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Review

A Review of Nitrogen Export and Its Eco-Environmental Significance in the Superficial Karst Desertification Zone

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Abstract: Epikarst is connected with a "binary" geological structure, and the hydrogeochemical processes are complex. Nutrients play an important role in the restoration of the surface ecosystem in the desertification area, which is prone to loss and leakage, and the transport and transformation of nitrogen is crucial to the growth and development of plants in the ecosystem and the safety of drinking water for the residents. In this study, we reviewed nitrogen research in the past 20 years in the "Web of Science (WOS)" and "China National Knowledge Infrastructure (CNKI)", and we reviewed nitrogen research in the following areas. From the results of the systematic review, (1) We found that nitrogen-related research literature has been growing over time, and the growth has been faster in the past five years, mainly in the fields of agriculture, public health, and environmental science; (2) In karst water systems, researchers are mostly concerned with the sources of nitrate, distribution characteristics, and pollution of karst water, and the dual isotope techniques of $\delta^{15}N$ and $\delta^{18}O$ are used to identify these; (3) In karst water systems, surface water bodies and underground rivers are the main objects of study, relatively few studies have been conducted on karst springs, and NO_3^--N, NO_2^--N, and NH_4^+-N are the main forms of nitrogen presence. The study of nitrogen in karst water systems ignores the unique subsurface leakage problem of karst areas, and the study of the relationship between nitrogen and nutrient leakage in karst water systems should be strengthened for karst desertification management and ecological restoration. This review may provide some insights for researchers working in related fields.

Keywords: rock desertification; superficial karst springs; nitrogen output; research progress

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1. Introduction

Epikarst is at the intersection of the lithosphere, atmosphere, biosphere, and hydrosphere, and all four circles can provide important karst dynamics. Therefore, the karst dynamic is strong, the dissolution and sedimentation chemistry is rapid, the carbon-water-calcium cycle is extremely active, and it is sensitive to environmental changes [1]. Karst itself has a unique "above-underground" binary three-dimensional structure geological background, the surface soil layer is thin and discontinuous, with the existence of a large number of bedrock fissures, making good above-underground connectivity and rapid changes in hydrological processes, but the spatial distribution of water and soil resources is not uniform [2,3]. Therefore, in karst areas where soluble rocks are more developed, there is not only surface loss at the surface, but also subsurface leakage, accordingly [4,5]. Nutrient leakage is part of the subsurface leakage in karst areas. Nutrient leakage degrades soils, decreases agricultural productivity, and enriches groundwater nutrients, to the extent that it causes a series of ecological and environmental problems, of which nitrogen pollution is one. With the growth of population and socio-economic development, the ecological environment of karst areas has been damaged, and rock desertification is the most serious ecological problem in karst areas [6]. Karst rock desertification refers to the manifestation form of soil erosion, large areas of bare bedrock, and land degradation caused by human disturbance in tropical and subtropical humid and subhumid climate conditions and under the natural background of karst development [7]. Even after the national "Ninth Five-Year Plan" to "Thirteenth Five-Year Plan" for the comprehensive management of karstic desertification, significant results have been achieved in the management of stone desertification, and there are numerous nutrient-related research results [8–10], but the problem of stone desertification is still serious, and the significance and impact of nutrient export from the epikarst on the desertification environment under different degrees of stone desertification is still unclear. However, the problem of karst desertification is still serious, and the pattern of subsurface nutrient leakage under different degrees of stone desertification is still unclear, and the significance and impact of nutrient export from the epikarst on the stone desertification environment still need to be further studied.

Karst desertification is a long-term problem that karst areas still need to face in the future, and it is also a problem that needs to be solved urgently for the sustainable development of the region. Exposed bedrock, sparse vegetation, thin soil layer, and scattered distribution are the characteristics of karst desertification. The stronger the degree of karst desertification, the poorer the water retention capacity of the surface, and the greater the nutrient loss (leakage), accompanied by water and soil loss (leakage) [11,12]. Due to the complexity of the karst subsurface structure and the inability to "generalize" it, groundwater systems in epikarst may have different sensitivities to different degrees of karst desertification. The surface karst springs are the direct presentation of the groundwater system in epikarst, an important node of the groundwater nitrogen cycle in the karst region, and the output end element of the karst groundwater nutrient leakage [8,13]. The nitrogen output of karst springs directly reflects the nitrogen pollution status in the karst groundwater system, and also supports the reflection of the environmental condition of the local surface and the environmental impact situation generated by human agricultural activities. By revealing the seasonal variation characteristics of nitrogen output from superficial karst springs in areas with different degrees of stone desertification, we can provide some scientific basis and theoretical support for studying the characteristics of subsurface nutrient leakage in karst areas with different degrees of stone desertification.

The karst region in southwest China has sufficient rainfall to provide sufficient dynamic conditions for soil loss (leakage), which, together with the thin soil layer, degradation of overlying vegetation, and unreasonable human production and living activities interference, makes the region enter into a vicious cycle of rock desertification, and the sustainable development of local economy and society is seriously restricted. Due to the existence of the stone desertification problem, the rocks are highly permeable, and surface soil nutrients (such as organic matter and nitrogen in the soil) are very easily lost, and rainfall erosion accelerates the loss of surface soil nutrients (leakage), which restricts the growth and development of vegetation [6,12]. Nitrogen, as the basic element of biological processes, is used in plants to produce chlorophyll molecules for photosynthesis and plant growth, providing necessary nutrition for the growth of vegetation, and is one of the important links of the nitrogen spatial cycle [14]. Therefore, strengthening the study of nitrogen in superficial karst springs in stone desertification areas can help advance the development of the theory of nitrogen cycles in groundwater systems in epikarst and reveal the process mechanism of nutrient leakage to the subsurface of stone desertification areas. Some of the soil nutrients are absorbed and used by the sparse vegetation, some are lost on the surface with rainfall erosion, and some are leaked into the groundwater system, due to the existence of bedrock fissures, pipes, and water fall holes, etc. In the long run, the thin and scattered surface soil layer becomes the main feature of the soil in rock desertification areas, which leads to the weak land productivity and restricted agricultural development and greatly affects the surface ecosystem. This study reviews the current status and main progress of nitrogen research in karst groundwater systems and provides research data to support the study of nitrogen transport and transformation in karst groundwater systems.

