Response of Gross Mineralization and Nitrification Rates to Banana Cultivation Sites Converted from Natural Forest in Subtropical China

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Abstract: Evaluations of gross mineralization (M\textsubscript{Norg}) and nitrification (O\textsubscript{NH4}) can be used to evaluate the supply capacity of inorganic N, which is crucial in determining appropriate N fertilizer application. However, the relevant research for banana plantations to date is limited. In this study, natural forest and banana plantations with different cultivation ages (3, 7, 10, and 22 y) were chosen in a subtropical region, and the \textsuperscript{15}N dilution technique was used to determine the gross M\textsubscript{Norg} and O\textsubscript{NH4} rates. The objective was to evaluate the effect of the conversion of natural forests to banana plantations on inorganic N supply capacity (M\textsubscript{Norg} + O\textsubscript{NH4}) and other relevant factors. Compared to other natural forests in tropical and subtropical regions reported on by previous studies, the natural forest in this study was characterized by a relatively low M\textsubscript{Norg} rate and a high O\textsubscript{NH4} rate in the soil, resulting in the presence of inorganic N dominated by nitrate. Compared to the natural forest, 3 y banana cultivation increased the M\textsubscript{Norg} and O\textsubscript{NH4} rates and inorganic N availability in the soil, but these rates were significantly reduced with prolonged banana cultivation. Furthermore, the mean residence times of ammonium and nitrate were shorter in the 3 y than in the 7, 10, and 22 y banana plantations, indicating a reduced turnover of ammonium and nitrate in soil subjected to long-term banana cultivation. In addition, the conversion of natural forest to banana plantation reduced the soil organic carbon (SOC), total N and calcium concentrations, as well as water holding capacity (WHC), cation exchangeable capacity (CEC), and pH, more obviously in soils subjected to long-term banana cultivation. The M\textsubscript{Norg} and O\textsubscript{NH4} rates were significantly and positively related to the SOC and TN concentrations, as well as the WHC and CEC, suggesting that the decline in soil quality after long-term banana cultivation could significantly inhibit M\textsubscript{Norg} and O\textsubscript{NH4} rates, thus reducing inorganic N supply and turnover. Increasing the amount of soil organic matter may be an effective measure for stimulating N cycling for long-term banana cultivation.

Keywords: banana plantation; \textsuperscript{15}N tracing; mineralization; nitrification; inorganic N supply and turnover

1. Introduction

Due to the high economic benefits it offers, the banana (Musa nana) has been widely cultivated as a food source of regional populations in subtropical and tropical regions around the world, occupying an extremely important position in local markets [1,2]. Banana plantation area and production have increased from approximately 4.55 million ha and 67.2 million tons in 2000 to 5.16 million ha and 117 million tons in 2019, respectively [3]. In China, banana plantation area and production amounted to approximately 0.36 million ha and 12.0 million tons in 2019 [3]. To increase banana growth and yield, a suitable
nitrogen (N) management strategy is required [4], since N is the main element limiting crop growth [5]. The current recommended rate of N fertilizer application for bananas is between 250 and 600 kg N ha\(^{-1}\), in the form of split application or basal application [6,7].

In soil, inorganic N, such as ammonium (NH\(_4^+\)) and nitrate (NO\(_3^−\)), are the main N forms available for crop uptake [8,9]. In previous studies, the net transformation method for determining changes in NH\(_4^+\) and NO\(_3^−\) concentrations has been widely used to evaluate soil N availability and its environmental effects, but this method cannot identify the production process [9,10]. In soil, inorganic N is produced mainly through the conversion of organic N to NH\(_4^+\) (i.e., mineralization) and the subsequent oxidation of NH\(_4^+\) to NO\(_3^−\) (i.e., nitrification) [11-13]. Thus, the determination of gross mineralization and nitrification rates using the \(^{15}\)N dilution technique can provide a better understanding of the process and intensity of inorganic N production, which has been widely conducted in various ecosystems (e.g., forest, agriculture, grass) [14-16]. However, the relevant information regarding changes in gross mineralization and nitrification is limited for banana plantations in tropical or subtropical regions. Considering the wide distribution of banana plantations around the world, the investigation of gross mineralization and nitrification rates as a means of evaluating inorganic N supply is crucial to guide N fertilizer application.

