cm soil stratum, with shrub (20a) and woodland (40a) increasing by 2.23 times and 2.02 times compared to cultivated land. Finally, in the 30-40cm soil stratum, the trend remained consistent with previous stratums, where shrub (20a) and woodland (40a) increased by 2.44 times and 1.94 times compared to cultivated land.

## Variation characteristics of soil nitrate nitrogen

**Fig. 4 Variation characteristics of soil nitrate nitrogen**

The nitrogen content in the soil showed variability ranging from 14.20-868.06 mg/kg across different types of vegetation restoration, with levels fluctuating between 53.84-868.06 mg/kg in 40a woodland, 120.02-680.38 mg/kg in 20a shrubs, 72.94-454.58 mg/kg in 15a shrub grassland, 14.08-676.29 mg/kg in 5a grassland, and 34.26-560.42 mg/kg in cultivated land. As time progressed, there was a trend of increasing soil nitrogen content with the duration of cultivation, reaching a peak on the 14th day. Furthermore, the nitrogen content was higher in the upper 0-10cm soil stratum compared to the deeper stratum (10-20cm, 20-30cm, and 30-40cm).

On the 14th day of cultivation, the highest concentration of soil $NO_3^-$-N was observed. In the top 0-5cm soil stratum, soil $NO_3^-$-N levels ranked as follows: 40a woodland > 20a shrubs > cultivated land > 15a shrub grassland > 5a grassland, with 40a woodland and 20a shrubs displaying a 0.44 and 0.39 times increase, respectively, compared to cultivated land. Moving to the 5-10cm soil stratum, the order shifted to 20a shrubs > cultivated land > 40a woodland > 15a shrub grassland > 5a grassland, with shrubs showing a 0.13 times increase compared to cultivated land in 20a. The trend continued in the 10-20cm soil stratum, with shrubs exhibiting a 0.46 times increase compared to cultivated land in 20 years. Transitioning to the 20-30cm soil stratum, soil $NO_3^-$-N levels ranked as 20a shrubs > 40a woodland > 15a shrubland > cultivated land > 5a grassland, where 40a woodland and 20a shrubs increased by 0.71 and 0.92 times, respectively, compared to cultivated land. The pattern persisted in the 30-40cm soil stratum, with 40a woodland and 20a shrubs showing a 0.17 and 1.36 times increase, respectively, compared to cultivated land.

## Vegetation restoration on net ammonification rate

**Fig.5 Effects of vegetation restoration on net ammonification rate**

The findings illustrated in Figure 5 show that the soil's net ammoniation rate displays a varying pattern throughout the growth period, initially dropping, then rising, followed by a decline before reaching stability. The 14th day marked the lowest level during cultivation, while the peak occurred on the 21st. Noteworthy is that ammoniation levels on the 35th day were lower than those on the 7th day. As the cultivation period advances, the soil's ammoniation impact diminishes, resulting in a decrease in the net ammoniation rate. Throughout cultivation, the net soil mineralization rate in 40a woodland, cultivated land, 5a grassland, 15a shrub grassland, and 20a shrub grassland increased in succession, averaging 7.36 mg/(kg·d), 11.18 mg/(kg·d), 21.11 mg/(kg·d), 21.71 mg/(kg·d), and 58.26 mg/(kg·d) respectively. The 15a shrub grassland and 20a shrub experienced growth of 0.94 times and 4.21 times, respectively, compared to cultivated land.

Throughout the initial seven days of planting, the soil AR size at varying depths displayed a consistent pattern: 20a shrubs > 5a grassland > 15 shrubland > cultivated land > 40a woodland. Mainly, 20a shrubs, 5a grassland, and 15a shrub vegetation boasted notably higher measurements than cultivated land. As cultivation advanced to 14 days, woodland in 40a portrayed the smallest measurement, differing from other plant types, while 20a shrubs displayed the highest measurement with no significant variance from the different vegetation. Progressing to day 21 of cultivation, the ranking of soil AR size within the soil stratum was as follows: 20a shrub > 40a woodland > 15a shrubland > cultivated land > 5a grassland, with a marked distinction between shrubs and cultivated land in 20a. By the 28th day, the sequence of soil AR size persisted consistently across the stratum: 20a shrubs > 15a shrubland > 5a grassland > cultivated land > 40a woodland, with considerable differences between shrubs and cultivated land in 20a. As planting approached day 35, the soil AR size in the 0-5cm stratum was as follows: 5a grassland > 15a shrub vegetation > cultivated land > 20a shrubs > 40a woodland, whereas in the 5-10, 10-20, 20-30, and 30-40cm stratums, the arrangement was: 20a shrubs > cultivated land > 5a grassland > 15 shrub vegetation > 40a woodland.

**Vegetation restoration on net nitrification rate**

**Fig.6 Effects of vegetation restoration on net nitrification rate**

The data presented in Figure 6 demonstrates a gradual decrease in the soil net nitrification rate as the duration of culture increases. The highest rate is observed after seven days of culture, while the lowest rate is recorded after 35 days. Furthermore, there is a continuous decline in the soil net nitrification rate as the culture period progresses. It is important to note that soil nitrification diminishes with extended culture time. Throughout the cultivation process, the net soil mineralisation rate decreases sequentially in a 40-year-old woodland, 15-year-old shrubland, a 20-year-old shrub, cultivated land, and a 5-year-old grassland, with average values of 10.63mg/(kg·d), 7.91mg/(kg·d), 6.81mg/(kg·d), 2.94mg/(kg·d), and 1.16mg/(kg·d) respectively. Woodland aged 40 years, shrubland aged 15 years, and shrubs aged 20 years show increases of 2.62 times, 1.69 times, and 1.32 times, respectively, compared to cultivated land.

During the initial 0-7 days of soil incubation, the size of soil NR in the 0-5 cm stratum followed the order 40a woodland > 15a scrub grassland > cropland > 20a shrub > 5a grassland, with 40a woodland significantly higher than cropland. In the subsequent 5-10, 10-20, and 20-30 cm stratums, the soil NR size overall ranked as 15a scrub grassland > 40a woodland > cropland > 20a shrub > 5a grassland, and 15a scrub grassland was notably higher than cropland. However, in the 30-40 cm stratum, no significant differences in vegetation recovery were observed.

