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Pathways and Drivers of Gross N Transformation in Different Soil Types under Long-Term Chemical Fertilizer Treatments

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Abstract: Microbial-mediated nitrogen (N) dynamics is not only a key process for crop productivity, but also a driver for N losses. Therefore, a better understanding of N dynamics and controlling factors in different soil types is needed to better manage N fertilization in crop fields. To achieve this, a ^{15}N tracing approach was used to quantify simultaneously occurring N transformation rates in four agricultural trials (>20 years chemical fertilizer application) with contrasting climatic and edaphic types (three upland soils and one paddy soil). The results showed that recalcitrant soil organic carbon (SOC) mineralization was the main source of NH_4^+ at all the sites, with rates ranging from 0.037 in fluvo-aquic soil to 3.096 $\text{mg kg}^{-1} \text{ day}^{-1}$ in paddy red soil. Autotrophic nitrification (O_{NH_4}) was the predominant NO_3^- production mechanism in the black and fluvo-aquic soils, whereas it was negligible in the upland and paddy red soils. Nitrification capacity, as an indicator of nitrate leaching risk, was in the order: upland red soil (1%) < paddy red soil (8%) < black soil (235%) < fluvo-aquic soil (485%), implying a high nitrate leaching risk in the last two soils. However, high microbial immobilization (41%) and abiotic adsorption (6%) decreased NO_3^- leaching in black soil. The partial least squares path modeling (PLS-PM) showed that SOC, temperature and pH were the main factors controlling nitrate immobilization, N mineralization and nitrification. In summary, even under similar chemical fertilization conditions, N transformation dynamics are expected to differ with respect to soil type. Therefore, N management strategies should be adjusted to soil type to control N losses and increase crop yield.

Keywords: soil type; gross N transformation; ^{15}N tracing; fertilizer application; paddy soil



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1. Introduction

Microbial-mediated nitrogen (N) dynamics is a key process in an agroecosystem, which not only provides ammonium and nitrate for crop growth, but also causes soil acidification, produces N_2O and causes nitrate leaching. Soil type, which is based on local climate, parent material and anthropological management, is one of the most important environmental factors governing microbial mediated N dynamics [1,2]. It affects the activity and abundance of soil microorganisms and functional genes [2,3], which control the internal N cycle. Paddy soil, which undergoes alternate submersion and drying, exhibits specific N dynamics [4]. However, how soil types and long-term flooding (in soils from the same parent material, but one is an upland soil and the other is a paddy soil) affect gross N dynamics in agricultural soil under long-term fertilization is still not clear.

Only a few studies have reported on gross N transformations in paddy soils [4,5]. For example, Lan et al. [6] investigated a summer rice–winter wheat rotation and took samples during the winter wheat season, whereas Nishio et al. [7] and Kader et al. [8] investigated N transformations under continuous waterlogged conditions. In fact, cyclic change of wet and dry conditions produces strong effects on gross N transformation [4]: with extensive flooding, mineralization slows down due to less efficient and incomplete decomposition [4];

nitrification is also low because nitrifiers are obligate aerobes [7]. In addition, these studies used one N pool model to simulate gross N rates.

Long-term fertilization, which largely changes soil properties (e.g., pH, SOC, C/N), strongly influences N dynamics in upland soil [9,10] and paddy soil [5]. Several studies have found that gross N mineralization, nitrification and immobilization are promoted by fertilization in nutrient-limited soils [9,10], because N and phosphate input increase aboveground biomass, and higher biomass provides nutrients and energy for microbes via root deposits or residue [10]. In contrast, long-term fertilization decreases gross N mineralization in a fertile soil because the microbes invest less energy in enzyme production to decompose polymers [11]. According to a literature survey, Booth et al. [12] found that gross N dynamics do not vary under fertilization. To reduce the effect of different managements (especially fertilization) on gross N dynamics, we collected soil samples from long-term fertilization experiment, which makes the soil physical and chemical properties relatively stable, and the managements were similar.

To address the knowledge gaps regarding N dynamics in different soil types that vary with long-term flooding or dry farming, and the difference in models with different N pools, we performed ^{15}N tracing studies to quantify the gross N transformation rates in four contrasting soils from long-term experiment sites (>20 years) in different parts of China, and analyzed affecting factors. We hypothesized that gross N mineralization-nitrification-immobilization would vary according to soil type. The main aims of the study were to test the hypothesis and elucidate pathways and affecting factors in different soil types.

