

Article

Combined Application of Organic Fertilizer and Quicklime Enhances Soil Nitrogen Retention in Acidified Apple Orchards

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Abstract

Soil acidification in apple orchards disrupts nitrogen (N) transformation and raises the risk of N loss. Using in situ ¹⁵N-urea microcosms in an acidified orchard (pH 4.46) in North China, we tested four treatments: N, phosphorus (P), and potassium (K) (CK, control); N, P, K plus organic fertilizer (NPKM); N, P, K plus quicklime (NPKL); and N, P, K plus organic fertilizer and quicklime (NPKML). We measured soil ammonium (AN), nitrate (NN), fixed AN (FAN), amino sugars and their ¹⁵N abundance to quantify fertilizer N distribution and the mechanisms of N retention. The results indicated that co-amendment with organic fertilizer and quicklime increased microbial necromass N (MNN) accumulation, with bacterial necromass N accounting for 83–87% of total MNN based on amino sugar biomarkers, suggesting a predominance of bacterial-derived residues in the retained fertilizer N pool. The NPKML treatment achieved the highest fertilizer N accumulation in the biological pool, maintained a stable pH of 6.20 and a total N content of 1.34 g kg^{−1}, and showed the highest FAN and MNN retention. Based on the observed variations in soil physicochemical indicators and N pool distribution, we infer that quicklime may enhance abiotic ammonium fixation through pH elevation, while organic fertilizer may promote microbial residue accumulation by supplying carbon substrates. Their combined application was associated with the highest N retention across both biotic and abiotic pools, suggesting a complementary effect that warrants further mechanistic investigation. This study provides a theoretical basis for and practical strategies for efficient N management in acidified orchards.

Keywords: acidified soil; organic and inorganic amendments; ¹⁵N isotope tracing; microbial necromass nitrogen; amino sugars



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1. Introduction

Nitrogen (N) is a fundamental nutrient for the growth and development of fruit trees, and its effective supply directly influences the yield and quality of apples [1,2]. Intensive apple orchards typically depend on large-scale N applications to secure production benefits [3]. However, the long-term practice of excessive water and fertilizer use has led to significant soil acidification (pH < 5.0), which acts as a critical bottleneck for the efficient utilization of N in orchards [4]. Soil acidification profoundly alters N transformation pathways by modifying the surface properties of clay minerals and inhibiting microbial

activity [5,6]. This alteration results in abnormal nitrification rates of ammonium N (AN) and the substantial accumulation of nitrate N (NN), thereby intensifying N loss processes such as leaching and denitrification [7]. Consequently, this situation not only leads to a marked decrease in the N fertilizer utilization rate but also contributes to ecological and environmental issues, including nitrate pollution of groundwater and greenhouse gas emissions [8,9]. Therefore, understanding the key processes of N immobilization in acidified orchards and developing effective N immobilization regulation measures are essential for the sustainable, high-quality, and efficient development of apple orchards.

Soil N immobilization, which is essential for N conservation and the minimization of N losses, involves two primary processes: biological and abiotic [10]. Biological immobilization relies on the “biological N pump”, where microorganisms transform inorganic N into stable residues such as amino sugars to form microbial necromass N (MNN) and build up soil organic N pools [11]. Notably, fungal-derived amino sugars decompose slowly to support long-term N retention, while bacterial amino sugars participate in rapid internal N cycling [12,13]. Abiotic immobilization primarily entails the lattice fixation of AN by clay minerals [14] and is based on the “chemical N pump”. Fixed AN (FAN), a vital component of the stable soil N pool, can contribute to over 15% of the total nitrogen (TN) in topsoil [15]. Its formation and release are directly influenced by soil physical and chemical properties, serving as a significant mechanism for abiotic N conservation [16]. In acidified soils, low-pH conditions result in the cation exchange sites of clay minerals being occupied by H^+ and Al^{3+} , which inhibits the abiotic fixation of AN [17]. Concurrently, acid stress markedly suppresses microbial activity, particularly the growth and metabolism of fungi, thereby diminishing the capacity for biological N immobilization [18]. Consequently, this leads to a reduction in total soil N immobilization and a marked increase in the risk of N loss.

Quicklime and organic fertilizer are widely utilized materials for amending acidified soils in agricultural production, yet they exhibit significant differences in their mechanisms for regulating soil N cycling [19]. Quicklime effectively neutralizes soil acidity and elevates pH levels, alleviating the inhibitory effects of acid stress on nitrifying microorganisms [20]. Additionally, it increases the availability of cation exchange sites on clay minerals, thereby enhancing the abiotic fixation of AN and dominating the “chemical N pump” [21,22]. Nevertheless, sole liming often overstimulates nitrification, causing nitrate N (NN) buildup and greater leaching risk [23]. In contrast, organic fertilizer supplies abundant organic carbon (C) and N substrates, stimulates soil microbial activity, and enhances the assimilation and retention of inorganic N by microorganisms [10,24,25]. This process encourages a shift in the microbial community toward a fungal-dominated structure, thereby establishing a stable biological retention system and dominating the “biological N pump” [12,26]. However, the impact of a single application of organic fertilizer on enhancing soil pH is limited, making it challenging to quickly mitigate the effects of severe acidification on abiotic N retention [27]. Other studies have investigated the effects of quicklime or organic fertilizer applied separately on N transformation in acidified soils [19,28]. Still, few studies have assessed their joint influences on both biotic and abiotic N retention, alongside the trade-offs between these two N-storage pathways in acidic orchard soils [29]. Furthermore, there is a notable absence of quantitative analyses utilizing in situ ^{15}N tracing technology, which hampers the ability to inform precise N management strategies in acidified orchards [30].

This work was conducted on severely acidified apple orchard soil in Qixia, Shandong. We adopted an in situ ^{15}N -urea microcosm incubation approach, using amino sugars as indicators of biological N retention and FAN as an indicator for abiotic N fixation. We

quantified the allocation of applied fertilizer N across biological, abiotic and labile inorganic N pools under single and combined lime-organic fertilizer treatments.

Working Hypothesis: (1) quicklime-induced pH elevation would concurrently alter nitrification intensity and clay-fixed ammonium pools, with measurable shifts in NN and FAN contents relative to unamended soil; (2) organic fertilizer input would supply labile carbon substrates to trigger biological buffering, driving distinct variation patterns in microbe-derived residual N pools; (3) co-application of quicklime and organic fertilizer would produce coordinated, mutually reinforcing increases in both biotic and abiotic N retention pools, showing correlated responses of MNN and FAN; (4) combined amendment would achieve the most favorable N retention profiles and the lowest N loss rates, forming a practical fertilization regime for severely acidified apple orchards. We acknowledge that the terms ‘biological N pump’ and ‘chemical N pump’ are used here as conceptual frameworks to interpret observed patterns in N pool distribution rather than as demonstrated mechanistic processes. Our experimental design allows us to quantify changes in N pools but does not directly measure the underlying biological or physicochemical processes. Therefore, all mechanistic interpretations are presented as hypotheses requiring further validation through targeted experiments.

This study aimed to evaluate treatment-induced changes in soil N pools and analyze correlative responses of N fractions to amendment practices, with four research focuses aligned with the above hypotheses: (1) assess how lime-driven pH shifts correlate with changes in nitrification and abiotic ammonium; (2) characterize the biological buffering effect of organic fertilizer and describe the corresponding variation patterns of microbe-associated N pools; (3) analyze coordinated shifts in biotic and abiotic N pools after co-amendment to identify the correlative linkage between the so-called biological N pump and chemical N pump; (4) propose practical N management schemes for acidified apple orchards based on observed N retention patterns across treatments. This research delivers new observational evidence for the coupled variation in biotic and abiotic N pools in acidic soils and provides empirical support for soil remediation and optimized N application in intensively acidified fruit orchards.

