

Production, Leaching and Transport of Nitrate Nitrogen in Dryland Soils and Control Measures: Postprint

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Abstract

How to improve nitrogen fertilizer use efficiency, reduce nitrogen losses and their environmental impacts while ensuring high crop yields has become a worldwide research topic. Nitrate leaching is one of the important pathways of nitrogen loss in dryland soils, a major cause of regional groundwater nitrate pollution, and has become a significant agricultural and ecological environmental issue in intensively farmed regions globally. Clarifying the production and leaching processes of soil nitrate nitrogen and their influencing factors in different cropping systems, and further proposing control measures, holds important agronomic and environmental significance. In this regard, scholars both domestically and internationally have conducted long-term, extensive research and accumulated abundant data. This paper reviews the microbially driven processes of nitrification in dryland soils, compares the advantages and disadvantages of currently most commonly used in-situ field monitoring methods for nitrate leaching, analyzes various factors affecting nitrate accumulation and leaching, summarizes corresponding control measures, and provides perspectives for future research: strengthening research on key microbial processes, mechanisms of nitrification in dryland soils and the regulatory roles of related driving factors will help enhance understanding of soil nitrogen cycling, and such understanding constitutes an important foundation for nitrogen optimization management and the development of nitrate leaching control technologies in dryland cropping systems; continuing to seek and establish an in-situ field monitoring method with less disturbance, simple maintenance, lower sample contamination, and especially suitable for long-term monitoring is particularly important for accurately revealing the leaching patterns and real-time fluxes of nitrate nitrogen in dryland soils; systematically conducting comprehensive studies on nitrate leaching in different dryland cropping systems, clarifying the main factors affecting nitrate accumulation and leaching, and proposing targeted control measures are more

consistent with field realities and hold great significance for reducing regional nitrate leaching risks.

Full Text

Review of Soil Nitrate Formation, Leaching Transport, and Control Measures in Upland Farming Systems

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Abstract: Improving nitrogen use efficiency while reducing nitrogen losses and their environmental impacts without compromising crop yields has become a leading global research priority. Nitrate leaching represents a primary pathway of nitrogen loss from upland soils and a major cause of regional groundwater nitrate contamination, posing severe agricultural and ecological challenges in intensively farmed regions worldwide. Understanding nitrate formation, leaching processes, and influencing factors across different cropping systems is essential for developing effective control strategies. This paper reviews the microbially-driven nitrification processes in upland soils, compares the advantages and limitations of commonly used in-situ monitoring methods for nitrate leaching, analyzes factors affecting nitrate accumulation and leaching, and summarizes corresponding control measures. Future research should focus on: (1) elucidating key microbial processes, mechanisms, and regulatory factors of nitrification in upland soils to enhance understanding of soil nitrogen cycling—knowledge that forms the foundation for optimizing nitrogen management and developing nitrate leaching control technologies; (2) developing less disruptive, low-maintenance, minimally contaminating in-situ monitoring methods suitable for long-term studies to accurately characterize leaching patterns and fluxes; and (3) conducting comprehensive studies across diverse upland farming systems to identify key factors influencing nitrate accumulation and leaching and propose targeted control measures that reflect field realities, thereby reducing regional nitrate leaching risks.

Keywords: Upland soil; Nitrification; Nitrate leaching; In-situ sampling method; Influencing factors; Control measures

Nitrogen fertilizers have played a critical role in global crop production, but meeting growing food demands has come at a severe environmental cost [1-2]. Consequently, improving nitrogen use efficiency while reducing nitrogen losses and their environmental impacts—without sacrificing crop yields—has emerged

as a worldwide research priority attracting widespread attention [1,3].