At present, the unreasonable rates of fertilization and tillage in banana plantations lead to low fertilizer use efficiency, reductions in yield and quality, etc. [2,17]. Moreover, in the past decade, large banana plantations have been abandoned due to the outbreak of banana wilt disease [18]. Consequently, excessive logging and forest clearing for new banana plantations have been enacted in order to meet the strong market demand for bananas [19]. Previous studies have found a high N retention capacity in the highly weathered soils of natural forests in subtropical or tropical regions, which exhibit high gross mineralization rates along with low gross nitrification rates [11,13]. During the conversion of forest to farmland, an input of N, preferentially provided in inorganic form, as well as frequent irrigation and tillage can greatly change the soil properties and biochemical environment (e.g., soil organic matter, water holding capacity and pH) [20-23], which may affect mineralization and nitrification rates, and N availability in soils. At the initial stage of banana cultivation via conversion from forests, tillage can increase the soil's porosity and subsequently the O\(_2\) diffusion into the soil, which could increase microbial abundance and activity, thus accelerating the decomposition of soil organic matter [24], suggesting a possible increase in mineralization rate. In addition, the application of organic fertilizer can increase the active organic matter sufficiently to stimulate an increase in mineralization rate [25]. On the other hand, increases in soil aeration and N fertilizer application during agricultural cultivation can stimulate increases in the abundance and activity of nitrifying microorganisms, such as ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) [26,27], thereafter possibly increasing the oxidation of NH\(_4^+\) to NO\(_3^−\). These results indicate that the short-term conversion of natural forests to banana plantations may increase the NO\(_3^−\) production rate in soils. In soil, NH\(_4^+\) is lost to the atmosphere through ammonia volatilization, but NO\(_3^−\) is more easily lost through leaching, runoff, and the emission of nitrogenous gases due to denitrification [28,29], especially in subtropical and tropical regions with high rainfall [30]. This may ultimately lower the sustainable supply capacity of soil inorganic N. Noticeably, this stimulating effect of mineral N fertilizer application on gross mineralization and nitrification rates may gradually decrease with the prolonged cultivation of banana, possibly due to the decline in soil quality [15,31]. For example, long-term rubber or oil cultivation sites converted from forests cause significant reductions in the organic carbon (C) and total N (TN) concentrations in soils, as well as the macro-aggregate levels, but cause increases in micro-aggregate [32,33]. Due to the decline in soil organic carbon (SOC) and macro-aggregate levels, the soil can become hard and compacted [34]. If this holds true for long-term banana cultivation as well, the rates of mineralization and nitrification and the inorganic N supply may be reduced as the reduction in substrate, the occurrence of soil compaction, and the input of organic N fertilizer prevent the effective conversion of N to a form available for plant uptake. Thus,
we hypothesized that (1) short-term banana plantations converted from natural forests cause increase mineralization and nitrification rates, thus stimulating inorganic N supply, and (2) long-term banana cultivation causes significant reductions in both rates, and reduce inorganic N supply.

To verify our hypotheses, soils were sampled from natural forests and banana plantations with different ages of cultivation (3, 7, 10, and 22 y) in the subtropical region of Southwestern China. The purpose of this study was to determine the mineralization and nitrification rates in soils using the $^{15}$N tracing approach, and thus to evaluate the effects of the conversion of natural forests to banana plantations on the soil’s inorganic N supply capacity.

2. Material and Methods

2.1. Site Description and Sample Collection

The studied sample sites were located in Gulingning Nature Reserve, Maguan County, Southeastern Yunnan Province, China (103°54′ E, 22°43′ N) (Figure 1). This region is characterized by a typical subtropical monsoon climate. The annual average temperature is between 18.2 and 22.2 °C, and the annual average precipitation is 1700 mm, which mainly occurs from May to October. The main tree species of the natural forest are Dipterocarpus tonkinensis, Pometia tomentosa, Altingia yunnanensis, Shorea chinensis var. kwangsiensis, Burretiodendron hsiennmu, Castanopsis fabri, Caryota urens, Lithocarpus truncatus, Fagus longipetiolata, Alnus nepalensis, Cunninghamia lanceolata, Dendrocalamus strictus, and Arenga pinnata. Four banana plantations with 3-, 7-, 10-, and 22-year cultivation ages were chosen, all of which were converted from natural forests. The slope (approximately 8°) and altitude (approximately 450–650 m) were relatively consistent between the natural forest and the four banana plantations. Approximately 2400 bananas ha$^{-1}$ were planted, and commercial organic fertilizer was applied at a rate of 36,000 kg ha$^{-1}$ y$^{-1}$ as the base fertilizer. Inorganic fertilizers were applied five to six times each year in a circular trench approximately 20 cm away from the banana plants. According to the field investigation, these plantations were fertilized with approximately 320–380, 220–240, and 150–530 kg ha$^{-1}$ y$^{-1}$ of N, phosphorus (P), and potassium (K), respectively. The organic fertilizer contained 3.1 g N kg$^{-1}$, 3.0 g P kg$^{-1}$, and 2.1 g K kg$^{-1}$. The soil of this area is a mixed soil deriving from carbonate rock and basalt weathering, and is classified as Latosol (US Soil Taxonomy), containing 18.5% clay, 65.6% sand, and 15.9% silt.