2. Materials and Methods

2.1. Study Site

The field experiment was conducted from October 2023 to September 2024 in an apple orchard in Qixia City, Shandong Province (120°74′ E, 36°20′ N). This study area experiences a warm-temperate, semi-humid continental monsoon climate characteristic of East Asia. The multi-year average temperature is 11.4 °C, annual precipitation ranges from 640 to 846 mm, and the average annual total sunshine hours is 2659.9 h. The experimental orchard consisted of 6-year-old Golden Delicious apple trees grafted onto dwarfing self-rooted rootstocks and was managed using a high-density planting approach. Due to prolonged management involving excessive irrigation and fertilization, the soil in the orchard had become severely acidified. The soil type under investigation was classified as Luvisols (World Reference Base) [31]. The basic properties of the topsoil (0–20 cm) are presented in Table 1.

Table 1. Basic properties of the tested soil.

pH	EC ($\mu\text{s cm}^{-1}$)	SOC (g kg^{-1})	TN (g kg^{-1})	C/N	Sand (%)	Silt (%)	Clay (%)	GluN (mg kg^{-1})	GalN (mg kg^{-1})	MurN (mg kg^{-1})
4.46 $\pm$ 0.05	232.06 $\pm$ 2.43	13.48 $\pm$ 0.18	1.19 $\pm$ 0.02	11.36 $\pm$ 0.02	37.35 $\pm$ 0.62	49.38 $\pm$ 0.63	13.27 $\pm$ 0.78	219.11 $\pm$ 7.23	126.49 $\pm$ 5.18	29.15 $\pm$ 1.94

Note: EC, electrical conductivity; SOC, soil organic carbon content; TN, total soil nitrogen content; C/N, carbon-to-nitrogen ratio; GluN, glucosamine; GalN, galactosamine; MurN, muramic acid. Data are presented as the mean $\pm$ standard deviation ($n = 3$).

2.2. Experimental Design

The experiment consisted of four treatments, each with three replicates. For each treatment, three apple trees exhibiting similar growth vigor were selected, and protective barriers were established between the trees to prevent cross-contamination of water and fertilizers. The specifics of each treatment are as follows: (1) CK (control): Application of N, P, and K fertilizers only; (2) NPKM: N, P, and K fertilizers combined with organic fertilizer; (3) NPKL: N, P, and K fertilizers supplemented with quicklime; (4) NPKML: N, P, and K fertilizers supplemented with both organic fertilizer and quicklime.

In all treatments, ^{15}N -labeled urea (^{15}N 99 atom%) was applied at a N application rate of 550 kg N hm^{-2} soil [32]. The application rates for phosphate fertilizer and potassium fertilizer were $247 \text{ kg P}_2\text{O}_5 \text{ hm}^{-2}$ and $110 \text{ kg K}_2\text{O hm}^{-2}$, respectively, and these were applied as a single basal fertilizer application. The organic fertilizer (composted cow manure) was applied at a rate of $45,000 \text{ kg hm}^{-2}$. The effective calcium content of quicklime was $\geq 90\%$, and it was applied at a rate of 2500 kg hm^{-2} .

On 22 October 2023, the autumn application of base fertilizer in the orchard involved the even distribution of organic fertilizer, quicklime, and phosphorus and potassium fertilizers across the plow layer soil. Subsequently, on 28 March 2024, in situ ^{15}N microcosm culture devices were established. A total of 490 g of fresh soil (based on dry weight) was weighed and mixed with ^{15}N -labeled urea, which contained 108 mg of pure urea N, along with phosphorus and potassium fertilizers in the appropriate proportions. This mixture was then placed into a PVC pipe with an inner diameter of 5.0 cm and a height of 25 cm. The bottom of the pipe was sealed with a nylon mesh ($75 \mu\text{m} \times 75 \mu\text{m}$), and the mixture was compacted to reflect the actual field bulk density, thereby forming a microcosm. The PVC pipes were vertically inserted under the crowns of apple trees in each treatment plot, ensuring that the pipe ends were flush with the ground surface. To prevent cross-contamination, the distance between PVC pipes within the same plot was maintained at $\geq 2 \text{ m}$.

2.3. Sample Collection and Processing

On 25 September 2024, coinciding with the apple harvest period, soil samples from the PVC pipes of each treatment were collected and promptly transported to the laboratory. A portion of the fresh soil was sieved through a 2 mm mesh, and then used for immediate determination of AN, NN, and their respective ^{15}N abundances. The remaining fresh soil was air-dried, ground, and subsequently sieved through a 100-mesh sieve to facilitate the assessment of TN, amino sugar and FAN contents, and their ^{15}N abundances. Concurrently, the fundamental properties of the soil, including pH, electrical conductivity (EC), and soil organic carbon (SOC), were measured.

2.4. Analytical and Determination Methods

2.4.1. Determination of Basic Properties of Soil

Soil pH was assessed using the potentiometric method with a soil-to-water ratio of 1:2.5. EC was determined using the conductivity method at a soil-to-water ratio of 1:5. SOC was quantified using the potassium dichromate oxidation method with external heating. TN was measured using the Kjeldahl method. The composition of soil particles was analyzed using a laser particle size analyzer (LS 13 320, Beckman Coulter, Inc., Brea, CA, USA), while bulk density was evaluated using the ring knife method.

2.4.2. Determination of Different Forms of N in Soil

AN and NN were quantified using the potassium chloride extraction and flow injection analyzer method [33], while FAN was assessed through the magnesium oxide distillation method [34].

2.4.3. Determination of Amino Sugars

Soil amino sugars, specifically glucosamine (GluN), galactosamine (GalN), and muramic acid (MurN), were quantified through acid hydrolysis followed by gas chromatography [35]. Soil samples underwent hydrolysis with 6 mol L⁻¹ HCl at 105 °C for 8 h. Subsequent steps included neutralization, centrifugation, and filtration, after which the amino sugars were derivatized and then assessed using gas chromatography (GC). The contents of individual amino sugars were calculated by the internal standard method [36], and the amount of total amino sugars (TAS) was calculated from the sums of the monomers of each amino sugar [37].

2.4.4. ¹⁵N Abundance Determination

The ¹⁵N abundances of AN, NN, and FAN were quantified using a stable isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) [32]. The ¹⁵N enrichment of each amino sugar was assessed via gas chromatography/mass spectrometry (GC/MS) and expressed as atom percent excess (APE) [38]. Additionally, the ¹⁵N recovery rates for each N pool and the distribution ratio of fertilizer N were calculated.

2.5. Calculations

The values of ¹⁵N recovery rates (R_{15N} , %), fertilizer N allocation ratio (A_i , %), and N loss rate (L_N , %) were calculated as follows [37,39,40]:

$$R_{15N}(\%) = \frac{C_{15N,s} \times W_{s,dry}}{M_{15N,applied}} \times 100\% \quad (1)$$

$$A_i(\%) = \frac{R_{15N,i}}{R_{15N,total}} \times 100\% \quad (2)$$

$$L_N(\%) = 100 - (A_{inorg} - A_{bio-immob} - A_{abio-immob} - A_{unknown}) \quad (3)$$

where $C_{15N,s}$ is the content of ¹⁵N in the sample, $W_{s,dry}$ is dry weight of the sample, $M_{15N,applied}$ is the total amount of applied ¹⁵N, $R_{15N,i}$ and $R_{15N,total}$ are the ¹⁵N recovery rate of the *i*-th N pool and the total ¹⁵N recovery rate, respectively, and A_{inorg} , $A_{bio-immob}$, and $A_{abio-immob}$ represent the sum of the distribution ratios of fertilizer N in the inorganic N pool, biological immobilization pool, and abiotic immobilization pool, respectively. It is worth noting that $A_{unknown}$ indicates that a considerable proportion of N is still lost through unknown pathways, and such N loss cannot be neglected.

MNN is a stable component of the soil organic N pool, which is closely related to the accumulation and retention of soil N [32]. Fungal-derived N (FNN) and bacterial-derived N (BNN) are important components of MNN, reflecting the contributions of fungi and bacteria to soil biological N immobilization respectively [41]. Combined with the content characteristics of soil amino sugars (GluN, GalN, and MurN), the values of FNN (mg kg⁻¹), BNN (mg kg⁻¹), and MNN (mg kg⁻¹) were calculated as follows [35,42]:

$$FNN(\text{mg kg}^{-1}) = \left[\frac{\text{GlcN}}{179} - 2 \times \frac{\text{MurN}}{251} \right] \times 179.17 \times 0.9 \quad (4)$$

$$BNN(\text{mg kg}^{-1}) = \text{MurN} \times 11 \quad (5)$$

$$MNN(\text{mg kg}^{-1}) = FNN + BNN \quad (6)$$

where amino sugars are used as biomarker substances to distinguish the sources of microbial residues: MurN is a unique marker component of the bacterial cell wall, GluN and GalN are important components of the fungal and bacterial cell walls, respectively [36].