2. Materials and Methods

The literature search was conducted on the basis of the China Knowledge Network (CNKI) database (https://www.cnki.net/, accessed on 11 March 2023) and the Web of Science (WOS) core database (https://www.webofscience.com/, accessed on 11 March 2023). Firstly, we entered "nitrogen, nitrate" as the search terms in the search item "subject", and used the key words "karst, groundwater" to restrict the search in the database of the China Knowledge Network. The search deadline was March 2023; the search time range was the maximum time range of the China Knowledge Network; the literature range was all journal databases. Then, in the Web of Science core database, we entered "nitrogen, nitrate" in the search item "subject" for the first search, and used the terms "groundwater, karst" to restrict the search. The results were screened and selected directly by the search terms. The deadline for the search was March 2023. Finally, the above-mentioned Chinese and English literature were manually screened and selected, according to the purpose and content of the study on "nitrogen export". Through the above search and screening, a total of 177 papers were obtained: 96 Chinese journals, 1 conference paper (domestic conference), 22 master's and 4 doctoral theses, respectively; 81 English journals.

3. Results

3.1. Chronological Distribution of the Literature

An analysis of the statistical literature shows that studies on nitrogen in karst regions have an early origin and that China conducted research in this area before other countries. The analysis of Figure 1 shows that the period 2003–2023 can be roughly divided into three phases. In the first stage, 2003–2010, during which there were sporadic articles published in China and no literature on nitrogen in karst springs in other countries, the total number of literature did not exceed 15 articles, and the number of literature increased by no more than 3 articles per year in the initial stage; in the second stage, 2011–2018, the number of literature in China showed an up-and-down trend, and the number of literature in other countries was small; in general, the growth period was still slow; in the third stage, 2019–2023, the number of published literature in China began to enter a surge, showing a rapid growth trend, and the number of literature in other countries was also in the growth state; the number of average annual literature is more than 20, and the content of the study gradually increases in depth.

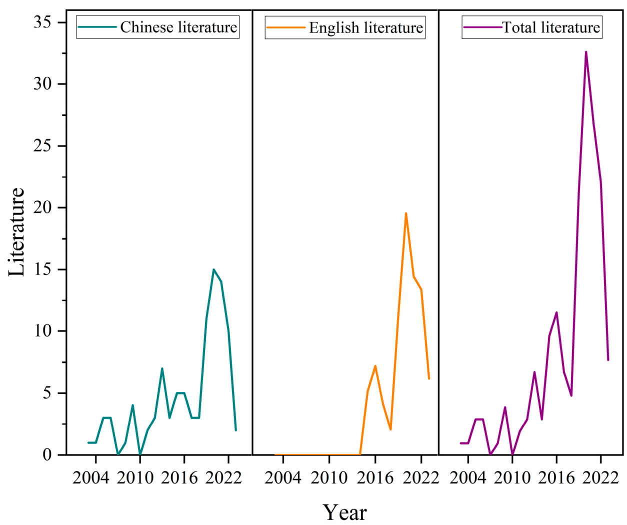

Figure 1. Annual distribution of literature research.

3.2. Literature Type Distribution

The types of studies of all the literature were classified and summarized according to the study of chemical characteristics of karst spring water, characteristics of nitrogen output variation, nitrogen export estimation, significance, and impact of nitrogen export in the environment, as well as nitrogen transport variation (Figure 2). Among them, the number of literatures about hydrochemistry studies of surface karst water bodies accounted for 5.6% of the total literature, the literature about nitrogen migration in karst water accounted for 29.9%, the literature about nitrogen export variation characteristics accounted for 12.4%, the literature about nitrogen export estimation accounted for 7.9%, and the nitrogen export and environmental significance accounted for 44.1% of the related literature. Among them, the studies related to the environmental significance and impact of nitrogen output dominate. In recent years, many scholars have begun to study the nutrient transport of karst springs in depth to study the influence mechanism, focusing on the characteristics of nutrient loss and flux estimation of karst springs under the combined effect of rainfall, soil, vegetation, and topography, and combining the more mature $\delta^{15}N$ and $\delta^{18}O$ isotope techniques to identify nitrogen sources in karst water, so as to reveal the influence mechanism and environmental significance of karst nitrogen export.

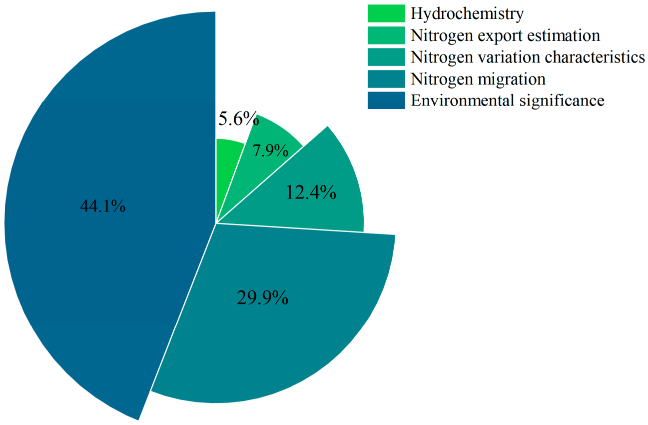

Figure 2. Classification of karst spring literature types.

3.3. Regional Distribution of the Literature

The studies related to karst water bodies showed obvious geographical characteristics (Figure 3). Among all the 177 reviewed papers, North America and Europe have more studies on karst spring nitrogen in the United States, Ireland, and Germany, among which the United States has the highest number of papers, up to 19, accounting for 47.5% of the total literature, followed by Mexico, Canada, Austria, and other regions with less than 5 papers; the number of papers in the three countries accounts for 15% of the total literature. There are more studies on karst water in southwest China, and the number of articles in Guizhou, Guangxi, and Chongqing are more than 20. The most articles in Guizhou are 27, while there are relatively few studies in other provinces. After the above data analysis, it was found that the nitrogen research of karst water has obvious coupling with developed agricultural regions, and the existing research in other regions has some reference significance for the research of karst spring and their nutrient migration.