Moving on to the 0-14 days of incubation, in the 0-5 cm stratum, the soil NR size was 40a woodland > 5a grassland > 15a shrub meadow > 20a shrub > cropland, with 40a woodland significantly surpassing cropland. In the subsequent stratum (5-10, 10-20, and 20-30 cm), the soil NR size followed the sequence 20a shrub > 40a woodland > cropland > 15a shrub meadow > 5a grassland, with 20a shrub showing significant superiority over cropland. The 30-40 cm stratum did not exhibit significant differences in vegetation restoration. As for the 0-21 days of incubation, the 0-5 cm stratum displayed a soil NR size order of 40a woodland > 20a shrub > 15a shrub meadow > cropland > 5a grassland, with 40a woodland significantly outperforming cropland. In the subsequent stratum (5-10, 10-20, 20-30, and 30-40 cm), the overall soil NR size trended as 20a shrub > 40a woodland > 15a shrub meadow > cropland > 5a grassland. Lastly, at 0-28 days of incubation, the 0-5 cm stratum showed a soil NR size sequence of 40a woodland > 15a shrub meadow > 20a shrub > cropland > 5a grassland, with 40a woodland significantly higher than cropland. In the subsequent stratum (5-10, 10-20, and 20-30 cm), the overall soil NR size ranked as 40a woodland > 20a shrub > 15a shrub meadow > cropland > 5a grassland. In the stratum of soil measuring 30-40 cm, there was no notable variance in the regeneration of plant life. Following an incubation period of 0-35 days, the ranking of NR size in the soil stratum of 0-5 cm was as follows: 15a meadow of shrubs > 40a forested area > 20a shrubbery > 5a grassy meadow > cultivated land. For the stratum of soil measuring 5-10 and 10-20 cm, the NR size ranking was: 20a shrubbery > 15a meadow of shrubs > 5a grassy meadow > 40a forested area > cultivated land. Nonetheless, in the stratum of soil measuring 20-30 and 30-40 cm, no significant disparities were noted in vegetation regeneration.

**Vegetation restoration on net nitrogen mineralisation rate**

**Fig. 7 Effects of vegetation restoration on net nitrogen mineralisation rate**

The nitrogen mineralisation rate in the soil reflects the changes in inorganic nitrogen over time. This research evaluated the nitrogen mineralisation rate at different time points, ranging from 0-7 to 0-35 days. Figure 7 shows a clear downward trend in nitrogen mineralisation for soil undergoing vegetation restoration, reaching a peak of 46.86±7.55 mg/(kg·d) on the 7th day in a 40-year-old forest area. The ranking of nitrogen mineralisation rates in the 0-40cm soil stratum is as follows: shrub grassland aged 15 years > woodland aged 40 years > cultivated land > shrubland aged 20 years > grassland aged five years. The decline in nitrogen mineralisation is linked to more extended cultivation periods, leading to a slower nitrogen mineralisation process in the soil. Throughout cultivation, the nitrogen mineralisation rates in woodland aged 40 years, shrubland aged 15 years, shrubland aged 20 years, grassland aged five years, and cultivated land decreased progressively, with average values of 10.64 mg/(kg·d), 7.93 mg/(kg·d), 6.87 mg/(kg·d), 2.95 mg/(kg·d), and 1.18 mg/(kg·d) respectively. Comparatively, woodland at 40 years, shrubland at 15 years, and shrubland at 20 years showed nitrogen mineralisation rates that were 8.01 times, 5.72 times, and 4.82 times higher than cultivated land. To sum up, vegetation restoration can potentially boost soil nitrogen mineralisation rates.

During the initial seven days of cultivation, the maximum soil NMR value recorded was (46.86±7.55) mg/(kg·d) in a 40a woodland area, while the minimum value was negative in a 5a grassland region. In the 0-40cm soil stratum, the NMR values followed the order: 15a shrub grassland > 40a woodland > cultivated land > 20a shrub > 5a grassland. Specifically in the 0-5cm soil stratum, the sequence of soil NMR values was 40a woodland > 15a shrubland > cultivated land > 20a shrub > 5a grassland, with 40a woodland showing significantly higher values compared to cultivated land and 5a grassland exhibiting negative values. In the 5-10cm and 10-20cm soil stratum, the soil NMR trends were consistent with those observed in the 0-40cm soil stratum. However, there was no significant difference between the 20-30cm and 30-40cm soil stratum.

During the initial two weeks of planting, the soil NMR levels varied from (38.85±14.00)mg/(kg·d) in the 40a forest area to a low of (4.74±4.04)mg/(kg·d) in 5a grassy terrain. The sequence of nitrogen mineral rates in the 0-40cm soil stratum was 40a woodland > 20a shrub > 5a grassland > 15a shrubland > farmland. The soil NMR hierarchy in the top 5cm soil stratum was 40a woodland > 5a grassland > 15a shrubland > 20a shrubs > farmland. Transitioning to the 5-10cm soil stratum, the soil NMR performance showed 20a shrubs > 40a woodland > 15a shrubland > farmland > 5a grassland. In the 10-20cm soil stratum, the soil NMR pattern demonstrated 20a shrubs > farmland > 40a woodland > 15a shrubland > 5a grassland. Notable variances were noted between 20a shrubland, 40a woodland, and farmland in the 20-30cm and 30-40cm soil segments.

During the initial 21 days of planting, the highest soil NMR value recorded was 40a woodland (26.33±10.34) mg/(kg·d). In the 0-40cm soil stratum, the sequence of soil NMR values observed was 40a woodland > 20a shrubs > land under cultivation > 15a shrub grassland > 5a grassland. For the soil stratum of 0-5cm, the soil NMR values were similar to those in the 0-40cm stratum, with 40a woodland exhibiting significantly higher values compared to cultivated land. In the soil stratum of 5-10cm, 10-20cm, 20-30cm, and 30-40cm, the sequence of soil NMR values followed as 20a shrub > 40a woodland > 15a shrub grassland > land under cultivation > 5a grassland.

