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Effects of Heavy Metals on Nitrogen in Soils of Different Ecosystems in the Karst Desertification of South China

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Abstract: Nitrogen, as a crucial limiting nutrient in terrestrial ecosystems, plays a vital role in determining land quality. Heavy metals, as drivers of soil substance transformation, are important indicators for assessing ecosystem function. Currently, the relationship between soil nitrogen and heavy metals in karst desertification areas remains unclear. Therefore, this study focuses on the soil of grassland, forest, and agroforestry ecosystems in a karst desertification area to investigate the relationship between heavy metals and nitrogen distribution using ecological stoichiometry. The findings revealed the following: (i) Total nitrogen (TN) and available nitrogen (AN) exhibited the trend of agroforestry * > forest > grassland, while soil microbial biomass nitrogen (SMBN) showed the trend of forest * > grassland * >> agroforestry; (ii) *Chromium* (Cr), *Ferrum* (Fe), *Niccolum* (Ni), and *Plumbum* (Pb) showed the trend of agroforestry * > grassland > forest, while *Cuprum* (Cu) demonstrated the trend of agroforestry > grassland > forest, and *Zincum* (Zn) exhibited the trend of grassland > forest * >> agroforestry. The Nemerow comprehensive pollution index were 0.77 for grassland, 0.69 for forest, and 0.94 for agroforestry; (iii) The sensitivity of soil nitrogen and heavy metals ranked as grassland > agroforestry > forest. The research findings aim to provide a scientific reference for karst desertification control, ecological protection and restoration, and enhancement of ecosystem function.

Keywords: karst; desertification; soil; nitrogen; heavy metals

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1. Introduction

The karst area in south China is one of the most important karst landforms in the world, and rocky desertification (KRD) is a severe environmental issue in these areas [1]. KRD is characterized by land degradation, soil erosion, and vegetation degradation, resulting in low productivity and loss of biodiversity [2,3]. Soil characteristics in KRD areas differ significantly from those in other regions, with factors such as soil fossilization, shallow soil depth, poor water retention capacity, heavy metal pollution, and nutrient deficiency posing significant threats to the environment and agriculture [4–6]. The main causes of KRD include overgrazing, deforestation, inappropriate land use, and climate change [7,8].

Soil heavy metal pollution is a common environmental issue in KRD areas, mainly caused by anthropogenic activities, such as mining, smelting, and agriculture [9,10]. The accumulation and toxic effects of heavy metals can result in a decline in soil quality, reducing soil fertility and water retention capacity. High concentrations of heavy metals have toxic effects on plants, soil microorganisms, and other organisms. They can disrupt plant growth and development, leading to reduced crop yield and quality. These impacts can have negative consequences on agricultural production and the stability and sustainability of ecosystems [11,12]. Nitrogen is an essential nutrient for plant growth and ecosystem functioning, and its cycling is closely linked to soil microbial activity and plant productivity [13,14]. However, soil heavy metals can affect nitrogen cycling by altering soil microbial communities, adsorbing nitrogen, and changing nitrogen transformation pathways [15]. Therefore, understanding the effects of soil heavy metals on nitrogen cycling is crucial for the sustainable management of KRD areas.

The relationship between soil heavy metals and nitrogen has garnered significant research interest in recent years. On one hand, soil heavy metal pollution can inhibit the activity of nitrogen-fixing bacteria, which play an important role in the nitrogen cycle. This can lead to reduced nitrogen availability in the soil and negatively impact plant growth [16,17]. On the other hand, high levels of nitrogen in soil can lead to decreased accumulation of heavy metals. This is because nitrogen can stimulate plant growth, which can in turn lead to increased uptake of heavy metals from the soil. Additionally, nitrogen fertilizers can also increase soil pH, which can facilitate the release and mobility of heavy metals in soil [18–20]. While some studies have explored the effects of heavy metal pollution on the nitrogen cycle and the potential impact of high nitrogen levels on heavy metal accumulation in soil, the understanding of the complex interactions between these two factors remains limited. Further clarification is needed on the mechanisms underlying the effects of heavy metal accumulation on the form and efficiency of soil nitrogen, as well as the impact of soil nitrogen morphology on heavy metal accumulation. These research gaps highlight the need for additional studies to deepen our understanding of the complex interactions between soil heavy metals and nitrogen.

