

Article

Divergent Response of the Supply Capacity and Turnover of Inorganic Nitrogen to Pitaya Cultivation in the Subtropical Karst Region of Southwest China

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Abstract: Determining the availability and supply capacity of soil inorganic nitrogen (N) can effectively guide the appropriate application of N fertilizers during crop cultivation. However, the mechanism underlying soil inorganic N production remains unknown for cash crops in karst regions. In this study, the rates of organic N mineralization to ammonium (NH_4^+) and NH_4^+ nitrification to nitrate (NO_3^-) were determined using a ^{15}N tracing technique to evaluate the supply capacity of inorganic N in soils from woodland and pitaya plantations with different cultivation years (3, 9, and 15 years) in the subtropical karst region of China. The conversion of woodland to pitaya plantations significantly decreased the content of soil organic carbon (SOC), total N, calcium (Ca), and magnesium (Mg), along with the soil pH and cation-exchange capacity (CEC), but significantly increased the content of available potassium, available phosphorus, iron, and aluminum, in a more pronounced fashion with the increasing length of pitaya cultivation. The conversion of woodland to pitaya plantations has not significantly changed soil NH_4^+ and NO_3^- content, but this land use has resulted in divergent effects on mineralization and nitrification rates. Compared to woodland ($5.49 \text{ mg N kg}^{-1} \text{ d}^{-1}$), pitaya cultivation significantly reduced the mineralization rate to $0.62\text{--}2.38 \text{ mg N kg}^{-1} \text{ d}^{-1}$. Conversely, the nitrification rate significantly increased from $4.71 \text{ mg N kg}^{-1} \text{ d}^{-1}$ in soil under woodland to $9.32 \text{ mg N kg}^{-1} \text{ d}^{-1}$ in soil under 3-year pitaya cultivation, but this rate decreased to $1.74 \text{ mg N kg}^{-1} \text{ d}^{-1}$ under 15-year cultivation. Furthermore, the mean residence time of inorganic N was significantly higher in long-term than in short-term pitaya plantations, indicating the decline in inorganic N turnover with the increasing length of pitaya cultivation. Taken together, long-term pitaya cultivation could significantly decrease the supply capacity and turnover of inorganic N in soil. The Ca, Mg, SOC, and total N content, as well as CEC, were significantly and positively related to the mineralization rate, but negatively related to the mean residence time of NH_4^+ and NO_3^- , suggesting that the incorporation of organic matter can accelerate the soil inorganic N supply and turnover for long-term pitaya plantation in subtropical regions.

Keywords: pitaya cultivation; ^{15}N tracing; mineralization rate; nitrification rate; mean residence time of inorganic N



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1. Introduction

Karst landforms developed from carbonate rock are widely distributed in China and occupy an area of approximately $3.44 \text{ million km}^2$ [1]. Due to their geological specificity, limited arable land resources exist in karst regions. Thus, sloped woodland or shrubland is often deforested to cultivate common crops (e.g., beans, corn). However, in addition to low economic income, this cropping pattern inevitably induces some negative environmental

and ecological effects, such as large-scale “rocky desertification” in karst regions [1,2]. Thus, the cultivation of high-yield and economically sustainable crops is urgently needed in order to promote local farmers’ income. Due to inherent ecological benefits (e.g., barren resistance, easy growth, soil, and water conservation) and high economic costs, pitaya (*Hylocereus undatus*) has been introduced and planted in the subtropical karst regions of southwest China [3]. Statistical data show that the pitaya cultivation area in China has increased from approximately 3×10^3 ha in 2011 to 4×10^4 ha in 2017 [4]. Noticeably, the slow growth and low yield of pitaya frequently hinder the sustainable development of this crop [5,6]. In addition to climate factors and management practices, soil fertility is a critical factor affecting pitaya productivity.

As the key indicator of soil fertility, nitrogen (N) is an important nutrient element that limits crop growth and yield [7,8]. With the exception of small-molecule organic N, ammonium (NH_4^+) and nitrate (NO_3^-) are the main forms of N for crop uptake [9,10]. In previous studies, NH_4^+ and NO_3^- content have been widely measured to assess the availability of N, which can indicate whether the available N supply in soil satisfies the growing demand of crops [11,12]. However, this method cannot differentiate the mechanisms underlying inorganic N production [13,14]. In soil, inorganic N needs to be produced through the mineralization of organic N to NH_4^+ , and the subsequent nitrification of NH_4^+ to NO_3^- [14,15]. Therefore, the supply process of inorganic N in soil can be clearly quantified by the determination of mineralization and nitrification, which is of particular importance for proposing appropriate fertilizer management for pitaya cultivation. However, the intensity of mineralization and nitrification, along with the affecting factors in subtropical karst regions, remains unknown.