After nitrogen fertilizer application, three primary fates await: crop uptake, soil retention, and loss (via ammonia volatilization, leaching, nitrification-denitrification, and runoff). These pathways are interconnected and influenced by numerous factors including crop characteristics, soil texture, agricultural practices, and meteorological conditions, forming the common basis for studying both agronomic and environmental effects of nitrogen fertilizers. As a major form of soil inorganic nitrogen and an important product of fertilizer transformation, nitrate-nitrogen levels reflect actual soil nitrogen supply capacity [4-5]. While ammonium-nitrogen carries a positive charge and is readily adsorbed by negatively charged soil colloids, nitrate-nitrogen is negatively charged and most susceptible to leaching. Upland soils are predominantly distributed in arid, semi-arid, or semi-humid regions where annual precipitation typically falls below evaporation. Early research suggested minimal nitrate leaching under such climatic conditions, but mounting evidence demonstrates that leaching intensity is primarily governed by the magnitude of individual or several rainfall and irrigation events when substantial nitrate accumulation occurs [4,6]. Therefore, nitrate leaching constitutes an important nitrogen loss pathway in upland soils [6-7], represents a significant cause of regional groundwater nitrate pollution [8-9], and has become a major agricultural and ecological problem in intensive farming regions globally [10-12]. Clarifying nitrate formation, leaching processes, and influencing factors across different cropping systems is crucial for developing control measures with important agronomic and environmental implications. Extensive long-term research by scholars worldwide has generated substantial knowledge in this field. This paper provides a comprehensive review of nitrification processes, in-situ monitoring methods, influencing factors, and control measures for nitrate leaching in upland soils.

1. Pathways of Soil Nitrate-Nitrogen Production

Aside from direct application of nitrate fertilizers, soil nitrate primarily originates from nitrification of fertilizer ammonium and soil ammonium, making nitrification and mineralization capacity critical for nitrate production and accumulation [13]. Nitrification is a vital process in soil nitrogen cycling that not only determines nitrogen availability but also relates to environmental issues including soil acidification, nitrate leaching, and nitrous oxide (N_2O) emissions [14]. The process is generally considered a two-step transformation of NH_4^+ to NO_2^- and subsequently to NO_3^- [15], with ammonia oxidation as the rate-limiting step driven by chemolithoautotrophic microorganisms. For decades, ammonia-oxidizing bacteria (AOB) were considered the primary drivers of soil ammonia oxidation. However, groundbreaking research over the past decade has challenged this conventional view [16-17]. The discovery of ammonia-oxidizing archaea (AOA) *amoA* genes and successful isolation of AOA strains with ammonia-oxidizing capacity suggest AOA may play important roles in soil nitrification. This finding has attracted widespread attention, rapidly becoming a hot topic

in soil nitrogen cycling research. Studies confirm that AOA are widely distributed across terrestrial ecosystems, including agricultural [18-19], forest [20], and grassland soils [18], often outnumbering AOB, which further suggests their potential importance in ammonia oxidation.

Nevertheless, research in high-nitrogen-input agricultural systems reveals that despite higher AOA amoA gene abundance, AOB dominate the ammonia oxidation process [21]. Similar conclusions from New Zealand grassland soils [22] confirm AOB's predominant role in nitrification. Thus, although AOA amoA abundance often exceeds AOB in most soils, this does not necessarily indicate greater functional significance, and AOA's ecological functions require further exploration and validation. Kinetic studies of the archaeon *Nitrosopumilus maritimus* demonstrate that AOA possess extremely high substrate affinity for ammonium, with minimum substrate concentrations for ammonia oxidation far lower than AOB [23], indicating competitive advantages in oligotrophic environments. Subsequent research has gradually confirmed AOA's ammonia-oxidizing function: in acidic red soils, AOA abundance and community composition respond more significantly to long-term fertilization than AOB, suggesting potentially greater contributions to nitrification in acidic soils [19]. Zhang et al. [24] verified that two AOA functional genes (amoA and hcd) assimilate ^{13}C -labeled CO_2 during autotrophic ammonia oxidation, providing direct evidence for AOA involvement in soil nitrification. Based on this principle, microcosm experiments further confirmed AOA's dominant role in ammonia oxidation in acidic soils [25]. Recently, an acidophilic archaeon (*Nitrosotalea devanaterra*) was successfully cultured from acidic soils, capable of growth and ammonia oxidation at pH 4.5 [26], providing important insights into AOA contributions to soil nitrification.