2. Materials and Methods

2.1. Site Description and Experimental Design

Four long-term experimental sites (three upland soils and one paddy soil) in typical crop production areas in China were selected. The four sites cover a wide range of geographical areas and climate conditions. They are at Gongzhuling (124°48' E, 43°30' N, black soil, Chernozem) in Jilin Province (north-east), Zhengzhou (113°41' E, 35°00' N, fluvo-aquic soil, Calcaric-Fluvisols) in Henan Province (central China), Qiyang (111°52' E, 26°45' N, upland red soil, Ferralic Cambisol soil), and Wangcheng (112°80' E, 28°37' N, paddy red soil, Ferralic Cambisol soil) in Hunan Province (south). The upland long-term experiments have been conducted since 1990, and the paddy land experiment began in 1980 (Wangcheng). The soils in Qiyang and Wangcheng (paddy) originated from the same parent material (quaternary red clay). The typical climate conditions and the soil properties are shown in Table 1.

Table 1. Soil properties and meteorological data of the four study sites.

	Black Soil Chernozem (WRB) *	Fluvo-Aquic Soil Calcaric-Fluvisols	Upland Red Soil Ferralic Cambisol	Paddy Red Soil Ferralic Cambisol
Parent material	Quaternary sediments	River Alluvium	Quaternary red clay	Quaternary red clay
Clay mineral type #	Montmorillonite	Hydromica Montmorillonite	Kaolinite	Kaolinite
Clay content (%)	31.1	20	43.9	38.7
SOC (g kg^{-1})	12.6	7.3	8.7	21.3
Total N (g kg^{-1})	1.34	0.67	1.02	2.11
pH	5.9	7.9	4.6	5.4
Temperature ($^{\circ}\text{C}$)	4.5	14.4	18	17
Rainfall (mm)	525	700	1255	1370

* Soil taxonomy based on the World Reference Base (WRB).

Chemical fertilizer treatments (combined application of N, phosphate and potassium) corresponding to those used by local farmers were selected for this study. The treatments had three replicates. The plot size was around 43 m². The crop rotation practices and fertilizer application rates at each site are shown in Table 2. One-third of the N was applied

as basal fertilizer (together with P and K) and the other two-thirds were applied at the beginning of the jointing stage.

Table 2. Fertilizer application rates for each growing season at the four sites (kg ha^{-1}).

N Treatments	Black Soil	Fluvo-Aquic Soil		Upland Red Soil		Paddy Red Soil	
	Maize	Wheat	Maize	Wheat	Maize	Early Rice	Late Rice
N (urea)	165	165	188	90	210	165	165
P (calcium superphosphate)	36	36	41	16	37	45	45
K (potassium chloride)	68	68	78	30	70	120	120

2.2. ^{15}N Tracing Experiment

The gross N dynamics were analyzed after collecting soil samples at the end of September 2016. At each site, thirty soil cores were collected to form one composite sample. After sieving (2 mm), the homogenized samples were separated into two parts, one for the ^{15}N tracing study and the other for the soil properties analysis. Soil chemical properties, including labile and recalcitrant pools of C and N, were analyzed [13].

For the ^{15}N tracing, 100 g of air-dry soil was put into 500 mL plastic beakers and packed to a bulk density of 1.0 g cm^{-3} . The soil was adjusted to 40% water filled pore space (WFPS), and preincubated for 1 week at 20°C in the dark (covered with Parafilm with five pin pricks for air exchange). On the 8th day, an aliquot of differentially ^{15}N labeled ammonium nitrate (60 atom % excess $^{15}\text{NH}_4\text{NO}_3$ and $\text{NH}_4^{15}\text{NO}_3$) was uniformly sprayed on the soil at a rate of $50 \mu\text{g N g}^{-1}$. It was thoroughly mixed in and then the soil was repacked. The repacked soil moisture content was 50% WFPS. The final soils were incubated at 20°C in the dark. After 3 h, 1, 2, 4, 7, 10, 15, 20, 25, 30, and 35 days, 12 soil samples (3 replicates $\times$ 4 soil types $\times$ 10 sample times) were extracted and analyzed for mineral N (NH_4^+ , NO_3^-) and their ^{15}N enrichment levels.