In karst regions, the soils are characterized by high pH, calcium (Ca), and magnesium (Mg) [16,17]. In the undisturbed natural ecosystems (e.g., woodland) of karst regions, high Ca levels can stimulate the accumulation of soil organic matter, which may result in high mineralization and nitrification rates in the soil [18,19]. When woodland is converted to pitaya plantations, the mineralization and nitrification processes may be significantly altered due to the changes in management practices (e.g., tillage and fertilization) and soil properties. The conversion of woodlands to pitaya plantations can greatly decrease organic N content if organic fertilizer cannot be applied [20,21]. If this is the case, pitaya cultivation may decrease the mineralization in the soil due to the decrease in organic N. On the other hand, the intensive application of N fertilizer, combined with tillage, may stimulate the nitrification of NH_4^+ to NO_3^- by increasing the abundance or activity of ammonia-oxidizing archaea (AOA) and/or bacteria (AOB) [22–24]. Noticeably, such stimulatory effects may be inhibited with the increasing duration of pitaya cultivation, because long-term crop cultivation can significantly decrease soil organic matter, causing the formation of a heavy clay texture. This may inhibit mineralization and nitrification rates, reducing the supply capacity and turnover of inorganic N in soil [19,21], even when large amounts of mineral N fertilizer are applied to pitaya plantations. Thus, we hypothesized that long-term pitaya cultivation would decrease the supply capacity and turnover of inorganic N by reducing mineralization and nitrification rates in karst regions.

To verify this hypothesis, woodland and pitaya plantations with different cultivation durations were chosen from the subtropical karst region of southwest China, and the mineralization and nitrification rates in soils were determined using the ^{15}N tracing technique. The objective of this study was to clarify the changes in inorganic N supply capacity and turnover in soils, along with the influencing factors.

2. Materials and Methods

2.1. Sites and Soil Sample Collections

The study site was located in the Guohua karst experimental site ($23^\circ 22'$ N, $107^\circ 23'$ E), Pingguo County, Guangxi Province, southwest China (Figure 1). The study region has a subtropical monsoon climate, with an annual average temperature of approximately $19\text{--}21.5^\circ\text{C}$, annual average evaporation of 1572 mm, and relative humidity of above 80%.

The soil type was classified as Alfisol developed from limestone according to the USDA soil taxonomy. Most of the peasants in this region live in mountainous karst regions with poor crop production and living conditions. Local people can only grow plants on slope lands that have been deforested, resulting in rocky desertification problems. To promote the restoration of barren hill vegetation, reduce water and soil loss, and increase farmers' income, pitaya was introduced in this area in 2003.

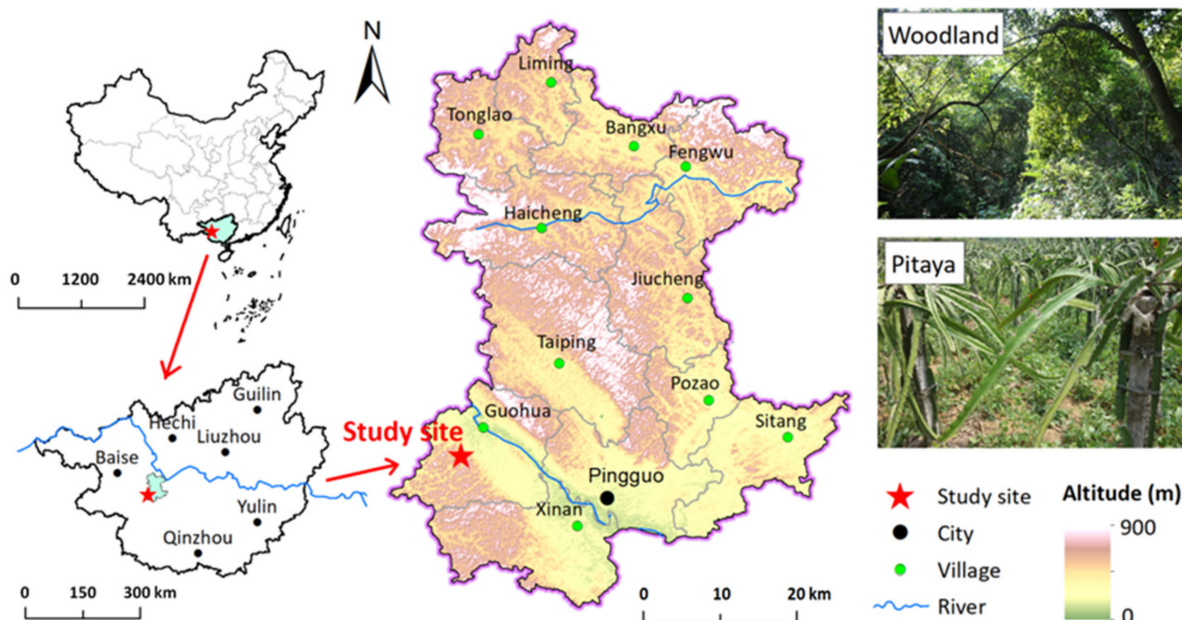


Figure 1. Schematic diagram of the study site.

