Nitrogen

Review

Advances in the Study of NO_3^- Immobilization by Microbes in Agricultural Soils

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Abstract: The extensive application of nitrogen (N) fertilizers in agriculture has resulted in a considerable accumulation of N in the soil, particularly nitrate (NO_3^-), which can be easily lost to the surrounding environments through leaching and denitrification. Improving the immobilization of NO_3^- by soil microorganisms in agriculture is crucial to improve soil N retention capacity and reduce the risk of NO_3^- loss. In this paper, we reviewed the significance of microbial immobilization of soil NO_3^- in soil N retention, the techniques to quantify soil gross microbial NO_3^- immobilization rate, and its influencing factors. Specifically, we discussed the respective contribution of fungi and bacteria in soil NO_3^- retention, and we clarified that the incorporation of organic materials is of vital importance in enhancing soil microbial NO_3^- immobilization capacities in agricultural soils. However, there is still a lack of research on the utilization of NO_3^- by microorganisms of different functional groups in soil due to the limited techniques. In the future, attention should be paid to how to regulate the microbial NO_3^- immobilization to make soil NO_3^- supply capacity match better with the crop N demand, thereby improving N use efficiency and reducing NO_3^- losses.

Keywords: NO_3^- loss; microbial immobilization; organic materials; fungi; bacteria

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1. Introduction

N is an essential nutrient for plant growth and a limiting factor in agricultural production [1]. Thus, the application of N fertilizers is crucial for achieving high crop yields and quality. Over the last few decades, China has achieved remarkable success in agricultural production, with per capita grain, meat, and egg production exceeding the global average as early as 1998 [2]. This success is largely attributed to the increased application of N fertilizers. However, due to the repeated application of high levels of N fertilizer, the mismatch between soil N supply, crop demand, and the low N use efficiency of crops, a large amount of N remains in the agricultural soils. Once the residual N leaves the agricultural soils, it can contribute to environmental pollution. Soil ammonium (NH_4^+) is easily adsorbed by negatively charged soil colloids due to its positive charge, thus remaining in the soil [3]. In contrast, NO_3^-, with its negative charge, can quickly move with soil water beyond the root zone if not promptly absorbed by crops [4]. It can then diffuse into the atmosphere or water bodies through denitrification or leaching, ultimately posing a threat to human health [5]. In croplands with high N application, residual N in the soil predominantly exists as NO_3^- [2], and studies have also found that high N inputs increased the proportion of NO_3^- in the residual N [6]. Additionally, in neutral or alkaline soils, NH_4^+ is easily converted into NO_3^- through strong microbial nitrification within a short period [7], making NO_3^- the dominant form of inorganic N in these soils [8]. Excessive NO_3^- in drinking water has been linked to various human diseases, such as gastric cancer [9,10]. Therefore, for agricultural soils with high NO_3^- contents or where NO_3^- is the predominant form of inorganic N, it is crucial to implement suitable management practices that reduce soil NO_3^- concentrations while maintaining productivity to mitigate environmental risks.

In recent years, innovative N fertilizers, such as slow/controlled-release fertilizers, nitrification inhibitors, and urease inhibitors, have gained increasing popularity. These fertilizers can slow or control N release in the soils, thereby better aligning N availability with crop demands while reducing nitrification rates, ammonia (NH_3) volatilization, N_2O emissions, and NO_3^- concentrations in runoffs and leachates [11,12]. However, these fertilizers are 2 to 5 times more expensive than the traditional chemical N fertilizers, which limits their broader application. Additionally, although applying nitrification inhibitors to slow down the autotrophic nitrification of NH_4^+ is a conventional method to reduce NO_3^- concentrations in agricultural soils, in alkaline soils, extending the retention time of NH_4^+ can result in increased NH_3 volatilization [13], contamination from nitrification inhibitors, and potential adverse effects on crop quality [14].