In July, 2020, three natural forest sites and three sites for each banana plantation type were selected as spatial replicates. The distance between each site exceeded 300 m. Five plots (about 1 × 1 m) were randomly established at intervals of 20 m for each site. Due to the high exposure rate of carbonate rock (20%), the soil layer was relatively thin (<40 cm). After removing the litter layer, the soils were sampled at the banana plantations’ cultivation horizon using a hand auger (5 cm diameter) to a 0–15 cm depth, and all subsamples were mixed to form one composite sample. The sampling method for the natural forest was identical to that of the banana plantations. Fresh soil was passed through a 2 mm sieve after removing litter, plant roots, stones, and other impurities. All soils were immediately placed in a covered cooler with ice for transport to the laboratory. Subsequently, the soils were divided into two constituent parts. A portion of fresh soil was used to determine gross mineralization and nitrification rates and bacterial or archaeal amoA abundance, and the other portion of soil was air-dried to determine its basic physicochemical properties.
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2.2. Gross Mineralization and Nitrification Rates

The gross mineralization and nitrification rates were quantified using $^{15}$N pool dilution techniques [14,35,36]. A series of 30 g samples of fresh soil (oven-dried) were weighted into 250 mL Erlenmeyer flasks for each soil, and these were subsequently pre-incubated at 25 °C for 24 h. After the pre-incubation, 1 mL of $^{15}$NH$_4$NO$_3$ or NH$_4^{15}$NO$_3$ (10 atom% $^{15}$N excess) solution, containing 1.5 mg NH$_4^+$–N kg$^{-1}$ and 1.5 mg NO$_3^-$–N kg$^{-1}$, was evenly applied to the soil in each Erlenmeyer flask. Noticeably, the amount of applied NH$_4$NO$_3$ was relatively higher in this study compared to that previously reported [14,32]. Due to the rapid conversion of NH$_4^+$ to NO$_3^-$ in soils under natural forest conditions with a high pH [30,31], the application of a small amount of NH$_4$NO$_3$ can lead to a relatively low NH$_4^+$ content in the soil after 24 h of incubation, which cannot satisfy the determination criteria of $^{15}$N. As such, NH$_4$NO$_3$ was applied at the relatively high rate of 50 mg NH$_4^+$–N kg$^{-1}$ and 50 mg NO$_3^-$–N kg$^{-1}$. Consequently, the potential rather than actual gross nitrification rate was measured. Distilled water was added to adjust the soil’s moisture to 60% water holding.
capacity (WHC). Then, the flasks were capped with plastic film with small holes in it and incubated for 24 h at 25 °C. The soil samples in the Erlenmeyer flasks were extracted with 150 mL 2M KCl solution at 0.5 and 24 h after NH4NO3 application in order to determine the NH4+ and NO3− concentrations and the respective 15N atom% excess.

2.3. Analyses

Soil pH was determined using a SevenExcellence™ pH/mV detector. After carbonate removal using 1.0 M HCl, an elemental analyzer (Sercon Integra 2) was used to determine the TN and SOC concentrations. The neutral ammonium acetate exchange method was used to determine the soil’s cation exchange capacity (CEC) [37]. Total X-ray fluorescence spectroscopy was used to quantify the total calcium (Ca), magnesium (Mg), P, and K concentrations in the soil. The soil’s available K (AK) and available P (AP) were extracted with neutral NH4OAc and NH4F–HCl solutions, respectively, and subsequently determined via use of a flame photometer and a spectrophotometer. A continuous flow analyzer (Skalar, Breda, The Netherlands) was used to determine the NH4+ and NO3− concentrations in the extract. In addition, the KCl extracts were gradually distilled with magnesium oxide (MgO) and Devarda’s alloy so as to separate the pools of NH4+ and NO3− for 15N measurements [38]. In brief, 100 mL of KCl extract was steam-distilled with MgO to convert the NH4+ into ammonia (NH3), and then Devarda’s alloy was added, and it was distilled again to convert NO3− into NH3 through the reduction of NO3− to NH4+. The NH3 was trapped in a boric acid solution in a conical flask, acidified, and converted into ammonium sulfate ((NH4)2SO4) using 0.02 M H2SO4. According to our preliminary experiment, the recovery ratios of NH4+ and NO3− using the distillation method were 98–102% and 96–98%, respectively. The H2SO4 solution containing NH4+ was then evaporated to dryness at 80 °C in order to analyze the 15N atom% excess with an isotope mass spectrometer (Sercon Integra 2, SerCon Ltd., Crewe, UK).