Three pitaya plantations with different cultivation times (3, 9, and 15 years) were chosen, all of which had been converted from woodland, and the adjacent woodland was also chosen as a control. Both woodland and pitaya plantations were on land steeper than 10° at an elevation of approximately 410 m asl. The main tree species at woodland sites include *Zenia insignis*, *Melia azedarach*, *Apodytes dimidiata*, and *Choerospondias axillaris*, accompanied by shrubs including *Alchornea trewioides*, *Cipadessa cinerascens*, and *Vitex negundo*. The ground cover is occupied by grass communities dominated by *Nephrolepis auriculata*, *Eupatorium odoratum*, *Microstegium gratum*, and *Cymbopogon caesius*. Four pitaya per pillar were arranged, maintaining $3\text{ m} \times 3\text{ m}$ spacing. Approximately 165, 136, 145, and $12,210\text{ kg ha}^{-1}\text{ y}^{-1}$ of N, P_2O_5 , K_2O , and sheep manure, respectively, were applied to the pitaya fields. The N, potassium (K), and phosphorus (P) content of the sheep manure were 6.5, 5.0, and 3.0 g kg^{-1} , respectively.

Three representative sites ($50\text{ m} \times 50\text{ m}$) were randomly established as the spatial replication for the woodland and each pitaya plantation, and the distance between each site was over 50 m. At each site, five plots with 20 m intervals between them were staked out. After removing the litter of topsoil, two soil cores that were 5 cm in diameter were set up from the 0–15 cm layer in each plot and, subsequently, all of the soil cores from the five plots were composited to form one sample per site. Consequently, there were 12 soil samples in total to be studied. Stones, plant roots, and residuals were removed from the sampled soils, which were then passed through a 2 mm sieve. Part of soil the samples was air-dried for the determination of the basic soil properties, and other fresh soils were used to determine the abundances of AOA and AOB, as well as the rates of mineralization and nitrification.

2.2. ^{15}N Labelling Experiment

A ^{15}N labelling experiment in the laboratory was performed according to a previously published method [18,21]. There were two ^{15}N treatments ($^{15}\text{NH}_4\text{NO}_3$ and $\text{NH}_4^{15}\text{NO}_3$), with three replicates for each treatment and two incubation times. A series of fresh soil (30 g of oven-dried basis) was weighed in 250 mL Erlenmeyer flasks and pre-incubated for 24 h in darkness at 25 °C. After the pre-incubation, the $^{15}\text{NH}_4\text{NO}_3$ or $\text{NH}_4^{15}\text{NO}_3$ (10 atom% excess) solutions were evenly added to the soil surface of each flask, and the application rate of NH_4NO_3 reached $50 \text{ mg NH}_4^+-\text{N} (\text{kg dry soil})^{-1}$ and $50 \text{ mg NO}_3^--\text{N} (\text{kg dry soil})^{-1}$. Subsequently, distilled water was applied to adjust the soil moisture to 60% water-holding capacity (WHC). All flasks were sealed with plastic film containing small holes, and then incubated in darkness again at 25 °C. The soils in the flasks were extracted with 150 mL of 2 M KCl solution at 0.5 h and 24 h after the application of NH_4NO_3 . The KCl extracts were used to determine the content and ^{15}N atom% excess of NH_4^+ and NO_3^- with a continuous-flow analyzer (Skalar, Breda, The Netherlands) and a Sercon Integra2 isotope ratio mass spectrometer (Sercon Ltd., Crewe, UK), respectively. The detailed information on the preparation of ^{15}N samples of NH_4^+ and NO_3^- pools was presented by Xie et al. (2018) [14] and Garousi et al. (2021) [19].

2.3. Analyses of Soil Properties

The soil organic carbon (SOC) and total N were determined using a Sercon Integra2 elemental analyzer, when inorganic C in soil was removed with a diluted phosphoric acid solution. Soil pH was determined via extraction with a 1:2.5 (w:v) soil:water ratio, and subsequently analyzed using SevenExcellence pH/mV and Conductivity Meters. After removing organic material and carbonates using hydrogen peroxide and hydrochloric acid, respectively, the soil texture—including sand (50–2000 μm), silt (2–50 μm), and clay (<2 μm)—was analyzed using a laser particle characterization analyzer (Beckman Coulter LS-230, Brea, CA, USA) [19]. The cation-exchange capacity (CEC) was determined via the ammonium acetate method [25]. The content of total K, P, Ca, Mg, iron (Fe), and aluminum (Al) in the soil were analyzed using total X-ray fluorescence (XRF) spectroscopy. Available P and available K were determined via the NaHCO_3 extraction method [26] and the NH_4OAc extraction method [27], respectively. After the extraction with the citrate bicarbonate dithionite system, free Fe and Al oxides were measured using ICP-AES [28].