The above formulas quantitatively distinguish the contribution of fungi and bacteria to soil N retention by calculating the N content derived from their residues, and total MNN is the sum of fungal- and bacterial-derived N, which comprehensively reflects the total level of soil biological N immobilization [43].

2.6. Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted using SPSS 27.0 software, with Duncan's multiple comparison method applied to assess the significance of differences among treatments ($p < 0.05$), the ANOVA hypotheses were formally tested before applying the Duncan multiple comparison procedure. Pearson correlation analysis was utilized to examine the relationships between soil properties and N indicators. Data were visualized using Origin 2024 software.

3. Results

3.1. Effects of Different Treatments on Soil Properties

Significant differences were observed in the responses of soil properties to the different treatments (Table 2). Compared to the control (CK), soil pH in the NPKL and NPKML treatments increased significantly ($p < 0.05$) by approximately 1.8, reaching values of 6.24 and 6.20, respectively. In contrast, no significant change in soil pH was detected in the NPKM treatment ($p > 0.05$). EC increased significantly in all treatments compared to the initial soil; however, no significant differences were found among the treatments ($p > 0.05$). Additionally, there were no significant differences in EC, SOC and C/N among the treatments ($p > 0.05$), suggesting that the treatments did not significantly affect the SOC pool under short-term cultivation. TN content followed the order of NPKML > NPKL > NPKM > CK, with the TN level in the NPKML treatment being significantly higher than that in CK ($p < 0.05$).

The responses of soil inorganic N and FAN content to the different treatments exhibited significant differences. AN content demonstrated a notable decreasing trend with increasing soil pH, following the order of CK > NPKM > NPKL > NPKML. Additionally, AN content in NPKL and NPKML treatments was significantly lower than that in CK ($p < 0.05$). In contrast, the NN content in the NPKL treatment was significantly higher than that in CK ($p < 0.05$), whereas the NN contents in the NPKM and NPKML treatments decreased significantly ($p < 0.05$). Furthermore, FAN content exhibited a significant increasing trend with rising soil pH, following the order of NPKML > NPKL > NPKM > CK. Notably, FAN content in the NPKML treatment was significantly higher than that in CK ($p < 0.05$), showing that soil pH was the core factor driving its regulation.

Table 2. Basic properties of the harvest stage soil.

Treatment	pH	EC ($\mu\text{s cm}^{-1}$)	SOC (g kg^{-1})	TN (g kg^{-1})	C/N	AN (mg kg^{-1})	NN (mg kg^{-1})	FAN (mg kg^{-1})
CK	4.43 $\pm$ 0.07 b	251.90 $\pm$ 7.23 a	13.49 $\pm$ 0.29 a	1.20 $\pm$ 0.05 b	11.27 $\pm$ 0.62 a	19.07 $\pm$ 1.90 a	79.15 $\pm$ 5.79 ab	133.36 $\pm$ 7.24 b
NPKM	4.46 $\pm$ 0.02 b	263.07 $\pm$ 9.04 a	14.07 $\pm$ 0.61 a	1.25 $\pm$ 0.05 ab	11.36 $\pm$ 0.94 a	15.67 $\pm$ 1.29 b	74.77 $\pm$ 4.53 b	136.97 $\pm$ 7.09 ab
NPKL	6.24 $\pm$ 0.05 a	259.34 $\pm$ 4.38 a	13.39 $\pm$ 1.00 a	1.27 $\pm$ 0.10 ab	10.58 $\pm$ 0.89 a	12.23 $\pm$ 0.81 c	86.01 $\pm$ 7.35 a	142.08 $\pm$ 5.42 ab
NPKML	6.20 $\pm$ 0.03 a	259.37 $\pm$ 3.03 a	14.10 $\pm$ 0.79 a	1.34 $\pm$ 0.06 a	10.54 $\pm$ 1.10 a	11.52 $\pm$ 0.61 c	73.93 $\pm$ 3.71 b	147.63 $\pm$ 7.40 a

Note: EC, electrical conductivity; SOC, soil organic carbon content; TN, total soil nitrogen content; C/N, carbon-to-nitrogen ratio. Data are presented as the mean $\pm$ standard deviation, different lowercase letters indicate significant differences between treatments ($p < 0.05$, $n = 3$).

3.2. Fertilizer N-Derived Inorganic N

The inorganic N pool of each treatment was predominantly composed of NN, with the fertilizer N distribution ratio ranging from 32.52% to 46.69%, which was significantly higher than that of AN (6.21–10.39%). FAN was the second-largest component of the inorganic N pool, accounting for 12.10–17.36% (Figure 1). Organic fertilizer application exerted a pronounced inhibitory effect on the conversion of fertilizer N into NN. Specifically, the NPKM and NPKML treatments decreased the fertilizer N distribution ratio of NN by more than 10% relative to the CK and NPKL treatments ($p < 0.05$), demonstrating that organic fertilizer effectively suppressed the nitrification process and reduced the accumulation of fertilizer-derived NN. Meanwhile, quicklime application significantly reduced the distribution ratio of fertilizer-derived AN. Compared with the CK and NPKM treatments, NPKL and NPKML treatments lowered the AN distribution ratio by over 30% ($p < 0.05$), implying that quicklime promoted the further conversion of AN into other N fractions, including NN and FAN, thereby accelerating the turnover of fertilizer-derived AN in soil inorganic N pools.

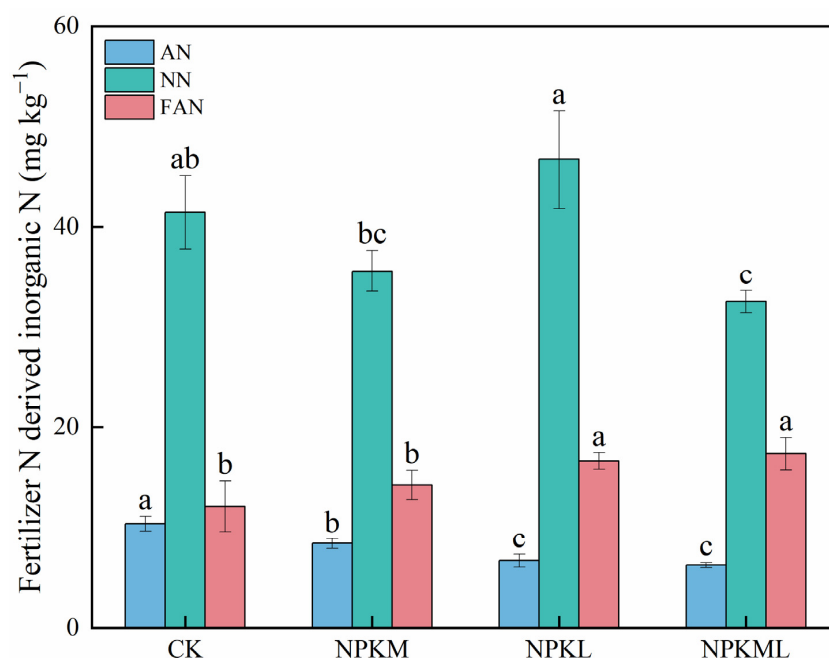


Figure 1. Distribution of fertilizer N within the inorganic N pool (AN, NN, FAN) across various treatments. AN, ammonium N; NN, nitrate N; FAN, fixed ammonium N. CK: conventional N, P, and K fertilizer treatment; NPKM: N, P, and K combined with organic fertilizer; NPKL: N, P, and K combined with quicklime; NPKML: N, P, and K combined with both organic fertilizer and quicklime. Different lowercase letters denote significant differences among treatments ($p < 0.05$, $n = 3$).

