Nitrogen Mineralization, Soil Microbial Biomass and Extracellular Enzyme Activities Regulated by Long-Term N Fertilizer Inputs: A Comparison Study from Upland and Paddy Soils in a Red Soil Region of China

Sehrish Ali [1,†], Kailou Liu [2,†], Waqas Ahmed [3], Huang Jing [4], Muhammad Qaswar [5], Christian Kofi Anthonio [1], Ali Akbar Maitlo [1], Zhang Lu [4], Lisheng Liu [4] and Huimin Zhang [1,4,*]

[1] National Engineering Laboratory for Improving Quality of Arable Land, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China; Sehrishbakhsh788@gmail.com (S.A.); ckanthonio@gmail.com (C.K.A.); aamaitlo@sau.edu.pk (A.A.M.)

[2] National Engineering and Technology Research Center for Red Soil Improvement, Jiangxi Institute of Red Soil, Nanchang 331717, China; kailouliu322@gmail.com

[3] Guangdong Provincial Key Laboratory for Radionuclides Pollution Control and Resources, School of Environmental Science and Engineering, Guangzhou University, Guangzhou 510006, China; drwaqas@gzhu.edu.cn

[4] National Observation Station of Qiyang Agri-Ecology System, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Qiyang 426182, China; huangjing@caas.cn (H.J.); zhanglu01@caas.cn (Z.L.); liulisheng@caas.cn (L.L.)

[5] Department of Environment, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium; mqaswar2@gmail.com

* Correspondence: zhanghuimin@caas.cn

† Sehrish Ali and Liu Kailou equally contributed to this article.

Abstract: A long-term experiment (38 years) was conducted to elucidate the effects of long-term N addition on the net N mineralization in both paddy and upland soils, based on their initial soil N status, with and in connection with soil microbial biomass and N cycling extracellular enzyme activities. Two treatments without N addition CK (No fertilizer) and K (inorganic potassium fertilizer) and two treatments with N addition (inorganic nitrogen fertilizer) and NK (inorganic nitrogen and potassium fertilizer) were placed in incubation for 90 days. Results showed that the total N and soil organic carbon (SOC) contents were higher in the treatments with N application compared to the treatments without N in both paddy and upland soils. The SOC content of paddy soil was increased relative to upland soil by 56.2%, 45.7%, 61.1% and 62.2% without N (CK, K) and with N (N and NK) treatments, respectively. Site-wise, total N concentration in paddy soil was higher by 0.06, 0.10, 0.57 and 0.60 times under the CK, K, N and NK treatments, respectively, compared with upland soil. In paddy soil, soil microbial biomass nitrogen (SMBN) was higher by 39.6%, 2.77%, 29.5% and 31.4%, and microbial biomass carbon (SMBC) was higher by 11.8%, 11.9%, 10.1% and 12.3%, respectively, in CK, K, N and NK treatment, compared with upland soil. Overall, compared to upland soil, the activities of leucine-aminopeptidase (LAP) were increased by 31%, 18%, 20% and 11%, and those of N-acetyl-b-D-glucosaminidase (NAG) were increased by 70%, 21%, 13% and 18% by CK, K, N and NK treatments, respectively, in paddy soil. A significantly linear increase was found in the NO_3^--N and NH_4^+-N concentrations during the 90 days of the incubation period in both soils. NK treatment showed the highest N mineralization potential (N_o) along with mineralization rate constant, k (NMR) at the end of the incubation. SMBC, SMBN, enzyme activities, NO_3^--N and NH_4^+-N concentrations and the N_o showed a highly significant ($p \leq 0.05$) positive correlation. We concluded that long-term N addition accelerated the net mineralization by increasing soil microbial activities under both soils.

Keywords: nitrogen mineralization; extracellular enzymes; upland and paddy soil; soil microbial biomass; long-term N addition

1. Introduction

Nitrogen (N) mineralization potential (N_o) of soil has been described as the extent of soil organic N that is capable of being mineralized [1]. N mineralization is a key mechanism influencing the fertility of soil [2,3] and is needed to be mastered and understood when it comes to meeting the N demands of the crops [4]. Crops use N in the form of: (i) mineral N application; (ii) decomposition of organic amendments or plant residues; and (iii) soil organic N mineralization [5]. N mineralization produces NH_4^+-N and NO_3^--N, which are taken up by the plants, and also controls the supply and magnitude of the mineral N from the soil system to the plants as well [6,7].

Fertilization is a popular recommended practice to improve and sustain the fertility of the arable lands and is also assumed to increase the soil's capacity of the nutrient supply and maintaining soil organic carbon (SOC) levels, which helps to sustain higher crop yields [8]. It controls soil microbial activities and the relevant functions which play an important role in soil nutrient cycling and soil organic matter decomposition [9]. Significant impacts of soil-available nutrients on N mineralization have been well documented in several studies [10,11].

Soil microorganisms and microbial extracellular enzymes involved in N cycling play a crucial role in sustaining soil quality, ecosystem processes, including the acquisition of essential soil nutrients [12], decomposition of organic matter [13], nitrogen (N) cycling and carbon (C) cycling [14]. These are measured as microbial biomass nitrogen (SMBN) and carbon (SMBC), primarily known as "biological traits", which are key indicators of soil health [15,16] and are also stated as the sensitive indicators of organic matter decomposition and N mineralization [17,18]. Nutrient supply in the soil is mainly governed by the microbial biomass nutrient pool [19,20] and plays a vital role in the nutrient storage capability of the soils [21]. It improves the soil quality and also serves as a source of plant-available nutrients [22]. Soil microbial activities are strongly affected by nutrient availability. For example, some studies have shown that N fertilizer addition can increase the enzymatic activity, microbial biomass and soil C mineralization rate [23,24].

Soil extracellular enzymes which are involved in N mineralization (NAG and LAP) are usually taken as the sensitive N mineralization indexes and can also contribute to providing an insight into the vital soil biochemical processes [25]. All the above processes are mediated by the soil enzymes, which are straight away produced from the viable microbial cells, or they can be produced from those enzymes which, after their production, get stable in the soil system but remain catalytic [26]. Microbial nutrient demands reflected by enzyme activities are explored by the stoichiometry of elements with different microbial communities [11,27]. Microorganisms residing in the soil can get the nutrients by producing the extracellular enzymes as a result of SOM decomposition [28].

The southern region of China's mainland occupies the farmlands mainly with red upland and paddy soils, having a significant proportion of the overall country's grain production. Generally, the red upland soil is considered a "low fertility" soil endangered with erosion problems [29]. Within the same geomorphic unit, paddy soils have a relatively good fertility status with higher contents of organic matter compared with upland soils [30,31]. Paddy soil is often subjected to the rotation of wetting and drying cycles, thereby affecting the physicochemical properties of soil, which can be less observed in the case of upland soils [32–34].