2.4. Bacterial or Archaeal amoA Abundance Analysis

The FastDNA® Spin Kit for Soil (MP Biomedicals, OH, USA) was used to extract soil DNA, which was subsequently stored at –20 °C until use. A spectrophotometer (Nanodrop ND-2000, NanoDrop Technologies, DE, USA) was used to quantify soil DNA quantity and purity. The abundances of bacterial (AOB) and archaeal (AOA) amoA genes were determined via the quantitative PCR method on a real-time detection system (Bio-Rad CFX96, Laboratories Inc., Hercules, CA, USA). The primers of bacterial and archaeal amoA genes were amoA-1F/amoA-2R and Arch-amoA-F/Arch-amoA-R, respectively [39,40]. Detailed information about the quantitative PCR analysis can be obtained from Zhu et al. (2018) [41].

2.5. Data and Statistical Analyses

The gross mineralization and nitrification rates, expressed as mg N kg−1 d−1, were calculated via the equation of Kirkham and Bartholomew (1954) [35], as follows:

\[
\text{Mineralization (M}_{\text{Norg}}\text{)} = \frac{[\text{NH}_4^+]_0 - [\text{NH}_4^+]_t}{t} \times \frac{\log \left( \frac{\text{APE}}{\text{APE}_0} \right)}{\log \left( \frac{\text{NH}_4^+]_0}{\text{NH}_4^+]_t} \right) \tag{1}
\]

where \( t \) is the incubation time (day), \([\text{NH}_4^+]\) is the NH4+ concentration (mg N kg−1), and APE is the 15N atom% excess of NH4+.

\[
\text{Nitrification (ONH}_4\text{)} = \frac{[\text{NO}_3^-]_0 - [\text{NO}_3^-]_t}{t} \times \frac{\log \left( \frac{\text{APE}}{\text{APE}_0} \right)}{\log \left( \frac{\text{NO}_3^-]_0}{\text{NO}_3^-]_t} \right) \tag{2}
\]
where \( t \) is the incubation time (day), \([\text{NO}_3^-]\) is the \( \text{NO}_3^- \) concentration (mg N kg\(^{-1}\)), and APE is the \( ^{15}\text{N} \) atom\% excess of \( \text{NO}_3^- \).

The mean residence times of \( \text{NH}_4^+ \) (MRT \( \text{NH}_4^+ \)) and \( \text{NO}_3^- \) (MRT \( \text{NO}_3^- \)), expressed as \( d \), were calculated following the equation of Corre et al. (2007) [42].

\[
\text{MRT } \text{NH}_4^+ = \frac{c(\text{NH}_4^+)}{\text{M}_{\text{Norg}}}
\]

\[
\text{MRT } \text{NO}_3^- = \frac{c(\text{NO}_3^-)}{\text{O}_{\text{NH}_4}}
\]

where \( c(\text{NH}_4^+) \) and \( c(\text{NO}_3^-) \) are the initial \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) concentrations (mg N kg\(^{-1}\)) in the studied soils, respectively. If the MRT value of a certain N pool is high, this indicates low turnover.

The supply capacity of inorganic N was calculated by \( \text{M}_{\text{Norg}} + \text{O}_{\text{Norg}} \) [13]. SPSS 23 software (SPSS, Chicago, IL, USA) was used to analyze the relationships between soil properties, AOA and AOB abundances, and \( \text{M}_{\text{Norg}} \) and \( \text{O}_{\text{Norg}} \) rates. Analysis of variance (ANOVA) was used to compare the differences in soil properties, AOA and AOB abundances, and \( \text{M}_{\text{Norg}} \) and \( \text{O}_{\text{Norg}} \) rates between natural forests and banana plantations at the \( p = 0.05 \) level.