2.4. Determination of Archaeal *amoA* and Bacterial *amoA* Abundances

A FastDNA[®] Spin Kit for Soil (MP Biomedicals, Cleveland, OH, USA) was used to extract the soil DNA, which was subsequently quantified and purified using a NanoDrop ND-2000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The quantitative PCR method was carried out to determine the genes of archaeal *amoA* (AOA) and bacterial *amoA* (AOB) using a CFX96 Optical Real-Time Detection System (Bio-Rad, Laboratories Inc., Hercules, CA, USA). The Arch-*amoA*F/Arch-*amoA*R [29] and *amoA*1F/*amoA*2R [30] were the primers of the AOA and AOB genes, respectively. The detailed information of DNA extraction, along with the amplification and quantitation of PCR, was obtained from the works of Garousi et al. (2021) [19] and Wang et al. (2015) [31].

2.5. Statistical Data Analyses

According to the changes in content and atom% of NH_4^+ in the $^{15}\text{NH}_4\text{NO}_3$ treatment or NO_3^- in the $\text{NH}_4^{15}\text{NO}_3$ treatment between times t_1 (0.5 h) and t_2 (24 h), the mineralization and nitrification rates [32], respectively, were calculated as follows:

$$M_{\text{Norg}} = \frac{[\text{NH}_4^+]_{t_1} - [\text{NH}_4^+]_{t_2}}{t_2 - t_1} \times \frac{\log\left(\frac{\text{APE}_{t_1}}{\text{APE}_{t_2}}\right)}{\log\left(\frac{[\text{NH}_4^+]_{t_1}}{[\text{NH}_4^+]_{t_2}}\right)} \quad (1)$$

where M_{Norg} is the mineralization rate ($\text{mg N kg}^{-1} \text{ d}^{-1}$), while $[\text{NH}_4^+]_{t1}$, $[\text{NH}_4^+]_{t2}$, APE_{t1} , and APE_{t2} are the content (mg N kg^{-1}) and ^{15}N atom% excess of NH_4^+ at time 0.5 h and 24 h, respectively.

$$O_{\text{NH}_4} = \frac{[\text{NO}_3^-]_{t1} - [\text{NO}_3^-]_{t2}}{t2 - t1} \times \frac{\log\left(\frac{\text{APE}_{t1}}{\text{APE}_{t2}}\right)}{\log\left(\frac{[\text{NO}_3^-]_{t1}}{[\text{NO}_3^-]_{t2}}\right)} \quad (2)$$

where O_{NH_4} is the nitrification rate ($\text{mg N kg}^{-1} \text{ d}^{-1}$), while $[\text{NO}_3^-]_{t1}$, $[\text{NO}_3^-]_{t2}$, APE_{t1} , and APE_{t2} are the content (mg N kg^{-1}) and ^{15}N atom% excess of NO_3^- at time 0.5 h and 24 h, respectively.

The mean residence times (d) of NH_4^+ (MRT NH_4^+) and NO_3^- (MRT NO_3^-) were calculated using the equation developed by Corre et al. [33], which can indicate the turnover of inorganic N.

$$\text{MRT NH}_4^+ = \frac{c(\text{NH}_4^+)}{M_{\text{Norg}}} \quad (3)$$

$$\text{MRT NO}_3^- = \frac{c(\text{NO}_3^-)}{O_{\text{NH}_4}} \quad (4)$$

where $c(\text{NH}_4^+)$ and $c(\text{NO}_3^-)$ are the initial content of NH_4^+ and NO_3^- (mg N kg^{-1}) in the soil, respectively.

Differences in soil properties, AOA and AOB abundances, and N-cycling rates between pitaya plantations and woodlands were estimated by analysis of variance (ANOVA) at the $p < 0.05$ significance level. Redundancy analysis (RDA) was used to determine the relationships between soil properties, AOA and AOB abundances, and N-cycling rates, using Canoco 5.