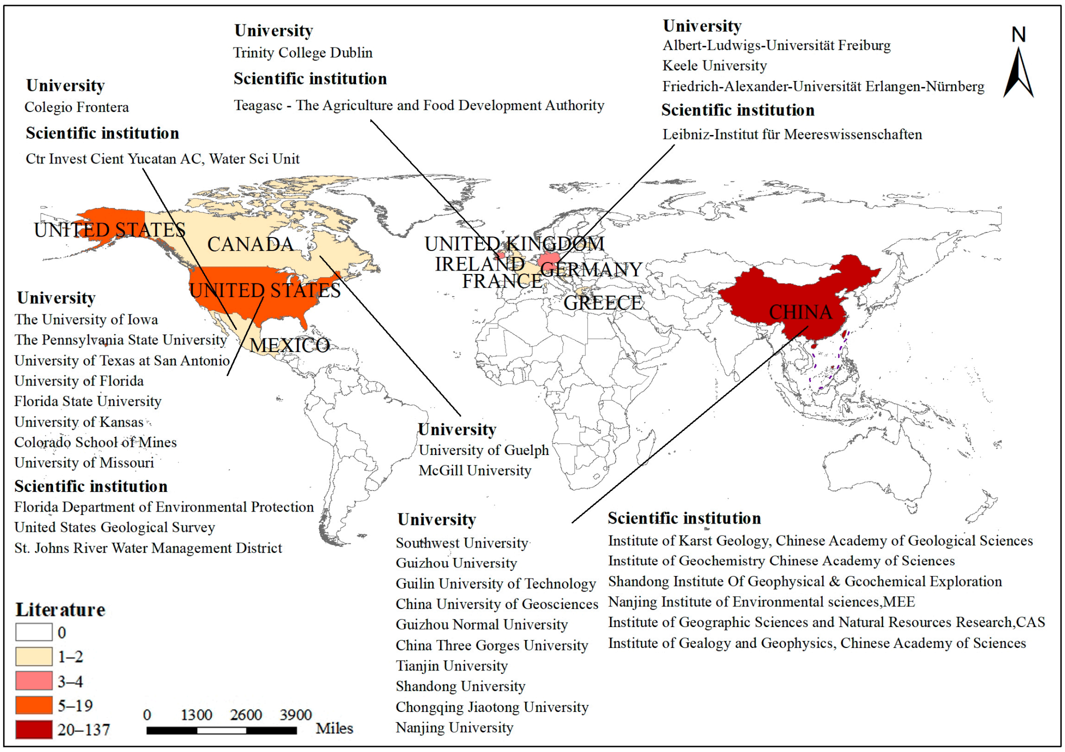

Figure 3. Regional and institutional distribution of research on karst water (due to space limitations, only countries and research institutions with more than two articles, and some with one article, in the literature are marked in the figure).

3.4. Unit Distribution of the Literature

Among the 177 Chinese and foreign literature retrieved, in China, the research literature on nitrogen in karst water bodies was mainly generated by universities and research units with strong karst fields, such as the Institute of Karst Geology, Chinese Academy of Geological Sciences, Southwest University, Guizhou University, Guilin University of Technology, Guizhou Normal University, China University of Geosciences, and Institute of Geochemistry Chinese Academy of Sciences, with 7 units with more than 3 publications, and 2 only from the General Administration of Geology and Mines of China, and only one unit including 26 units, such as Guangxi Zhuang Autonomous Region General Station of Geological and Environmental Monitoring, Guangzhou Institute of Water Science, Guizhou Institute of Engineering and Applied Technology, and Guizhou University of Nationalities. Among the 81 English literatures retrieved, there were 29 institutions, and the top four units were the University of Florida, Florida State University, Trinity College Dublin, Ireland, and the U.S. Geological Survey (3 articles each). The remaining 25 institutions published 2 or fewer papers (Figure 4).

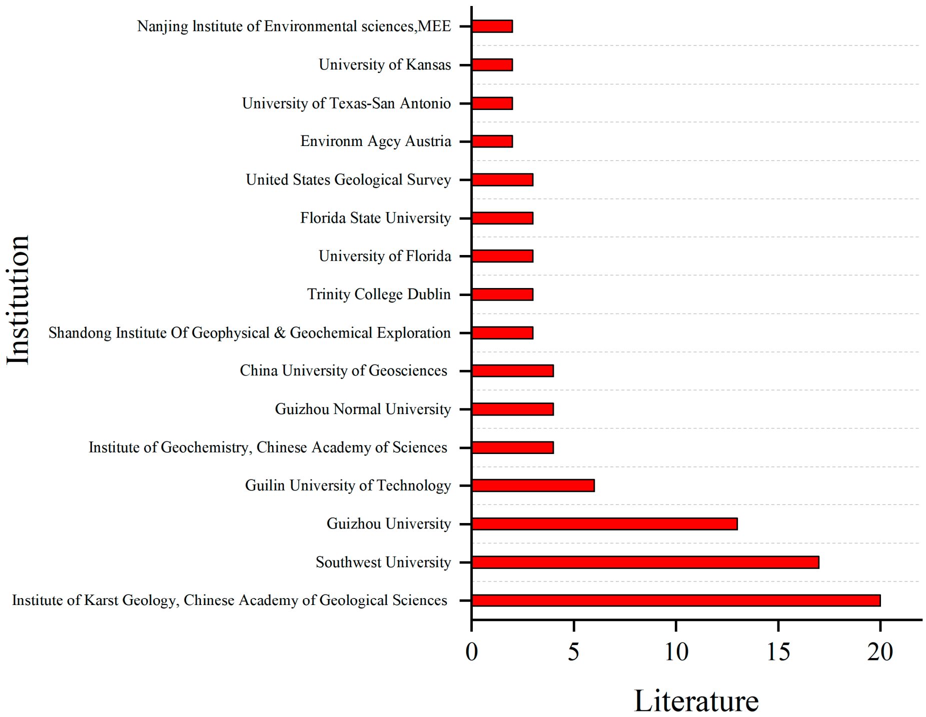

Figure 4. Distribution of research institutions of foreign and Chinese literature.