These findings suggest that the two ammonia-oxidizing microbial groups may occupy different ecological niches with distinct metabolic processes [27-28]. AOA likely adapt better to extreme oligotrophic environments (acidic, low-nitrogen), while AOB predominantly function in neutral to alkaline soils. However, despite revolutionary discoveries—including AOA amoA genes [16], successful AOA cultivation [26], and verification of AOA participation in nitrification [24]—our understanding of AOA contributions remains limited. Deepening our comprehension of AOA ecological functions and metabolic characteristics under in-situ conditions is scientifically crucial for advancing knowledge of soil nitrification.

2. Field In-Situ Research Methods for Nitrate Leaching

From a nitrogen availability perspective, nitrate accumulated below the root zone is largely inaccessible to crops [29]. In upland soils with deep water tables, capillary rise of nitrate is limited, and the lack of active carbon sources in deep soil layers rarely creates conditions suitable for denitrification, making microbial immobilization or denitrification of this nitrate unlikely [30]. The primary fate of this nitrate is continued downward movement. Consequently, nitrate movement out of the root zone is considered leaching loss. Nitrate movement in

soil is a chaotic, intermittent process rather than following conventional “piston flow” theory [31], complicating real-time monitoring and quantitative research. Current sampling methods each have advantages and limitations, and no universally applicable method has been identified [32]. This section reviews several widely used international methods for in-situ monitoring of soil nitrate leaching.

2.1 Soil Profile Sampling Method

The soil core sampling method involves using augers to collect stratified soil samples at specific depths and calculating nitrate accumulation from measured concentrations. This approach typically requires deep sampling using undisturbed soil augers to obtain intact cores. The method offers high convenience, and spatial variability can be addressed through multiple sampling points. However, due to strong temporal variability in nitrate accumulation, movement, and leaching, monitoring dynamic changes requires repeated sampling, creating substantial workload and causing permanent damage to experimental plots. Even after backfilling, preferential flow through created macropores remains unavoidable [33].

Soil profile sampling directly reveals nitrate accumulation and movement between soil layers [4]. The method can collect soil columns several meters or even tens of meters deep, effectively studying nitrate distribution, retention, accumulation, and migration in soil profiles [34-35]. In upland soils, nitrate leaching can be estimated from differences in nitrate accumulation below the root zone between fertilized and unfertilized plots based on crop root distribution and soil water movement patterns [5]. However, while this method directly monitors soil nitrate concentrations, it cannot quantify leaching flux without complementary water flux measurements using techniques like water balance calculations [36-37]. Results also differ substantially from those obtained by lysimeter and suction cup methods [36].

2.2 Soil Solution Extraction Method (Suction Cups)

The soil solution extraction method, commonly called suction cups, is currently the most widely used in-situ technique for monitoring nitrate leaching. The system typically consists of three components: a porous suction cup (made of ceramic, sintered glass, or stainless steel) [38], a vacuum pump, and a collection bottle. Installation requires burying the cup at a specific depth, often with mud injection to ensure close contact with soil. Soil solution is extracted under negative pressure when the cup’s matric potential falls below the soil’s matric potential. Compared to lysimeter methods, suction cups can extract soil solution under relatively dry conditions, collecting both gravitational and capillary water. However, because these water fractions have different residence times in soil, microbial processes like mineralization and nitrification significantly affect their chemical composition (e.g., nitrate and ammonium concentrations), representing a key reason for differences between suction cup and lysimeter methods [39].