3. Results

3.1. Soil Properties and AOA/AOB Abundances

The soil properties were different between the woodlands and pitaya plantations (Table 1). Compared to woodlands ($72.5 \text{ mg C kg}^{-1}$ and $6.83 \text{ mg N kg}^{-1}$), pitaya cultivation significantly decreased the content of SOC and TN to $12.8\text{--}22.3 \text{ mg C kg}^{-1}$ and $1.47\text{--}2.87 \text{ mg N kg}^{-1}$, respectively ($p < 0.05$). The soil C/N ratio, WHC, and CEC also decreased significantly after the conversion of woodlands to pitaya plantations ($p < 0.05$). Compared to woodlands (6.52), the soil pH did not significantly change in short-term pitaya plantations (3 years) ($p > 0.05$), but significantly decreased to 5.57 in long-term pitaya plantations (15 years) ($p < 0.05$). The content of Ca and Mg in soils under woodlands was about 6.05–17.7 and 4.83–6.48 times higher than in soils under pitaya cultivation, respectively. Conversely, pitaya cultivation greatly increased the available K, available P, total K, P, Fe, Al, free Fe, and Al oxides content as well as the silt composition, with significant differences between woodlands and the 15-year pitaya plantation ($p < 0.05$). No significant differences in clay and/or clay compositions were found in any of the studied soils ($p > 0.05$). In comparison to woodlands (8.42 and $16.0 \text{ mg N kg}^{-1}$, respectively), pitaya cultivation increased NH_4^+ and NO_3^- content to $12.2\text{--}22.5 \text{ mg N kg}^{-1}$ and $21.4\text{--}27.2 \text{ mg N kg}^{-1}$, respectively, but the difference was not significant ($p > 0.05$). The content of both Ca and Mg was significantly and positively correlated with SOC and TN content, WHC, and CEC, suggesting the importance of Ca and Mg in maintaining soil conditions.

In all of the studied soils, AOA abundance was significantly higher than AOB abundance ($p < 0.05$, Table 1). Compared to woodland (6.70×10^7 and 2.54×10^6 copies g^{-1} , respectively), the abundance of AOA and AOB in soil under 3-year pitaya cultivation significantly increased to 4.19×10^8 and 1.36×10^7 copies g^{-1} , respectively. However, the abundance of AOA and AOB significantly decreased with prolonged pitaya cultivation, with the lowest values found in soil under the 15-year pitaya cultivation.

Table 1. Soil properties under woodland and three pitaya plantations ($n = 12$).

Properties	Woodland	3-Year Plantation	9-Year Plantation	15-Year Plantation
SOC (g C kg ⁻¹)	72.3 ± 6.64 a	22.3 ± 2.11 b	19.9 ± 2.62 b	12.8 ± 1.33 c
TN (g N kg ⁻¹)	6.83 ± 0.70 a	2.87 ± 0.70 b	2.30 ± 0.3 bc	1.47 ± 0.13 c
C/N	10.6 ± 0.14 a	7.97 ± 1.19 b	8.71 ± 0.87 b	8.68 ± 0.18 b
pH	6.52 ± 0.39 a	6.47 ± 0.44 a	6.10 ± 0.09 b	5.57 ± 0.39 b
NH ₄ ⁺ (mg N kg ⁻¹)	8.42 ± 5.62 a	12.2 ± 4.82 a	22.5 ± 13.2 a	19.1 ± 8.73 a
NO ₃ ⁻ (mg N kg ⁻¹)	16.0 ± 0.81 a	21.4 ± 2.74 a	26.3 ± 14.4 a	27.2 ± 11.8 a
WHC	1.12 ± 0.11 a	0.81 ± 0.02 b	0.71 ± 0.06 bc	0.68 ± 0.04 c
Clay (<2 µm) (%)	53.6 ± 0.46 a	50.9 ± 0.67 a	52.0 ± 1.43 a	54.4 ± 2.94 a
Silt (2–50 µm) (%)	39.2 ± 2.16 a	45.5 ± 2.08 a	42.4 ± 4.46 a	42.4 ± 2.82 a
Sand (50–2000 µm) (%)	7.13 ± 2.06 a	3.59 ± 1.61 a	5.58 ± 3.21 a	3.19 ± 0.82 b
CEC (cmol kg ⁻¹)	58.1 ± 2.43 a	24.9 ± 2.63 b	19.6 ± 1.90 b	20.3 ± 4.83 b
Available K (mg kg ⁻¹)	92.9 ± 19.6 b	322 ± 67.3 b	372 ± 54.7 ab	434 ± 31.1 a
Available P (mg kg ⁻¹)	2.63 ± 0.31 b	71.7 ± 17.1 a	83.1 ± 13.8 a	90.0 ± 13.7 a
K (g kg ⁻¹)	3.63 ± 1.67 c	10.7 ± 0.06 b	10.5 ± 0.35 b	13.2 ± 0.33 a
P (g kg ⁻¹)	1.90 ± 0.32 b	2.91 ± 0.27 a	2.63 ± 0.24 a	3.36 ± 0.55 a
Ca (g kg ⁻¹)	56.4 ± 12.3 a	8.00 ± 0.66 b	6.30 ± 0.58 c	3.18 ± 0.20 c
Mg (g kg ⁻¹)	33.3 ± 6.37 a	5.71 ± 0.34 b	4.45 ± 0.99 b	4.57 ± 0.75 b
Fe (g kg ⁻¹)	62.3 ± 6.58 b	69.8 ± 5.07 b	77.0 ± 9.50 ab	86.8 ± 4.18 a
Al (g kg ⁻¹)	34.2 ± 6.22 c	83.4 ± 4.96 b	119 ± 14.6 a	118 ± 14.8 a
Free Fe oxide (g kg ⁻¹)	41.7 ± 2.84 b	62.3 ± 4.57 a	59.5 ± 7.25 a	61.3 ± 3.80 a
Free Al oxide (g kg ⁻¹)	12.8 ± 2.21 b	15.8 ± 2.00 a	16.5 ± 0.15 a	17.4 ± 0.61 a
AOA abundance ×10 ⁷ amoA gene copies (g dry soil) ⁻¹	6.70 ± 0.90 c	41.9 ± 0.53 a	23.1 ± 8.41 b	20.4 ± 2.77 b
AOB abundance ×10 ⁶ amoA gene copies (g dry soil) ⁻¹	2.54 ± 0.15 b	13.6 ± 1.17 a	3.24 ± 0.46 b	1.88 ± 0.90 b