In the soil microbial N cycle, the primary consumption ways of NO_3^- are through denitrification, dissimilatory reduction, and immobilization. Denitrification and dissimilatory reduction require specific anaerobic conditions and result in the production of intermediate compounds such as N_2O and NO, which contribute to N loss from agricultural soils and have detrimental effects on atmospheric quality [15]. Microbial immobilization of NO_3^-, however, is both environmentally friendly and beneficial for maintaining soil N levels [16]. It involves soil microbes using NO_3^- as a N source and transforming it into microbial biomass [17,18], thereby reducing NO_3^- accumulation in the soil. NO_3^- immobilized by soil microorganisms can be mineralized and released as inorganic N for use by the current or subsequent crop, or it may enter the stable organic N pool in the soil for long-term storage [19]. This process not only helps to reduce NO_3^- losses through runoff, leaching, and denitrification but also maintains high levels of N in the soil, ensuring the sustainable management of NO_3^- [16,20–24]. Microbial NO_3^- immobilization has long been overlooked [25,26] due to the traditional view that microorganisms preferentially utilize NH_4^+ as a N source [27–29]. Nevertheless, the importance of microbes in utilizing NO_3^- has been demonstrated in natural ecosystems, and studies found that enhancing microbial NO_3^- immobilization is an efficient and promising way to increase the rate of NO_3^- consumption [16,30,31], thus lowering the risk of environmental pollution from NO_3^- related losses through runoff, leaching, and denitrification.

2. Research Methods for Microbial NO_3^- Immobilization Rates

Here, in this article, we discuss the methods that have been most commonly used and developed in recent years.

2.1. Soil Gross Microbial NO_3^- Immobilization Rates

The ^{15}N stable isotope technique is the only method capable of precisely quantifying the rate of NO_3^- immobilization by microbes (Table 1). The ^{15}N dilution method, proposed firstly by Kirkham and Bartholomew (1954) [32], was one of the traditional methods for determining microbial NO_3^- immobilization rates in soils. This method assumes that denitrification and dissimilatory NO_3^- reduction to ammonium (DNRA) are negligible under aerobic conditions [21,33], thereby allowing the total NO_3^- consumption rate to be directly interpreted as the gross microbial NO_3^- immobilization rate. Since NO_3^- loss through runoff and leaching are not considered in this method, the microbial NO_3^- immobilization rate obtained by this method may be overestimated. The microbial biomass ^{15}N recovery method is theoretically the most accurate, but the complete extraction of microbial biomass N during fumigation is difficult, and the conversion factors used for correction are inconsistent [34]. Therefore, many studies have suggested that the NO_3^- immobilization rates quantified by this method may lead to large discrepancies from actual results [35]. In the soil organic ^{15}N recovery method, the gross microbial NO_3^- immobilization rate is determined by the differences in ^{15}N recovered from the KCl-extracted residue soil during incubation after ^{15}N addition, divided by the total amount of labeled $^{15}NO_3^-$ added [36]. The advantage of this method lies in its direct consideration of changes in N pool content and the abundance before and after immobilization, thereby minimizing the calculation

errors caused by gaseous losses during denitrification or DNRA processes. Through experimental research, Chen et al. (2020) [37] confirmed the similarity and feasibility of these three methods for measuring the microbial immobilization rate of NO_3^- when applying recalcitrant plant residues into soils. However, when calculating the gross immobilization rate of NO_3^- by soil microorganisms with the addition of easily decomposable C sources, the results from the ^{15}N dilution method were significantly higher than those from the other two methods. Nevertheless, considering the convenience of data collection, the organic ^{15}N recovery method is more accurate and time-efficient.

Table 1. Comparison of methods for measuring the gross microbial NO_3^- immobilization rates.