3.3. Fertilizer N-Derived Amino Sugars

The fertilizer N-derived TAS exhibited significant variations in response to different improvement measures, generally following the order: NPKML > NPKM > NPKL > CK (Figure 2). Notably, TAS content in the NPKML treatment was significantly greater than that in the other treatments, while the CK treatment displayed the lowest content among all treatments, with significant differences observed ($p < 0.05$). Further analysis of the contributions of the amino sugar components indicated that GluN served as the principal component driving the increase in TAS content. Its concentration in the NPKML treatment was significantly higher than that in the CK treatment, establishing it as the main contributor to the accumulation of TAS. Although GalN and MurN demonstrated an upward trend with the optimization of treatment measures, their contributions to the increase in TAS

were both lower than that of GluN. This indicated that these observations suggest greater production of fungal-derived amino sugars contributed to N buildup within biological immobilization pools, and such N storage was reflected in elevated TAS alongside abundant bacterial-derived residual N biomarkers. As the predominant constituent of TAS, GluN serves as a valuable proxy for quantifying residual N sequestered in microbial residues. This result may further corroborate the activating effect of the improvement measures on the N assimilation functions of soil microorganisms, particularly the synergistic interaction between bacteria and fungi in amino sugar synthesis, thereby establishing a foundation for enhancing N biological immobilization capacity.

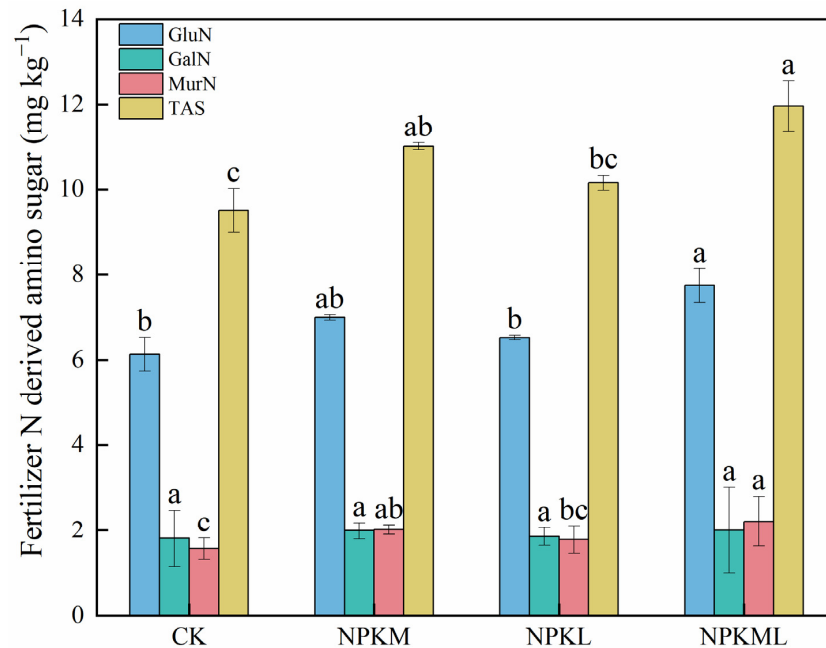


Figure 2. Fertilizer N-derived amino sugar components across various treatments. GluN: glucosamine; GalN: galactosamine; MurN: muramic acid. CK: conventional N, P, and K fertilizer treatment; NPKM: N, P, and K combined with organic fertilizer; NPKL: N, P, and K combined with quicklime; NPKML: N, P, and K combined with both organic fertilizer and quicklime. Distinct lowercase letters denote significant differences among treatments ($p < 0.05$, $n = 3$).

3.4. Fertilizer N-Derived MNN

MNN serves as a fundamental pool in the biological immobilization of fertilizer N, elucidating the primary mechanism of biological immobilization through its distribution across various microbial groups (Figure 3). In all treatments, BNN constituted 83% to 87% of MNN, significantly surpassing the proportion of FNN. This finding demonstrates that bacterial necromass constituted the major fraction of microbe-derived residual N sequestered from applied fertilizer, based on amino sugar quantification rather than direct measurement of microbial metabolic activity. BNN concentrations differed significantly across treatments. The NPKML group recorded BNN at 25.5 mg kg^{-1} , representing a 41.7% elevation relative to CK ($p < 0.05$). BNN values were also higher in NPKM and NPKL than CK, though the magnitude of increase was smaller ($p < 0.05$). These residual N data indicate that co-amendment promoted greater accumulation of bacterial cellular debris derived from fertilizer N; however, this observation cannot confirm synergistic stimulation of live bacterial assimilation activity. By contrast, FNN concentrations showed no statistical disparities among all treatments ($p > 0.05$). This residual-based observation implies that fungal cellular debris derived from fertilizer N did not accumulate to varying extents under the tested amendment regimes, though we lack direct measurements of fungal N uptake

activity to verify shifts in their metabolic capacity. The FNN/BNN ratio (Figure 4) illustrates the relative proportions of fungal versus bacterial residual N pools retained from fertilizer inputs, with no significant inter-treatment variation detected ($p > 0.05$), consistent with the stable share of fungal necromass observed across all treatments. Overall, the combined application of organic fertilizer and quicklime resulted in the maximum accumulation of fertilizer N in the biological immobilization pool.

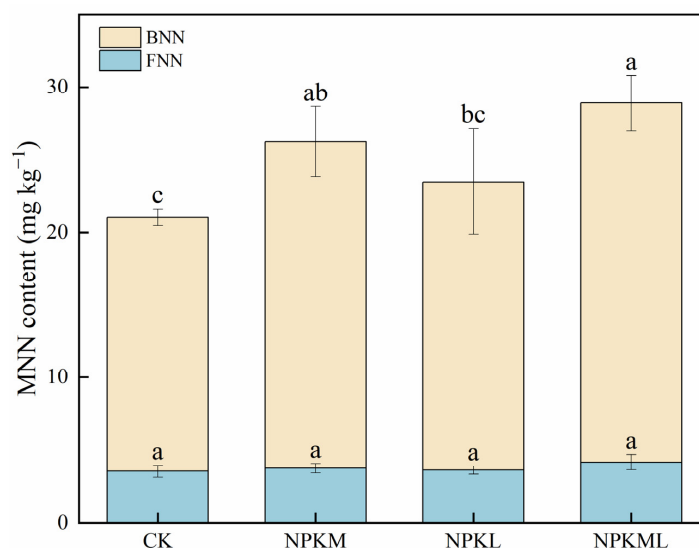


Figure 3. Distribution characteristics of fertilizer N in microbial necromass N (MNN) across various treatments. FNN, fungal necromass N; BNN, bacterial necromass N. CK: conventional N, P, and K fertilizer treatment; NPKM: N, P, and K combined with organic fertilizer; NPKL: N, P, and K combined with quicklime; NPKML: N, P, and K combined with both organic fertilizer and quicklime. Distinct lowercase letters denote significant variances between treatments ($p < 0.05$, $n = 3$).

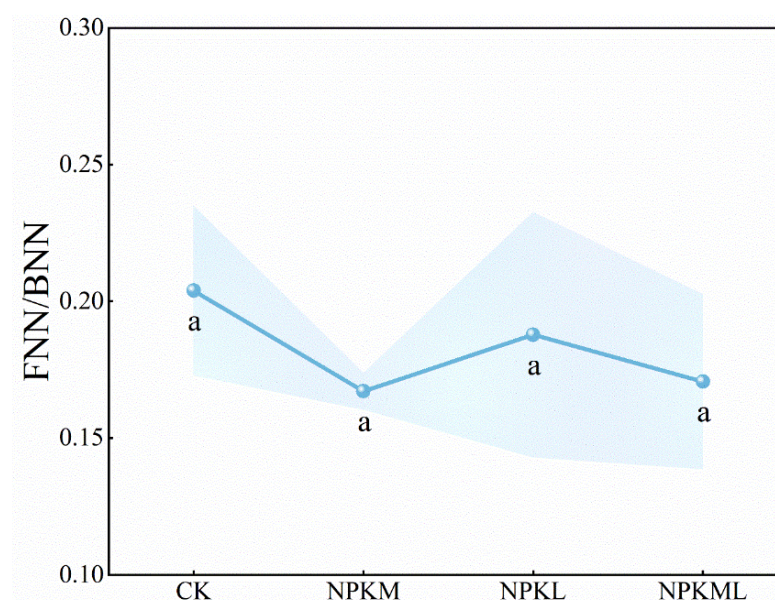


Figure 4. Ratios of fertilizer N-derived fungal necromass N (FNN) to bacterial necromass N (BNN) across the different treatments. The ratio reflects the relative contributions of fungi and bacteria to microbial necromass N (MNN). CK: conventional N, P, and K fertilizer treatment; NPKM: N, P, and K combined with organic fertilizer; NPKL: N, P, and K combined with quicklime; NPKML: N, P, and K combined with both organic fertilizer and quicklime. The blue shaded area indicates the range of standard deviation. Different lowercase letters denote significant differences among treatments ($p < 0.05$, $n = 3$).