3.5. Research Phase Division

According to the annual distribution of research literature, it can be seen (Figure 1) that the study of nitrogen in karst waters began at the beginning of the 21st century and has been developing for nearly 20 years. Combining the background of research on the changing stages of hydrochemistry influencing factors and nitrogen pollution in springs in the epikarst, groundwater, and spring in the epikarst during this period, the study of nitrogen export from karst spring was classified into three stages, i.e., the starting period, the slow development period, and the rapid growth period (Table 1).

Table 1. Division of karst spring research stages.

Research Phase	Related Progress	Research Background
Start-up period (2003–2010)	Few relevant literatures were found, there were gaps in several years, and the research content was mainly focused on groundwater flow characteristics, with less research on isotope-tracing nitrogen sources, which was at the preliminary exploration stage.	With the change of karst subsurface hydrological process as the research background, the study of nitrogen loss characteristics of karst groundwater is still in the initial stage.
Slow development period (2011–2018)	The number of relevant journal publications increased, but the growth rate was slow, and the maximum number of articles per year was not more than 10. The research content began to initially involve nitrogen pollution and hydrochemistry of rock karst groundwater, and the isotope research gradually deepened.	As research has progressed and researchers have paid more attention and importance to the study of karstic groundwater, many researchers have conducted water quality monitoring and hydrochemistry studies, and use isotopes to use sources of contamination.
Rapid Growth Period (2019–2023)	Studies on hydrochemistry and solute (nutrient) transport in karst springs, the role of material cycling, as well as nitrogen flux estimation in karst groundwater and nutrient loss have increased and are growing rapidly. Research methods are gradually being quantified and scientificized.	The role of karst groundwater in the hydrological process of watershed is getting more and more attention, and it is also one of the important water sources in agriculture, tourism, and life, and the study of nutrient transport in karst groundwater is urgent.

4. Research Progress and Landmark Results

4.1. Nitrogen Cycle and Karst Desertification

In karst ecosystems, nitrogen cycling and stone desertification are related, and nitrogen transformation processes, such as nitrification and denitrification, are the basic nitrogen cycling processes, and nitrogen plays an important role in the growth and development of vegetation, while different levels of karst desertification lead to different rates of nutrient leakage, which restrict the development of vegetation in karst stone desertification areas. The excessive nitrogen input and loss may further increase the vulnerability of stone desertification ecosystems.

Nitrogen (N) is an important element in biogeochemical cycles and plays a key role in regulating ecosystem structure and function [15–17]. The nitrogen cycle refers to the process of nitrogen cycling in nature, which is the basic material cycle of the biosphere in the ecosystem. Zhao et al. (2021) found, in the research progress of soil nitrogen cycle in grassland from 2010 to 2020, that the current domestic and international research in nitrogen cycle is gradually increasing, and concluded that the nitrogen cycle is the most basic cyclic process in the grassland ecosystem, and biological nitrogen fixation, mineralization, nitrification, and denitrification are its main cyclic processes [18]. Martin et al. (2019) proposed a global nitrogen cycle model including nitrate (NO_3^-) to study the transformation between different forms of N in oceanic anoxic zones (ODZs). They assessed the contribution of denitrification and anaerobic ammonia oxidation to N loss, and concluded that low N availability limits carbon uptake in the surface ocean [19]. Xu et al. (2016) conducted a study that showed that climate change may significantly alter the nitrogen balance and cycle connecting the geosphere, biosphere, and atmosphere, and create considerable challenges and raise new issues that threaten environmental security [20]. Bechmann et al. (2014) also argue that anthropogenic acidification, caused mainly by nitrogen emissions, leads to an abnormal balance of natural biogeochemical cycles in terrestrial ecosystems and will lead to serious ecological management and agricultural sustainability problems, while excess nitrogen inputs to land are another major cause of global water and soil pollution, further increasing ecosystem vulnerability, including biological vulnerability, the rapid loss of biodiversity, and severe degradation of ecosystem functions [21,22].

The attention to nitrogen in karst areas is also not low, with a relative concentration of studies mainly on soils and vegetation. Zhang et al. (2018) studied vegetation communities in three typical stages of karst crest depressions in northwestern Guizhou by using spatial instead of temporal methods, and found that there were significant differences in soil nitrification and denitrification potentials among different vegetation communities, and that organic carbon, nitrate-nitrogen content, and soil microbial biomass nitrogen were the key common factors affecting soil nitrification and denitrification potentials in karst crest depressions [23]. Zeng (2018) combined isotope techniques and modeling tools to discriminate the main sources of substances and their contributions in the precipitation of the Houzhai River karst agricultural sub-basin in Puding County, Guizhou Province, analyzed the spatial and temporal differences in wet deposition of nitrogen, and estimated the source contribution of wet deposition of nitrate and the amount of nitrogen deposition [24]. Ren (2019) investigated the transformation process and spatial and temporal distribution characteristics of nitrogen at the soil-water interface in a karst watershed, as well as the spatial and temporal distribution of nitrogen and loss during the drenching process under simulated rainfall conditions. They did this by designing a soil column experiment with fertilization process and simulated rainfall, using nitrogen and oxygen double isotopes ($\delta^{15}N$ and $\delta^{18}O$), and comparing the loss of different fertilizers (urea and manure) in the soil [25]. Musgrove (2016) investigated the Edwards Karst Aquifer in central Texas and concluded that long-term increases in NO_3^- concentrations cannot be attributed to hydrologic conditions, and that land application of septic tanks and treated wastewater may be a source of increased NO_3^- loading, results that highlight the vulnerability of the Karst Aquifer to NO_3^- [26].

Organic carbon and nitrogen, as the energy source and main nutrient elements of soil microorganisms, drive the transformation and circulation of nutrients in soil and influence the physicochemical properties and productivity of soil [27]. In karstic stone desert areas, land use has a significant effect on the N conversion rate. Soil texture and organic matter stability are important influencing factors of inorganic N supply, and the application of active organic fertilizer in agricultural plantations may be an effective practice to increase unstable organic carbon and improve soil structure to accelerate N cycling and inorganic N supply [28].