Method	Principle	Calculation		Advantages/Disadvantages	References
		Formula	Explanation		
^{15}N Pool Dilution Method	After labeling the NO_3^- pool with ^{15}N, as other unlabeled N forms are converted into the labeled ^{15}N form, the ^{15}N abundance in this N pool decreases. The gross microbial NO_3^- immobilization rate is then determined by analyzing the temporal changes in NO_3^- concentration and atomic percentage excess.	$F_i = \frac{[(Q_i - Q_{i+1}) \times \ln(A_i / A_{i+1})]}{[(t_{i+1} - t_i) \times \ln(Q_i / Q_{i+1})]}$; $I_{NO3} = F_i - \left[\frac{(Q_{i+1} - Q_i)}{(t_{i+1} - t_i)}\right]$	where Fi is the gross nitrification rate; *i* is the *i*th measurement; Q and A are the amount and ^{15}N atom% excess of NO_3^-, respectively.	Advantages: Simple experimental procedure and calculation method. Disadvantages: Be overestimated due to the lack of consideration for denitrification and DNRA.	[21,32,33]
Microbial Biomass ^{15}N Recovery Method	After the NO_3^- pool is labeled with ^{15}N, the ^{15}N concentration in microbial biomass increases as the labeled NO_3^- is immobilized by microorganisms.	$I_{NO3} = \frac{(N_{fum} Ae_{fum}) - (N_{nfum} Ae_{nfum})]}{Ae_{NO3}}$	where N_{fum} and N_{nfum} are the measured N amount in digested fumigated and non-fumigated samples, respectively; Ae_{fum} and Ae_{nfum} are ^{15}N atom% excess in extracts of fumigated and non-fumigated samples, respectively; Ae_{NO3} is average ^{15}N atom% excess of soil NO_3^- at the beginning and end of the incubation.	Advantages: Theoretically, this approach provides the most accurate results. Disadvantages: The experimental procedure is complex, making it challenging to obtain precise results.	[34,35]
Soil Organic ^{15}N Recovery Method	After labeling the NO_3^- pool with ^{15}N, the ^{15}N concentration in soil organic N increases as the labeled NO_3^- is immobilized by microorganisms.	Org $^{15}N_i$ = Org N_i × Ae_{OrgaNi}; $I_{NO3} = \frac{1}{d}\sum_{i=1}^{n} \frac{Org^{15}N_{i+1} - Org^{15}N_i}{\frac{Ae_{i+1} + Ae_i}{2}}$	where Org $^{15}N_i$ is the amount of ^{15}N in the KCl-extracted residue soil, d is the number of days of incubation, Org N_i is the measured amount of N in the washed soil residue, Ae_{OrgaNi} is the ^{15}N atom% excess in the washed soil residue, and Ae_i is the ^{15}N atom% excess of NO_3^-.	Advantages: Simple experimental procedure with minimal errors. Disadvantages: The incubation period should not be prolonged to avoid re-mineralization, which could increase the error.	[36,37]

2.2. NO_3^- Immobilization Rates by Fungi and Bacteria

Fungi and bacteria are two major microbial populations that utilize NO_3^- in soils [16,31,38,39]. However, studies distinguishing the respective contribution of fungi and bacteria to immobilize NO_3^- are relatively rare due to limitations in research techniques (Table 2), and thus, our understanding of the NO_3^- immobilization by fungi and bacteria largely remains at a qualitative stage [22,40]. The microbial selective inhibitor method has traditionally been used to differentiate the potential of fungi and bacteria in retaining NO_3^- [39]. For instance, Myrold et al. (2017) used protein synthesis inhibitors—such as chloramphenicol to inhibit bacteria and cycloheximide to inhibit fungi—and discovered that the ability of soil bacteria to assimilate NO_3^- was comparable to that of fungi [41]. However, due to the low specificity of selective inhibitors and the difficulty in controlling their dosages, the accuracy and reliability of the results obtained by this method are relatively low [42,43]. In recent years, Li et al. (2019) developed the "amino sugar stable isotope probing" (AS-SIP) technique to distinguish the contributions of soil fungi and bacteria to NO_3^- immobilization [16,38,42,44]. According to this method, the rate of accumulation of newly synthesized amino sugars derived from soil fungi (^{15}N-GluN) and bacteria (^{15}N-Mur), following short-term incubation with 15N-labeled NO_3^-, is regarded as proxies of their respective NO_3^- immobilization rate [31]. However, due to the difficulty in obtaining accurate turnover rates of different N-containing components in microbial cells, AS-SIP still cannot accurately obtain the accurate immobilization rates of NO_3^- by soil fungi and bacteria [44]. To address these issues, Li et al. (2021) proposed to integrate the gross microbial NO_3^- immobilization rate obtained from experiments with the synthesized rates of ^{15}N-GluN and ^{15}N-Mur [44]. By constructing a linear regression model and applying the least squares method, it is possible to back-calculate the conversion coefficients between the synthesized rates of ^{15}N-GluN and ^{15}N-Mur and their actual immobilization rates, ultimately estimating their individual NO_3^- immobilization rate [16,44]. Since this method does not require the additional use of fungal or bacterial selective inhibitors, it provides results that are closer to the actual situation [16,38,44]. However, the method assumes that the utilization of NO_3^- by other groups, such as archaea, can be ignored, leading to potential discrepancies between the measured rates of fungal and bacterial NO_3^- immobilization and the actual situation. Additionally, this method cannot distinguish the specific microbial species involved in NO_3^- immobilization. Although the ^{15}N-DNA-SIP technique has been applied to identify specific microbial groups involved in NO_3^- immobilization [45], NO_3^- immobilization rates by specific microbial groups cannot be quantified by this method. Therefore, future research should focus on how to quantify the NO_3^- immobilization rates of different microbial communities.