2.3. Determination of Mineral N Concentration and ^{15}N Enrichment

The soil in each beaker was extracted with 2 M KCl (1:3 W/V). Each suspension was filtered through GF/D glass-fiber papers (General Electric Biological Technology Co. Ltd., Hangzhou, China). The filtrates were stored at -20°C prior to measuring concentration and ^{15}N enrichment of NH_4^+ and NO_3^- .

The NH_4^+ and NO_3^- concentrations in the extracts were determined by automated continuous flow analysis (AA3, Seal Analytical, Norderstedt, Germany). The $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ enrichment levels in the extracts were determined by a modified acid diffusion procedure of Letif et al. [14]. For $^{15}\text{NH}_4^+$ diffusion, 0.3 g MgO was added to 20 mL extract in a bottle. The bottle was capped with an acidified filter disk ($10 \mu\text{L } 2.5 \text{ mol L}^{-1}$ oxalic acid) hanging on the cap and shaken at 180 rpm on a shaker at 30°C for 24 h. For $^{15}\text{NO}_3^-$ diffusion, 0.3 g MgO was added to 20 mL extract. The bottle, without cap, was shaken at 180 rpm on a shaker at 30°C for 24 h. Then 0.3 g Devarda's alloy was added and capped with an acidified filter disk ($10 \mu\text{L } 2.5 \text{ mol L}^{-1}$ oxalic acid) hanging on the cap. The bottle was shaken at 180 rpm on a shaker at 30°C for 24 h. Then all filter disks were placed in a desiccator to dry over concentrated H_2SO_4 . The dried filter disks were put into tin capsules and the total N and atom % ^{15}N were determined by a Vario PYRO cube elemental analyzer (Elementar, Langenselbold, Germany) coupled to an isotope ratio mass spectrometer (IsoPrime 100, Isoprime Ltd., Stockport, UK). The recovery rate for standard solutions using the same procedure was more than 95% for both $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$.

2.4. Quantification of Gross N Transformation Rates

Gross N dynamics, including two N pools and ten transformation rates, were quantified using the Ntrace model [15]. The model used a Markov Chain Monte Carlo Metropolis algorithm (MCMC-MA) to optimize N dynamic parameters (both rates and kinetics; [14]). A

parameter optimization process simultaneously optimized observed NH_4^+ , NO_3^- , $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ concentrations (eight sets of measured data together). Variance of the individual observations was considered in the misfit function. The optimization result was evaluated using the Akaike Information Criterion (AIC). The MATLAB (Version 7, The MathWorks Inc., Massachusetts, USA) and Simulink (Version 7, The MathWorks Inc.) were used to program the MCMC-MA [15].

2.5. Calculations and Statistical Analyses

Gross N transformation rates that match first-order and Michaelis-Menten kinetics were calculated by integrating the rates over the whole period divided by the total time. Zero-order kinetics, which do not change with time, were directly obtained from simulations. A one-way ANOVA was used to test whether the rates are significantly different. Pair-wise comparisons of the rates for all soil type combinations were calculated using the Holm-Sidak test by SigmaPlot (Version 12.5, Systat Software Inc., San Jose, CA, USA).

To explain how soil types and soil properties affect gross N dynamics in different environments, we used partial least squares path modeling (PLS-PM) to statistically quantify multivariate (cause and effect) relationships among observed and latent variables [16]. The model was run first to find insignificant parameters, then it was re-run (omitting insignificant parameters) to quantify relationships among the significant parameters. The path coefficients and the coefficients of determination (R^2) were calculated by software R (Version 3.3.3, package “plsrm” created by Gaston Sanchez) and validated by 1000 bootstraps.

3. Results

3.1. Inorganic Nitrogen Pool Sizes and ^{15}N Enrichment

Changes in inorganic N pool sizes were shown to be soil-type-dependent. For example, the NH_4^+ pool decreased sharply in the fluvo-aquic soil, whereas it increased continuously in the paddy soil (Figure 1). The Ntrace-model-optimized data matched well with the observed NH_4^+ and NO_3^- concentrations and their ^{15}N enrichments for all sites (Figure 1). The coefficient of determination (R^2) for the observed values and the model simulation was larger than 0.96.