3. Results
3.1. Soil Physical and Chemical Properties, AOA and AOB Abundances

The conversion of natural forest to banana plantations reduced the SOC, TN, and CaO concentrations, as well as the WHC, CEC, and pH, more significantly as the cultivation ages increased (Table 1), but this process significantly increased the AK and AP concentrations. In all the studied soils, \( \text{NO}_3^- \) dominated the inorganic N pool with \( \text{NO}_3^-/\text{NH}_4^+ \) ratios of 2.31 (natural forest) and 3.47–8.15 (banana plantations). The difference in \( \text{NH}_4^+ \) concentration between natural forests and banana plantations was not significant due to the high variation; however, the highest \( \text{NO}_3^- \) concentration (70.9 mg N kg\(^{-1}\)) was found in soil under 10 y banana cultivation conditions. The other three banana cultivation conditions manifested concentrations of 17.1–46.2 mg N kg\(^{-1}\) (\( p < 0.05 \)). The SOC and TN concentrations were significantly positively related to CaO, CEC, and pH (\( p < 0.05 \)), and a significant and positive relationship was also found between CaO and pH (\( p < 0.05 \)) (Table 2).

### Table 1. Physical and chemical properties of soils under natural forest and banana plantation conditions with different cultivation ages.

| Parameter \(^a\) | Natural Forest | 3 y \(^b\) | 7 y | 10 y | 22 y |
|------------------|----------------|---------|-----|------|------|
| SOC (g C kg\(^{-1}\)) | 34.6 ± 6.29 a | 32.9±3.14 a | 21.4±1.47 b | 20.0±2.36 b | 19.7±3.34 b |
| TN (g C kg\(^{-1}\)) | 3.25 ± 0.18 a | 2.92 ± 0.18 a | 2.06 ± 0.06 b | 1.88 ± 0.09 b | 1.99 ± 0.29 b |
| pH | 6.75 ± 0.09 a | 6.29 ± 0.08 b | 5.05 ± 0.39 c | 4.65 ± 0.22 cd | 4.30 ± 0.16 d |
| WHC | 0.92 ± 0.15 a | 0.73 ± 0.02 b | 0.69 ± 0.02 bc | 0.67 ± 0.01 c | 0.58 ± 0.03 d |
| CEC (cmol kg\(^{-1}\)) | 18.4 ± 1.25 a | 15.6 ± 0.85 b | 11.3 ± 0.07 c | 10.6 ± 0.38 c | 11.5 ± 0.49 c |
| CaO (%) | 8.39 ± 3.13 a | 3.94 ± 0.52 b | 1.77 ± 0.48 c | 1.45 ± 0.46 c | 1.54 ± 0.53 c |
| AP (mg kg\(^{-1}\)) | 3.27 ± 0.51 c | 173 ± 26.0 a | 136 ± 10.3 b | 197 ± 18.2 a | 159 ± 19.6 a |
| AK (mg kg\(^{-1}\)) | 236 ± 103 b | 783 ± 227 a | 848 ± 331 a | 800 ± 87.1 a | 761 ± 74.8 a |
| \( \text{NH}_4^+ \) (mg N kg\(^{-1}\)) | 9.45 ± 0.36 a | 6.35 ± 2.54 a | 15.0 ± 8.41 a | 10.2 ± 5.34 a | 4.85 ± 0.7 a |
| \( \text{NO}_3^- \) (mg N kg\(^{-1}\)) | 21.9 ± 1.41 c | 29.7 ± 6.79 c | 46.2 ± 3.55 b | 70.9 ± 12.7 a | 17.1 ± 5.36 c |
| \( \text{NO}_3^-/\text{NH}_4^+ \) | 2.31 ± 0.07 b | 5.08 ± 1.63 c | 3.65 ± 1.53 ab | 8.15 ± 4.07 a | 3.47 ± 0.78 ab |
| AOA abundance \( \times 10^7 \) \( \text{amoA} \) gene copies (g dry soil\(^{-1}\)) | 11.6 ± 1.23 a | 14.4 ± 2.45 a | 5.55 ± 3.23 b | 3.66 ± 1.45 b | 1.45 ± 0.29 b |
| AOB abundance \( \times 10^5 \) \( \text{amoA} \) gene copies (g dry soil\(^{-1}\)) | 19.6 ± 3.20 a | 24.6 ± 4.98 a | 6.48 ± 3.13 b | 11.6 ± 2.64 b | 9.14 ± 3.78 b |