Table 2. Comparison of methods for measuring fungal and bacterial NO_3^- immobilization rates.

Method	Principle	Calculation		Advantages/Disadvantages	References
		Formula	Explanation		
Microbial Selective Inhibition	Through the manipulation of adverse culture conditions or the addition of specific agents, the growth of non-target microorganisms in the sample can be suppressed, thereby promoting the preferential growth of target microorganisms.			Advantages: It enables the quantification of NO_3^- immobilization rates for fungal or bacterial communities. Disadvantages: The low specificity of selective inhibitors and the difficulty in controlling their dosage may compromise the accuracy and reliability of the outcomes.	[39,41]
^{15}N-AS-SIP	Soil amino sugars, serving as markers of microbial residues, are characterized by their stability and origin specificity. Glucosamine primarily originates from fungi, while muramic acid is exclusively derived from bacteria. Based on this, the synthesis rate of ^{15}N-labeled amino sugars specific to fungi and bacteria during short-term incubation can be used to indicate the NO_3^- immobilization rates of these microorganisms.	APE = (Re − Rc)/[1 + (Re − Rc)] × 100; CL = CT × APE/100	where Re is the isotope ratio of the incubated samples, Re = [A(F + 1)/A(F)]; Rc is the corresponding ratio obtained from the original soil; CL is the content of the ^{15}N-labeled portion of GluN and MurN; CT is the concentration of each amino sugar.	Advantages: It allows for the quantification of NO_3^- immobilization rates by fungal or bacterial communities. Disadvantages: It neglects the potential contribution of other microbial groups, like archaea, to NO_3^- immobilization, resulting in potential inaccuracies in the findings.	[16,38,44,46]
^{15}N-DNA-SIP	By employing ^{15}N stable isotopes to trace the microorganisms participating in NO_3^- immobilization, the immobilization process is directly linked to specific microbial species, thereby identifying the specific functional microorganisms that are directly involved in soil NO_3^- immobilization.			Advantages: It enables the direct identification of specific microbial species that participate in NO_3^- immobilization. Disadvantages: It is unable to provide a quantitative measurement of the NO_3^- immobilization rate.	[45]

3. Factors Influencing Microbial Immobilization of NO_3^-

The immobilization of NO_3^- is driven by microbial processes, so any factors that affect microbial activity will consequently influence soil microbial NO_3^- immobilization. Key factors that affect NO_3^- immobilization in agricultural soils include soil microbial communities, the availabilities of N and soil organic C, the ratio of C to N, soil pH, and temperature.