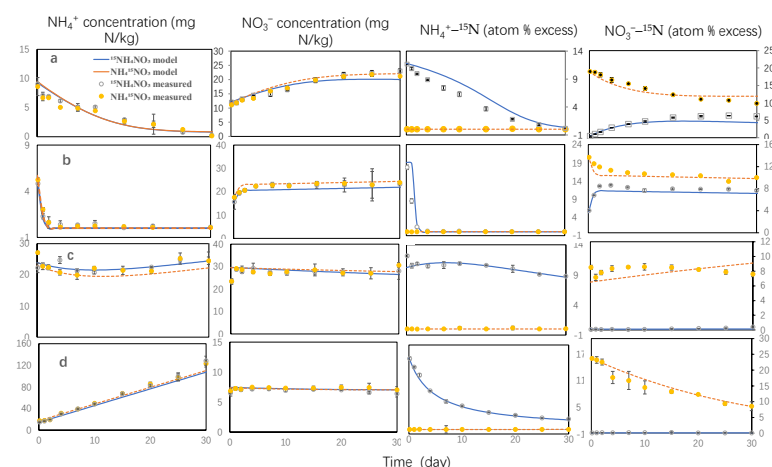


Figure 1. Measured and modeled concentrations of NH_4^+ , NO_3^- , and their ^{15}N enrichment in black soil (a), fluvo-aquic soil (b), upland red soil (c), and paddy red soil (d) under aerobic incubation conditions.

3.2. Gross N Transformation Rates

Gross N transformation rates varied with soil type ($p < 0.05$, Table 3). Recalcitrant organic matter mineralization (M_{Nrec}) was the main source for NH_4^+ production in all the sites ($>76\%$ NH_4^+ production), with the rate ranging from 0.037 (fluvo-aquic soil) to $3.096 \text{ mg kg}^{-1} \text{ day}^{-1}$ (paddy soil). Labile organic matter mineralization (M_{Nlab}) contributed 27% and 24% to NH_4^+ production in black soil and fluvo-aquic soil, respectively, whereas

it contributed less than 5% in the other soils. Ammonium immobilization (I_{NH4}) was only significant in upland red soil, and contributed 36% to NH_4^+ production.

Table 3. Gross N transformation rates ($mg\ N\ kg^{-1}\ day^{-1}$) for the different soil types (Mean $\pm$ SD).

	Black Upland Soil		Fluvo-Aquic Upland Soil		Red Upland Soil		Red Paddy Soil	
	K *	Mean	K	Mean	K	Mean	K	Mean
M_{Nrec}	0	$0.145 \pm 0.005\ c$	0	$0.037 \pm 0.003\ d$	0	$0.724 \pm 0.035\ b$	0	$3.096 \pm 0.020\ a$
I_{NH4}	1	$0.003 \pm 0.001\ b$	1	$0.000 \pm 0\ b$	1	$0.272 \pm 0.086\ a$	1	$0.003 \pm 0.002\ b$
M_{Nlab}	1	$0.054 \pm 0.009\ a$	1	$0.012 \pm 0.002\ b$	1	$0.036 \pm 0.297\ b$	1	$0.017 \pm 0.009\ b$
O_{Nrec}	0	$0.000 \pm 0.000\ b$	0	$0.007 \pm 0.000\ b$	0	$0.002 \pm 0.008\ b$	0	$0.238 \pm 0.004\ a$
I_{NO3}	1	$0.193 \pm 0.014\ b$	1	$0.000 \pm 0.000\ c$	2	$0.010 \pm 0.006\ c$	1	$0.259 \pm 0.005\ a$
O_{NH4}	2	$0.467 \pm 0.012\ a$	2	$0.223 \pm 0.016\ b$	2	$0.000 \pm 0.000\ c$	1	$0.010 \pm 0.001\ c$
A_{NH4}	1	$0.000 \pm 0.000\ b$	1	$0 \pm 0\ b$	1	$1.204 \pm 0.128\ a$	1	$0.000 \pm 0.005\ b$
R_{NH4}	1	$0 \pm 0\ b$	1	$0 \pm 0\ b$	1	$0.715 \pm 0.045\ a$	1	$0 \pm 0\ b$
A_{NO3}	1	$0.029 \pm 0.009\ b$	1	$0 \pm 0\ c$	1	$0.110 \pm 0.013\ a$	1	$0 \pm 0\ c$
R_{NO3}	1	$0.039 \pm 0.002\ a$	1	$0 \pm 0\ b$	1	$0.040 \pm 0.003\ a$	1	$0 \pm 0\ b$
NC (%)		235		485		1		8
AIC		5359		3600		583		207
R2		0.96		0.99		0.96		0.99