Experimental research on the N addition to soil has been mainly discussed in many previous studies [3,35–37]; however, its influence on the soil N dynamics and relevant mechanisms for the responses of some important N cycling processes based on the N status of soil after long-term fertilization are poorly understood. N mineralization usually occurs after a system reaches N saturation or when there is already considerable N deposition, which indicates that the responses of soil N dynamics to the fertilizer input might largely be dependent on soil N status [38]. N mineralization responds to N-saturated soil to a greater extent than in N-limited soil, but it might respond to N-limited soils after long-term fertilizer application. However, the validity of this opinion is still not well confirmed.

Studies in N-limited soils have reported both positive [39] and negligible effects [40–42] and also produced inconsistent results. Most importantly, previous experiments lacked consistency in the experimental methods and soil type; thus, the comparability of their results was weak. There is still a scarcity of information on the comparison of long-term fertilization among different soil types with the same climate and environmental conditions with different soil N concentrations in a single study.

In the current study, a long-term upland, as well as paddy soil under different fertilization, was studied with a focus on: (i) how N mineralization varies based on the initial N status (without N and with N) of upland as well as paddy soil, and (ii) to evaluate the differences between upland and paddy soils in terms of enzymatic activities and soil microbial biomass under long-term fertilization having the same climatic condition. It was hypothesized that long-term N addition would enhance soil extracellular enzyme activities and soil microbial biomass linked to the N-mineralization by enhancing N availability in both the soil conditions.

2. Materials and Methods

2.1. Site Description and Experimental Setup

A long-term upland soil under different fertilization (28°21′6″ N, 116°10′21″ E and 28°21′9″ N, 116°10′33″ E, respectively) was located in Jinxian, Jiangxi Province of China. The mean annual temperature (MAT) is 18.1 °C with 260 frost-free days in a calendar year. A typical subtropical climate prevails in the area having a mean annual precipitation (MAP) of 1727 mm. The long-term upland soil experiment was started in 1986 with early maize (*Zea mays* L.) (April–July), and later maize (July–October) cropping patterns in each year. Before the initiation of the experiment, the soil contained about 36% of clay and was categorized as "red soil" in the Chinese soil classification or as typical "Plinthosols" [43]. The parent material is quaternary red clay with dominant kaolinite minerals. At the start of experiment in 1981, soil characteristics in the plough horizon (0–20 cm) were: pH 6.01; SOC 9.38 g kg^{-1}; total nitrogen (N) 0.97 g kg^{-1}, potassium (K) 15.8 g kg^{-1} and phosphorus (P) of 0.97 g kg^{-1}. The available N, P and K contents were 60.1 mg kg^{-1}, 12.8 mg kg^{-1} and 102.2 mg kg^{-1}, respectively.

Within the same geomorphic unit, a long-term paddy soil experiment was initiated in 1981, with a double rice cropping pattern having early rice (Oryza sativa) (April–July) and later rice (July–November). The parent material and its dominant minerals in paddy soil were the same as in upland soil, with 25% clay content and classified as "Stagnic Anthrosols". The initial soil characteristics before the start of the experiment were pH 6.92; SOC 16.31 g kg^{-1}; total N 1.45 g kg^{-1}, total P 0.46 g kg^{-1} and total K of 10.7 g kg^{-1}. The available N, P and K contents were 145 mg kg^{-1}, 4.17 mg kg^{-1} and 80.51 mg kg^{-1}, respectively. Depending on the initial N status of the soil, in the year 2019, we selected the following two sets of treatments without N application, (i) no fertilizer (CK), (ii) potassium fertilizer (K), and with N application, (iii) N fertilizer (N), (iv) combined N and K fertilizers (NK), from upland as well as paddy soil. Both experiments were randomized in a complete block design (RCBD), replicated three times with a plot size of 22 m^2 in upland and 47 m^2 in paddy soil. Inorganic fertilizers were applied as urea for nitrogen (N) and potassium chloride for potassium (K) at both the experimental sites. The amount of added N and K fertilizer are given in (Table 1). Before each season, 40% of both the chemicals (N and K fertilizer) were applied before the sowing of maize crop, the remaining N and K fertilizer were applied about 40 days after sowing. On the other hand, 60% of N fertilizer and 50% of K fertilizer were applied before the transplantation of rice seedlings in paddy soil, and the rest was applied at the tillering stage. Both upland and paddy soil followed conventional management practices such as pest management and irrigation.

Table 1. The Annual rate (kg ha^{-1}) of synthetic nitrogen (N) and potassium (K) fertilizer addition and organic fertilizer applied in the various fertilization treatments.

| Sites | Treatments | Fertilization | | N Status |
		N	K	
Upland	CK	0	0	Without N
	K	0	60	Without N
	N	60	0	With N
	NK	60	60	With N
Paddy	CK	0	0	Without N
	K	0	75	Without N
	N	90	0	With N
	NK	90	75	With N

CK, no fertilizer; K, synthetic potassium; N, synthetic nitrogen and NK, synthetic nitrogen and potassium.

2.2. Sampling and Laboratory Analysis

Surface soil (0 cm to 20 cm) samples from each plot were collected after harvesting in the year 2019, both paddy and upland soils. It consisted of randomly collected four core samples from every plot at the harvest stage. The collected samples from each site were mixed thoroughly into one sample that represented each replicate of the four treatments and then carried to the laboratory immediately. After sieving (<2 mm), all the debris, gravel and visible roots were removed by manual hand-picking. After that, the prepared soil was split into two parts. Before chemical analyses, one part was sieved through a 0.25 mm sieve after air drying. The respective sub-samples were used to analyze soil pH, and the moisture content of soil samples was calculated by the gravimetrical method (difference of the soil weight before and after drying at 105 °C). The second part of the soil sample was kept at 4 °C to analyze soil microbial and enzymes activities and to use the soil in the incubation experiments as well.

2.3. Soil Chemical Analyses

The SOC content of the soil was assessed by using the oxidation method [44]. Soil total N, total P and available P contents were determined by the method described by [45–47], respectively. Pre-incubated, moist soil samples were used for the measurement of SMBN and SMBC by the chloroform fumigation extraction method of [48], followed by the eventual 0.5 M K_2SO_4 extraction. The N and C in extraction were calculated as SMBN or SMBC = (N or C in a fumigated solution—N or C in an unfumigated solution)/KE, where KE is 0.45 for MBC [49] and 0.57 for MBN [50].

The potential soil extracellular enzymes, N-Acetyl-glucosaminidase (NAG, N-acquiring enzyme) and leucine-aminopeptidase (LAP, N-acquiring enzyme), were determined by following the [51] method. We selected these enzymes because it is believed that the biogeochemical activities and rates of microbial metabolism are dependent on the enzyme activity potentials and are termed as the main soil microbial nutrient requirement indicators [52]. Concisely, a homogenous soil suspension was made by taking 1.0 g soil (fresh) with 100 mL of newly prepared buffer (50 mM) of sodium acetate ($C_2H_3NaO_2$). The pH of the prepared buffer was adjusted according to the mean of the tested soil (Table 2). After that, 200 μL acetate buffer, soil suspension and substrates (50 μL) were carefully dispensed into 96-well black microplates that were incubated in the dark for 4 h at 25 °C. At last, the microplate reader (SynergyH1, BioTek, Winooski, VT, USA) was used to quantify the fluorescence intensity, with an excitement of 365 nm and 450 nm emission filters.