3.1. Soil Microbes

Current studies to disentangle NO_3^- immobilization by different functional groups are still at a qualitative stage. For example, *Bacillus*, *Burkholderia–Caballeronia–Paraburkholderia,* and Micrococcaceae are found to be primary bacteria governing NO_3^- utilization, and *Penicillium*, *Chaetomium*, *Aspergillus*, and *Fusarium* were dominant fungi to immobilize NO_3^- [45]. While NO_3^- immobilization rates by fungal and bacterial groups can be quantified. As the two major microbial groups in soil, fungi and bacteria have been shown to play vital roles in utilizing soil NO_3^- [16,31,38,39]. However, their relative contributions to NO_3^- immobilization are distinct [16,39,43], owing to their differences in physiology, morphology, survival strategies, and abundance [40,47–50]. Studies found that the biomass and species composition of soil fungi and bacteria significantly affect the microbial immobilization of NO_3^- [16,38,51]. Although the immobilization rates of NO_3^- by fungi and bacteria are positively correlated with their respective PLFA biomass [16,38], their contributions to NO_3^- immobilization vary across different environments. Compared to bacteria, fungi are more favored to grow in undisturbed or lightly disturbed forest and grassland soils due to their hyphal networks [52], and their ability to degrade lignin enables them to degrade complex organic compounds, such as leaf litter [53,54]. Furthermore, due to their higher C/N ratio, fungi tend to prioritize the decomposition of organic materials with a high C/N ratio to sustain the inner stoichiometric balance [55,56]. Thus, some studies suggested that fungi may be the dominant microbial community that utilizes NO_3^- in natural ecosystems [16,31]. In agricultural soils, the contributions of fungi and bacteria to NO_3^- immobilization are inconsistent. Agricultural soils typically have lower organic C content than forest soils, which reduces the availability of C and energy sources for both fungi and bacteria, thus leading to lower microbial biomass, activity, and NO_3^- immobilization capacity [16]. Agricultural practices, such as tillage and fertilization, can disrupt fungal growth [57,58], impairing their ability to utilize NO_3^- [31]. Wang et al. (2024) demonstrated that the immobilization of NO_3^- in calcareous cropland soils is predominantly driven by bacteria rather than fungi, and this might be related to the higher bacterial biomass [59]. There are also studies reporting that fungi are the dominant microbes that immobilize NO_3^- in agricultural soils [31,52,60,61], and this might be due to fungi's preference for acidic environments [22,52]. These findings indicate that improving soil conditions, particularly for fungi, could enhance NO_3^- immobilization and N retention in agricultural soils [31,62].

3.2. Soil N Availability

The effect of soil N availability on microbial NO_3^- immobilization is contingent upon whether microbial growth is constrained by N [63]. When microbial activity is limited by N, the soil microbial immobilization rates of NO_3^- increase with the availability of N. While it decreases with the increase in N availability when the limiting factor shifts from N to C [64]. Excessive N in the soil can reduce the ratios of fungal to bacterial biomass and activity, inhibit the activity of β-glucosidase and phenol oxidase, and enhance non-biological NO_3^- retention while weakening microbial retention of NO_3^- [65]. Additionally, the forms of soil N affect microbial NO_3^- immobilization. Generally, it is assumed that the presence of NH_4^+ in the soil can inhibit the uptake of NO_3^- into microbial cells [66] and suppress the synthesis or activity of NO_3^- reductase and assimilatory enzymes [67–70], due to the fact that microbial immobilization of NO_3^- requires more energy compared to NH_4^+ [28]. Research suggests that soil microorganisms begin to utilize NO_3^- only when

the concentration of NH_4^+ falls beneath 2 mg N/kg [68]. However, due to the spatial heterogeneity of in-situ agricultural soils, microenvironments with low or near-zero NH_4^+ concentrations exist. Furthermore, N fertilizers applied in practice are often rapidly nitrified, converting NH_4^+ to NO_3^- [7], resulting in NO_3^- concentrations frequently exceeding those of NH_4^+. Moreover, nitrifying bacteria and heterotrophic bacteria strongly compete for NH_4^+. Therefore, when NH_4^+ levels are insufficient to meet microbial N requirements, microorganisms shift to utilizing NO_3^- [51].