M_{Nrec} : mineralization of recalcitrant organic nitrogen to NH_4 ; I_{NH4} : immobilization of NH_4 to recalcitrant organic nitrogen; M_{Nlab} : mineralization of labile organic nitrogen to NH_4 ; O_{Nrec} : oxidation of recalcitrant organic nitrogen to NO_3 ; I_{NO3} : immobilization of NO_3^- to recalcitrant nitrogen; O_{NH4} : oxidation of NH_4^+ to NO_3^- ; A_{NH4} : adsorption of NH_4^+ ; R_{NH4} : release of adsorbed NH_4^+ ; A_{NO3} : adsorption of NO_3^- ; R_{NO3} : release of adsorbed NO_3^- ; NC: nitrification capacity = NH_4^+ oxidation / ($M_{Nrec} + M_{Nlab}$); * K = kinetics: 0 = zero-order, 1 = first-order, 2 = Michaelis-Menten. AIC: Akaike Information Criterion; R2: coefficient of determination. Different lowercase letters indicate significant differences between soil types ($p < 0.05$).

Nitrate production was mainly associated with NH_4^+ oxidation (O_{NH4}) in the black and fluvo-aquic soils, whereas it was neglectable in the red soils. Nitrification capacity ($O_{NH4}/(M_{Nrec} + M_{Nlab})$), an indicator of nitrate leaching risk, was in the order: fluvo-aquic (485%) > black soil (235%) > paddy red soil (8%) > upland red soil (1%). However, in the black soil, NO_3^- immobilization contributed 41% of NO_3^- production (Nit_{tot}), which decreased leaching risk.

3.3. PLS-PM Analysis

The PLS-PM results showed that gross N transformation rates varied significantly due to changes in temperature, soil pH and SOC contents (including SOC and recalcitrant OC). Labile N and clay content were key reflective indicators for soil type, although they had no significant impacts on gross N dynamics. Mineralization of recalcitrant organic nitrogen (M_{Nrec}) was regulated by SOC (0.55). Nitrification (O_{NH4}) of NH_4^+ was regulated by temperature (−0.48) and pH (0.39). Nitrate immobilization was regulated by SOC (0.86). The prediction power (GoF, Goodness of Fit) of the structural model for the correlations was higher than 0.74 (>0.7 is considered to be very good).

4. Discussion

Nitrogen dynamics are considerably influenced by both broad-scale environmental conditions and local-scale soil heterogeneity [17]. Different soil types, as the combined results of climate, parent material and long-term anthropological management (such as paddy soil), showed different transformation rates and pathways (Table 3). This supported our hypothesis. Further PLS-PM analysis showed that the differences in temperature, soil pH and SOC, instead of N (total N and recalcitrant N), were the main reasons for the variations of gross N mineralization, nitrification, and immobilization in the four soil types.

4.1. Patterns of Gross N Mineralization Rates and Affecting Factors

Gross N mineralization rates differed significantly between the soil types, ranging from 0.037 in fluvo-aquic soil to 3.096 $mg\ kg^{-1}\ day^{-1}$ in paddy soil. This result agrees

with Nishio et al. [7], who showed that gross N mineralization and immobilization rates were higher in paddy soil than in upland soil which had been converted from paddy soil. They further suggested that high SOC and high partially decomposed SOC in paddy soils, but not microbial population, were the main reasons for the high mineralization rates in paddy soil.