During the initial 28 days of plant growth, the woodland area measuring 40 acres displayed notably larger soil NMR dimensions in the top 5cm stratum than other vegetation types. As the cultivation progressed up to day 35, the soil NMR dimensions in the top 5cm stratum were ranked in the following order: shrub grassland spanning 15 acres > woodland area of 40 acres > shrub area of 20 acres > grassland area of 5 acres > cultivated land. Moving down into the 5-10, 10-20, 20-30, and 30-40cm soil stratum, the overall soil NMR dimensions were observed in the series: shrub area of 20 acres > shrub grassland spanning 15 acres > woodland area of 40 acres > grassland area of 5 acres > cultivated land.

Throughout the incubation period among different plant regenerations, ammonification rates in the soil were significantly lower compared to nitrification rates, showing a rising pattern that contrasted with soil nitrification rates. The primary method of net mineralisation in the soil occurred via soil nitrification, a result that aligned with the documented soil nitrification process.

## Effects of environmental factors on vegetation restoration

**Fig.8 Effects of environmental factors on nitrogen mineralization**

**Abbreviations: The heat map's rectangular shapes depict correlations between soil physicochemical factors. The line's thickness represents Mantel's r-test correlation coefficient magnitude (solid line for positive, dashed line for negative correlation). The line's colour indicates Mantel's p-test value (grey for *$P < 0.05$, cyan for **$P < 0.01$, orange for ***$P < 0.001$). These correlations examine various organic nitrogen fractions and environmental factors such as AR (net ammonification rate), NR (net nitrification rate), NMR (net nitrogen mineralisation rate), SOC (organic carbon), pH, BD (looseness), STP (total porosity), Cl (clayey grains), Si (silt grains), Sa (sandy grains), SMC (water content), TN (total nitrogen), AN (alkali dissolved nitrogen), AP (quick phosphorus), AK (quick potassium), $NH_4^+$-N**

**(ammoniacal nitrogen), and $NO_3^-$-N (nitrate-nitrogen).**

Analysis of the Mantel test revealed that positive correlations existed between AR and TN, TP, TK, AN, AK, SOC, Si, $NH_4^+$-N; NR and TN, TP, TK, Si, BD, STP, SMC; NMR and TN, TP, TK, AN, AK, SOC, Si, Sa, pH. In contrast, AR, NR, and NMR exhibited negative correlations with $NO_3^-$-N and Ap. The findings suggest that soil mineralization processes in the study area were primarily influenced by TN, TP, TK, and Si, with ammonium ($NH_4^+$-N) having the most significant impact on AR, BD on NR, and AN and SOC on NMR. Moreover, $NH_4^+$-N showed positive relationships with TK, AK, and C, while $NO_3^-$-N was positively associated with TN, AP, and SOC.

# Discussion

## Effects of vegetation restoration on inorganic nitrogen

Subtropical and tropical forests are typically seen as limited in phosphorus, in contrast to nitrogen-limited temperate and boreal forests (Elser et al., 2007). Recent research by Zhang et al. (2015) and Lan et al. (2020) has indicated that areas of karst lands in subtropical regions may display conditions akin to nitrogen limitation as vegetation starts to regenerate. The detection of $NO_3^-$-N as the primary form of inorganic nitrogen in this investigation is in line with the results of Hu et al. (2021), showing a 19.38% rise in shrubs versus cultivated areas. This rise in $NO_3^-$-N is linked to the recovery of vegetation, resulting in the buildup of plant debris and roots in the ground, improving soil permeability, bacterial and microbial processes, and eventually increasing nitrogen concentrations. The higher levels of $NO_3^-$-N in the woodlands in comparison to grassland samples, as demonstrated in this study, are in agreement with earlier studies by Xing (2013) contrasting grassland ecosystems with a greater variety of trees, shrubs, and grasses(Li et al., 2019). Woodlands often contain more apoplastic substances with lesser C/N ratios, leading to greater mineral N in the surface soil (Saikh et al., 1998; Yimer et al., 2007). The research findings indicated a notable contrast in nitrate N levels between the upper and lower strata of the soil. This difference was ascribed to the presence of a rich oxygen environment, abundant organic matter, and a diverse array of microorganisms in the topsoil, which facilitated the process of nitrification. In the soil stratum ranging from 0-40 cm, the ranking of levels of soil ammonium nitrogen was as follows: woodland > shrub-grassland > shrub > grassland > arable land, with increases of 4.89, 2.03, 0.58, and 0.41 times respectively compared to arable land. These variations were tied to the continuous breakdown of decomposing plant matter during vegetation restoration, the ongoing mineralization of organic nitrogen in the soil, the accumulation of inorganic nitrogen, and the increase in $NH_4^+$-N content.

## Effects of vegetation restoration on nitrogen mineralization

Restoring vegetation has a significant capacity to uptake and utilize ammonium and nitrate nitrogen in the soil. According to Loeb et al. (2009), the speed at which nitrogen undergoes mineralization plays a critical role in providing these essential nutrients. Over time, the soil gradually accumulates various nitrogen species, building up adequate nitrogen reserves. The rate of net mineralization acts as a crucial metric for evaluating soil N efficacy, as observed by Sainju et al. (2006). Their research showed that inorganic nitrogen concentrations in the soil ranged from 14.50 to 869.36 mg/kg under different vegetation restoration conditions. As the incubation period increased, the levels of inorganic nitrogen in the soil generally followed a trend of increase, decrease, and stabilization, reaching a peak on the 14th day, in line with nitrate nitrogen patterns. The negatively charged nature of $NO_3^-$-N limits its adsorption and utilization in the soil, leading to most $NO_3^-$-N remaining in the soil solution without being utilized. Soil nitrification processes result in the consumption of ammonium nitrogen from the soil and external fertilizers, diminishing losses from ammonia volatilization while accumulating more $NO_3^-$-N in the soil. The study highlighted that the upper 0-10 cm soil stratum exhibited higher concentrations of inorganic nitrogen compared to the deeper stratum. Surface soils have a higher capacity to absorb external organic N, fostering rapid accumulation rates. As soil depth increases, permeability gradually decreases, resulting in slower ageing and decomposition of soil organic matter. This, subsequently, diminishes the availability of organic matter for decomposition and plant uptake, causing a reduction in microbial populations and activity, which could potentially lower the rate of N mineralisation (Xue et al., 2013; YAN, 2012). Soil surface temperature variations have been shown to impact microbial activity, with deeper soil stratum exhibiting reduced susceptibility (Koga et al., 2001). The research demonstrates that $NO_3^-$-N is the primary form of inorganic nitrogen. Initially, vegetation absorbs $NO_3^-$-N, leading to a significantly lower net residual of $NO_3^-$-N in densely vegetated regions compared to sparsely vegetated areas (Li et al., 2017). The loss rate of $NO_3^-$-N exceeded that of $NH_4^+$-N, and the relatively high $NH_4^+$-N levels in soils across various locations helped maintain soil nitrogen levels (Sainju et al., 2006). Soil inorganic N levels increased gradually during vegetation mineralization recovery but decreased as the recovery period lengthened. This was due to the gradual stabilization of organic matter and apoplastic material in the soil and decreased microbial activity during vegetation restoration, resulting in a slowdown of mineralization processes. As a result, the rate of inorganic nitrogen concentration increased and decreased as the restoration period became longer.