4.2. Nitrogen Cycling in the Epikarst

In the epikarst, water is the main driving force of nitrogen migration, and the process of nitrogen migration is mainly the process of surface nitrogen loss, formed by rainfall leaching and washing of surface nitrogen, and the process of surface nitrogen leaking through karst pipes, fissures, and pores, with water and soil after precipitation leaching. Soil organic nitrogen, agricultural fertilization, and domestic sewage are the main sources of nitrogen in karst springs, and nitrogen input and output play an important role in the ecological environment of karst areas.

4.2.1. Nitrogen Morphology and Material Sources in the Epikarst

The nitrogen form of surface karst springs is mainly dominated by nitrate nitrogen, ammonia nitrogen, and nitrite nitrogen. Kuypers et al. (2018) concluded that triple nitrogen (nitrate, nitrite, and ammonium nitrogen), a typical contaminant of groundwater, is widely present in soil and groundwater in many areas [29]. Triple nitrogen is involved in the natural nitrogen cycle, and its main roles include nitrification, denitrification, and allochthonous reduction, which need to be mediated by microorganisms. Lyu et al. (2019) experimentally demonstrated that, after entering the soil, it was rapidly adsorbed and reduced in the shallow surface layer of the envelope soil layer, and the starting concentrations on different soil profiles decreased with increasing depth of the soil profile, and the content in groundwater increased rapidly [30]. The sources of nitrogen in groundwater mainly include soil organic nitrogen mineralization, surface eutrophic water recharge, agricultural nitrogen fertilization, and domestic sewage discharge.

4.2.2. Nitrogen Transport and Export Processes in the Epikarst

With the fluctuation of groundwater level, influenced by the original accumulation of nitrogen in the fluctuating zone, the size of the medium particles in the fluctuating zone, and the hydrodynamic conditions, the triple nitrogen shows different migration characteristics, and there is a strong leaching process of the rising water level on the contaminants in the packaged gas zone, which is conducive to the lateral migration in the groundwater. Rasiah et al. (2013) monitored nitrate and ammonia nitrogen in 39 monitoring wells in the Mulgrave River basin, Canada, during 2 consecutive periods of abundance and depletion, and found that the concentrations of nitrate and ammonia nitrogen in groundwater increased with increasing groundwater levels during the rainy season, fluctuated during the peak of the rainy season, and decreased with decreasing water levels after the rains stopped. The concentrations could increase to nearly 50 times higher during the high-water-level period than during the low-water-level period, and the leaching of nitrogen increased with the increase of nitrogen fertilizer application [31]. Nitrification-denitrification is the process that occurs coupled and is the key role in controlling the transformation of tri-nitrogen migration. Denitrification is the process of reduction to N_2 ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). Denitrification is a relatively complex parthenogenic anaerobic process, and it is generally accepted that bacteria are the dominant denitrifying population. The process of nitrogen transformation in karst water is shown in Figure 5.

4.2.3. Environmental Impact of Nitrogen Loss in the Epikarst

In the southwest karst area, carbonate aquifers develop various forms and scales of underground river pipes, caves, fissures, and pores, with high spatial inhomogeneity and anisotropy, rapid transformation of the "three waters", coupled with soil erosion and rock desertification, resulting in the lack of sufficient soil layer protection on the surface, permeable soil, good irrigation and drainage conditions, and shallow water table, etc., which will cause surface nitrogen leakage to the underground. Zhang et al. (2007) demonstrated that multi-interface hydrological processes controlled by the soil-epikarst structure jointly drive dual surface and subsurface soil erosion, which further exacerbates the risk of nitrogen loss in karst areas [4]. Nitrate in tri-nitrogen is easily soluble in water and difficult to be adsorbed by soil, and is the most dominant form of nitrogen in groundwater, mainly by exogenous input carried by water. Soil nutrients migrate with water, and the nutrients absorbed and used by vegetation are subsequently reduced, which, in turn, affects the growth and development of overlying vegetation, and leads to weakened ecosystem services.

4.3. Hydrochemistry Composition of Karst Springs

At present, there are a large number of research results on surface karst springs, and the relevant studies on spring water chemistry show that the water chemistry of springs has a large correlation with the surface ecology, but it is mainly controlled by the lithology of the surface bedrock and the influence of water-rock interaction, so the hydrochemistry composition of springs has differences, and, in terms of the source of spring water, the hydrogen and oxygen isotope technique is the main identification method, and rainfall is the main recharge of karst spring source.

Karst spring water fraction is influenced by climate, geomorphology, and other factors, mainly recharged by atmospheric precipitation, and the main hydrogen and oxygen isotopes are used as the main discriminatory method. Li (2013) monitored rainfall and karst springs over a two-year period, and the slope of the spring correlation fit was close to the global atmospheric precipitation line equation, indicating that the karst spring source is mainly from rainfall [32]. Rainfall infiltration through water-rock and other processes to form karst water, after the mixing of old and new water and spring outcropping time, showing the complexity of karst spring formation. Huang (2015) conducted hydrogen and oxygen isotope analysis of Heilongtan yielding: (a) Local atmospheric precipitation lines were established by hydrogen and oxygen isotopes, indicating that the springs originated from atmospheric precipitation and recharged the springs mainly in summer. (b) Gaussian mixing model analysis indicated that the recharge of Qingshui Lake originated from the Wildcat Mountain area and from infiltration recharge during runoff. In the rainy and dry seasons, the type of recharge is different. The small water pool is currently identified as being recharged by the Permian tuff aquifer in the north, and possibly by the pore fissure water from the basalt hills in the northeast [33]. Hydrogen and oxygen stable isotopes mainly reveal the water mixing patterns of karst springs [29], aquifer water transport modeling, recharge processes [34], elevation of recharge areas, karst spring water sources, and pollution [35].