Soil heterogeneity substantially affects solution collection. Soil type, required sample volume, and water content primarily determine sampling suction and duration [40], but spatiotemporal variability introduces uncertainty and practical difficulties. Continuous sampling responds more rapidly to events like rainfall or irrigation, exhibits lower solute adsorption to cups, and yields more representative samples than intermittent sampling, though maintenance and contamination issues persist [38]. The impact of suction cups on natural soil moisture conditions remains unclear [40]. Applied suction alters soil matric potential, distorting water flow fields and affecting sample collection. In natural soils, the collected sample volume and sampling area are temporally and spatially discontinuous [41], preventing quantitative measurement of solute flux [32]. Therefore, water flux must be simultaneously monitored and calculated, commonly using water balance methods [42] or modeling approaches [6,43].

2.3 Pan Lysimeter Method

The pan lysimeter method involves installing collection pans in soil to gather leachate over time, calculating nitrate leaching from measured volume and concentration [32]. This approach collects soil water moving downward under gravity and is widely used for in-situ soil solution monitoring [44-45]. However, installation is complex, particularly for large pans in undisturbed field soils [46]. Installation disrupts original soil pore structure, creating a soil-water-air interface (capillary barrier) above and below the pan where surface tension impedes water infiltration. Water only enters the pan when soil above the interface becomes saturated, potentially causing divergent flow that reduces collection efficiency [47]. Some studies have added divergent flow control walls to improve water collection efficiency [48].

Lysimeters (typically PVC or steel cylinders) are installed by pressing into soil, extracting intact cores, sealing the bottom with an outlet, and reburying [45]. Installation of undisturbed soil lysimeters is also complex (Fig. 1 [Figure 1: see original paper]), often requiring heavy machinery that substantially disturbs experimental areas [49]. The lysimeter wall must tightly contact surrounding soil while minimizing anthropogenic impacts on the microenvironment [50]. This method has long been plagued by preferential flow along sidewalls [46,51], and bottom saturation may cause denitrification, affecting nitrate concentrations [32,50].

Since upland soils rarely reach saturation, nitrate can still migrate slowly downward under gravitational and matric potentials under unsaturated conditions. Nitrate retained in pans may undergo transformations or upward migration. In upland soils, lysimeter methods generally yield underestimates of solute flux [32].

2.4 Other In-Situ Research Methods

The ion exchange resin method (resin bags) utilizes exchange properties of resins to absorb solutes like nitrate. Resin bags of known area are buried at specific depths, retrieved after a period, and extracted to determine total absorbed nitrate for calculating leaching flux. This simple, maintenance-free method is suitable for long-term monitoring and widely used in field studies [52-53]. However, limitations include: (1) quantifying solute flux only over time periods without monitoring concentration dynamics; (2) requiring repeated soil excavation that is labor-intensive and permanently damages plots; and (3) differences in moisture characteristics between resin bags and surrounding soil that affect natural flow fields and solute flux.

The capillary wick sampler method uses capillary action to collect and monitor soil solutes and fluxes, commonly employing materials like glass fiber or rock wool [47-48]. While collection efficiency is relatively high [47], some studies suggest potential overestimation of leaching flux [48]. Field installation is complex, causes substantial soil disturbance, and suffers from preferential flow issues. Additionally, bromide serves as an excellent tracer for solute transport studies due to low background levels, resistance to chemical/biological transformation, and similarity to NO_3^- as a monovalent anion. Many field experiments use bromide to study nitrate transport [6,31,36], and research shows consistent transport behavior between bromide and nitrate [6,54], enabling bromide as a tracer for in-situ nitrate migration studies.

3. Influencing Factors and Control Measures for Nitrate Leaching

Two fundamental conditions determine nitrate leaching [10]: nitrate accumulation in soil and water flux through the soil profile. Nitrate accumulation and leaching result from integrated processes of nitrogen transformation, crop uptake, soil retention, and loss, influenced by multiple factors including nitrogen application rate, fertilizer type, and application method [55-56]; irrigation amount and rainfall [6,42]; phosphorus/potassium and organic fertilizer application [57]; soil texture and moisture characteristics [10]; crop rotation systems [58]; and other management practices [59].