Identical letters within a row indicate no significant difference in the same soil properties between woodland and pitaya plantations at $p > 0.05$.

3.2. N-Cycling Rates

Compared to woodland (5.49 mg N kg⁻¹ d⁻¹), pitaya cultivation significantly decreased the M_{Norg} rate to 2.38–0.62 mg N kg⁻¹ d⁻¹—a change that was more obvious with the increase in the duration of pitaya cultivation. A higher O_{NH_4} rate (9.32 mg N kg⁻¹ d⁻¹) was observed in soil under the 3-year pitaya cultivation than in woodland (4.71 mg N kg⁻¹ d⁻¹), while this rate significantly decreased to 3.91 mg N kg⁻¹ d⁻¹ in soil under the 9-year pitaya cultivation and 1.74 mg N kg⁻¹ d⁻¹ in soil under the 15-year pitaya cultivation (Figure 2).

Compared to woodland (1.63 d), pitaya cultivation significantly increased the mean residence time of NH₄⁺ to 5.06–32.8 d, with the highest value in soil under the 15-year pitaya cultivation. Conversely, a lower mean residence time of NO₃⁻ in soil under the 3-year pitaya cultivation (2.70 d) was found than that in woodland (5.01 d), while this rate significantly increased to 15.3 d in soil under the 15-year pitaya cultivation (Figure 3).

The correlation analyses showed that SOC, total N, Ca, and Mg content, along with CEC, were significantly and positively correlated with the mineralization rate, but negatively correlated with the mean residence time of NH₄⁺ and NO₃⁻ (Figure 4), indicating that the decline in soil quality negatively affected the turnover of inorganic N.

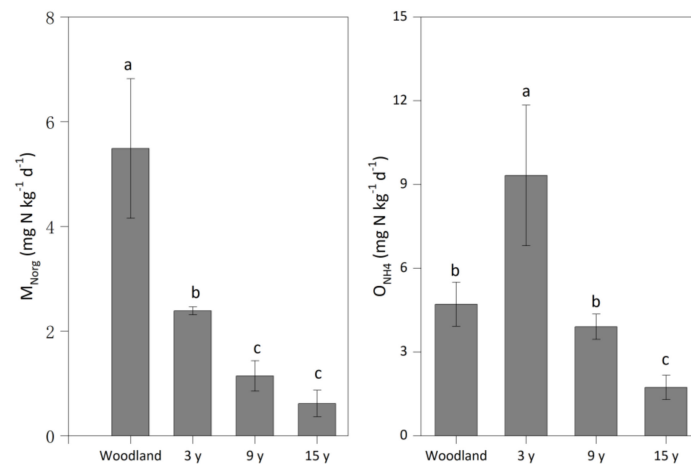


Figure 2. The mineralization (M_{Norg}) and nitrification (O_{NH4}) rates in soils under woodland and three pitaya plantations. Error bars depict standard deviations. Identical letters for M_{Norg} or O_{NH4} indicate no significant differences between woodland and pitaya plantations at $p > 0.05$.