The ionic characteristics of karst springs are limited by water-rock interaction and are related to the rate of rock weathering, with Ca^{2+}, Mg^{2+}, and HCO_3^- as the major ions. The hydrochemistry of karst springs is usually used for the study of hydrogeochemical processes in karst areas, where karst water-rock interactions are strong and karst spring hydrochemistry is governed by water-rock interactions [36]. Wang et al. (2020) showed that the karst water in the spring domain is a weakly alkaline freshwater, Ca^{2+}, Mg^{2+}, HCO_3^-, and SO_4^{2-} are the main ions in the groundwater, mainly from the weathering dissolution of calcite, dolomite, and gypsum, and Na^+ and Cl^-, mainly from the dissolution of a small amount of rock salt [37]. The hydrochemistry types of karst groundwater in the spring area are mainly HCO_3-Ca-Mg and HCO_3-SO_4-Ca-Mg types. The main controlling factor in the evolution of the main hydrochemistry components of groundwater is rock weathering. Ca^{2+}, Mg^{2+}, SO_4^{2-}, and HCO_3^- in groundwater mainly originate from carbonate rocks containing calcite ($CaCO_3$), dolomite ($CaMg(CO_3)_2$), and dissolution of sulfate rocks ($CaSO_4$-$2H_2O$), and part of SO_4^{2-} comes from pyrite oxidation. Wang et al. (2020) compared and analyzed the main anion and cation concentrations, δ D and $\delta^{18}O$ composition and TDS concentration values, and found that the K^+ and Na^+ ion contents showed that clastic rocks > carbonate and clastic interbedded rocks > pure carbonate rocks; the Ca^{2+} and HCO_3^- ion contents were the opposite, and the groundwater chemistry type was mainly HCO_3-Ca-type; the TDS contents showed that pure carbonate rocks > carbonate and clastic interbedded rocks > clastic rocks, and the abundant season > dry season; the mixed effect of different water-bearing rock groups on groundwater TDS contents was obvious; the hydrogen and oxygen stable isotopes in the system all fell into the same category > carbonate rock and clastic rock interstratification > clastic rock, abundant season > dry season; mixing of different water-bearing rock groups has an obvious effect on groundwater TDS content; stable isotopes of hydrogen and oxygen of groundwater in the system all fall near the atmospheric precipitation line in the Anshun area, indicating that the recharge of these karst descent springs still comes from atmospheric precipitation [38]. Lin et al. (2011) selected surface karst springs under three different land use practices (scrub, arable land, and stone desert land) in the karst mountains of Nanchuan as the study object, and, by measuring the hydrochemistry indexes of the three springs for one hydrological year, we found that: the proportion of HCO_3^- + Ca^{2+} of the nine main ions measured in the three springs were 87.1%, 71.8%, and 62.9%. The HCO_3^- concentration and the proportion of HCO_3^- + Ca^{2+} to the nine major ions measured in the springs decreased gradually from scrub spring→cultivated spring→karst desertification spring [39].

The surface ecology is correlated with the hydrochemistry of karst springs, and some of the ion concentrations are affected by it and the water quality is threatened. The epikarst is in the zone where the atmosphere, biosphere, lithosphere, and hydrosphere intersect, and its water resources are not only influenced by its own spatial structure and atmospheric precipitation, but also closely related to the vegetation ecology. The chemical composition of water in the springs also varies according to different vegetation ecology. According to Liang (2003) and Yao (2006), who studied the geochemistry of surface karst spring water in Luota, Hunan Province, different ecological environments have obvious effects on the water chemical components of epikarst water, and the degree of vegetation and soil cover is positively correlated with the high and low chemical component content of spring water [40,41]. Deng et al. (2007) studied the hydrochemistry of three superficial karst springs in Lalalan Dentang, Dongwang, and Pingguo Xiaolonghe, and the results showed that the pH, conductivity, HCO_3^-, and total anion content of Lalalan Dentang spring were higher, further confirming that the hydrochemistry of the spring was positively correlated with the vegetation overlying it [42]. Zou (2019) monitored the old spring for one year. Its overlying vegetation was pepper; the NO_3^- and SO_4^{2-} mass concentrations in the spring were higher after pepper tree planting, and sulfuric and nitric acids were involved in the karst process and caused important effects on the quality of the old spring [43]. Zhao (2015) conducted a year of monitoring of the Bai Shu Wan Spring, Lanhuagou Spring, and Hougou Spring, and concluded that the chemical properties of karst springs differed significantly under different land use types. Cations in all three karst springs were dominated by Ca^{2+}, with lower concentrations of Mg^{2+}, K^+, and Na^+, but anion concentrations differed significantly. Cypress Bay Spring is less affected by human activities, and the anions in the spring are dominated by HCO_3^-; Lanhuagou Spring area and Hougou Spring area are affected by nitrogen fertilizer and coal and iron residue, and the NO_3^- concentration and SO_4^{2-} concentration in the spring are higher [44].

4.4. Seasonal Variation of Nitrogen in Surface Karst Springs and Factors Influencing It

Rainfall has a leaching effect on the soil layer of the surface karst zone, which can carry a large amount of material into the karst springs in the process of water transport; therefore, the nitrogen export from the springs is affected by rainfall with spatial and temporal changes, and, due to the instability of nitrite and ammonia nitrogen, the nitrogen is cyclically transformed under nitrification and denitrification, and the nitrogen export from the karst springs is mainly in the form of nitrate nitrogen, ammonium nitrogen, and nitrite nitrogen.

Nitrate nitrogen, ammonia nitrogen, and nitrite nitrogen are the main nitrogen export forms of karst water. The nitrogen forms of surface karst springs are mainly nitrate nitrogen, ammonia nitrogen, and nitrite nitrogen. Zeng (2017) monitored the Houzhai River basin, a typical karst agricultural sub-basin in Qianzhong, to explore the morphological characteristics of nitrogen export from the karst sub-basin, and the results showed that the nitrogen content of water bodies in the Zhai River karst agricultural sub-basin was higher than that of major rivers in China, and the nitrogen export from the Houzhai River basin was mainly in the form of nitrate nitrogen, with an average of 95.2% of the total export of nitrate nitrogen from the basin [45]. Kuypers et al. (2018) concluded that triple nitrogen (nitrate, nitrite, and ammonium nitrogen), a typical contaminant of groundwater, is widely present in soil and groundwater in many areas [29]. Qiao (2020) revealed the seasonal variation pattern of nitrogen in karst groundwater through the response of the major nitrogen, i.e., nitrate nitrogen, ammonia nitrogen, and nitrite nitrogen, in karst water systems to rainfall, in the influence of nitrogen on rainfall [46].