Table 2. Soil enzymes assayed for potential activity along with their enzyme commission number (EC), abbreviation, substrate and incubation time.

Enzyme	Abb.	Substrate	EC	Substrate Conc. (nM)	Inc. Time
Leucine-aminopeptidase	LAP	L-Leucine-7-amido-4-methylcoumarin hydrochloride	3.4.11.1	400	45 min
N-acetyl-b-D-glucosaminidase	NAG	4-Methylumbelliferyl N-acetyl-b-D-glucosaminide	3.2.1.30	300	4 h

MUB denotes methylumbelliferyl.

2.4. Soil Incubation and N Mineralization Measurements

The soil N mineralization experiment was done by following the classical method of [1]. Concisely, 15 g of prepared soil and sand quartz (acid-washed) were put in polyvinyl chloride leaching tubes in a 1:1 ratio. The leached samples were taken at 0, 10, 20, 35, 50, 70 and 90 days intervals by adding 0.01 M $CaCl_2$ (100 mL) followed by the N-free solution (25 mL). Sample tubes were incubated for consecutive 90 days at 25 °C. The mineral N content (NH_4^+-N and NO_3^--N) was analyzed by using a continuous flow analyzer (Foss FIASTAR 5000 Analyzer) after the leaching.

Soil cumulative N mineralization was estimated by the total sum of mineral N after the completion of incubation. N mineralization potential (N_o) was calculated by implying the pseudo-first-order kinetic model [53] as shown below:

$$N_{min} = N_o \left(1 - e^{-kt}\right) \tag{1}$$

where, N_{min} = cumulative mineralized N (mg kg^{-1}), N_0 = mineralizable N potential (mg kg^{-1}) and k = net mineralization rate constant, NMR (d^{-1}). The equation fitting for N mineralization was done by the global fit curve wizard in origin-pro (Systat Software, Inc., Chicago, IL, USA).

2.5. Statistical Analysis

Statistical analysis was done by using SPSS 20.0 (SPSS, Chicago, IL, USA). Analysis of variance (ANOVA) was used, subsequently assessed by the least significant difference (LSD) test at $p \leq 0.05$ level for each variable to calculate the significant variations among the different treatments. Linear regression between soil extracellular enzyme activities, microbial biomasses and the N mineralization was done in R-software (ggplot2-package). Pearson correlations between soil properties and N mineralization in both soils were also analyzed. Variance partitioning analysis (VPA) was performed in R-software in order to check the proportional contributions of different factors affecting the net N mineralization.

3. Results

3.1. Soil Chemical Properties and Nutrient Content

Long-term N addition affected soil chemical properties in both upland and paddy soils, except soil pH (Table 3). Soil pH ranged from 4.91 to 4.34 and from 5.22 to 5.15 in the upland and paddy soil, respectively. After 38 years of long-term experiment, the SOC content of paddy soil was higher relative to upland soil by 56.2%, 45.7%, 61.1% and 62.2% in without N (CK, K) and with N (N and NK) treatments, respectively. On average, the SOC was 10.9 g kg^{-1} and 17.2 g kg^{-1} in the upland and paddy soils, respectively. Meanwhile, the maximum SOC content was attained under the N treatment, followed by NK, K and CK. Analysis of variance showed significant differences ($p < 0.05$) among the soil type, fertilization, as well as their interactions.

Soil total P and available P were not affected by the fertilization in both the soils; however, the effect of soil type was significant among them. Compared with upland soil, total P and available P under paddy soil were decreased by 37.6%, 30.2%, 31.2% and 68.9%, 67.5%, 66.6% and 67.6% with CK, K, N and NK treatments, respectively. Analysis of variance showed significant differences ($p < 0.05$) of total P among the soil type and fertilization, while fertilization was non-significant ($p > 0.05$) in the case of available P.

Moreover, the interactive effect of both soil type and fertilization was also non-significant ($p > 0.05$).

Total N concentrations were significantly influenced by the site, fertilization as well as their interaction (Table 3). N treatment showed the maximum N concentration of 2.18 g kg^{-1}, followed by NK (2.14 g kg^{-1}), K (1.55 g kg^{-1}) and CK (1.54 g kg^{-1}) under paddy soil. Overall site-wise, the N concentrations were increased by 0.06, 0.10, 0.57 and 0.60 times by the above treatments under paddy soil, compared with upland soil. It is likely that soil-available N significantly increases after N addition, providing adequate soil nutrients for plant growth. Analysis of variance showed significant differences ($p < 0.05$) among the soil type, fertilization, as well as their interactions.

Table 3. Effect of long-term fertilization on the soil pH, SOC, total P, available P and the total N concentrations in upland and paddy soils.

Sites	Treatments	pH	SOC (g kg^{-1})	Total P (g kg^{-1})	Averaged Total P (g kg^{-1})	Available P (mg kg^{-1})	Total N (g kg^{-1})
Upland	CK	4.91 ± 0.16	8.54 ± 0.23 f	1.64 ± 0.02	1.08A	10.7 ± 1.08	0.10 ± 0.12 d
Paddy	CK	5.22 ± 0.17	13.3 ± 1.73 b	0.51 ± 0.02		6.71 ± 0.65	1.54 ± 0.04 b
Upland	K	4.93 ± 0.26	9.76 ± 1.24 e	1.54 ± 0.01	1.02A	9.74 ± 0.84	0.16 ± 0.07 d
Paddy	K	5.25 ± 0.24	14.2 ± 0.98 b	0.50 ± 0.06		6.79 ± 0.33	1.55 ± 0.07 b
Upland	N	4.27 ± 0.12	12.9 ± 1.11 cd	1.38 ± 0.03	0.92B	9.37 ± 0.22	1.25 ± 0.02 c
Paddy	N	5.13 ± 0.20	20.8 ± 1.02 a	0.46 ± 0.02		6.44 ± 0.37	2.18 ± 0.02 a
Upland	NK	4.34 ± 0.13	12.6 ± 2.05 d	1.39 ± 0.02	0.92B	9.46 ± 0.76	1.28 ± 0.03 c
Paddy	NK	5.15 ± 0.16	20.5 ± 0.75 a	0.45 ± 0.06		6.42 ± 0.88	2.14 ± 0.08 a
	ANOVA						
	Soil type	ns	***		***	***	***
	Fertilization	ns	***		***	ns	***
	S × F	ns	***		Ns	ns	***

CK, no fertilizer; K, synthetic potassium; N, synthetic nitrogen and NK, synthetic nitrogen and potassium. Data followed by different lowercase letters denote significant differences ($p \leq 0.05$) based on interaction (S × F). Moreover, the uppercase letters denote significant differences ($p \leq 0.05$) based on fertilization (F) and "***" denotes the significance level.