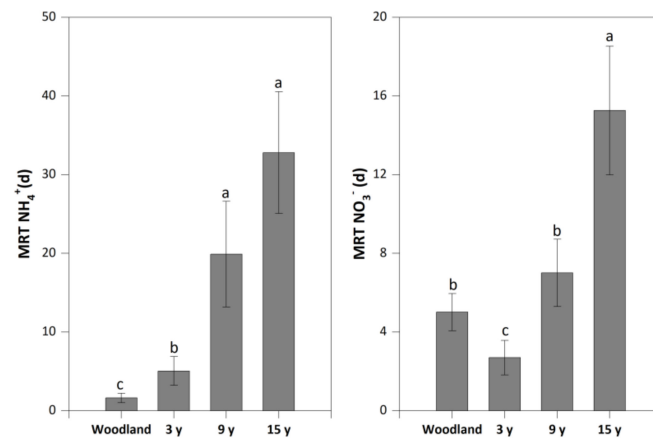


Figure 3. The mean residence time of NH_4^+ ($MRT NH_4^+$) and NO_3^- ($MRT NO_3^-$) in soils under woodland and three pitaya plantations. Error bars depict standard deviations. Identical letters for $MRT NH_4^+$ and $MRT NO_3^-$ indicate no significant differences between woodland and the three pitaya plantations at $p > 0.05$.

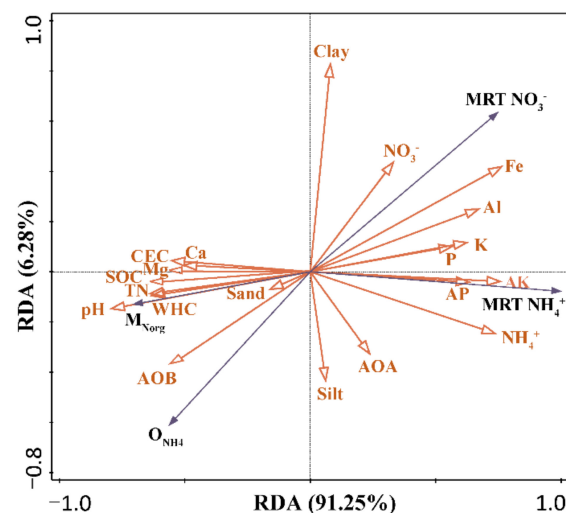


Figure 4. Redundancy analysis (RDA) of soil N-cycling rates, AOA and AOB abundance, and soil properties under woodland and pitaya plantations.

4. Discussion

In this study, pitaya cultivation greatly increased the content of inorganic N, total P, total K, available P, and available K. According to the classification standard of soil fertility from the Soil Survey Office of China (Table S1), both P and K content in soils under pitaya cultivation were at high levels, which could be attributed to the application of large amounts of mineral fertilizer. However, pitaya cultivation significantly decreased the SOC and TN content compared to woodland. The present study showed that the SOC and TN content decreased approximately five-fold in the 15-year pitaya plantation compared to woodland, which is consistent with the findings of previous studies conducted in other long-term crop plantations (e.g., bananas, citrus, rubber) in subtropical karst regions [21,34]. Due to the reduction in soil organic matter, soil may become heavily clayed in long-term pitaya plantations with high clay composition (>50%). These results suggest the possible decline in soil quality under long-term crop cultivation in karst regions, which may adversely affect soil biotic factors (e.g., microbial abundance and activity), thereby altering soil N cycling.

In this study, the mineralization rate in soils under woodland in the subtropical karst region reached $5.49 \text{ mg N kg}^{-1} \text{ d}^{-1}$, which was significantly higher than those in highly weathered red soils in non-karst regions of the same latitude ($1.01\text{--}4.63$; average $2.83 \text{ mg N kg}^{-1} \text{ d}^{-1}$) [18,35,36]. Furthermore, a higher nitrification rate was also found in our studied sites than in the red soil of woodlands in subtropical non-karst regions ($0.06\text{--}1.45$; average $0.76 \text{ mg N kg}^{-1} \text{ d}^{-1}$) [18,35,36]. This difference could be attributed to the characteristics of calcareous soils in karst regions. Calcareous soil is characterized by Ca enrichment, which is the important factor driving the changes in pH, SOC, and TN content, along with other properties (soil moisture, CEC, etc.). In undisturbed ecosystems in karst regions, high Ca levels can promote the accumulation of soil organic matter and the formation of macroaggregates [37–39], all of which can improve the soil quality and soil structure. In addition, soil pH is also positively correlated with Ca content. Consequently, the increase in the substrate (i.e., organic N) facilitates the occurrence of mineralization to produce more NH_4^+ . Previous studies have found a significant and positive correlation between pH and nitrification [22,35]. Under high pH conditions, NH_4^+ is easily oxidized to NO_3^- by the nitrifying microorganisms [35,40]. Due to high mineralization and nitrification rates, inorganic N content was relatively high, and was mainly dominated by NO_3^- in the studied soils under woodlands in the karst region (Table 1).