3.3. Soil Organic C Availability

The C and N cycles in soils are intricately interconnected [71,72]. Consequently, the capacity of soil microbes to immobilize NO_3^- is influenced by the availability of soil organic C, which serves as energy and electron acceptors for heterotrophic microorganisms. In natural ecosystems, soil C content is abundant, and microbial immobilization of NO_3^- is strong and has been identified as a crucial mechanism for N retention [73]. However, in agricultural ecosystems, long-term and frequent human activities have altered soil physicochemical properties, which significantly affected the N cycle and disrupted N retention mechanisms [22]. For example, in humid forest soils of southern China, 90% of NO_3^- produced by microbes can be immobilized by microbes, whereas this percentage drops to 10% in agricultural soils [52]. This is because the low organic C content in agricultural soils limits microbial utilization of NO_3^- [16,22,74]. Therefore, adding organic matter along with N fertilizers to agricultural soils holds significant potential for enhancing microbial NO_3^- retention capacities [22,30,36,37]. Therefore, some studies have suggested that soil microbial utilization capacities of NO_3^- are positively correlated with the amount of available C added to the soil in agricultural systems [16,59,74]. Nevertheless, Cheng et al. (2017) through a meta-analysis, found that microbial NO_3^- immobilization only increased significantly when easily decomposable C sources exceeded 500 mg C/kg in agricultural soils [74]. This may be because when the added C content is low, microbial communities prioritize C use for basic metabolic processes [75]. However, not all exogenous C added to the soil can promote microbial NO_3^- utilization, as the quality of the added C can also regulate microbial NO_3^- immobilization [74,76]. Some studies have demonstrated that easily decomposable organic C in the soil can meet the energy and metabolic needs of microbes, thereby stimulating NO_3^- uptake [77]. Moreover, the lignin content and lignin/total N ratio in organic C can affect its decomposition characteristics. Chen et al. (2022) [78] reported a logarithmic increase in microbial NO_3^- immobilization rates with increasing cellulose and hemicellulose content in exogenous C. These findings suggest that adding sufficient quantities of easily decomposable or complex organic C sources to agricultural soils can promote microbial NO_3^- retention. Additionally, it is noteworthy that recalcitrant organic C, such as phenolic compounds, can promote the abiotic immobilization of NO_3^- [77].

Adding organic C to agricultural soils can enhance NO_3^- utilization by both fungi and bacteria [16], with the extent of this enhancement closely associated with the quality of the organic C [38]. Generally, the addition of easily decomposable C sources to soil promotes bacterial proliferation more than fungal growth [79], potentially enhancing bacterial NO_3^- utilization more than fungal. Conversely, adding recalcitrant C sources to soil promotes fungal growth, but the small molecules released by fungi during the degradation of recalcitrant C can also be utilized by bacteria, potentially enhancing bacterial NO_3^- immobilization [80]. Li et al. (2020) found in laboratory incubation experiments that increasing the addition of plant residues (C/N = 25.2) significantly enhanced NO_3^- immobilization by both fungi and bacteria, with a more pronounced effect on fungal NO_3^- immobilization [38]. However, there is still no consensus on how organic C addition affects the relative roles of fungi and bacteria to assimilate NO_3^-, which may be attributed to the complexity of microbial community composition. Exogenous C inputs may influence microbial NO_3^- immobilization by altering microbial community structure and metabolism [16,51]. Therefore, future

studies are necessary to identify the particular fungal or bacterial species that play key roles in soil NO_3^- immobilization under exogenous C addition.

3.4. The Ratio of Soil C to N

The ratio of C/N in soils is a critical indicator of soil organic C quality, which can reflect the availability of C and N for microbial use, and is therefore often positively correlated with microbial NO_3^- immobilization [81]. A high C/N ratio (indicating more available C) encourages microbial immobilization of NO_3^-, as microbes will assimilate more N (including NO_3^-) to balance their nutrient intake for growth. As a result, microbes tend to immobilize more NO_3^- when degrading organic C with a higher C/N ratio [82]. Cheng et al. (2017), through a meta-analysis, also found that the addition of complex C sources with a C/N ratio greater than 18 significantly promoted microbial NO_3^- immobilization in agricultural soils [74]. Additionally, as discussed before, fungi tend to utilize organic matter with a high C/N ratio [55,56]; thus, adding complex organic matter with a high C/N ratio into agricultural soils may significantly promote fungal NO_3^- immobilization capacity [16,38]. However, this is not always true. As Chen et al. (2024) revealed when the C/N ratio exceeds the critical threshold that controls whether microbial NO_3^- immobilization occurs, it may no longer serve as an accurate index for assessing the quality of organic material [83].