The recalcitrant organic nitrogen was the main source for NH_4^+ production at all sites, which agreed with the findings reported by Müller et al. [18] and Zhang et al. [9] for grassland and agricultural soils. The most likely reason was that long-term fertilization without organic matter inputs caused a rapid decomposition of labile organic N, which meant that highly decomposed substances (e.g., recalcitrant humus C) became the main N sources available for decomposition. This process was similar to the observed time-dependent shift of mineralization in response to 10-year elevated CO_2 levels [19], i.e., mineralization of recalcitrant SOC enhances under long-term CO_2 enrichment or N input [19].

Recalcitrant organic matter mineralization was controlled by SOC (Figure 2). This observation agreed with Elrys et al. [1]. Many studies have shown that gross N mineralization rates are positively related to SOC concentrations [1,12,20]. This positive relationship was true in paddy soil, black soil and fluvo-aquic soil (Tables 1 and 3). However, in upland red soil (low in SOC), the relationship was negative. The reason might be the low soil C:N ratio and high Fe oxidation. Booth et al. [12] found that C:N ratios were negatively related to gross N mineralization rates, whereas Fe oxidation was positively related to gross N mineralization rates [21]. Fe oxidation in upland red soil is high due to its parental material; high rainfall and high temperatures enhance the production.

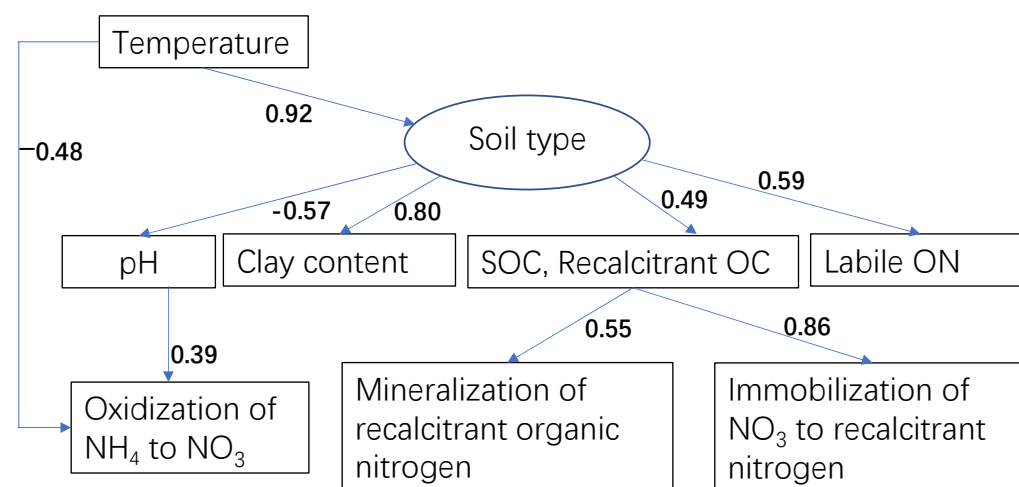


Figure 2. Partial least squares path model (PLS-PM) of the observed variables (measured) and latent variables (constructs). Path coefficients were calculated after 1000 bootstraps. Only significant path coefficients are shown in the figure ($p < 0.05$). The statistical parameter (GoF, Goodness of Fit) was 0.74, which means the structural model predicted the correlations very well.

4.2. Patterns of Gross N Nitrification Rate and Affecting Factors

High mineralization in red soil means more substrate (NH_4^+) for nitrification [22]. However, the nitrification rate was low in red soil. The first reason was that high abiotic adsorption of NH_4^+ in red soil decreased NH_4^+ availability (Table 3). Red soils originating from quaternary red clay, as were used in this study, have special charge properties [23]. Their variable negative charge is more than 10 times greater than other red soils (developed from purple soil and granite). Their positive charge is also significantly larger than that of other red soils. Therefore, the red soils used in the study can adsorb considerably larger amounts of NH_4^+ and NO_3^- [23]. The second reason was a high clay content (>40%) in the red soil, which provided more cation-exchange sites for NH_4^+ fixation. The third reason was that long-term flooding caused microorganisms that only adapted to anaerobic conditions to remain active during the dry condition [24], whereas nitrifiers are obligate

aerobes. Therefore, the short-term aerobic incubation (35 days) in red soils did not change NH_4^+ oxidization to NO_3^- (Table 3, [25]). This also agreed with a previous study that showed that NH_4^+ is the predominant inorganic N form in acidic and highly weathered subtropical soils [23].