3.5. Recovery of Fertilizer N in the Abiotic and Biotic Immobilization Pools

The distribution ratio of fertilizer N within the abiotic fixation pool serves as a direct indicator of the ammonium fixation capacity of clay minerals, and it revealed significant differences across the various treatments (Figure 5). The relative proportion of fertilizer N as AN in the NPKL treatment (3.02%) was significantly higher than that in the NPKML treatment (2.82%) ($p < 0.05$). The distribution ratio of fertilizer N in the inorganic N pool followed the order of NN > FAN > AN across all treatments, indicating that the improvement measures mainly regulated NN and FAN rather than AN. The distribution ratio of fertilizer N in FAN followed the order of NPKML > NPKL > NPKM > CK. Notably, the NPKML treatment exhibited a 43.4% increase compared to CK ($p < 0.05$), suggesting that the combined application of quicklime and organic fertilizer significantly enhanced the conversion of fertilizer N into FAN and improves the abiotic retention of N. In the NPKML treatment, the relative proportion of fertilizer N in FAN reached 7.87%, the highest among all treatments, representing a 46.2% increase compared to CK ($p < 0.05$). This finding indicated that the rise in soil pH, coupled with enhanced microbial activity, facilitated an increase in the ammonium fixation capacity of clay minerals, allowing for a greater conversion of fertilizer N into FAN. The N loss rate was ranked as CK > NPKM > NPKML > NPKL, demonstrating that both the individual and combined applications of quicklime significantly reduced N loss, while the effect of the individual application of organic fertilizer on loss reduction was relatively modest.

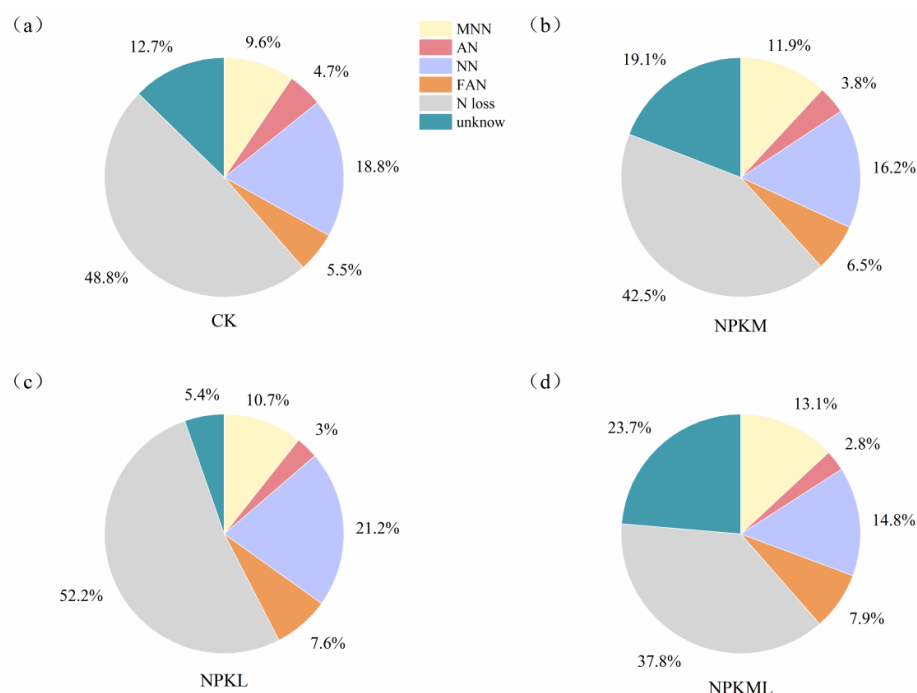


Figure 5. Distribution ratios of fertilizer N across various N pools and N loss rates under different treatments. Panels (a–d) correspond to CK, NPKM, NPKL, and NPKML, respectively. CK: conventional N, P, and K fertilizer treatment; NPKM: N, P, and K combined with organic fertilizer; NPKL: N, P, and K combined with quicklime; NPKML: N, P, and K combined with both organic fertilizer and quicklime. AN: ammonium N; NN: nitrate N; FAN: fixed ammonium N; and MNN: microbial necromass N. The values reflect the distribution ratios of fertilizer N within each N pool and the N loss rate (%).

3.6. Analysis of Influencing Factors and Correlations of N Immobilization

Soil pH was a fundamental factor influencing N retention, with variations in pH affecting the retention dynamics of different N forms (Figure 6). Overall, the retention

rate of NN was markedly the highest, exhibiting a median of approximately 17%, and the broadest data distribution range, indicating substantial dispersion. In contrast, the retention rate of microbial biomass N occupied a middle position, with a median of around 11% and data concentrated within the 9–14% range. FAN exhibited an intermediate retention rate, with a median value of roughly 7% and most observations ranging from 6% to 8%. AN had the lowest retention rate, with a median near 3%. Comparative analysis indicated that the gap between the NN and AN retention rates was the largest among all N pools. These patterns may imply that pH elevation could accelerate AN transformation and potential ammonia volatilization alongside stimulated nitrification, likely driving the conversion of AN to NN. Furthermore, partial overlap was observed between the distributions of MNN and FAN, tentatively suggesting that higher soil pH might be associated with simultaneous improvements in biotic and abiotic N retention potentials. Correlation analyses showed a positive relationship between biological and abiotic N retention indicators, though such covariation alone cannot confirm a true synergistic interaction between the two N retention processes.

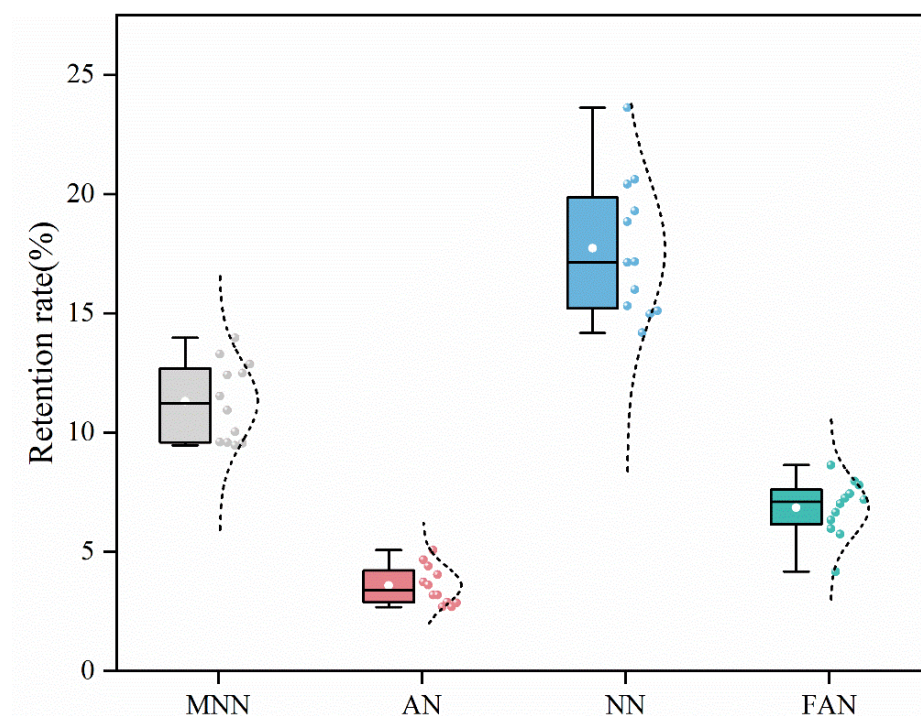


Figure 6. Retention rates of various forms of fertilizer N. MNN: microbial necromass N; AN, ammonium N; NN, nitrate N; and FAN, fixed ammonium N. The box in the figure depicts the interquartile range, while the horizontal line within the box indicates the median. Scatter points and dashed lines correspond to individual observations and probability distribution trends, respectively. The individual observations within each group adhere to a normal distribution. Notably, different treatments significantly influenced the retention rates: the retention rate for NN was the highest, followed by the middle retention rates for MNN and FAN, whereas the AN group exhibited the lowest retention rate.