3.2. Responses of Soil Microbial Biomass Nitrogen and Carbon to Nitrogen Additions

SMBC and SMBN were significantly higher with N treatments in both soils (Figure 1), compared with without N treatments. However, the comparison between without N and with N treatments was non-significant. The N treatment showed the maximum SMBC (494.3 mg kg^{-1} and 640.2 mg kg^{-1}) and SMBN (79.7 mg kg^{-1} and 87.6 mg kg^{-1}) among all other treatments in both soils, followed by NK, K and CK. Both SMBC and SMBN were increased by 39.6%, 2.77%, 29.5%, 31.4% and 11.8%, 11.9%, 10.1% and 12.3% in CK, K, N and NK treatment, respectively, under paddy soil, compared with upland soil. On average, the upland soil had 368.5 mg kg^{-1} and 66.4 mg kg^{-1}, whereas the paddy soil had 459.5 mg kg^{-1} and 74.1 mg kg^{-1} of SMBC and SMBN, respectively.

3.3. Soil Enzymatic Activities Associated with Soil N Mineralization

A significant difference in extracellular activities was observed under long-term with N and without N fertilization (Figure 2). The activities of LAP and NAG in the NK treatment were markedly increased by 105% and 203%, compared with without N treatment in the upland soil, whereas they were increased by 74% and 110%, compared to without N treatment in the paddy soil. Overall site-wise, in paddy soil, the activities of LAP and NAG were increased by 31%, 18%, 20%, 11% and 70%, 21%, 13% and 18% by CK, K, N and NK treatments, respectively, compared to the upland soil.
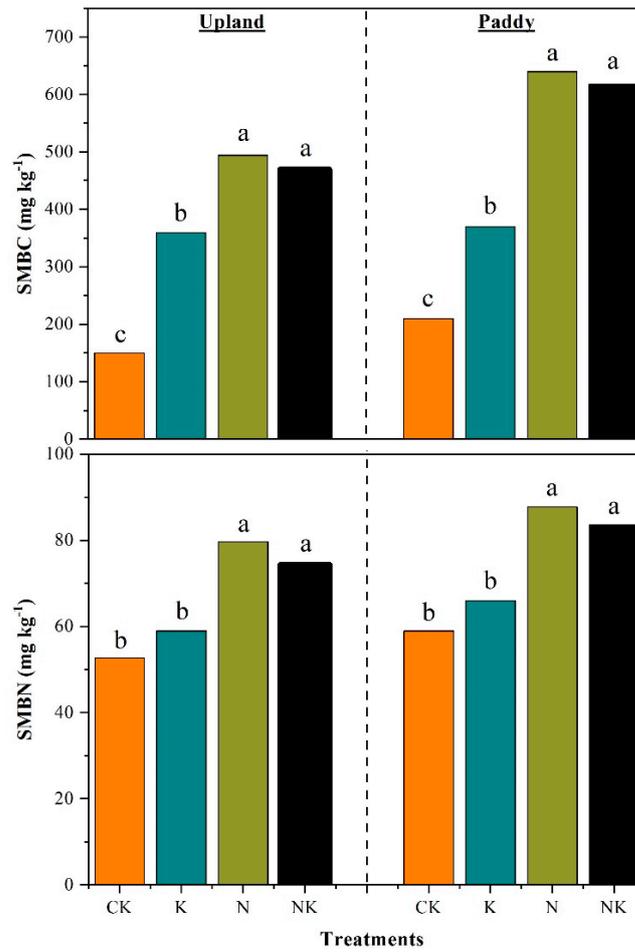
Figure 1. Soil microbial biomass C and N (SMBC, SMBN) concentrations under different fertilization treatments in upland and paddy soils. Notes: CK, no fertilizer; K, synthetic potassium; N, synthetic nitrogen and NK, synthetic nitrogen and potassium. Bars indicate the standard error, *n* = 3. Different letters indicate significantly different means at *p* < 0.05 using the least significance difference (LSD) test.
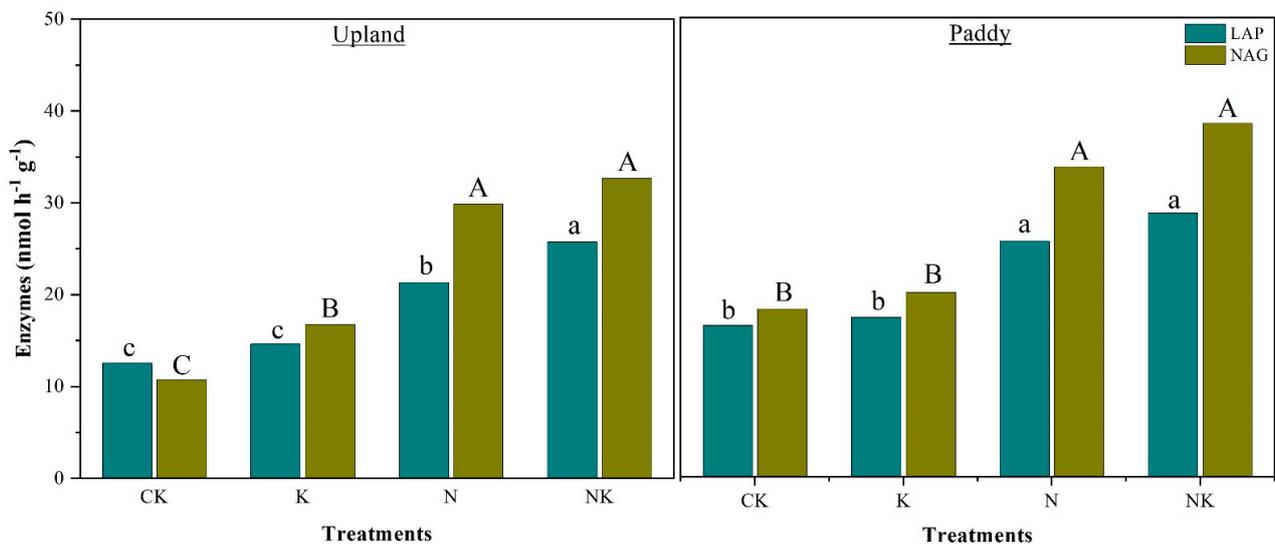
Figure 2. The effects of different fertilization treatments on the activities of extracellular enzymes in upland and paddy soils. Bars indicate the standard error, *n* = 3. Different letters indicate significantly different means at *p* < 0.05 using the least significance difference (LSD) test.