Soil pH was positively related with nitrification (Figure 2), because low pH of the soils suppresses nitrifier activity [26]. This result is comparable with Elrys et al. [21]. Many studies have shown that soil pH is the key property that significantly influences the ammonia-oxidizing archaea (AOA [27] and AOB [28]). However, AOA and AOB, as mediators of nitrification, contribute differently to different soils. In alkaline upland soils, AOB was the predominant controller [29]. In neutral upland soils, both AOA and AOB contributed equally to nitrification [28]. In acid paddy soil and upland soil, nitrification was controlled by AOA and AOB, respectively [30]. Furthermore, AOA and AOB show different responses to fertilizer application (e.g., AOA are not sensitive to mineral N, whereas AOB increase activity and growth under mineral N [31]). This suggests that when long-term mineral N interacted with the parent material, the response of gross N nitrification to long-term fertilization varies between soil types.

Heterotrophic nitrification (direct oxidization of recalcitrant organic matter, O_{Nrec}) mostly occurs in environments with a low pH and high organic C contents, such as forest soils [32]. In the paddy soil, it contributed more than 99% to total NO_3^- production (Table 3). This agreed with Xu et al. [4]. The reason may be that autotrophic nitrifiers are inhibited by long-term anaerobic conditions and low pH. Therefore, heterotrophic fungi and bacteria directly oxidize partially decomposed SOM to NO_3^- [25]. In addition, a high SOC (20.6 to 21.3 g kg⁻¹) and low pH (5.4) under long-term chemical fertilizer input may provide suitable conditions for heterotrophic nitrification in the paddy soil.

Nitrification capacity ($O_{\text{NH}_4}/(M_{Nrec} + M_{Nlab})$), an indicator of NO_3^- leaching risk, was in the order: fluvo-aquic soil (485%) > black soil (235%) > red paddy soil (8%) > red upland soil (1%). This implied that even under similar fertilization, leaching risk was different. In fluvo-aquic soils, a high nitrification rate, a low clay content and low SOM could lead to a greater risk of leaching. In black soils, although nitrification capacity is high, abiotic NO_3^- adsorption and high biotic immobilization of NO_3^- to recalcitrant N (41%) decreases the leaching risk [33]. In subtropical red soil, nitrate leaching is negligible due to low nitrification and high biotic/abiotic nitrate immobilization. Therefore, different N management methods have to be used to decrease leaching and increase N use efficiency.

4.3. Factors Affecting the Immobilization of NO_3^-

Nitrate immobilization appears to be very low in agricultural soils [34] except in alkaline purple soils, where immobilization is the dominant NO_3^- consumption process (29% of nitrification [20]). In this study, nitrate immobilization contributed 41.3% to nitrification in black soils, whereas it was neglectable in fluvo-aquic soil. The reason may be SOC, which is the predominant factor controlling NO_3^- immobilization (Table 3, [35,36]). In red soils (both upland and paddy), nitrate immobilization was higher than nitrification (Table 3). This result was similar to that derived by Niboyet et al. [37] in fertilized grassland. In paddy soil, this may be due to the fact that the immobilization not only includes the demand of NO_3^- as a nutrient for microbes, but also includes the demand of NO_3^- to serve as an electron acceptor, which is much greater than the demand for nutrient [25]. The reason for higher immobilization in upland red soil may be underestimated gross nitrification due to remineralization [37].

5. Conclusions

Gross N mineralization-immobilization-nitrification dynamics varied with soil type. Gross N mineralization rates in the acidic red soils (paddy and upland) were significantly higher than in the black soil and fluvo-aquic soil. However, the reverse was true for gross N nitrification rates, which were negligible in the red soils. Therefore, nitrate leaching risk was low in the red soils. However, high microbial immobilization of NO_3^- (41%) decreased

leaching risk in black soil. Soil organic carbon, temperature and pH were factors controlling the key dynamics. In summary, even under similar fertilizer application, different N management methods have to be used in different soil types and climate conditions to reduce N leaching and increase N use efficiency.

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