Pearson correlation analysis elucidated the intrinsic relationships between soil properties and N retention indicators (Figure 7). pH exhibited a highly significant negative correlation with AN ($r = -0.862$, $p < 0.01$), a significant positive correlation with FAN ($r = 0.774$, $p < 0.01$), and a weakly significant positive correlation with microbial biological retention (MNN) ($r = 0.362$, $p < 0.05$). AN demonstrated a significant negative correlation with FAN ($r = -0.746$, $p < 0.01$), indicating substrate competition between the transformation of AN and the formation of FAN. MNN showed a significant positive correlation

with FAN ($r = 0.647$, $p < 0.05$), suggesting that biological and abiotic N retention processes were not antagonistic but rather enhanced each other synergistically. EC did not exhibit a significant correlation with any N indicator, indicating that changes in soil salinity were not the primary driving factor for N retention in this experiment.

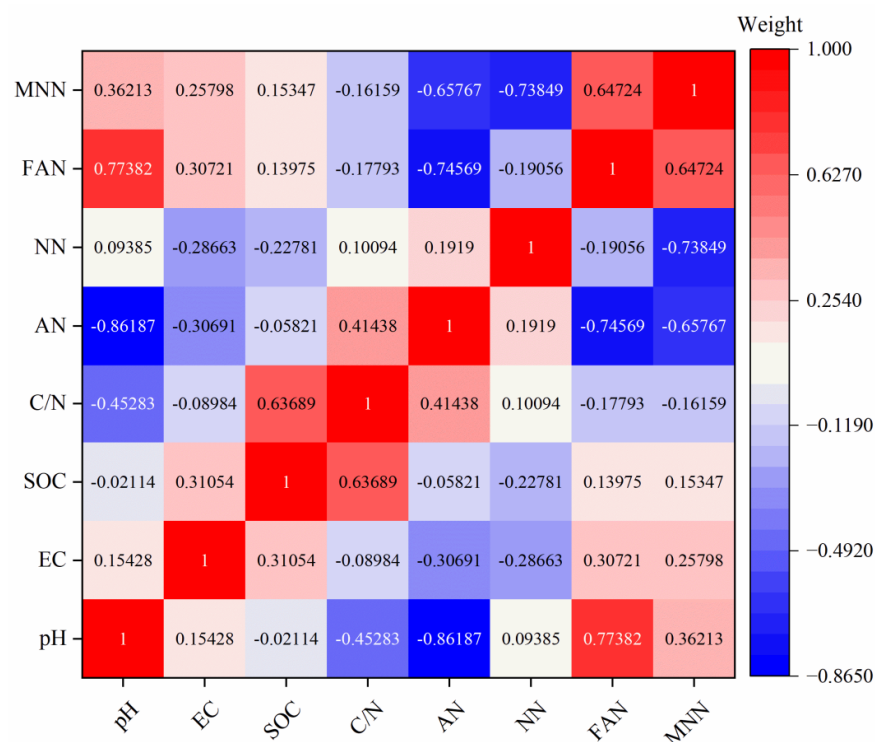


Figure 7. Pearson correlation analysis of soil chemical properties in relation to fertilizer N retention indicators. The red color signifies a positive correlation, while blue indicates a negative correlation. pH refers to soil acidity and alkalinity; EC, electrical conductivity; SOC, soil organic carbon; C/N, carbon-to-nitrogen ratio; AN, ammonium nitrogen; NN, nitrate nitrogen; FAN, fixed ammonium; MNN, microbial necromass nitrogen. The intensity of the color reflects the strength of the correlation: $|r| > 0.8$ denotes an extremely significant correlation, $0.5 < |r| < 0.8$ indicates a significant correlation, and $|r| < 0.5$ represents a weakly significant correlation.

3.7. Trade-Offs Between Soil Nitrogen Pool Status, Apple Yield and Residual Fertilizer N Under Different Soil Amendment Practices

To intuitively differentiate the synergistic and antagonistic effects of various improvement practices on soil N pool conservation, apple production, and fertilizer N retention, we employed four-quadrant scatter plots to quantify the trade-offs associated with each indicator relative to the CK treatment (Figure 8). Values exceeding “0” on the horizontal and vertical axes signify that the corresponding indicators improved compared to CK. Two dashed lines in the figure delineate the plot into four benefit zones. Figure 8a depicts the trade-off relationship between biotic and abiotic soil N pool indicators. The CK treatment serves as the reference benchmark, positioned at the origin of the coordinate system. All three amended treatments (NPKM, NPKL, and NPKML) are situated within the upper-right optimal zone, where both FAN and MNN increased concurrently. This finding suggests that the individual application of organic fertilizer or lime, as well as their combined application, can enhance soil N fixation capacity and the microbial N pool simultaneously, with the overall magnitude of improvement ranked as NPKML > NPKL > NPKM. Figure 8b illustrates the coupled variation pattern of apple yield and residual fertilizer N in soil, revealing a distinct trade-off differentiation among treatments. The NPKL treatment occupies the lower-left quadrant; while it resulted in a slight increase in apple yield, it significantly

reduced residual fertilizer N, creating a contradictory trade-off between yield enhancement and soil N sequestration. In contrast, both NPKM and NPKML are located in the upper-right synergistic zone, where they concurrently increased apple yield and retained a greater amount of fertilizer N in the soil. Notably, NPKML demonstrated superior performance in yield enhancement and soil N retention compared to NPKM. Integrating the findings from the two panels, sole lime amendment expanded both biotic and abiotic soil N pools but exacerbated the loss of exogenous fertilizer N. The application of single organic fertilizer achieved a balance among soil N pool preservation, fruit output, and fertilizer N retention; however, the extent of improvement across all indicators remained limited. The combined application of organic fertilizer and lime effectively elevated soil FAN, MNN, apple yield, and residual fertilizer N, representing the optimal amendment strategy that harmonizes soil N pool maintenance, stable fruit production, and efficient retention of fertilizer N.

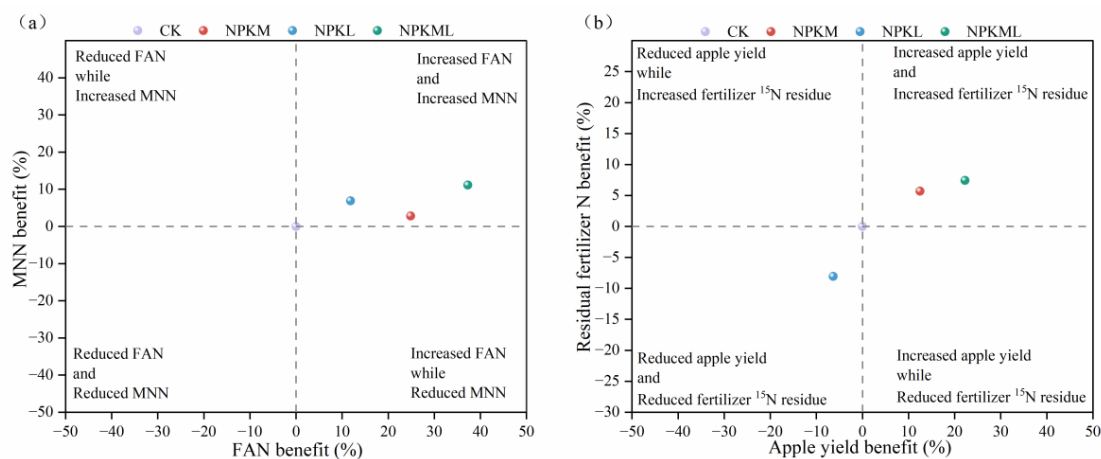


Figure 8. Trade-offs between the effects of the combined application of chemical fertilizer with organic manure and lime on soil nitrogen pool components, apple yield and residual fertilizer N. (a) Trade-off relationship between fixed AN (FAN) benefit and soil microbial necromass N (MNN) benefit; (b) Trade-off relationship between apple yield benefit and residual fertilizer N benefit.