3.4. Soil NO$_3^-$-N, NH$_4^+$-N Contents during Incubation Period

The incubation duration and the fertilization considerably influenced soil NO$_3^-$-N, NH$_4^+$-N contents and the net N mineralization in both paddy and upland soils (Figure 3). A significant effect was observed, demonstrating the variable concentration of NO$_3^-$-N and NH$_4^+$-N. A linear increase was observed in the NO$_3^-$-N concentrations during incubation; however, a more obvious increase was observed among all treatments after 20 days, and the maximum was observed in the NK treatment in both the soils (Figure 3A,B). NH$_4^+$-N concentration at first increased up to day 20 and then decreased but was relatively stable after day 70 of incubation (Figure 3C,D). Overall, after the 90 days of incubation, NO$_3^-$-N and NH$_4^+$-N contents under paddy soil were increased by 5.29%, 10.5%, 3.25% and 1.33% in CK, K, N and NK treatments, respectively, compared with the upland soil. The significant difference in the initial 20 days and the relatively stable mineralized N values at later stages reflected that the labile-N fraction and the intermediate fraction of applied N led to a wide-ranging k and N mineralization among both the soils with different fertilization.
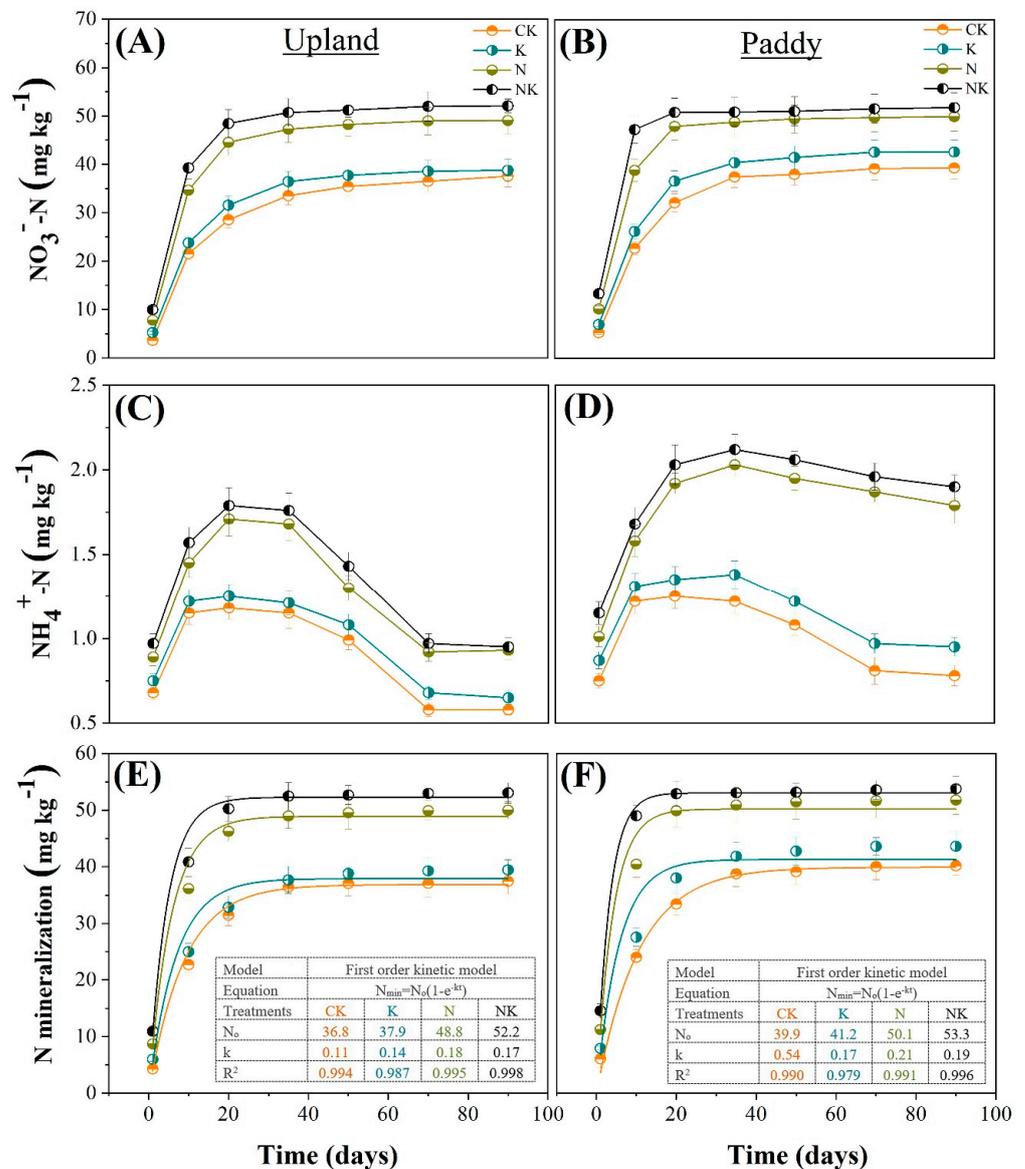
Figure 3. The concentrations of NO$_3^-$-N, NH$_4^+$-N and net N mineralization in different fertilization management practices after 90 days of incubation in upland (**A**,**C**,**E**) and paddy soils (**B**,**D**,**F**). The error bars denote the least significant difference values at the 0.05 level using the least significance difference (LSD) test.

3.5. N Mineralization Potential and Mineralization Rate Constant (k)

Total N mineralization, generally considered as the sum of NO_3^--N and NH_4^+-N, altered in a significant ($p \leq 0.05$) manner under both upland and paddy soils, with the incubation duration. N mineralization potential (N_0) ranged from 36.8–52.2 and 39.9–53.3 (mg kg^{-1}), NMR (k) ranged from 0.11–0.17 and 0.54–0.19 in upland and paddy soils, respectively. The net N mineralization has shown a significant curvilinear relationship with the time over 90 days of incubation after long-term with N and without N fertilization (Figure 3E,F). After the completion of incubation, the highest N mineralization (cumulative) was observed in the NK treatment (53.2 mg kg^{-1} and 53.3 mg kg^{-1}). An increase in the net N mineralization was observed by 3%, 31% and 39% in K, N and NK in upland soil, whereas it increased by 9%, 28% and 34%, respectively, in the paddy soil, compared with CK. Moreover, the N mineralization data fitted well ($R^2 = 0.97$–0.99) modeled by the pseudo-first-order kinetic model. N mineralization rate constant (k) was consistent with fertilization and has shown an increasing trend. Overall, after 90 days of incubation, the net N mineralization in paddy soil was increased by 5%, 11%, 4% and 2% in CK, K, N and NK treatment, compared with those of upland soil.

4. Discussion

Long-term N addition strongly affected the chemical and biological properties of soil. SOC content was higher with the N addition in both upland and paddy soils. This could be due to long-term N addition and slow decomposition of organic matter in the soil. This finding is also supported by earlier studies [54–56]. However, higher SOC in paddy soils can be explained as paddy soils are more fertile than upland soils, with 57.07% higher SOC content [30]. In fact, increasing evidence supports the theory that N fertilization enhances SOC sequestration in terrestrial ecosystems [57–59]. One mechanism for explaining the phenomenon is that the lignin-rich and aromatic compounds may become preserved from decomposition due to depressed oxidative activities under N fertilization [60]. Generally, SOC contents in paddy soils become stable after 30 years of rice cultivation in subtropical China. So, this increase of SOC with N treatments could be attributed to the increased underground biomass due to the introduction of modern rice varieties as well as increased nutrient inputs through rainfall and irrigation water [61]. Moreover, the soil organic matter enrichment in soil depends upon the SOC stabilization. Six et al. [62] presented three organic carbon stabilization mechanisms in the soil: (1) physical protection, (2) biochemical stabilization and (3) chemical protection. It has been observed that the biochemical stabilization of organic materials present in the soil system is primarily due to their complex chemical composition. This might be related to the lower aromatic/phenolic-C and higher O-alkyl-C intensities in paddy soil than in upland soil due to the presence of rice crop residues, compared to maize crop residues [63]. So, it is simply that the slower decomposition of maize residues than rice residues might be an inherent property of these crop residues [64].