\(^a\) SOC, soil organic C; TN, total N; WHC, water holding capacity; CEC, cation exchange capacity; AP, available P; AK, available K; AOA, archaeal \( \text{amoA} \) gene; AOB, bacterial \( \text{amoA} \) gene. \(^b\) 3, 7, 10, and 22 y represent the cultivation durations of the banana plantations that were converted from natural forests. Identical letters for the same value indicate that there were no significant differences in the soils under natural forest conditions and in the four banana plantations in the same region at \( p = 0.05 \).
Table 2. The relationships between soil properties, gross mineralization ($M_{\text{Norg}}$) and nitrification ($O_{\text{Norg}}$) rates, and archaeal amoA gene (AOA) and bacterial amoA gene (AOB) abundances ($n = 12$), under banana plantation conditions.

|        | NH$_4^+$ | NO$_3^-$ | $M_{\text{Norg}}$ | ONH$_4$ | MRT NH$_4^*$ | MRT NO$_3^-$ |
|--------|----------|-----------|-------------------|---------|-------------|-------------|
| SOC    | -0.09    | -0.22     | 0.87 **           | 0.70 *  | -0.39       | -0.39       |
| TN     | -0.22    | -0.34     | 0.91 **           | 0.72 ** | -0.58       | -0.51       |
| pH     | -0.21    | -0.14     | 0.87 **           | 0.83 ** | -0.53       | -0.48       |
| WHC    | 0.30     | 0.37      | 0.51              | 0.57    | 0.06        | 0.08        |
| CEC    | -0.34    | -0.47     | 0.96 **           | 0.87 ** | -0.64 *     | -0.65 *     |
| CaO    | -0.10    | -0.33     | 0.84 **           | 0.78 ** | -0.33       | -0.42       |
| AP     | -0.09    | 0.47      | -0.14             | 0.04    | 0.18        | 0.10        |
| AK     | -0.19    | 0.23      | -0.01             | 0.01    | -0.18       | -0.18       |
| AOA    | 0.04     | -0.15     | 0.83 **           | 0.79 ** | -0.25       | -0.29       |
| AOB    | -0.31    | -0.19     | 0.80 **           | 0.87 ** | -0.43       | -0.54       |

*, $p < 0.05$; **, $p < 0.01$.

The AOA and AOB abundances in soils under 3 y banana cultivation conditions were 1.2 times higher than those in natural forests, but these values gradually decreased as banana cultivation time lengthened. Both the AOA and the AOB abundances were significantly related to SOC, TN, WHC, CEC, CaO, and pH ($p < 0.05$) (Table 2).

3.2. Gross Mineralization ($M_{\text{Norg}}$) and Nitrification ($O_{\text{Norg}}$) Rates

Compared to those under natural forest conditions (1.70 and 6.30 mg N kg$^{-1}$ d$^{-1}$), the $M_{\text{Norg}}$ and $O_{\text{Norg}}$ rates were significantly increased to 3.23 and 11.9 mg N kg$^{-1}$ d$^{-1}$ in the soil under 3 y banana cultivation conditions, respectively, but decreased to 0.65–1.26 and 3.41–4.92 mg N kg$^{-1}$ d$^{-1}$ with the prolongation of banana cultivation (Figure 2). Contrastingly, the 3 y banana cultivation conditions lowered the residence times of NH$_4^+$ and NO$_3^-$ to 2.07 and 2.88 d, respectively, compared to those in natural forests (5.57 and 3.62 d), while long-term banana cultivation (>3 y) increased the residence times of NH$_4^+$ (5.83–15.8 d) and NO$_3^-$ (5.02–15.0 d) (Figure 3). Both the $M_{\text{Norg}}$ and $O_{\text{Norg}}$ rates in soils under banana cultivation conditions were significantly positively related to SOC, TN, WHC, CEC, and pH ($p < 0.05$) (Table 2). In addition, the $O_{\text{Norg}}$ rate was significantly positively related to AOA and AOB abundances ($p < 0.05$) (Table 2).
cultivation conditions were significantly positively related to SOC, TN, WHC, CEC, and pH ($p < 0.05$) (Table 2). In addition, the $O_{\text{Norg}}$ rate was significantly positively related to AOA and AOB abundances ($p < 0.05$) (Table 2).