Studying the effects of *Chromium* (Cr), *Cuprum* (Cu), *Ferrum* (Fe), *Niccolum* (Ni), *Plumbum* (Pb), *Zincum* (Zn), and other heavy metal elements in KRD typical areas on the nitrogen content, form, and availability of soil has significant academic value. First, it helps to deepen the understanding of the interaction mechanism between various elements in the soil environment and provides a scientific basis for the rational use of soil resources. Second, it can assess the impact of heavy metal pollution in the soil environment on soil fertility and plant growth, which provides theoretical and practical guidance for environmental protection and agricultural production. Third, it can provide some strategies and measures to improve soil fertility and crop yield, such as rational fertilization and adjusting soil pH to reduce the toxicity and influence of heavy metal elements and promote the absorption and utilization of nitrogen by plants. Last, studying the effects of heavy metal elements on the nitrogen content, form, and availability of soil can provide a reference for formulating soil pollution control and remediation technologies. By selecting appropriate remediation technologies and measures, the impact of heavy metal elements on the nitrogen content and availability of soil can be reduced, and the purpose of soil pollution remediation can be achieved.

2. Study Area and Sample

2.1. Study Area

The Salaxi area is situated in the southwest of Bijie city, Guizhou province, at coordinates 105°01′10″–105°08′39″ E and 27°11′08″–27°17′30″ N. It experiences a north subtropical humid monsoon climate, with an average annual temperature of 12 °C and an average annual rainfall of 984.40 mm. The altitude in the area ranges from 1495 m to 2200 m, with a relative elevation difference of 705 m. This area covers a total of 86.27 km^2, with the karst area accounting for 73.94%. Within the karst area, potential, mild, moderate, and intense rocky desertification occupy 30.91%, 22.26%, 8.57%, and 3.09% of the region, respectively. The region's topography is diverse, with broken terrain and varying landform types. Cultivated land is found on slopes, platforms, and mountain valleys, often forming mountain ladder soil and gully dam land. The dry soil has a shallow surface layer and low fertility. The area contains several landforms, including alternating rivers, funnels, blind valleys, sinkholes, skylights, dissolution depressions, and basins. The predominant soil type in the region is zonal yellow soil, with a small amount of yellow–brown soil and brown–black soil [21].

2.2. Sample

The natural grassland in the study area is rare and of poor quality, and the native vegetation has been basically destroyed; now, secondary vegetation is the main vegetation. Therefore, three ecological systems of grassland, agroforestry, and forest with similar site conditions, such as parent material (limestone), soil type (yellow soil), slope position (middle slope), slope (<25°), slope direction (southwest), and altitude (1700.12–1931.29 m), were selected as the research objects, and three sample plots with relatively uniform texture were arranged. The sample plots with relatively uniform soil texture were established within each ecological system. For each ecosystem, a total of 10 plots, each covering an area of 10 m × 10 m, were selected. Soil sampling was conducted within each plot, specifically targeting the 10–30 cm soil layer and considering the actual depth conditions. The five-point composite sampling method, known as the "plum blossom", "S", or "Z" method, was employed to collect representative soil samples. The distribution of the research area and sampling points is shown in Figure 1, and the sample site information is shown in Table 1.