4. Discussion

4.1. “Chemical Priming” Effect of Quicklime on Soil N in Acidified Orchards: Dual Regulation of Nitrification Activation and Abiotic Immobilization

The modification of N cycling in acidified orchard soils by quicklime is hypothesized to be primarily driven by a pH-mediated chemical priming effect [44]. This process swiftly neutralizes soil acidity while simultaneously activating nitrification and enhancing the abiotic fixation of AN [45]. Although these two functional shifts appear contradictory at first glance, the coordinated balance of N substrate distribution and transformation rates under liming may jointly improve the overall soil N retention capacity [46].

From the perspective of the nitrification activation mechanism, the low pH of acidified soil (pH = 4.46) inhibits the metabolic activity of nitrifying bacteria, whose optimal pH ranges from 7.0 to 7.5 [47]. This inhibition likely suppresses the accumulation of AN and obstructs the nitrification process [48]. Following the application of quicklime in this study, soil pH rose to approximately 6.2. This increase may partially alleviate the acid stress experienced by nitrifying microorganisms, such as ammonia-oxidizing bacteria and nitrite-oxidizing bacteria, thereby significantly enhancing their community abundance and metabolic activity [49,50]. Measured data showed significantly higher NN concentrations under the NPKL treatment relative to CK, which we interpret as observational evidence supporting the nitrification-stimulating potential of quicklime [40,51]. However, it is important to note that the pH increase induced by quicklime in the current study did not

reach the optimal range for nitrifying bacteria, resulting in insufficient over-activation of nitrification and thus leaving a substantial amount of AN substrate available for abiotic immobilization.

From the perspective of the mechanism enhancing abiotic immobilization, elevated soil pH may strengthen electrostatic adsorption sites on clay mineral surfaces, which could further promote the conversion of NH_4^+ from an exchangeable state to a fixed state [16]. Measured results recorded markedly higher FAN concentrations and a larger fraction of labeled fertilizer N allocated to the abiotic immobilization pool under NPKL than under CK [22].

In terms of the net effects, the activation of nitrification by quicklime resulted in a slight increase in NN accumulation [40]; however, the enhanced abiotic immobilization significantly mitigated the loss risk associated with nitrification [52]. In the NPKL treatment, the N loss rate was 11.0% lower than that in the CK treatment. From these observations, we infer that quicklime may exert a chemical priming effect that establishes a dual regulatory pathway combining nitrification activation and mineral ammonium immobilization. The core feature of this inferred mechanism is the dynamic balance between N transformation and retention modulated by pH adjustment, which may provide a chemical basis for stable N sequestration in severely acidified orchard soils [53,54].

4.2. “Biological Buffering” Effect of Organic Fertilizers on Soil N in Acidified Orchard Soils: Construction and Mechanism of a Bacteria-Dominated Immobilization Structure

The regulation of N retention in acidified soils by organic fertilizers is fundamentally based on the C-N-driven biological buffering effect [24,55]. By supplying ample organic C and N substrates, these fertilizers stimulate soil microbial activity and enhance the assimilation and retention of inorganic N by microorganisms [54]. Such inputs may also shift the community structure toward a bacterial-dominated N-retention functional group [24,54,55].

The “biological buffering” effect of organic fertilizers primarily manifests in the inhibition of initial N loss. In acidified soils, microbial activity is significantly suppressed by acid stress, leading to a diminished capacity for N assimilation from fertilizers [18]. Under such conditions, a large proportion of applied N persists in inorganic forms susceptible to leaching, ammonia volatilization and other loss pathways [18]. Upon the application of organic fertilizers, the abundant readily decomposable organic C provides essential energy sources for microorganisms. Organic N can be directly assimilated and utilized by these microorganisms, resulting in a marked increase in microbial biomass and metabolic activity [26]. Thus, the fertilizer-derived TAS in the NPKM treatment was significantly higher than that in the CK, indicating a substantial enhancement in the microorganisms’ ability to assimilate and immobilize fertilizer N. Additionally, colloidal substances such as humus present in organic fertilizers improve the soil’s pH buffering capacity, preventing sharp fluctuations in pH during the N transformation process [56]. This stabilization further supports the nitrification and assimilation functions of microorganisms and effectively reduces the accumulation of NN [12]. Consequently, the NN content in the NPKM treatment was significantly lower than that in the CK, thereby greatly diminishing the risk of N leaching [57].

Our results further demonstrate that BNN accounted for 83–87% of MNN across all treatments, while FNN remained consistently low, with no significant differences among treatments (Figure 4). This indicates that the biological immobilization structure promoted by organic fertilizers is strongly bacteria-dominated and that the contribution of fungi to fertilizer N immobilization was consistently minor and stable regardless of organic fertilizer or quicklime application. This finding does not contradict the well-known higher acid tolerance of fungi; rather, it suggests that under our experimental conditions (6-month in situ incubation), the rapid response and high efficiency of bacterial communi-

ties in utilizing fresh organic C substrates made bacteria the primary agents of immediate N immobilization [41,55,58]. Although bacterial residues decompose faster than fungal residues [59], the continuous input of organic C and N substrates from organic fertilizers sustains high bacterial turnover, effectively capturing fertilizer N in the short term and reducing N losses. In contrast, fungi, due to their slower growth and weaker competition for fresh labile carbon compared to fast-growing bacteria [57], contributed minimally to direct fertilizer N immobilization within the time frame of this study.

The relatively small and stable fungal contribution to MNN warrants further biological consideration regarding the role of fungi in N cycling and necromass dynamics. Fungi are generally considered more acid-tolerant than bacteria and are often dominant in low-pH soils [18]. However, in our study, fungi contributed minimally to fertilizer N retention despite the initial acidic conditions (pH 4.46). This apparent observation may be explained by several factors. First, the experimental duration (6 months) may have favored bacteria, which have shorter generation times and more rapid turnover rates, over fungi, which typically exhibit slower growth and longer residence times [59]. Second, the input of readily decomposable organic substrates (organic fertilizer) may have created a resource environment favoring fast-growing bacterial copiotrophs rather than fungi, which tend to be more competitive for complex and recalcitrant substrates [57]. Third, the rapid pH recovery induced by quicklime may have further disadvantaged acid-adapted fungi while benefiting neutrophilic bacteria [49]. Fourth, it is important to consider that our measurements reflect the net accumulation of fungal necromass (production minus decomposition), rather than direct fungal activity. A low FNN pool does not necessarily indicate low fungal activity but could instead reflect rapid fungal necromass decomposition, as fungi are known to be more susceptible than bacteria to grazing by soil fauna and to enzymatic decomposition [43]. The absence of a significant increase in FNN following organic fertilizer addition suggests that fungal residues may be turned over more rapidly than bacterial residues or that fungal communities were less responsive to the amendment treatments within the short-term experimental framework. This interpretation is consistent with the findings of Ren et al. [12], who reported that fungal residues contribute more to long-term N stabilization (years to decades), whereas bacterial residues are more dynamic in the short term. Given our 6-month incubation period, the detection of a significant fungal contribution may require longer-term observations. Future studies employing longer incubation periods and molecular approaches (e.g., fungal-specific PLFA or ITS sequencing) would be valuable to disentangle the relative roles of fungi versus bacteria in N retention and necromass dynamics over extended time scales.