Figure 2. $M_{\text{Norg}}$ and $O_{\text{NH4}}$ rates in soils under natural forest conditions and in banana plantations with different cultivation durations. Identical letters for $M_{\text{Norg}}$ and $O_{\text{NH4}}$ indicate there were no significant differences in soils under natural forest conditions and in the four banana plantations in the same region, at $p = 0.05$. $M_{\text{Norg}}$, the mineralization of organic N to NH$_4^+$; $O_{\text{NH4}}$, the oxidation of NH$_4^+$ to NO$_3^−$.

Figure 3. Mean residence times of NH$_4^+$ (MRT NH$_4^+$) and NO$_3^−$ (MRT NO$_3^−$) in soils under natural forest conditions and in banana plantations with different cultivation durations. Identical letters for MRT NH$_4^+$ and MRT NO$_3^−$ indicate there were no significant differences in soils under natural forest conditions and in the four banana plantations in the same region, at $p = 0.05$.

4. Discussion

4.1. Low Supply Capacity of Inorganic N in Soils under Natural Forest Conditions

The soil $M_{\text{Norg}}$ rates under natural forest conditions reached 1.70 mg N kg$^{-1}$ d$^{-1}$, which was significantly lower than the rates in other natural forests in tropical or subtropical regions, as reported by previous studies (2.29–9.20; average, 4.31 mg N kg$^{-1}$ d$^{-1}$) [11–13], suggesting a low inorganic N supply capacity in our studied soils under natural forest conditions. However, higher $O_{\text{NH4}}$ rates (6.30 mg N kg$^{-1}$ d$^{-1}$) were found in our studied soils.
than in the highly weathered soils under natural forest conditions in subtropical or tropical regions (0.06–1.97, average 0.75 mg N kg$^{-1}$ d$^{-1}$) [11–13], suggesting the rapid oxidation of NH$_4^+$ to NO$_3^-$ and inorganic N dominated by NO$_3^-$. This was supported by the high NO$_3^-$/NH$_4^+$ ratio (2.31) found in soil under natural forest conditions. Considering the high rainfall in this region, the NO$_3^-$ in soil can easily be lost through leaching and runoff, and thus inorganic N cannot be effectively conserved in soil. This result was inconsistent with those of previous studies conducted in natural forests in tropical or subtropical regions, in which high inorganic N supply and N retention capacities were found due to the high M$_{Norg}$ and low O$_{NH4}$ rates [11,12,28]. Our study, and other previous studies, have inferred a large variability in N dynamics under tropical or subtropical conditions.

The characteristics of the transformation of soil N in natural forests may be greatly related to differences in soil type. In this study, the soil was composed of a mixture of carbonate rock and basalt weathering, and was characterized by a relatively high Ca content. Calcium can react with organic matter to form stable calcium humate [43], which is more difficult to break down for soil organisms, thereby leading to a decline in M$_{Norg}$ rate even when the content of soil organic matter (SOM) is high. Previous studies have found that the O$_{NH4}$ rate is significantly positively related to pH in the soil [44,45]. Forest soil that has developed from basalt or granite in subtropical/tropical regions has a low pH (<4.5), which can inhibit the O$_{NH4}$ rate [11,13]. In this study, however, the soils were characterized by a relatively high pH (5.42), which could increase the abundance and activity of nitrifying microorganisms [46,47]. This might explain why a high O$_{NH4}$ rate was found in the studied soils under natural forest conditions. A high level of NO$_3^-$ production through O$_{NH4}$ in soil may increase the rate of denitrification in subtropical regions with high rainfall, thus subsequently increasing the nitrogenous gas emission potential [48]. Noticeably, the gross O$_{NH4}$ rate may be overestimated in this study due to the application of NH$_4^+$ to the soils. Further in situ experiments must be conducted to elucidate the actual dynamic changes in the NO$_3^-$ of soil under natural forest conditions.