## Factors Affecting Soil Nitrogen Mineralization

The Mantel test analysis revealed that TN, TP, TK, and Si exhibited positive correlations with soil AR, NR, and NMR (Fig. 8), consistent with the results reported by Li et al. (2019). On the other hand, pH negatively correlated with AR and NR, suggesting that an abundance of the substrate and favourable conditions can enhance soil N mineralization (Li et al., 2018), a critical factor in regulating N availability in soil (Wei et al., 2011). N transformations like mineralization and nitrification greatly influence soil N availability. Sa showed a negative correlation with AR and NR, supporting studies indicating that clay-rich fine-textured soils generally contain higher levels of microbial biomass, organic carbon, and nitrogen than coarse-textured soils, promoting overall N mineralization (Ding et al., 2021). Total N mineralization was notably higher in soils with elevated levels of fines and clays (Elrys et al., 2023). SMC was negatively correlated with soil $NO_3^-$-N, likely due to accelerated soil $NO_3^-$-N loss in moisture-rich soils (Srivastava et al., 2015). In addition, alkaline soils hinder soil organic matter decomposition and result in lower soil N mineralization (BELTRAN-HERNANDEZ et al., 1999), as supported by the negative relationship between pH and soil AR and NR parameters. Soil inorganic N levels play a crucial role in soil nutrient dynamics, with soil NMR indirectly affecting SOC and serving as a key indicator for evaluating soil fertility in revegetated ecosystems (Wei et al., 2009). The factors influencing soil N transformations differ between ecosystems, primarily due to variations in climate, vegetation type, and land use history (Burke, 1989; Li et al., 2014; Maithani et al., 1998).

## Conclusion

(1) There was a 19.38% rise in the 0-40 cm depth within 20 years for shrubs in the inorganic nitrogen, the primary form of nitrate nitrogen, compared to cultivated areas.

(2) As the duration of incubation increased, there was a clear trend in the soil $NH_4^+$-N levels - initially rising, then falling, followed by a spike, another decline, and ultimately reaching a stable state. On the 14th day of incubation, the $NH_4^+$-N content was at its lowest point, while it peaked on the 21st day. In contrast, the soil $NO_3^-$-N and total inorganic nitrogen exhibited an increase, decrease, and stabilization pattern, reaching their highest levels on day 14 of incubation. Furthermore, the $NO_3^-$-N, $NO_3^-$-N, and organic nitrogen levels in the upper 0-10 cm stratum of soil surpassed those in the deeper stratum (10-20 cm, 20-30 cm, and 30-40 cm).

(3) During the process of vegetation restoration, both the rate of nitrogen mineralisation in soil (NR) and the nitrogen mineralisation rate (NMR) experienced a gradual decrease with time. Soil NR and NMR levels saw a rise in regions that had been restored for 15 years with scrub grassland, 20 years with shrubland, and 40 years with woodland. Particularly, the presence of 15 years of scrub grassland and 20 years of shrubland also increased soil ammonium nitrogen (AR) levels.

(4) The analysis of the Mantel test showed that there were positive relationships between TN, TP, TK, and Si with soil AR, NR, and NMR. Among these, $NH_4^+$-N showed the most significant influence on AR, while BD had the greatest effect on NR. Additionally, AN and SOC had the most notable impact on NMR.