SMBC and SMBN were significantly higher with N treatments in both soils (Figure 1). The microbial biomass controls and regulates the N and C cycles, and soil management practices also control the size of the microbial biomass pool [65]. The readily metabolizable C and N, in addition to increasing root biomass and root exudates due to greater crop growth, are thought to be the most influential factors contributing to the increased microbial biomass [66]. Correspondingly, under long-term N addition, higher microbial metabolic activities might be due to the significant increase in the microbial biomass [67]. The essential SOC fractions and N become microbial components (i.e., proteins) after the soil organic matter mineralization, which were released by the microbial turnover at later stages [68]. Treatments with N application (N and NK) increased microbial N transformations because of faster decomposition with higher N concentrations when the soil moisture was higher in paddy soils [69].

A significant difference in extracellular activities was observed under long-term with N and without N fertilization (Figure 2). Generally, the N cycling enzymes LAP and NAG are

regulated by the C contents and N concentrations [28,70]. LAP showed significant positive correlations with SOC content and N mineralization, which suggests its substantial role in protein depolymerization [71]. LAP could be the limiting factor in the N mineralization process while considering the fact that the majority (>65%) of the total N content in plant and microbial cells is comprised of this protein [13]. The increased aminopeptidase activity could be due to the labile constituents after long-term N addition compared with those without N treatments [72]. Soil NAG plays a key role in the degradation of chitin. Proteins, chitin and peptidoglycan are the principal reservoirs of organic N [73]. With N treatments, increased NAG activity in upland and paddy soils, which suggests its production, substrate decomposition and its responses to the soil status might vary compared to those treatments without N. NAG has a direct link to organic matter mineralization in soil, as stated in some previous studies [74]. Maximum NAG activity with N soils might be due to the higher SOC content in the soil compared to without N soils. Thus, enzyme activities are enhanced after the long-term N addition, which also facilitates the accumulation of SOC and N contents in the soil that help to improve soil fertility. The incubation duration and the fertilization considerably influenced soil NO_3^--N, NH_4^+-N contents and the net N mineralization in both paddy and upland soils. This finding can be linked to the slower mineralization potential at the later stage of the incubation that caused a decreased temporal release of NH_4^+-N, indicating the recalcitrant portion of long-term N amendments, and due to the exponential nitrification process, the concentration of NO_3^--N increased [75]. It is generally known that net mineralization potential, which is measured by N_o, is a genuine predictor of the nutrient supply capacity, and it is also considered to be a sustainable approach to assess the soil microbial activities [76]. Moreover, N mineralization can be enhanced by fastening SOM degradation and microbial metabolism that can be boosted by a higher soil nutrient supply [43]. The decomposition of SOM, C and N mineralization is mainly facilitated by soil microorganisms [77]. This study indicates that the N mineralization potential rate after long-term N addition is significantly increased compared with those without N treatments, as also reported in some of the previous studies [78], suggesting that the N mineralization process was more affected by the fertilizer application practices than soil properties. Because of the variable changes in enzyme activities, microbial composition and higher nutrient availability, the mineralization rate was gradually increased [79]. The SOC stability governed by the protection mechanisms of different SOC fractions caused the variations in the N mineralization constant, which could be because of different long-term fertilizer regimes [9]. This study revealed relatively higher N mineralization rates compared with the previous research under a double-rice cropping pattern [80]. Higher N mineralization in N- and NK-treated soils has been shown to be due to the higher availability of carbon and nitrogen and higher enzyme activities [81,82]. This result was consistent with earlier studies [83], which may imply N transformation was affected more by the fertilizer application instead of soil properties in this study. These results were partly consistent with a previous study that showed that higher biomass and metabolic state of the microorganisms were followed by high rates of mineralization [84]. In the presence of K, NH_4^+-N concentrations increased 4.1 fold when N fertilizer was applied and 3.5 times in the absence of N application [85]. Response to K applications in both rice and wheat increases with N application, indicating that higher K rates are required at higher N rates [86]. Balancing the NPK ratio (especially N-K) by increasing the input of K fertilizers is a practical way to improve N agronomic efficiency [87]. The positive interaction of N and K may offer the opportunity for considerable savings in the cost of N fertilizer and food security for the rapidly expanding human population. Therefore, N and K fertilizers should be applied with optimal ratios at the right time and right rate according to the nutrient uptake pattern of the crops, soil nutrient status, soil texture and climate changes in order to reach the target yields with good quality and minimize K and N losses to the environment [86].

A linear relationship was observed among net N mineralization, soil microbial biomass and extracellular enzyme activities (Figure 4). SMBC, SMBN, LAP and NAG exhibited

a substantial ($p < 0.05$, $R^2 = 0.80$–0.98) positive relationship with net N mineralization in upland as well as paddy soil. Correlation heat map analysis further hints at the relationship between parameters and the net N mineralization in both upland and paddy soils (Figure 5). The two key factors consisting of the different soil parameters analyzed and two types of soils were studied. The results showed a complicated relationship between the studied parameters and the soil. For example, a significant positive correlation was noted between SOC, total N, NO_3^--N, NH_4^+-N and the net N mineralization ($p < 0.01$). Similarly, there were positive correlations between total P, available P and the net N mineralization. However, no significant correlation was identified between pH and the net N mineralization. Moreover, both soils had a significant positive correlation ($p < 0.01$) between SOC, total N, NO_3^--N, NH_4^+-N and the net N mineralization, while they had a negative relationship with pH, total P and available P ($p > 0.05$). These complex relationships mean that environmental impacts and soil types strongly influence net N mineralization.

The following two reasons can explain the difference between the two soils or the cropping systems. The first reason is that the upland soil (i.e., maize cropping system) is under rain-fed agriculture, while paddy soil (i.e., rice cropping system) is well irrigated, so it is less affected by seasonal drought. The second is that with 57% higher SOC content, paddy soil is more fertile than upland soil. Paddy soils have contrasting water management and soil properties to upland soils, indicating a lower mineralization rate of SOM under waterlogged conditions.