Compared to woodland, pitaya cultivation significantly reduced the soil mineralization rate, and this effect was more obvious with the increase in the duration of pitaya cultivation. When pitaya had been planted for more than 9 years, the mineralization rate was lower than $1 \text{ mg N kg}^{-1} \text{ d}^{-1}$. Similar results have also been found for other crops (e.g., citrus, rubber) cultivated for more than 7 years in karst regions, with mineralization rates of $0.61\text{--}1.34 \text{ mg N kg}^{-1} \text{ d}^{-1}$ [19,21]. These results suggest that long-term crop cultivation can greatly decrease the mineralization rate in soil in karst regions, indicating a decline in soil inorganic N supply, which supports our hypothesis. The decreased organic matter with the increasing duration of pitaya cultivation may explain the low mineralization rates in karst regions, because significant and positive relationships between mineralization rate, SOC, and total N content were found in this study (Figure 4). When woodland is converted to pitaya plantations, the changes in management practices—including tillage, fertilization, and the reduction in coverage due to the removal of understory vegetation—can lead to the rapid loss of organic matter, thereby reducing the substrate for mineralization [24,41]. In addition, the soil is heavily clayed after long-term crop cultivation in karst regions, which may decrease the microbial abundance and activity involved in the mineralization of organic N [19,21].

Generally, the application of N fertilizers can increase nitrification rates by stimulating the abundance and/or activity of soil-nitrifying microorganisms, especially under high pH conditions [22,42]. Thus, an increased nitrification rate can be expected in soils amended with mineral N fertilizer. Indeed, a high nitrification rate ($9.32 \text{ mg N kg}^{-1} \text{ d}^{-1}$) was observed in the soil under 3-year pitaya cultivation. However, this was not the case

for the 15-year pitaya plantation, which had an extremely low nitrification rate (only $1.74 \text{ mg N kg}^{-1} \text{ d}^{-1}$), even though a large amount of N fertilizer was applied (Table 1). Similar results were also found in other crop plantations (e.g., rubber, citrus) with long-term cultivation [19,21]. An important mechanism underlying the reduced nitrification rate may be that low soil organic matter content in the long-term pitaya plantations can destroy the soil structure, causing the soil to become clayey [19,21]. This could restrict the diffusion of oxygen and, in turn, inhibit the stimulatory effect of N fertilizer on nitrification in soil [43]. In addition, with the increasing duration of pitaya cultivation, the increase in soil iron and aluminum oxide content could also have an adverse impact on nitrifying microorganisms, resulting in a decline in the nitrification rate [14,44]. In this study, long-term pitaya cultivation significantly decreased the abundances of AOA and AOB compared to short-term pitaya cultivation, and the abundance of AOB was greatly positively correlated with the nitrification rate (Figure 4), further supporting the above speculation. All of these results suggest that long-term pitaya cultivation can significantly deteriorate soil quality (e.g., the decline in SOC and TN content, clayed texture, and the increase in Fe and Al oxides) and, subsequently, decrease the supply capacity of inorganic N by decreasing mineralization and nitrification rates. Noticeably, 15-year pitaya cultivation decreased soil pH by one unit compared to woodlands. Considering that nitrification is an important process in the release of hydrogen (H^+) [45], the continuous occurrence of nitrification caused by N fertilizers, combined with the direct loss of Ca, could be responsible for the significant decline in pH during pitaya cultivation.

Noticeably, long-term pitaya cultivation significantly increased the mean residence time of inorganic N in soil, indicating a decline in inorganic N turnover. In this case, large amounts of N fertilizer in the form of NH_4^+ or NO_3^- may not be effectively recycled, and may subsequently be lost in long-term pitaya plantations. Therefore, some appropriate management practices are especially imperative to regulate soil mineralization and nitrification processes, and to stimulate the turnover of inorganic N. Based on the positive relationships between mineralization rate, the mean residence time of inorganic N, and the content of organic C and total N, the application of organic fertilizers may accelerate inorganic N turnover in soil. Further work is needed to study whether organic matter can improve the deteriorated soil environment to stimulate N cycling.

5. Conclusions

The conversion of woodland to pitaya cultivation significantly changed the basic soil properties, causing declines in the SOC, TN, Ca, and Mg content as well as in the pH levels in the pitaya plantations. In addition, pitaya cultivation greatly decreased the mineralization and NH_4^+ turnover rates, decreasing the inorganic N supply capacity, and this effect was more pronounced with the increasing duration of pitaya cultivation. Conversely, short-term pitaya cultivation significantly stimulated nitrification and NO_3^- turnover rates, while long-term pitaya cultivation inhibited both rates. Overall, long-term pitaya cultivation significantly reduced the supply capacity and turnover of soil inorganic N. Thus, appropriate management practices must be developed in the future in order to increase soil inorganic N turnover in long-term pitaya plantations.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/land11060781/s1>, Table S1: Classification of soil fertility from Soil Survey Office of China (1992).

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