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# Figure 1

Table 1 Basic Information of Sample Sites

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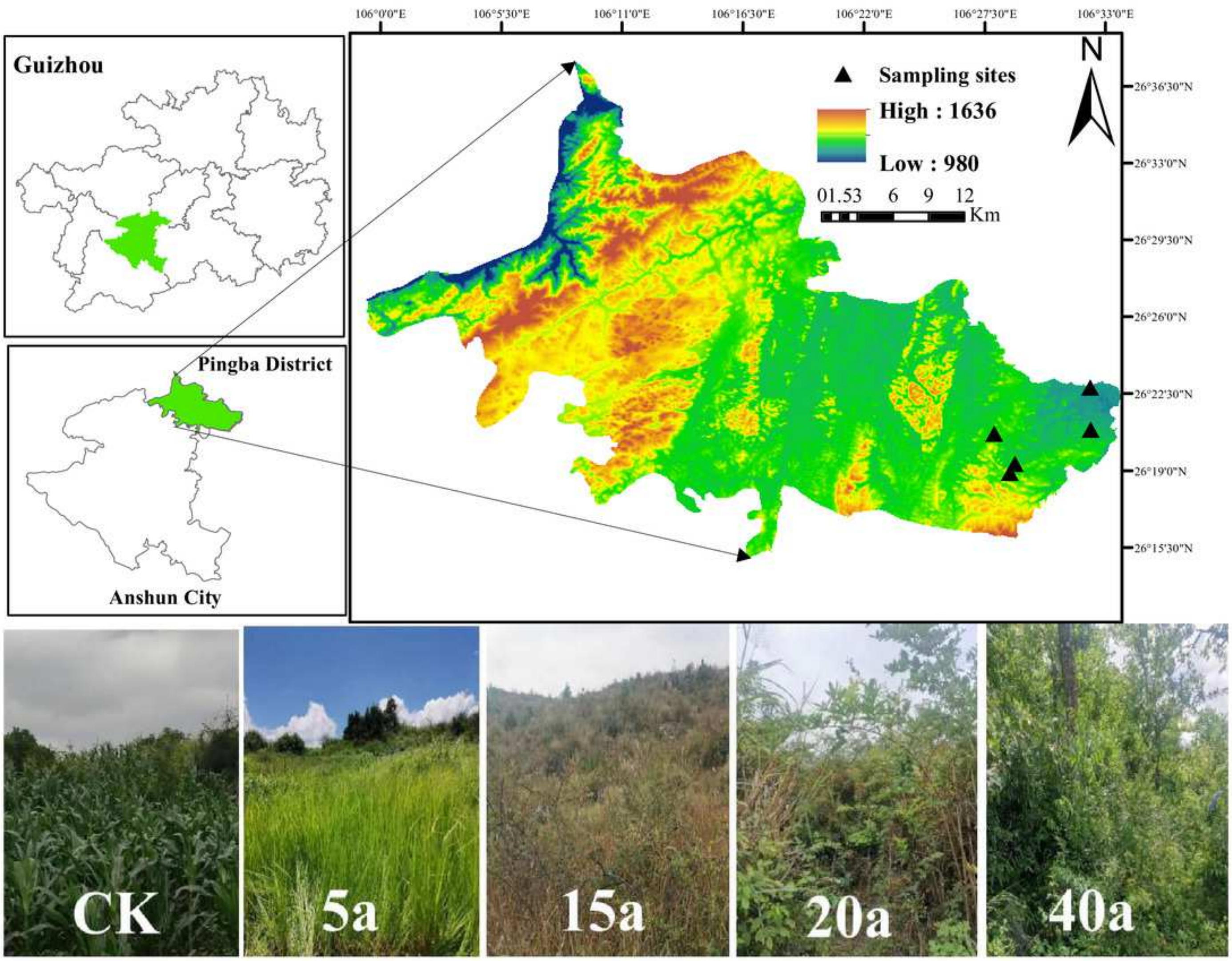
| Sample type | Recovery years / a | Altitude/m | Longitude and latitude | Major vegetation |
|---|---|---|---|---|
| farmland | 0 | 1211 | 26° 20′ 52″ N, 106° 32′ 18″ E | *Zea mays* |
| grassland | 5 | 1217 | 26° 20′ 59″ N, 106° 32′ 18″ E | *Imperata cylindrical* |
| | 15 | 1285 | 26° 20′ 5″ N, 106° 27′ 56″ E | *Imperata cylindrical* |
| shrubs | 20 | 1289 | 26° 18′ 54″ N, 106° 28′ 39″ E | *Pyracantha fortuneana*、*Artemisia annua*、*Rubus idaeus L.*、*Zanthoxylum simulans* |
| woodland | 40 | 1223 | 26° 19′ 19″ N, 106° 29′ 6″ E | *CatalpabungeiC.A.Mey* *Celtis sinensis Pers* |