In anaerobic conditions, microbial activities and the decomposition of SOM are relatively slower than in aerobic conditions [88]. Figure 6 shows the proportional contributions based on results from variation partitioning analysis (VPA), explaining the contribution of biotic factors (SMBC, SMBN, LAP, NAG), abiotic factors (pH, SOC, total N, total and available P) and N content (NO_3^--N and NH_4^+-N) to the net N mineralization. The biotic factors and N contents explained most of the variations related to net N mineralization based on the variance portioning analysis. The greater proportion of variation was accounted by biotic rather than by abiotic factors (Figure 7) was likely because biotic factors such as soil microorganisms are directly involved in the decomposition of soil organic matter and stimulate microbial activities [89]. The total variance of net N mineralization was explained by the abiotic and biotic factors linked with the improved environmental conditions, such as soil microbial biomass generally increased, and such increases considerably enhanced the metabolism of microorganisms [90,91], ultimately stimulating the activities of soil microbes [8].
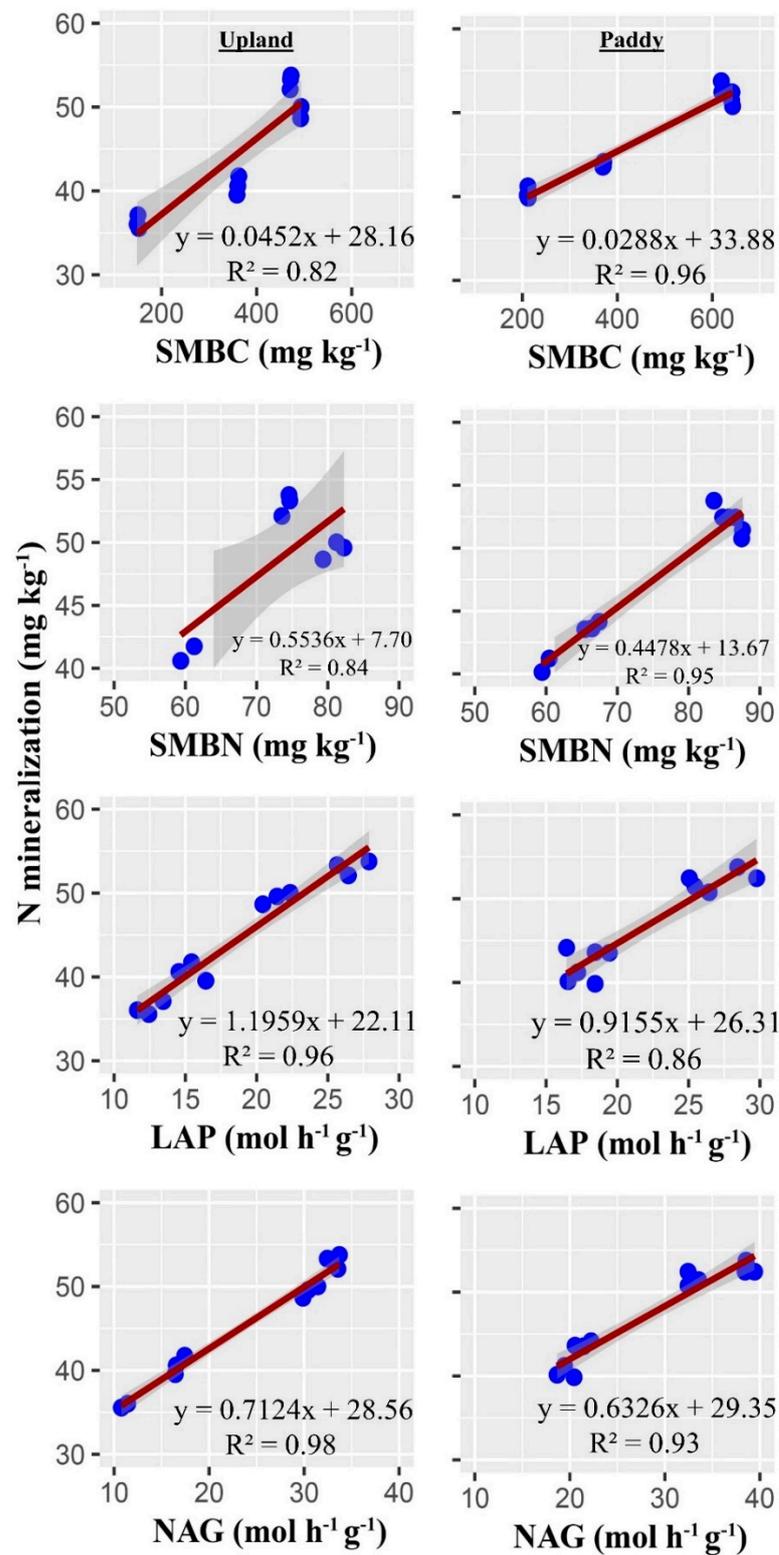
Figure 4. Relationship between net N mineralization and SMBC, SMBN, LAP and NAG under long-term upland and paddy experiment. The shaded area indicates the range at 95% confidence intervals. Permutation *p* values less than 0.05 and 0.01 were considered significantly different at $p < 0.05$ and $p < 0.01$.
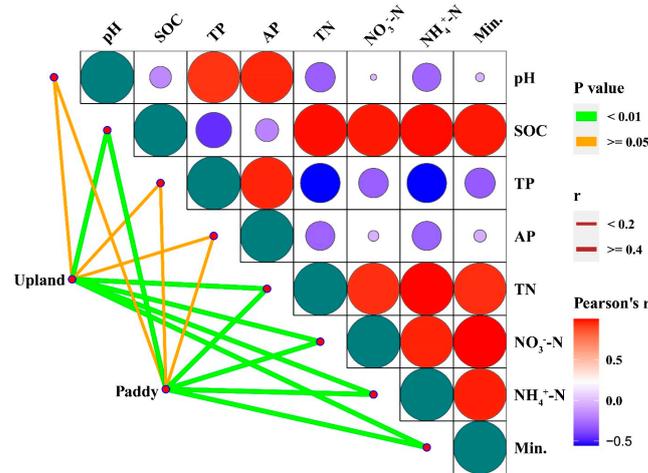
Figure 5. Correlation heatmap analysis between soil properties and the net N mineralization in upland and paddy soils.
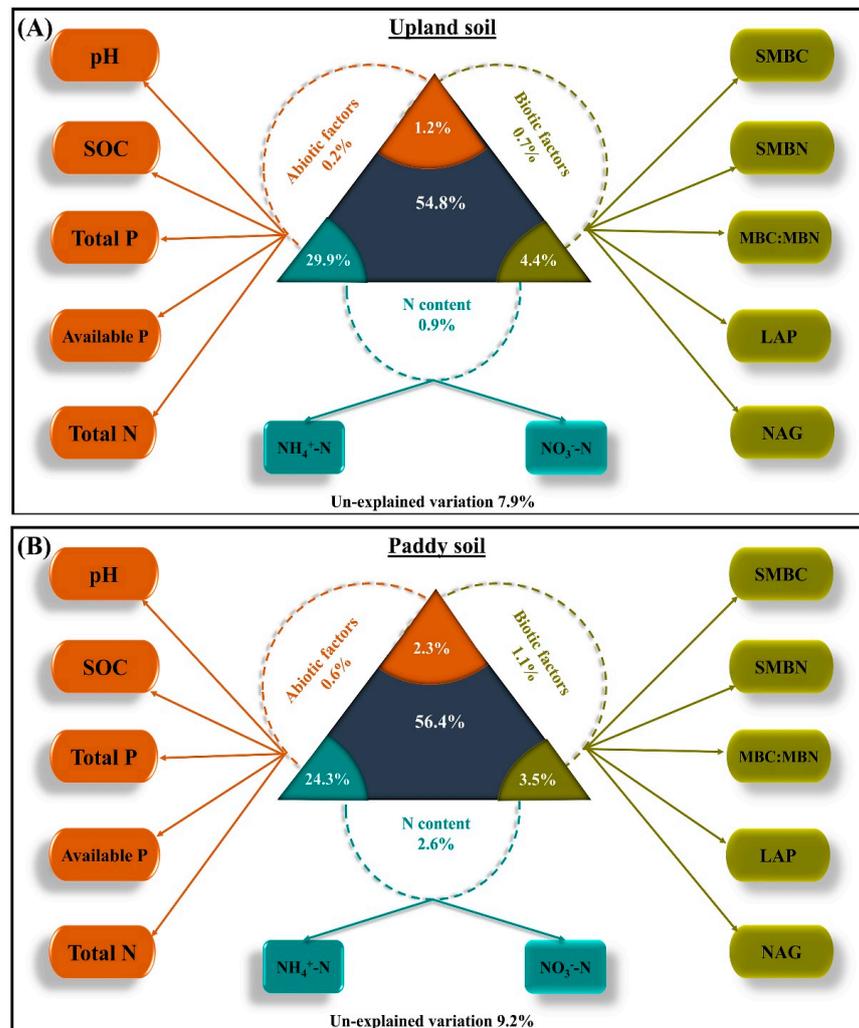