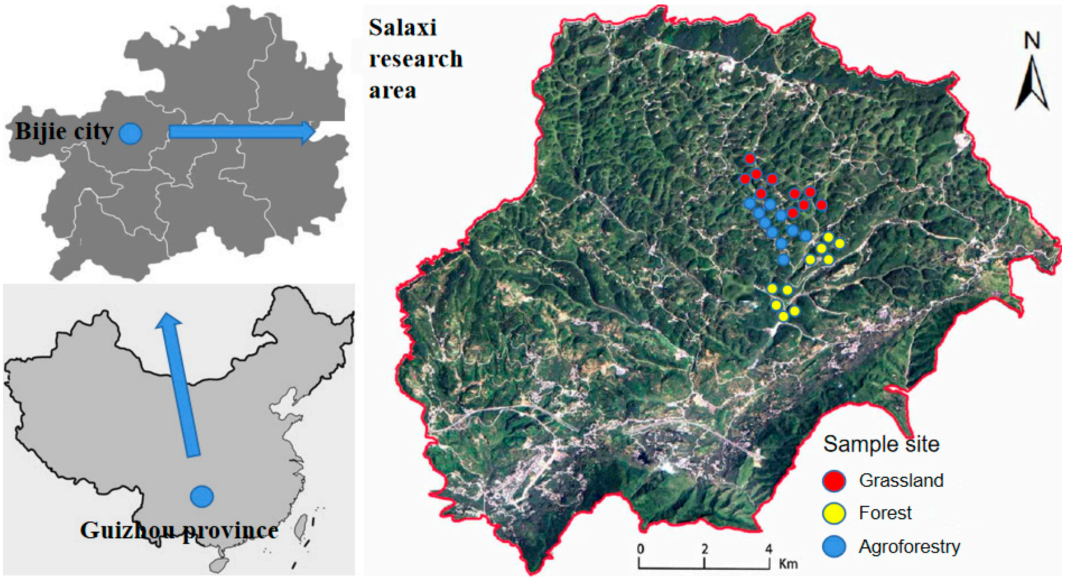

Figure 1. Distinguishing the layout of the study area and sample sites.

Table 1. Information on different ecosystem sites.

Type	Main Vegetation	Latitude and Longitude	Altitude
Grassland	Grass	105°5′23″ E 27°15′02″ N	1931.29 m
Forest	Walnut and Thorn pear	105°5′48″ E 27°14′52″ N	1836.26 m
Agroforestry	Masson's pine	105°5′24″ E 27°14′16″ N	1700.12 m

Information on different ecosystem sites are average data.

During the collection period from September to October 2022, 0.3 kg samples were collected from each sampling point for each ecosystem. The collected samples were mixed together and divided into three portions, with each portion weighing 1 kg. The samples were then placed in sterile plastic bags and shipped back to the laboratory in refrigerated boxes. Once in the laboratory, the samples were air-dried and sieved to remove plant roots, stems, and gravel. The sieve size used was 2 mm. After sieving, the samples were placed in sealed bags for indoor analysis. It is important to ensure that the samples are collected and processed carefully to avoid contamination and ensure accurate results.

3. Materials and Methods

Determination of soil nitrogen and heavy metals: Total nitrogen (TN) was determined using the semi-trace Kjeldahl method. Available nitrogen (AN) was determined using the alkali-hydrolyzed method. Soil microbial biomass nitrogen (SMBN) was extracted by chloroform fumigation. Cr, Cu, Fe, Ni, Pb, Zn were determined using flame atomic absorption spectroscopy.

The differences in nitrogen and heavy metal content in soils of different ecosystem types were analyzed using one-way analysis of variance (ANOVA) or Kruskal–Wallis analysis of variance (ANOVA), and the relationship between them was analyzed using the Spearman correlation and redundancy analysis based on the Bray–Curtis distance (db-RDA). The above data analysis was carried out in Origin 2022 and R3.3.4. Soil heavy metal pollution was typically assessed using various methods, including the single factor index and Nemerow comprehensive pollution index, as outlined in the "Soil Environmental Quality Agriculture Standard for Risk Control of Land and Soil Pollution" (GB 15618-2018). The single factor index focuses on individual metal concentrations in the soil, while the Nemerow comprehensive pollution index considers multiple factors, including metal concentrations, background values, and environmental standards [22,23].