# Figure 2

Samplingsites

Samplingsites



# Figure 3

Fig. 2 Effects of vegetation restoration on inorganic nitrogen

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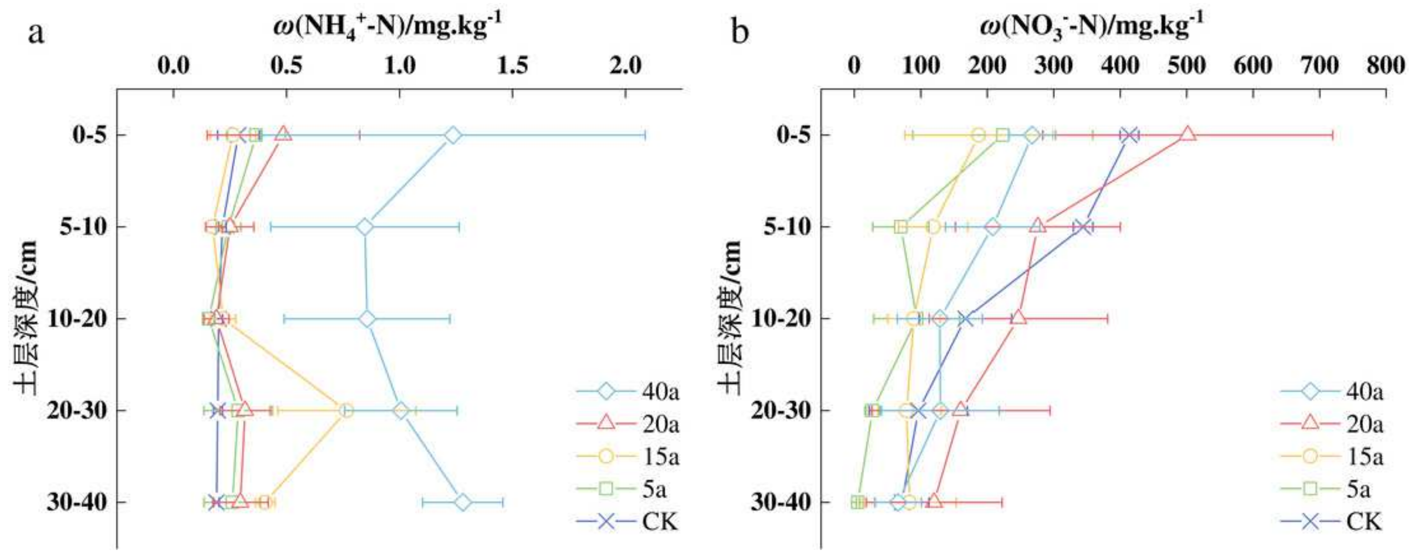
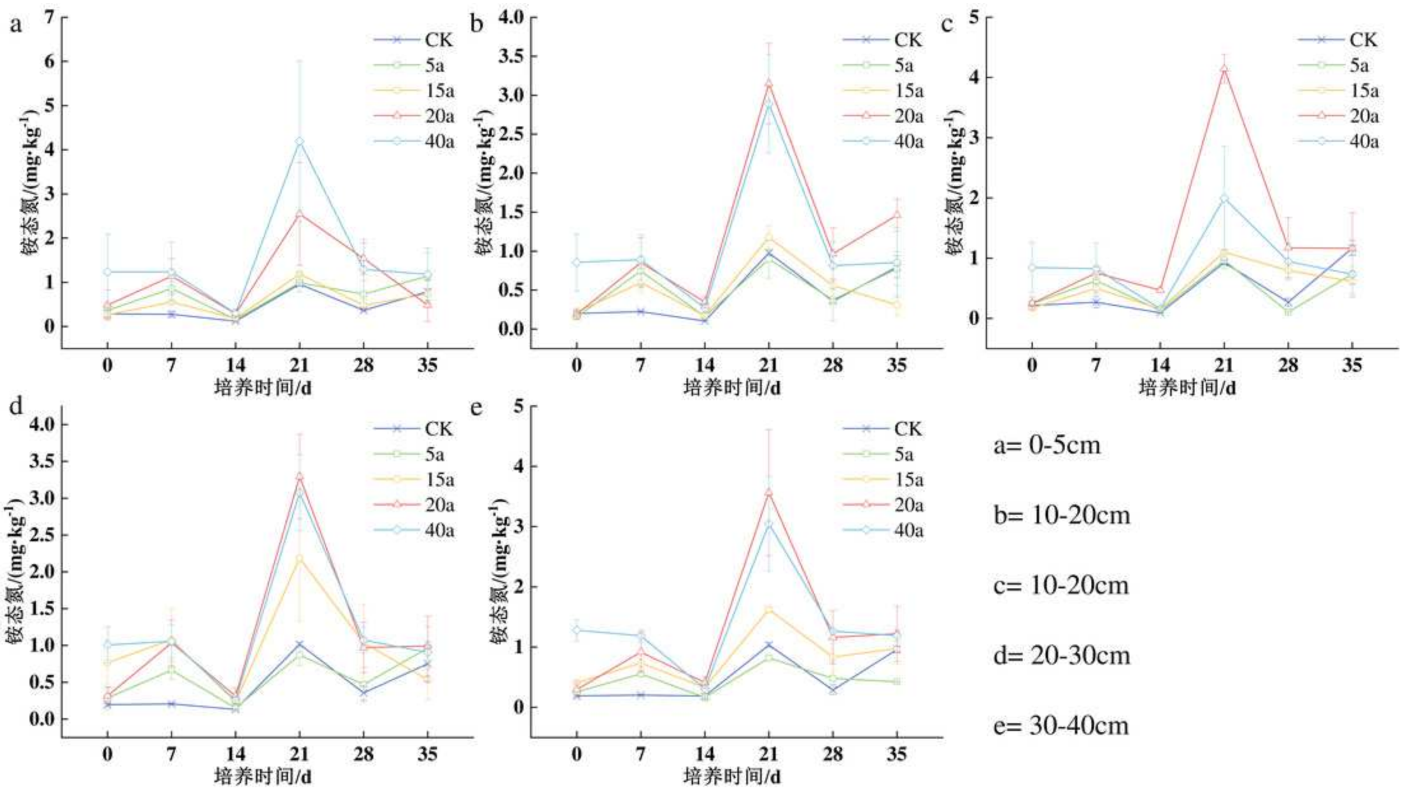


# Figure 4

Fig. 3 Variation characteristics of soil ammonium nitrogen

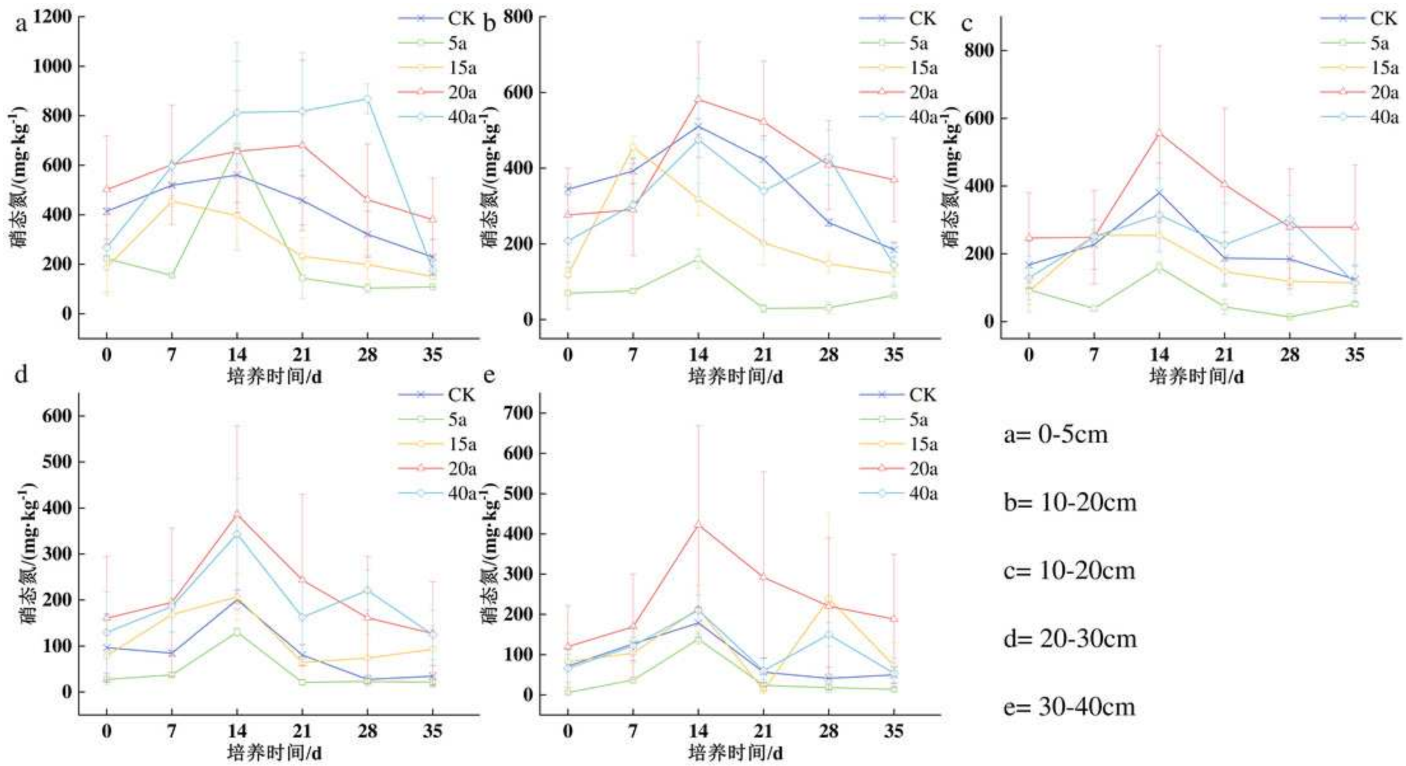
Fig. 3 Variation characteristics of soil ammonium nitrogen