The transformation cycle of nitrogen under nitrification and denitrification, etc., and the transport of water bodies is the main driving force of its migration. The transformation of nitrogen refers to the mutual transformation of various forms of nitrogen in a system under certain conditions, and its transformation process, mainly includes the mineralization of organic nitrogen, nitrification, denitrification, assimilation, and being adsorbed. In groundwater systems, the transformation processes of nitrogen are mainly the first three. Triple nitrogen is involved in the nitrogen cycle in nature, and its main roles include nitrification, denitrification, and allochthonous reduction, which need to be mediated by microorganisms [47]. Zhang (2015) investigated the effect of iron ions in groundwater on the migration and transformation of triple nitrogen through static experiments and dynamic soil column simulation experiments, and found that iron ion concentration had a significant effect on the transformation of triple nitrogen, i.e., with the increase of Fe^{2+} concentration, the conversion of nitrate nitrogen to ammonia nitrogen was favored, and the concentration of nitrate nitrogen in solution showed a decreasing trend, while the concentration of ammonia nitrogen increased [48]. The migration and transformation characteristics of nitrogen in the saturated upper soil layer show that the migration and transformation of nitrate are inseparable from the movement of groundwater, and the most influential effects on nitrogen migration and transformation are nitrification and denitrification. In addition, the more abundant the carbon source in the contaminated soil and water system, the more intense the nitrification and denitrification are, and the response depends largely on the carbon to nitrogen ratio (C/N) in the water. Under the same environmental conditions of migration transformation, the soil purification capacity was increased, with the increase of clay particle content in soil particles, where the increase of soil denitrification rate was the decisive factor for acid picker removal [49]. Infiltration recharge from atmospheric precipitation is the main way for groundwater to receive supplemental water, and the main carrier of downward migration of soluble nitrogen [50]. Zhu (2017) explored the relationship between nitrogen loss characteristics from the perspectives of rainfall, runoff processes, and the ratio of “new and old water”, based on hydrogen and oxygen stable isotopes, and showed that the ratio of “old water” showed a significant positive correlation with nitrate nitrogen, ammonium nitrogen concentration, and total loss, and “old water” may be the main medium for nitrogen transport in the soil-epikarst system in karst slopes [51].

There are spatial and temporal differences in nitrogen in karst systems, and spatial differences are the main starting point. Wang (2013) concluded that the water environment response to land use structural changes is an important guide to the sustainable use of soil and water resources and water environment management in watersheds, and the spatial distribution characteristics of "three nitrogen" in surface water and groundwater in his study area are mainly influenced by their sources [52]. In a study of nitrate sources in the Chishui River basin, Ren (2019) found that river nitrate concentrations were higher during the abundant water period than during the dry water period, and that nitrate concentrations were higher in the upper and middle reaches than in the lower reaches, which coincided with the distribution of karst landscapes in the basin, indicating that nitrogen loss was higher in karst areas than in non-karst areas [25]. The results of Liu (2016) showed that there was a significant difference between the rainy and dry seasons of groundwater nitrogen in the study basin, and the exceedance of nitrate-nitrogen reached 57.14% and 52.94% in the rainy and dry seasons, respectively. The content of nitrogen in groundwater and soil was significantly higher in the rainy season than in the dry season [53].

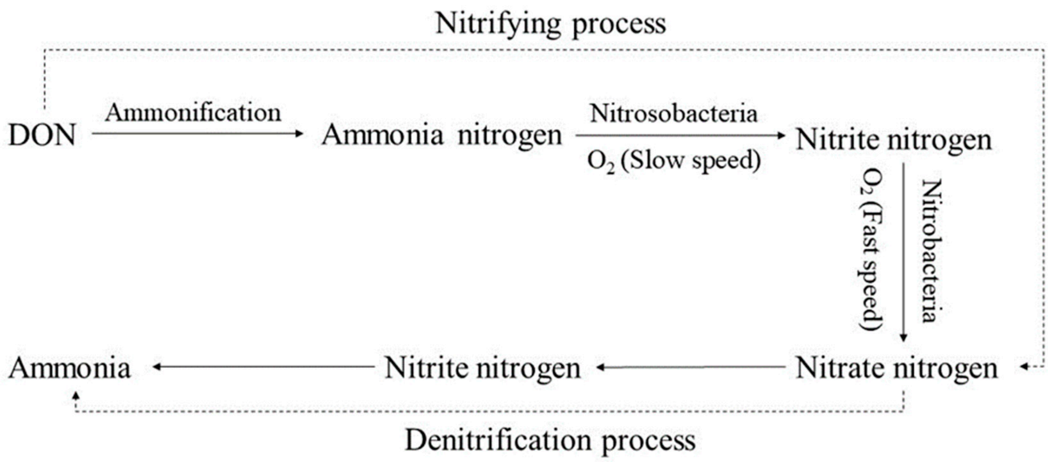

Figure 5. Transformation process of nitrogen in karst water body [52].

4.5. Estimation of Nitrogen Export from Karst Springs

There are very few studies related to the estimation of nitrogen output from karst springs, and the studies on nitrogen estimation in karst water systems mostly focus on rivers, mostly from the perspective of nitrogen sources and spatial and temporal distribution characteristics. the nitrogen and oxygen isotope technique is the main discriminatory method, and the nitrogen in karst water systems mainly comes from soil organic nitrogen, chemical fertilizers, manure water, and domestic sewage.