4. Results

4.1. Descriptive Statistics of Soil Nitrogen and Heavy Metals

Soil samples in the study area were analyzed and tested, and descriptive statistics were made on the experimental data (Table S1) to obtain the minimum, maximum, mean, standard deviation and coefficient of variation in soil nitrogen and heavy metals, respectively. The results are shown in Table 2. The arithmetic mean (mean) is an important parameter for reflecting the central tendency of a sample data set, providing a simple and clear quantitative indicator to describe the central position of the data. The coefficient of variation (CV) is an important parameter for describing the degree of variation in a sample. When CV $\leq$ 0.1, it indicates weak variability; when 0.1 < CV $\leq$ 0.9, it indicates moderate variability; and when CV > 0.9, it indicates high variability. In the grassland, the CV for AN and SMBN are both $\leq$0.1, indicating weak variability. On the other hand, the CV for TN, Cr, Cu, Fe, Ni, Pb, and Zn are between 0.1 and 0.9, indicating a moderate level of variability. In the forest, the CV for SMBN, Cr, Fe, and Zn are all $\leq$0.1, indicating weak variability. On the other hand, the CV for TN, AN, Cu, Ni, and Pb are between 0.1 and 0.9, indicating a moderate level of variability. In the agroforestry, the CV for TN, SMBN, Fe, and Zn are all $\leq$0.1, indicating weak variability. On the other hand, the CV for AN, Cr, Cu, Ni, and Pb are between 0.1 and 0.9, indicating a moderate level of variability.

Table 2. Descriptive statistics of soil nitrogen and heavy metals.

Ecosystem	Parameter	TN	AN	SMBN	Cr	Cu	Fe	Ni	Pb	Zn
Grassland	max	1.94	105.45	13.90	171.90	69.68	38.73	80.34	47.25	169.70
	min	1.39	77.39	11.90	121.60	31.43	20.45	43.33	26.15	120.30
	mean	1.73	91.63	12.80	143.30	51.09	25.72	59.21	30.85	142.58
	SD	0.20	9.04	0.63	18.24	11.83	7.87	14.44	6.02	17.93
	SE	0.06	2.86	0.20	5.77	3.74	2.49	4.57	1.90	5.67
	CV	0.11	0.10	0.05	0.13	0.23	0.31	0.24	0.20	0.13
Forest	max	2.04	117.77	14.40	155.90	58.97	22.08	58.03	37.41	143.90
	min	1.43	82.22	12.90	108.80	17.63	19.61	35.35	13.43	107.80
	mean	1.81	103.87	13.50	135.42	32.97	20.51	44.42	28.82	130.73
	SD	0.21	12.27	0.43	13.71	17.73	0.96	8.38	9.17	12.98
	SE	0.07	3.88	0.14	4.33	5.61	0.30	2.65	2.90	4.10
	CV	0.12	0.12	0.03	0.10	0.54	0.05	0.19	0.32	0.10

Table 2. *Cont.*

Ecosystem	Parameter	TN	AN	SMBN	Cr	Cu	Fe	Ni	Pb	Zn
Agroforestry	max	2.95	185.77	11.60	192.40	87.94	23.87	113.50	43.15	125.30
	min	2.45	113.28	10.12	145.60	17.63	20.61	45.45	31.32	101.30
	mean	2.68	146.92	10.62	165.47	52.01	22.38	73.26	38.10	109.83
	SD	0.16	22.23	0.51	18.44	27.63	1.06	26.76	4.69	8.32
	SE	0.05	7.03	0.16	5.83	8.74	0.34	8.46	1.48	2.63
	CV	0.06	0.15	0.05	0.11	0.53	0.05	0.37	0.12	0.08

TN and Fe content unit: g/kg; AN, SMBN and other heavy metal content unit: mg/kg.

4.2. Evaluation of Soil Heavy Metal Pollution

The single-factor pollution indices of heavy metals in the soils of three different ecosystems were calculated using the single-factor analysis method, and the results are shown in Table 2. The single-factor pollution indices of Cr in grassland and forest were less than 1, indicating no pollution, while the pollution index of Cr in agroforestry was between 1 and 2, indicating mild pollution. The single-factor pollution indices of Cu in these three ecosystems were less than one, indicating no pollution. The pollution indices of Fe in these three ecosystems were less than one, indicating no pollution. The single-factor pollution indices of Ni in grassland and forest were less than one, indicating no pollution, while the pollution index of Ni in agroforestry was between one and two, indicating mild pollution. The single-factor pollution indices of Pb in these three ecosystems were less than one, indicating no pollution. The single-factor pollution indices of Zn in these three ecosystems were less than one, indicating no pollution. Furthermore, the comprehensive pollution indices of heavy metals in the soils of three different ecosystems were calculated using the Nemerow comprehensive pollution index method, and the results are shown in Table 2. The comprehensive pollution indices of grassland, forest, and agroforestry were less than one, indicating no pollution.