3.1 Nitrogen Application Rate: A Critical Factor for Accumulation and Leaching

Nitrate leaching involves mineralization of organic nitrogen, nitrification of soil or fertilizer ammonium, crop uptake, microbial immobilization, denitrification, and downward migration with percolating water [55,60] (Fig. 2 [Figure 2: see original paper]). Nitrogen application rate significantly affects nitrate accumulation and leaching [7,10]. Under insufficient nitrogen application, added nitrogen fails to meet crop demand and microbial immobilization, depleting soil nitrogen pools and maintaining low nitrate levels with negligible leaching. Under

balanced nitrogen application, supply and demand roughly equilibrate, maintaining appropriate nitrate levels without environmental problems. Excessive nitrogen application far exceeds crop uptake and soil retention capacities, creating large nitrate surpluses that can cause severe leaching losses under certain conditions [55]. Therefore, rational nitrogen rate and application method are key to ensuring crop yields while reducing nitrate accumulation and leaching [10,55].

High nitrogen loss rates and environmental pressure from agricultural production represent global challenges. In developed countries with abundant land, strategies to reduce nitrogen application through improved fertilization techniques or lower yield targets (e.g., organic agriculture) have been adopted to mitigate environmental pressures like nitrate leaching [49,56,61]. Extensive research has reached consensus that: (1) strictly controlling nitrogen rate to reduce inputs, avoid large nitrate accumulation, and decrease leaching risk represents an important development direction [3,10]; and (2) optimized nitrogen application technologies [62], mechanized fertilization [63], appropriate fertilizer types, novel fertilizers [64], and nitrification inhibitors [65] can effectively reduce nitrate leaching.

3.2 Water Supply: A Necessary Condition for Leaching

Percolating water plays a critical transport role in nitrate migration and leaching. Whether nitrate leaches from the root zone depends on soil moisture characteristics, irrigation amount, precipitation frequency, and intensity [66-67]. Water supply—particularly from single or several irrigation/rainfall events—is a necessary condition for nitrate leaching and a key factor determining regional leaching flux [6,68]. Under insufficient water, surplus nitrogen accumulates in the root zone; when water supply exceeds field capacity, accumulated nitrate leaches below the root zone toward groundwater. This process is influenced by soil texture and groundwater depth but primarily by irrigation and rainfall. Under saturated conditions, nitrate moves rapidly through soil profiles [69], with both leaching amount and migration depth increasing significantly with irrigation amount [6,70]. Notably, increased irrigation and rainfall raise soil water content, directly diluting nitrate concentrations in soil solution. However, soil moisture also affects nitrification and denitrification processes; increased moisture reduces aeration, potentially enhancing denitrification while weakening nitrification, thereby altering soil nitrate content [71].

Excessive water and nitrogen inputs are major causes of nitrate leaching in intensive agricultural regions [4,7]. After excessive nitrogen application, surplus nitrate accumulates at various soil profile depths with high leaching risk, while excessive irrigation and heavy rainfall drive nitrate migration to groundwater. Numerous studies confirm that optimized irrigation is crucial for reducing nitrate migration and leaching [6,42], including: (1) limited irrigation, particularly restricting single irrigation intensity, which plays an important role in controlling nitrate leaching [6]; and (2) optimized irrigation technologies such as real-

time soil moisture monitoring [62], drip and micro-sprinkler irrigation [72], and short-furrow irrigation [73]. These improvements meet crop water demand while greatly reducing irrigation amounts and nitrate leaching.

3.3 Soil Texture: Important Influence on Retention, Transport, and Leaching

Soil texture significantly affects nitrate retention, transport, and leaching, representing a major cause of differences among soil types [74]. Effects manifest in two aspects: (1) Different textures develop varying pore structures, affecting matric potential and water content, which influence nitrate concentration, retention time, and denitrification potential [10,75]. Research shows nitrate content follows the pattern: loam > sandy loam > loamy sand, increasing with silt content [76]. Fine-textured soils with well-developed medium-small pores exhibit greater suction at equivalent water content, enhancing nitrate retention. Although water and nitrate availability are lower than in coarse soils, retention is more persistent, facilitating nitrate accumulation. (2) Water characteristic parameters vary greatly among textures, affecting water movement and consequently nitrate transport and leaching. Water preferentially flows through large macropores [32], with greater transport depths in coarse-textured soils, making them more prone to water and nitrogen loss [77]. Increased clay content impedes nitrate migration. Soil bulk density and organic matter content also affect vertical transport, with lower bulk density and higher organic matter facilitating faster nitrate movement [69].