4.2. Response of M$_{Norg}$ and O$_{Norg}$ Rates to Banana Cultivation

Previous studies found significantly positive relationships between SOC and TN concentrations and M$_{Norg}$ rate [9,13,15], indicating that the SOM level is a critical driver of M$_{Norg}$. Although the conversion of natural forests to banana plantations reduces the SOC and total N concentrations (Table 1), large variations in M$_{Norg}$ rate were found in banana plantations with different cultivation durations. Compared to natural forest conditions, short-term banana cultivation greatly increased the M$_{Norg}$ rate, but this rate gradually decreased with prolonged banana cultivation. This result supports our hypothesis, and may be attributed to changes in SOM quality and quantity [25,32]. The conversion of natural forests to banana plantations significantly reduces soil organic matter, thus reducing the substrate of M$_{Norg}$. At the initial stage of banana cultivation, however, organic fertilizer application and the separation of stable calcium humate via reductions in Ca$^{2+}$ protection can increase the soil’s labile organic matter content [49,50], which could stimulate the mineralization of organic N to NH$_4^+$, and even a decline in TN concentration (Table 1). When the labile organic matter is gradually consumed through continuous banana cultivation, the stimulating effect on M$_{Norg}$ may not offset the decline in M$_{Norg}$ caused by the reduction in TN content. The mechanisms by which organic N quality affects M$_{Norg}$ need investigating in the future.

Generally speaking, management measures (e.g., tillage and fertilization) can increase the soil O$_{NH4}$ rate [12,13]. Indeed, 3 y banana cultivation increased the O$_{NH4}$ rate to 11.9 mg N kg$^{-1}$ d$^{-1}$ compared to natural forest conditions, but this was not the case for the long-term (>3 y) banana cultivation assessed in this study, wherein the O$_{NH4}$ rate was significantly reduced. This result supported Hypothesis 1. The different responses of O$_{NH4}$ to various banana cultivation conditions may be related to changes in soil properties (e.g., pH, SOC, structure) [13,31]. Nitrogen fertilizer can increase the abundance and activity of nitrifying microorganisms, in order to increase the O$_{NH4}$ rate in soils with high pH when
natural forests are converted to banana plantations. However, due to the reductions in SOC and total N concentrations with the increasing duration of crop cultivation (Table 1), the reduction in macro-aggregates and the increase in micro-aggregates can cause the surface soil layer to become hard and compacted [34], which can reduce soil permeability and thus inhibit nitrifying microbial abundance and activity [31]. This inhibiting effect may be more obvious in soils composed of carbonate rock, which is characterized by a lower content of acid-insoluble matter and a heavy texture [51,52]. In addition, after long-term banana cultivation, the concentrations of SOC and TN, as well as the water holding capacity, CEC, and pH, are also reduced, thus deteriorating the soil condition, and all of these factors may have an adverse effect on the growth of nitrifying microorganisms and thus inhibit $\text{O}_{\text{NH}_4}$ [15,31]. Indeed, the $\text{O}_{\text{NH}_4}$ rate was found to be significantly positively related to the abundance of AOA and AOB, as well as the SOC, TN, CEC, WHC, and pH (Table 2), which supports the above speculation.

Due to the decline in the $\text{M}_{\text{Norg}}$ and $\text{O}_{\text{NH}_4}$ rates, long-term banana cultivation significantly reduces inorganic N supply capacity, while increasing the resident time of inorganic N, compared to short-term banana cultivation. This result is consistent with Hypothesis 2, implying the reduced turnover of inorganic N in soils under long-term banana cultivation conditions in subtropical regions. According to previous studies [11,13,31], as well as our present result that $\text{M}_{\text{Norg}}$ and $\text{O}_{\text{NH}_4}$ rates were positively related to SOC and TN concentrations, organic N fertilizer should be recommended over mineral N fertilizer to stimulate the supply and turnover of inorganic N in long-term banana plantations.

5. Conclusions

The present study highlights the fact that short-term (3 y) banana cultivation causes increased mineralization and nitrification rates, as well as increasing the turnover rate for inorganic N in the soil, but these rates are significantly reduced with the prolongation of banana cultivation. These results, combined with those of the previous studies, suggest the rapid reduction in soil inorganic N supply when natural forests are converted to economic crop plantations. In such cases, soil N cycling is blocked, and the applied N fertilizers cannot be effectively converted into a form of N that is available for banana uptake, suggesting low N use efficiency and high N loss potential. This is mainly attributed to the reduction in soil quality, i.e., reduced soil organic matter content, and high clay content. Considering the prevalence of banana cultivation worldwide, developing appropriate management measures in future will be necessary in order for banana plantations in specific regions to increase their inorganic N supply and turnover.

Author Contributions: Conceptualization, X.Q. and L.Y.; investigation, X.Q., C.Y., and L.Y.; formal analysis, C.Y. and E.M.; original draft preparation, X.Q. and L.M.; writing—review and editing, X.Q., L.M. and T.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from the National Natural Science Foundation of China (41661051, 42067008) and Guangxi Natural Science Foundation of China (2017GXNSFBA198034; 2018GXNSFBA138042).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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