4.3. One-Way ANOVA of Soil Nitrogen and Heavy Metals

Soil samples from three different ecosystems were analyzed for their nitrogen and heavy metal contents using Shapiro–Wilk normality tests and one-way ANOVA or Kruskal–Wallis ANOVA with a significance level of $p < 0.05$. TN, AN, SMSN, and Cr followed a normal distribution, so one-way ANOVA was used. However, Cu, Fe, Ni, Pb, and Zn did not follow a normal distribution, so Kruskal–Wallis ANOVA was used. The results are presented in Figure 2. The analysis revealed that the TN and AN content of the agroforestry were significantly higher than those of the grassland and the forest. The forest had slightly higher TN and AN content than the grassland, but the difference was not significant. The SMBN content of the forest was significantly higher than that of the grassland and agroforestry. The grassland had a significantly higher SMBN content than the agroforestry. The concentrations of Cr and Pb in the agroforestry were significantly higher than those in the grassland and forest. The grassland had slightly higher Cr and Pb content than the forest. The Cu content of the agroforestry was slightly higher than that of the grassland and forest. The grassland had slightly higher Cu content than the forest. The Fe and Ni in the agroforestry were significantly higher than those of the forest and usually higher than those of the grassland. The grassland had slightly higher Fe and Ni content than the forest. The Zn content of the grassland and forest were significantly higher than that of the agroforestry. The grassland had slightly higher Zn content than the forest.