3.4 Organic Fertilizer Application: Controversial Effects on Leaching

The effect of organic fertilizer on nitrate leaching remains controversial. Some studies suggest organic fertilizer reduces leaching primarily through lower nitrogen inputs, with no reduction observed when organic nitrogen rates exceed chemical fertilizer levels [50,78-79]. However, other research [53] shows that at equivalent nitrogen rates, organic fertilizer reduces nitrate leaching to only 18-23% of chemical fertilizer levels, with similar results even at much higher organic nitrogen application rates [80]. Mechanisms for reduced leaching include: (1) Organic nitrogen as the main form in organic fertilizers requires gradual mineralization and nitrification, reducing leaching risk [53]; (2) Organic fertilizer increases high C/N ratio organic matter, stimulating microbial populations and communities that facilitate nitrate immobilization and potentially enhance denitrification losses [53]; and (3) Balanced nutrient composition in organic fertilizers significantly improves soil fertility, promoting crop growth and nitrogen uptake [82].

Combined organic-inorganic fertilization is widely recognized as an effective measure for ensuring high yields, improving nitrogen use efficiency, and reducing nitrate leaching [53]. Balanced application of phosphorus, potassium, and other nutrients further promotes plant uptake of accumulated nitrogen, reducing soil solution nitrate concentration and leaching risk [4,10,83].

3.5 Other Agronomic Measures: Optimization for Enhanced Nitrogen Uptake

Different crops vary substantially in nitrogen demand and uptake capacity, with crop configuration and rotation systems affecting nitrate accumulation and leaching [75,84]. Numerous studies confirm that intercropping, cover cropping, and catch crops effectively reduce nitrate leaching [84-85]. Primary mechanisms include: (1) Increased nitrogen uptake and water consumption by cover or intercropped crops reduce soil nitrate accumulation and water flux; and (2) Altered C/N ratios and inorganic nitrogen content affect mineralization and immobilization processes, influencing nitrogen utilization by subsequent crops. Unreasonable rotation systems constitute an important factor in nitrate leaching [4], with summer fallow significantly increasing leaching compared to wheat-maize rotations [86]. Rational rotation—including appropriate crop configuration (intercrops, cover crops, deep-rooted species) and minimizing fallow periods—represents an important leaching reduction measure [84].

Straw return is generally considered beneficial for reducing nitrate accumulation and leaching [75] through two mechanisms: (1) As an exogenous carbon source, straw increases soil C/N ratio and microbial activity, stimulating immobilization of inorganic nitrogen to organic forms and reducing net mineralization and leaching risk. However, long-term straw return may increase nitrogen mineralization and exacerbate leaching [10,87]. (2) Carbon regulates microbial denitrification, and straw return may increase denitrification losses [88-89], reducing leaching losses—a complex process influenced by multiple factors. Optimizing straw return practices to promote nitrate immobilization into soil organic nitrogen pools warrants further research in nitrate-accumulating upland soils.

Biochar amendment has also proven effective in reducing nitrate leaching. With well-developed pore structure, high specific surface area, and acidic/alkaline functional groups, biochar can directly adsorb soil nitrogen through physical and chemical processes, increasing nitrogen retention and reducing leaching risk [90-92]. However, mechanisms of nitrogen adsorption remain unclear, with substantial variation among biochars from different feedstocks [92]. Biochar also increases water-holding capacity, reducing water percolation [93], and may decrease organic matter decomposition and mineralization while reducing microbial activity [94] or inhibiting nitrification through ammonium adsorption [95], potentially lowering leaching risk. Conversely, some studies show biochar increases ammonia-oxidizer abundance and activity, altering community structure [96] and promoting nitrification rates [93,96], indicating substantial uncertainty in biochar's effects on nitrification that requires further investigation.