# Figure 5

Fig. 4 Variation characteristics of soil nitrate nitrogen

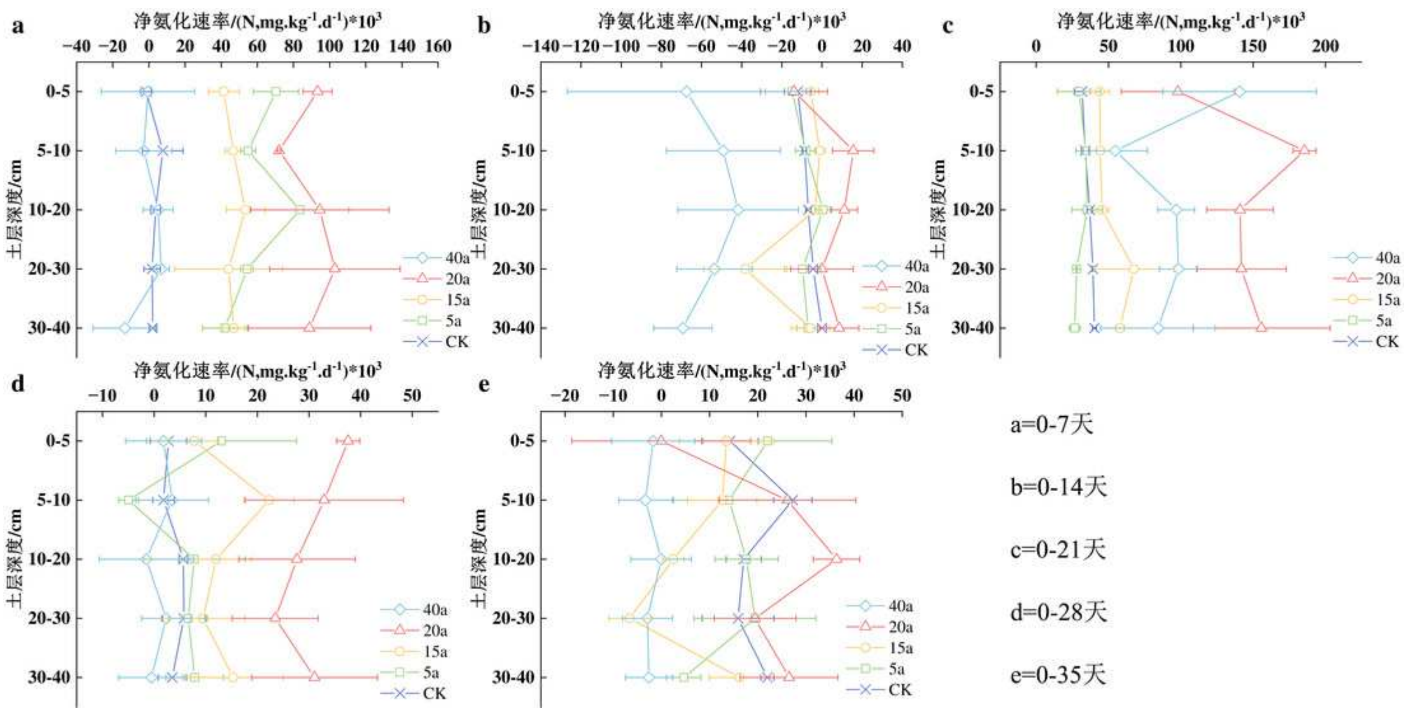
Fig. 4 Variation characteristics of soil nitrate nitrogen