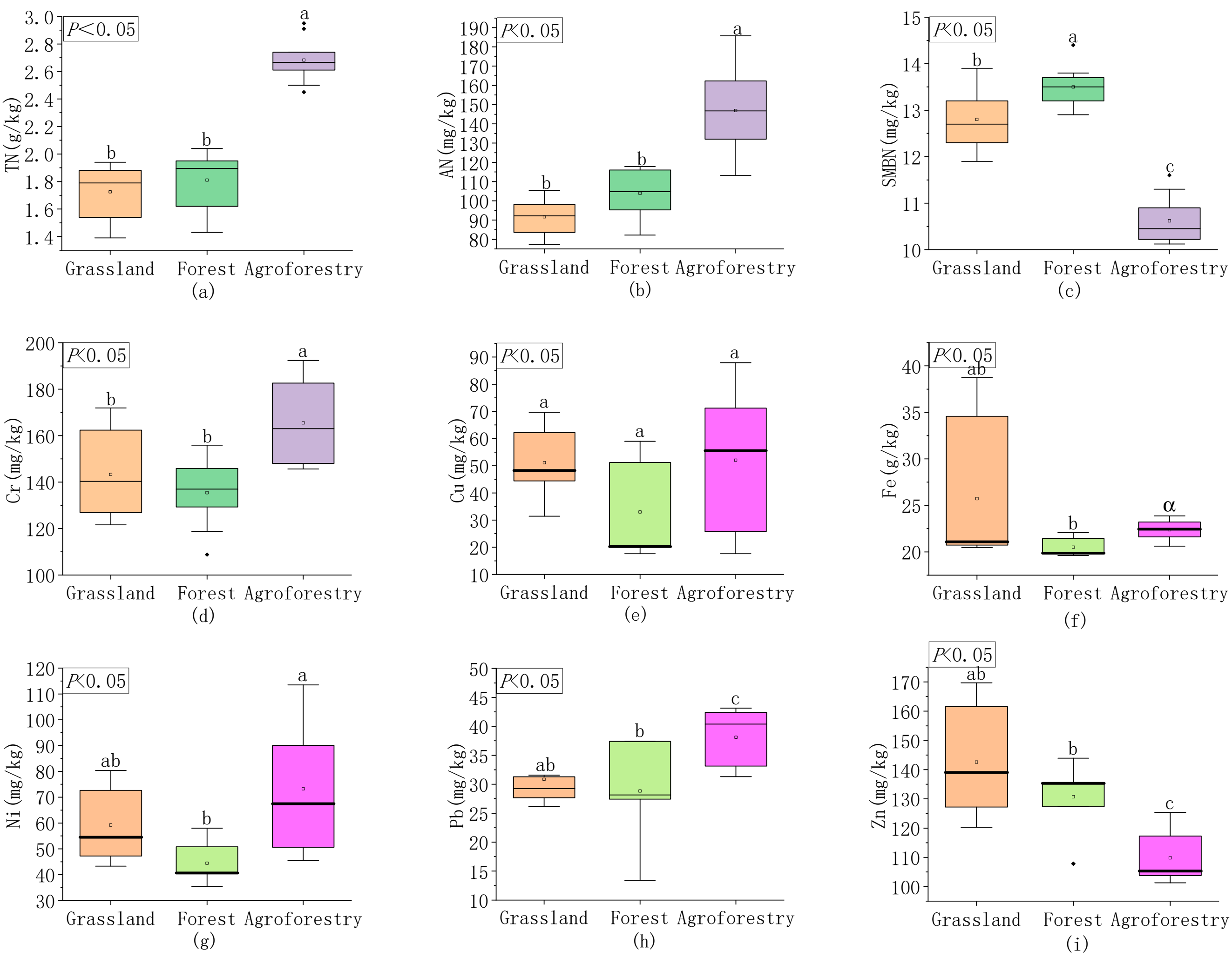

Figure 2. Soil nitrogen and heavy metals in different ecosystem types. (**a**) TN one-way ANOVA; (**b**) AN one-way ANOVA; (**c**) SMSN one-way ANOVA; (**d**) Cr one-way ANOVA; (**e**) Cu Kruskal-Wallis ANOVA; (**f**) Fe Kruskal-Wallis ANOVA; (**g**) Ni Kruskal-Wallis ANOVA; (**h**) Pb Kruskal-Wallis ANOVA; (**i**) Zn Kruskal-Wallis ANOVA. "a", "b", and "c" represent significant differences among different groups, and they are significantly different from other groups. "ab" indicates the presence of interaction effects between two or more groups, meaning that the differences between them cannot be attributed solely to individual group effects.

4.4. Correlation Analysis of Soil Nitrogen and Heavy Metals

The Spearman correlation coefficient was used to analyze the three ecosystems, and the results are shown in Figure 3. In the grassland, there is a strong correlation between soil nitrogen and heavy metals. Specifically, TN is negatively correlated with Cr, Cu, Ni, Pb, and Zn, with correlation coefficients of −0.77, −0.82, −0.76, 0.68, and −0.76, respectively. Similarly, there is a significant negative correlation between AN and Cr, Cu, Ni, and Zn, with correlation coefficients of −0.70, −0.73, −0.85, and −0.73, respectively. SMBN also showed a negative correlation with Cr, Cu, Ni, and Zn, with correlation coefficients of −0.86, −0.78, −0.89, and −0.88, respectively. In the forest, only SMBN showed negative correlation with Cu, with correlation coefficients of −0.73. In the agroforestry, only SMBN showed negative correlation with Cr and Pb, with correlation coefficients of −0.74 and −0.68, respectively.