Tillage timing significantly affects nitrate accumulation and leaching, with avoiding early tillage during fallow periods in winter-rainfall regions helping reduce accumulation and leaching risk [97-98]. Tillage methods also influence leaching. Conventional tillage alters soil physical, chemical, and biological properties, promoting mineralization and potentially increasing nitrate concentration and

leaching flux compared to no-till [99-100]. However, other studies find no-till improves soil structure, increases organic matter and water storage, but also enhances earthworm populations. Earthworm activity increases macropores, leading to higher water flux and nitrate leaching [101].

Future Research Directions

Nitrate accumulation and leaching in upland soils are influenced by integrated anthropogenic and environmental factors. Given the severe hazards and remediation difficulties of groundwater nitrate contamination, with large-scale restoration technologies still lacking worldwide, source control of nitrate leaching is particularly crucial. This requires simultaneous efforts in technical research and policy development, with technical priorities including:

1. **Microbial Mechanisms of Nitrification:** While substantial research has examined metabolic mechanisms and contributions of AOB and AOA, considerable controversy remains. Elucidating key microbial processes, mechanisms, and regulatory factors across different soils will greatly enhance understanding and provide the foundation for optimizing nitrogen management and developing leaching control technologies. AOA are ubiquitous and abundant in terrestrial ecosystems, yet our understanding of their contributions remains limited. Although some AOA have been isolated from marine and hot spring environments, few have been cultured from soils, requiring strengthened cultivation efforts. Notably, physiological characteristics under pure culture likely differ substantially from natural conditions, necessitating validation of culture-based results. The relative contributions of AOB and AOA to nitrification remain a hot topic in soil nitrogen cycling ecology. Understanding AOA ecological functions and metabolic characteristics under in-situ conditions is scientifically important, and cross-application of in-situ analytical methods such as stable isotope probing, secondary ion mass spectrometry, and high-throughput sequencing may prove crucial for revealing AOA functions.
2. **In-Situ Sampling Methodology:** As a highly variable continuum in time and space, soil presents challenges for monitoring nitrate's chaotic movement. Current in-situ methods each have advantages and limitations, but none adequately address these challenges, and no universally applicable method exists. Experimental design must comprehensively consider objectives, requirements, and soil characteristics to select appropriate methods. Continued development of less disruptive, low-maintenance, minimally contaminating methods suitable for long-term monitoring is essential for accurately revealing leaching patterns. Soil heterogeneity and preferential flow substantially increase sample variability, making representative sampling difficult. Samples strictly represent only point-specific, instantaneous conditions, making it challenging to characterize field-scale or temporal variations and introducing calculation errors. Therefore, selecting appropriate methods, determining sampling numbers and distri-

butions, and integrating geostatistical methods or modeling technologies represent important research directions.

3. **Comprehensive System Studies:** Nitrate retention, accumulation, migration, and leaching result from integrated processes affected by numerous factors under field conditions. Most research has focused on single factors, with few comprehensive studies. Systematic research across different upland farming systems to identify key factors affecting regional nitrate accumulation and leaching and propose targeted control measures would better reflect field realities and significantly reduce regional leaching risks. This represents an important direction for regional agricultural non-point source pollution prevention. Numerous field experiments confirm that only with good crop growth can applied nitrogen be efficiently utilized; otherwise, severe losses occur. Optimization of integrated agronomic management is key to ensuring efficient nitrogen use and reducing accumulation and leaching risks, including optimized water-nitrogen management, rational rotation systems, improved tillage practices, straw management, and other agronomic measures. Only through simultaneous optimization of these factors can high yields, high nitrogen use efficiency, reduced nitrogen losses, and minimized environmental impacts be achieved.

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