The source of nitrogen in karst springs is mainly external environmental input, and the method of nitrogen and oxygen isotope tracing provides a convenient way to discern the source of nitrogen. The sources of nitrogen in karst springs mainly include soil organic nitrogen mineralization, surface eutrophic water recharge, agricultural nitrogen fertilization, and domestic sewage discharge. Xu et al. (2014) determined the source of nitrate in water bodies based on the distribution of nitrogen sources and land use types, combined with hydrochemistry characteristics; this method is easy to operate, but can only identify nitrogen sources in a macroscopic manner [54]. In a study of groundwater in the eastern part of Guilin, Wang et al. (2013) found that NO_3^--N pollution in shallow groundwater in the suburbs of the eastern part of Guilin was more serious. The $\rho(NO_3^-$-N) in surface water is low and there is no (NO_3^--N) pollution; the higher $\rho(Cl^-)$ and EC levels indicate more seriousness of pollution in local areas, indicating the complexity of pollution sources and pathways [52]. Gutiérrez et al. (2018) investigated nitrate pollution in karst areas, using the nitrogen and oxygen dual isotope technique, whose accurate "fingerprinting" not only traces nitrate sources, but also combines multiple stable isotopes to resolve nitrate transformation processes, and, in addition, combines source resolution models to quantitatively identify the contribution of different sources of nitrate in water bodies [55]. Zhao et al. (2020) demonstrated that nitrate was mainly derived from chemical fertilizers, soil organic nitrogen, and manure effluent, using the nitrogen-oxygen dual isotope technique. The transformation process of nitrate in waters of the basin was mainly dominated by nitrification, and biogeochemical processes, such as ammonia volatilization and denitrification, did not have a fractionating effect on nitrate nitrogen and oxygen isotope values [56]. Zhang et al. (2019) also used nitrogen and oxygen isotopes to identify surface and groundwater nitrate sources and, simultaneously, quantified the contribution of each source, using the SIAR model, to demonstrate that local water nitrate originated from fertilizers and human wastewater [57]. Shen (2019) comprehensively analyzed the sources, transport processes, and transformation mechanisms of nitrate in karst subsurface river systems by means of hydrochemistry, environmental isotopes, and groundwater retention time, and found that the main sources of nitrate in groundwater were ammonium fertilizer, soil nitrogen, and domestic sewage or manure [58]. A comparison of nitrate identification methods and sources for karst water systems is shown in Table 2.

Table 2. Methods and sources of nitrate identification in karst water system. ("—" in the table indicates no value).

Study Subjects	Identification Method	$\delta^{15}N$/‰	Nitrogen Source
Surface water	Nitrogen and oxygen isotope technology	5.18~8.24	Soil organic nitrogen, Sewage and Manure, Fertilizer [59]
Spring	Nitrogen and oxygen isotope technology	3.7~17.0	Atmospheric precipitation, Soil organic nitrogen, Manure water [60]
Underground river	Nitrogen and oxygen isotope technology	2.05~23.76	Rainfall/Fertilizer, Soil organic nitrogen, Manure sewage [61]
Groundwater/surface water	Cl^- combined with NO_3^-/Cl^-	—	Fertilizer [56]

Nitrogen estimation of karst water is mainly studied by rivers, and there are very few estimates of nitrogen fluxes from karst springs, due to the influence of complex factors, such as karst geomorphology. Liang et al. (2021) took Xialao Creek, a small karst watershed in the middle and upper reaches of the Yangtze River divide in western Hubei, as the study object, to explore the spatial and temporal characteristics of river nitrogen and phosphorus concentrations and output loads. The results showed that the total nitrogen and total phosphorus concentrations in the Xiadun Creek were (1.46 ± 0.05) mg/L and (0.02 ± 0.04) mg/L, respectively, which were generally lower than those in other rivers within the Yangtze River basin [62].

4.6. Environmental Significance and Impact of Nitrogen Export from the Epikarst

The focus of relevant studies on nitrogen in the water system of the surface karst belt is basically on water body nitrogen pollution, and not enough attention is paid to the stone desert environment in karst areas, ignoring the problem of subsurface leakage in karst stone desert areas, where nitrogen, as one of the nutrient elements necessary for vegetation growth, has a constraining effect on vegetation growth and development, and the relevant studies have not paid attention to the impact and significance of nutrient leakage on the surface ecological environment.

The research related to karst water environmental problems is most common in nitrogen pollution, and groundwater is its main research object. The study of the transformation process and mechanism of nitrogen in groundwater has been a hot spot in the study of nitrogen pollution problems, and many scholars at home and abroad have conducted in-depth studies on it. Nitrate pollution is one of the most common environmental problems faced by karst springs or karst groundwater. Studies on groundwater nitrogen pollution problems mainly include field investigations, indoor simulation tests, model simulations, and vulnerability evaluation of nitrogen pollution [63]. Lu et al. (2016) applied multivariate statistical methods, such as the domain method and geostatistical methods, to identify the characteristics of spatial and temporal distribution of different forms of nitrogen in groundwater in the basin, and analyzed the effects of land use type, groundwater burial depth, and surface water on nitrogen in groundwater. The results showed that the nitrogen pollution in regional groundwater was not optimistic, with 29.73% of the samples having excess nitrate nitrogen content (10 mg/L $\leq NO_3^-$-N $\leq$ 20 mg/L), and 27.03% of the samples showed serious exceedances (NO_3^--N $\geq$ 20 mg/L), while changes in land use types were also the main influencing factors for groundwater infiltration and water quality in agricultural areas [64]. Nitrogen export from karst groundwater is inextricably linked to the phenomenon of nutrient leakage. The heterogeneity of the above-ground-subsurface space in karst areas is an innate condition for soil and water leakage, which enters underground rivers through fissures, water fall holes, and shafts under rainfall erosion. Jiang et al. (2014) considered soil-water leakage as the process of surface soil leaking downward to underground rivers through karst channels, such as water fall holes and karst fissures, under the action of mechanical erosion and chemical dissolution of water flow in karst areas where the surface and underground double-layered spatial structures are developed. Nutrients in the soil are lost with soil and water seepage after rainfall washing, erosion, and dissolution [65].

5. Conclusions

Nitrogen-related studies in epikarst have been increasing over time, but they mostly focus on nitrogen loss, vegetation utilization, and migration and transformation of "three nitrogen" in the superficial soils or small river basins, etc. Less attention has been paid to superficial karst springs, and most of them focus on nitrogen spatial and temporal distribution characteristics and nitrogen sources. There are few studies on the correlation between nitrogen and the degree of stone desertification from the perspective of the degree of stone desertification. There are numerous studies on nutrient loss from the surface, but there is almost a gap in the study of nutrient leakage from karst areas through surface karst springs, so more in-depth research is still needed. The study of nitrogen transport changes in karst ignored the interconversion of nitrogen in soil, epikarst, and the uptake and utilization of nitrogen by overlying vegetation, as well as the influence of karst desertification. Studies on the environmental significance of nitrogen export from surface karst springs have mostly focused on nitrogen pollution; however, they have rarely combined nitrogen pollution with the environmental response to stone desertification, ignoring the importance of nitrogen loss through surface karst springs to surface ecosystems.

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