# Figure 6

Fig.5 Effects of vegetation restoration on net ammonification rate

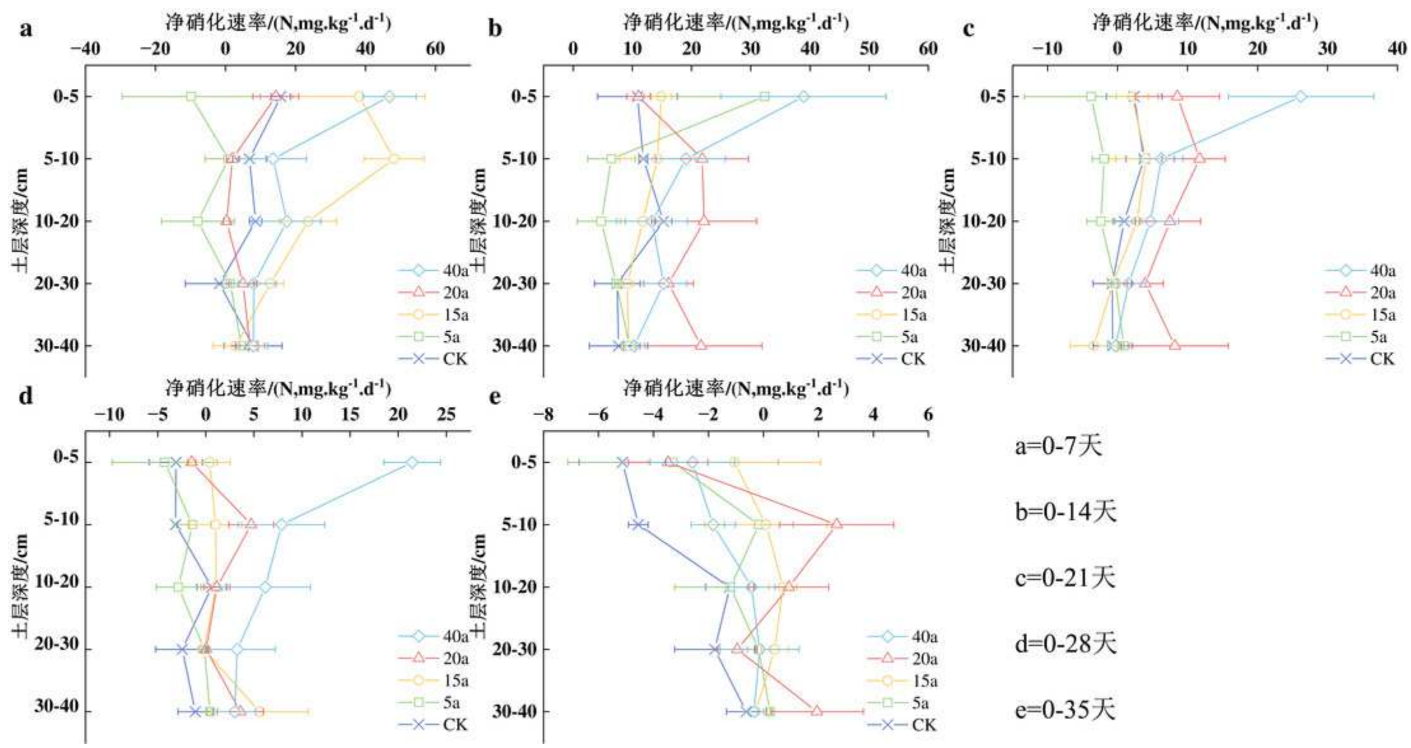
Fig.5 Effects of vegetation restoration on net ammonification rate



# Figure 7

Fig.6 Effects of vegetation restoration on net nitrification rate

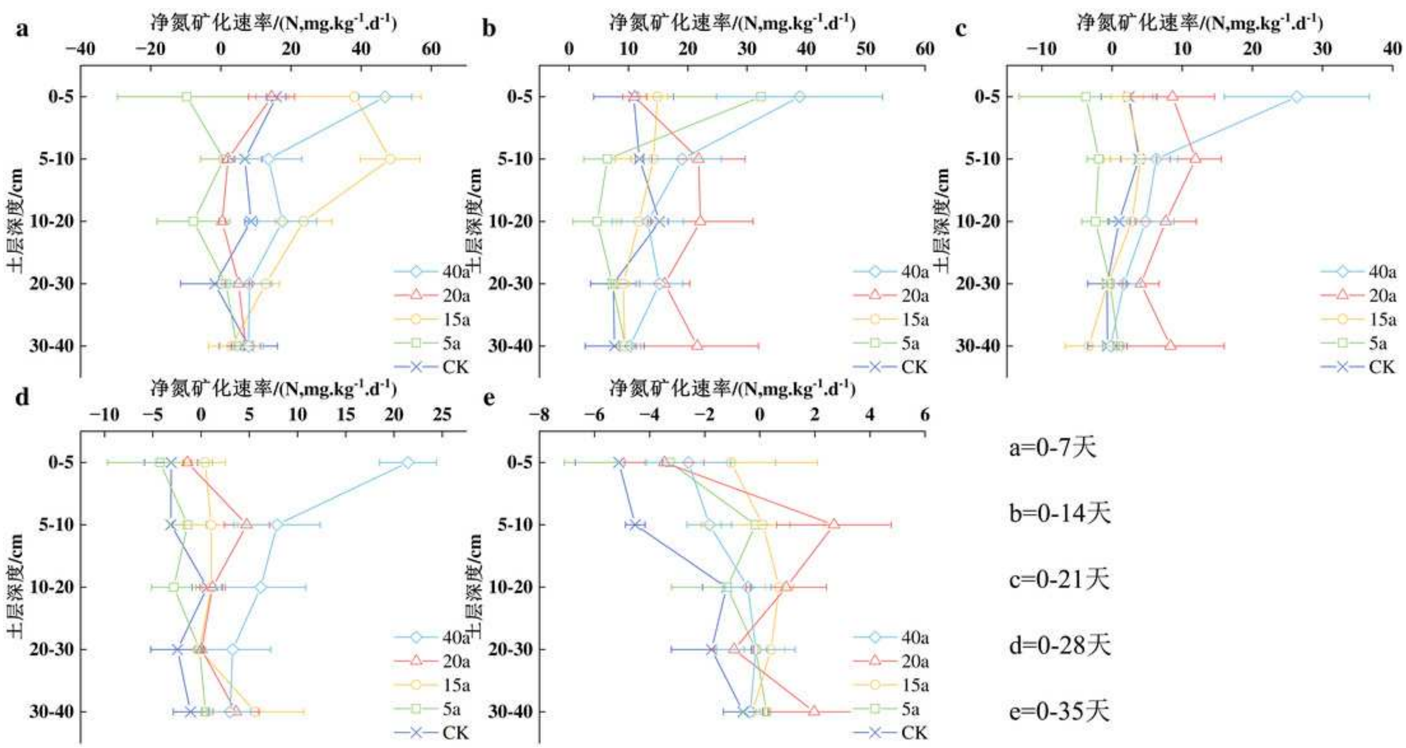
**Fig.6 Effects of vegetation restoration on net nitrification rate**



# Figure 8

Fig. 7 Effects of vegetation restoration on net nitrogen mineralisation rate

Fig. 7 Effects of vegetation restoration on net nitrogen mineralisation rate



# Figure 9

Fig.8 Effects of environmental factors on nitrogen mineralization

**Fig.8 Effects of environmental factors on nitrogen mineralization**