4.3. Combined Application of Organic Fertilizer and Quicklime Enhances N Retention Through Complementary Mechanisms

Measured data indicated that the NPKML treatment concurrently increased both biological (MNN) and abiotic (FAN) N pools [60]. We interpret this concurrent improvement as potentially arising from complementary actions of the two amendments, which may address each other's limitations. Organic fertilizer may mitigate the risk of excessive nitrification activation induced by quicklime through the biological buffering effect of microbial assimilation [61]. Concurrently, quicklime improves the environmental conditions for microorganisms by increasing pH, thereby potentially enhancing the fixation efficiency of mineralized N derived from organic fertilizer [24]. Together, the two amendments may complement each other's respective limitations, and their combined application appeared to maximize overall N retention efficiency (Figure 9).

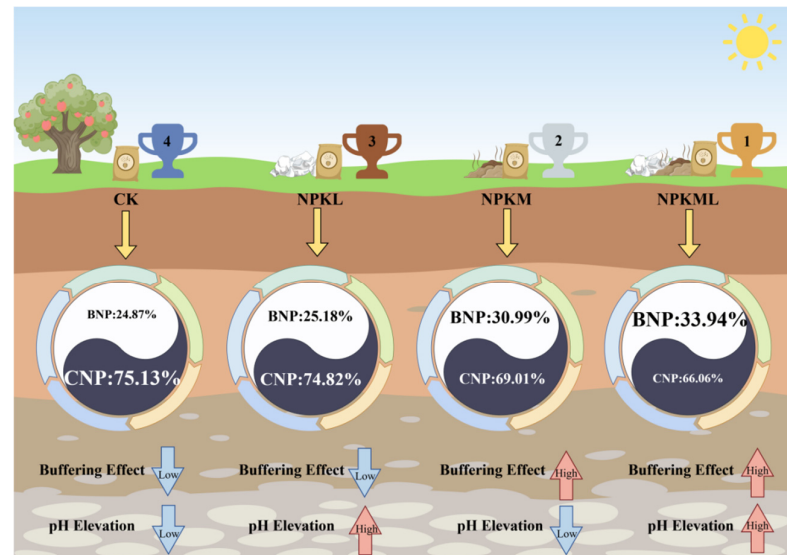


Figure 9. Conceptual diagram illustrating how the buffering effect and pH elevation drive the relative contributions of the biological N pump (BNP) and chemical N pump (CNP) to fertilizer N retention under different treatments. CK: conventional N, P, and K fertilizer treatment; NPKM: N, P, and K combined with organic fertilizer; NPKL: N, P, and K combined with quicklime; NPKML: N, P, and K combined with both organic fertilizer and quicklime.

Organic fertilizer effectively mitigates the risk of excessive nitrification activation induced by quicklime, thereby preserving adequate substrates for abiotic fixation [62]. While the exclusive application of quicklime can enhance abiotic fixation, it can also activate nitrification, which converts a portion of AN into NN, consequently diminishing the availability of ammonium-fixing substrates [63]. The combined application of organic fertilizers markedly enhances microbial activity [64]. Measured results showed significantly lower NN concentrations under NPKML compared with NPKL, with the fraction of fertilizer N recovered as NN reduced by 15.3%. This reduction may not only minimize the risk of N leaching but also preserve sufficient AN substrates for fixation by clay minerals.

Conversely, the application of quicklime raises soil pH, optimizing the microbial habitat, and may improve the fixation efficiency of N derived from organic fertilizers [65]. The use of organic fertilizers alone has a limited impact on enhancing soil pH [66]. Following the combined application of quicklime, soil pH increased to approximately 6.2, which may alleviate the inhibitory effects of acid stress on microorganisms. Notably, fungal metabolic activity appeared to be improved under this pH range [18], allowing microorganisms to more effectively assimilate and utilize the C and N substrates released during the mineralization of organic fertilizers, which may further boost biological retention capacity. Additionally, the elevated pH improves the ammonium fixation capacity of clay minerals [67], enabling the rapid fixation of AN released from organic fertilizers, thereby reducing ammonia volatilization losses [17]. This creates a dual N retention pathway integrating both biological and abiotic fixation of mineralized organic N.

Moreover, Pearson correlation analysis revealed a significant positive correlation between MNN and FAN ($r = 0.647$, $p < 0.05$), indicating that increases in biological and abiotic N retention occurred in a coordinated manner rather than through substrate competition. This positive covariation may reflect independent but simultaneous responses to improved soil conditions (pH elevation and carbon substrate availability) [51,67], or it may suggest that enhanced microbial activity indirectly promotes abiotic fixation through microbially metabolism-induced modifications of the soil microenvironment [68]. We caution, however, that correlation alone does not demonstrate a causal or synergistic interaction between these two processes. Nevertheless, based on our comparative measurements, the NPKML

treatment achieved the highest total biological and abiotic N retention among all treatments, with a 62.5% increase in TN retention efficiency compared to the CK treatment. We propose this enhanced performance as a working hypothesis—the “dual retention framework”—that requires further validation through targeted experiments (e.g., selective inhibition, dual isotope labeling, or functional gene analysis). Our current data support the observational pattern of superior N retention under co-amendment but do not directly demonstrate the proposed mechanistic coupling.

4.4. Practical Insights into High-Efficiency N Management in Acidified Apple Orchards

Based on the results of this study and the current production conditions in intensive apple orchards, we propose a fundamental strategy for effective N management in acidified apple orchards based on combining organic fertilizers and quicklime to create a dual N retention system consisting of a chemical N pump and a biological N pump. By integrating scientifically informed fertilization timings and methods, this approach aims to improve both soil acidification and N use.

As validated by the four-quadrant trade-off scatter plots (Figure 8), the combination of quicklime + organic fertilizer is the best approach to improve severely acidified apple orchards (pH 4.5) in North China. The recommended application strategy is 2500 kg quicklime hm^{-2} and 45,000 kg decomposed organic fertilizer hm^{-2} . Besides N, P, and K fertilizers, the solution can rapidly raise soil pH to the optimal range of 6.0–6.5, increase biological and non-biological N fixation, reduce N loss by over 10% and increase N fertilizer utilization by 15–20%. Figure 8b further reveals the intrinsic trade-off defects of single-amendment treatments: sole quicklime application (NPKL) rapidly alleviates soil acidity yet triggers intensified nitrification and exogenous fertilizer N loss, falling into the antagonistic quadrant with elevated apple yield but drastically reduced residual soil fertilizer N. Quicklime alone can quickly adjust soil acidity, but it risks activating nitrification. By contrast, the single organic fertilizer amendment (NPKM) lands in the synergistic zone of Figure 8b but delivers marginal improvements across soil N pools, fruit yield and fertilizer N retention with a prolonged soil remediation cycle and weak capacity to neutralize severe soil acidification. Organic fertilizer alone has long improvement cycle and limited acid adjustment, so neither option is suitable as a standalone solution for severely acidified orchards.

5. Conclusions

In this study, an in situ ^{15}N tracing technique was used to investigate changes in N pool distribution in response to the quicklime and organic fertilizer application in a severely acidified apple orchard soil. Quicklime raised soil pH from 4.46 to approximately 6.2, alleviated microbial acid stress, and enhanced both nitrification and abiotic ammonium fixation. Organic fertilizer exerted a C-N-driven “biological buffering” effect, increasing microbial N assimilation. Amino sugar biomarkers revealed that BNN accounted for 83–87% of MNN, meaning bacterial residues constituted the major fraction of microbe-sequestered fertilizer N, while fungal residual contributions remained low and stable across all treatments. Co-amendment with quicklime and organic fertilizer jointly improved overall soil N retention via the coupled regulatory framework of the so-called “chemical N pump” and “biological N pump”, balanced soil N fractions and mitigated N loss risks. For severely acidified orchards with a pH below 4.5, we recommend the application of 2500 kg hm^{-2} quicklime plus 45,000 kg hm^{-2} decomposed organic fertilizer. However, this trial was conducted in a single Luvisol apple orchard over one growth cycle. Our results cannot be generalized to soils, climates or long-term orchards with different acidification levels without further validation. Moreover, we only measured microbial residues using

amino sugar markers, with no molecular assays performed to characterize in situ microbial metabolic activity. Future research should focus on long-term field experiments, employ molecular biological techniques to elucidate microbial N immobilization mechanisms, and evaluate the adaptability of this strategy to different climatic conditions and soil types.

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