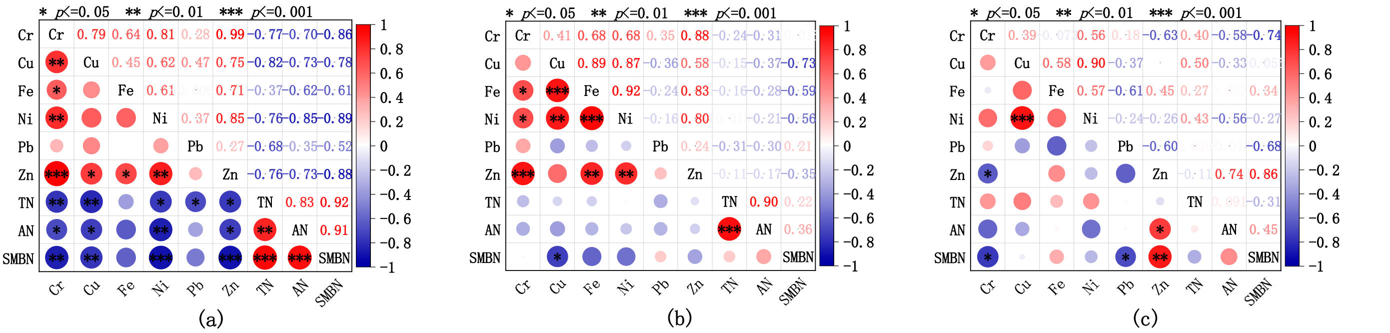

Figure 3. Spearman correlations of soil nitrogen and heavy metals in different ecosystem types. (**a**) Grassland; (**b**) Forest; (**c**) Agroforestry.

Furthermore, db-RDA was conducted to determine the soil nitrogen and heavy metals in the three ecosystems, and the results are shown in Figure 4. In the grassland, heavy metals explained 91.72% of the variation in soil nitrogen. Axis-1 explained 86.55% of all information, and Axis-2 explained 5.17%. Among the heavy metals, 74.6% (F = 23.6, p = 0.004) was explained by Ni, 10.2% (F = 5.4, p = 0.046) by Cr, 3.1% (F = 0.9, p = 0.328) by Pb, 2.4% (F = 0.7, p = 0.498) by Fe, 2.0% (F = 0.6, p = 0.588) by Zn, and Cu explained 1.7% (F = 0.9, p = 0.466). Ni and Cr were the dominant factors affecting soil nitrogen. In the forest, heavy metals explained 63.63% of the variation in soil nitrogen. Axis-1 explained 43.17% of all information, and Axis-2 explained 20.45%. Among the heavy metals, 26.2% (F = 2.8, p = 0.086) was explained by Ni, 15.6% (F = 1.9, p = 0.186) by Pb, 9.1% (F = 0.8, p = 0.526) by Cr, 9.6% (F = 0.8, p = 0.446) by Cu, 5.3% (F = 0.6, p = 0.588) by Fe, and Zn explained 0.2% (F < 0.1, p = 0.994). Ni and Pb were the major factors affecting the soil nitrogen. In the agroforestry, heavy metals explained 78.17% of the variation in soil nitrogen. Axis-1 explained 48.34% of all information, and Axis-2 explained 29.83%. Among the heavy metals, 45.2% (F = 6.6, p = 0.002) was explained by Zn, 10.3% (F = 1.6, p = 0.260) by Pb, 9.6% (F = 2.4, p = 0.192) by Fe, 8.8% (F = 1.5, p = 0.280) by Cu, 7.6% (F = 1.4, p = 0.294) by Ni, and Cr explained 6.2% (F = 1.1, p = 0.334). Zn and Pb were the major factors affecting soil nitrogen.