Figure 6. Venn diagrams showing the proportional contributions based on results from variation partitioning analysis (VPA), explaining the contribution of biotic factors, abiotic factors and N content to the net N mineralization in upland (**A**) and paddy soils (**B**). The effect of biotic factors (brown circle); the effect of abiotic factors (red circle); the effect of soil N content (blue circle); and their combined effects and respective variations explained are in areas overlapped by the different colored circles.
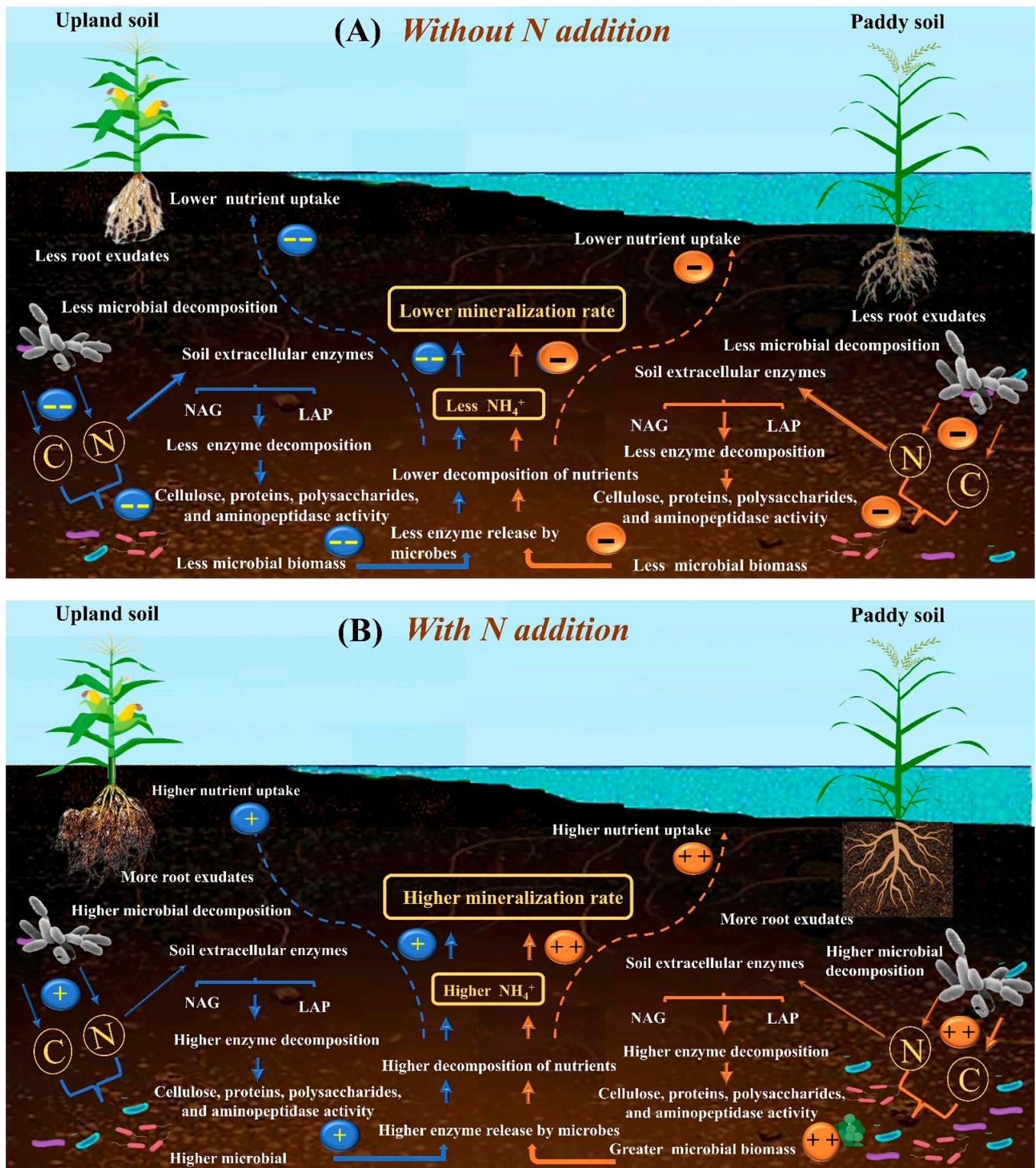
Figure 7. A diagram illustrating N content, microbial biomass and nutrient availability in soil and how soil organic matter, microbial biomass and extracellular enzymes influence N mineralization processes. Blue arrows show the processes in upland soil, while orange arrows show processes in paddy soil. (**A**) The figure represents the transformation processes in "without N addition" soils, "-" and "--" indicate the comparison between the soil microbial biomass, enzyme activity and mineralization rate in upland and paddy soils, respectively. (**B**) The figure represents the transformation processes in "with N addition" soils, while "+" and "++" indicate the comparison between the microbial biomass, enzyme activity and mineralization rate in upland and paddy soils, respectively.

5. Conclusions

Long-term N addition significantly affected SOC and N concentration in both paddy and upland soils. The substantial increase in the microbial biomass, extracellular enzyme activity and the net N mineralization in soil with N treatments (N and NK) were obvious and relatively higher in the paddy soil compared with upland soil. Net N mineralization had a strong relationship with SOC, SMBN, SMBC, extracellular enzymes, NO_3^--N and NH_4^+-N concentrations. Among all other soil properties, soil total N was the restraining factor. Moreover, further research is required to fully comprehend the mechanisms underlying the higher effectiveness of long-term N additions over the others under certain conditions such as soil pH, crop type and the type of experiment.

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