3.5. Soil pH

Soil pH plays a vital role in controlling soil microbial NO_3^- immobilization by influencing related microbial activity [31,84], enzyme activity [84], microbial diversity, and nutrient availability [85]. Soil pH can directly influence the heterotrophic microbial populations involved in NO_3^- immobilization via influencing related microbial activity and diversity. Fungi, as the important microbial groups in immobilizing NO_3^- in both natural ecosystems and agricultural ecosystems, exhibit a preference for acidic soils [52]. The activities of fungi and soil enzymes, such as β-*N*-acetylglucosaminidase, decrease with increasing soil pH [31,86]. Therefore, fungi are quite sensitive when soil pH changes [31,87,88]. Soil pH affects microbial preference for N [85]. Under acidic conditions, fungi are more likely to utilize NO_3^- [31]. Acidic conditions also facilitate the decomposition and mineralization of organic matter, thereby providing energy for microbial NO_3^- immobilization in the soil. While under neutral or slightly alkaline conditions, microbes preferentially utilize NH_4^+ [89]. Additionally, Elrys et al. (2022), through a meta-analysis, demonstrated that microbial NO_3^- immobilization rate was enhanced with increasing pH in humid subtropical soils that usually have a lower pH [90]. This effect is probably because an increase in soil pH enhances the population of ammonia-oxidizing bacteria, which in turn enhances microbial mineralization rates, thereby increasing the production of NH_4^+, the substrate for NO_3^- production [91,92]. They also reported that microbial NO_3^- immobilization rates were inhibited as pH increased in Mediterranean soils, which typically have high soil pH.

3.6. Temperature

The activities of microorganisms involved in N transformation are also affected by temperature [93,94]. As global warming increases the surface temperature, microbially mediated N cycling processes have been demonstrated to change a lot [95]. For example, increased temperature significantly stimulated soil N mineralization rate and nitrification rate [93], thereby increasing the substrate for microbes to utilize NO_3^-. In contrast, Dai et al. (2020) [95] found that elevated temperature shifts N cycling from microbial immobilization to enhanced mineralization, nitrification, and denitrification in terrestrial ecosystems. These results suggested that the effect of elevated temperature on soil microbial NO_3^- immobilization still needs further research.

4. Conclusions and Perspectives

Soil microbial NO_3^- immobilization is one of the key processes of the N cycle in agricultural soils, which plays a promising role in enhancing soil N retention and improving N

fertilizer use efficiency. Implementing N management strategies that enhance soil microbial NO_3^- immobilization is a crucial strategy for improving the soil's ability to retain N and minimizing NO_3^- losses from agricultural soils. Therefore, research focused on understanding and enhancing soil microbial NO_3^- -assimilation is of significant importance. In this review, we first discussed and compared the techniques to quantify soil gross microbial NO_3^- immobilization rate as well as fungal and bacterial NO_3^- immobilization rates, and then we summarized important factors that influence microbial NO_3^- immobilization. Specifically, we emphasized that the incorporation of organic materials is a promising management practice to promote soil microbial NO_3^- immobilization capacities in agricultural soils. For the better management of NO_3^- in agricultural soils, future research should focus on (1) The process of microbial immobilization of soil NO_3^- is largely carried out by a limited number of functional microorganisms. These microorganisms can be identified through ^{15}N-DNA-SIP technology, yet there remains an absence of techniques to quantitatively assess their NO_3^- assimilation rates. (2) How can the regulation of microbial immobilization of NO_3^- in soil be optimized to better match soil NO_3^- supply with crop demand, thereby improving nitrogen use efficiency and reducing nitrogen losses?

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