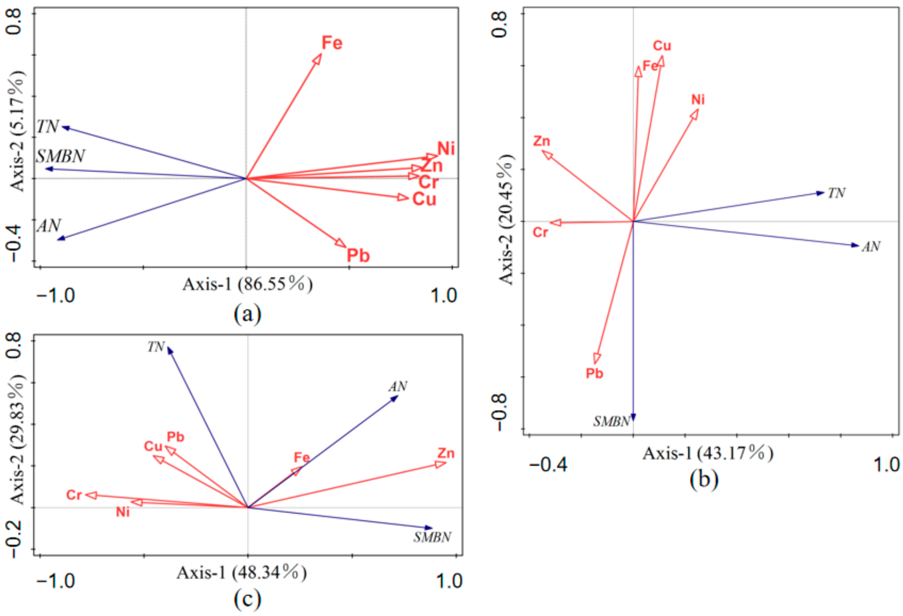

Figure 4. The ordination diagram of db-RDA with soil nitrogen and heavy metals in different ecosystem types. (**a**) Grassland; (**b**) Forest; (**c**) Agroforestry.

5. Discussion

5.1. Soil Nitrogen

Influenced by the unique geological and environmental conditions, soil nitrogen input is relatively limited, and the efficiency of nitrogen cycling is restricted in the KRD areas [24]. TN refers to the total amount of all forms of nitrogen in the soil, including organic nitrogen and inorganic nitrogen. It serves as an important indicator of the nitrogen status and nitrogen supply capacity of the soil [25]. AN refers to the portion of nitrogen in the soil that is readily accessible and can be effectively absorbed and utilized by plants. It includes both ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-). It serves as a crucial indicator for assessing soil fertility and availability of nitrogen supply [26]. SMBN refers to the amount of nitrogen stored within the microbial biomass present in soil. It plays a crucial role in indicating the nitrogen utilization and transformation capacity of soil microorganisms, which in turn reflects the state and efficiency of soil nitrogen cycling. Higher SMBN content typically indicates the presence of more abundant and active microbial communities, along with higher levels of soil organic matter. These conditions are associated with improved soil structure and enhanced ecological functions [27].

Based on the analytical outcomes (Figure 2), the agroforestry system exhibited significantly higher levels of TN and AN compared to the grassland and forest systems, suggesting a stronger nitrogen supply capacity and higher nitrogen availability in agroforestry soil. The forest had slightly higher TN and AN content than the grassland, but the difference was not statistically significant. Therefore, it can be inferred that there is no significant difference in terms of nitrogen supply capacity and nitrogen availability between the grassland and forest systems based on nitrogen content. Regarding SMBN content, the forest system showcased significantly higher levels when compared to the grassland and agroforestry systems. This implies that the forest soil harbors relatively greater microbial biomass nitrogen. Similarly, the grassland system exhibited significantly higher SMBN content than the agroforestry system, indicating an elevated microbial biomass nitrogen in grassland soil. Therefore, it can be inferred that grassland and forest systems may have richer and more active microbial communities, higher levels of soil organic matter, and higher nitrogen use efficiency compared to the agroforestry system. These factors contribute to the overall nitrogen cycling and availability in the respective ecosystems, highlighting the importance of natural vegetation and undisturbed ecosystems in maintaining robust nitrogen dynamics [28].

As natural ecosystems that are less affected by human activities, both grassland and forest systems receive nitrogen inputs from various sources, such as atmospheric deposition, biological fixation, decomposition of organic matter, and rock weathering. However, when comparing the soil nitrogen content between grassland and forest systems, the forest system generally exhibited higher levels of TN, AN and SMBN, with significant differences observed in SMBN [27,29]. There are several factors that contribute to the higher TN, AN and SMBN content in forest soil. First, forest systems often have higher vegetation coverage and a greater diversity of plant species. The presence of a dense and diverse plant canopy leads to increased organic matter input through leaf litter, root exudates, and plant debris. The decomposition of this organic matter releases nitrogen compounds into the soil, resulting in higher TN and AN levels [30]. Second, forests typically possess more developed and deeper root systems compared to grasslands. The extensive root networks of trees and understory plants facilitate efficient nutrient uptake, including nitrogen, from the soil. The deeper root systems enable access to deeper soil layers where nitrogen may be more abundant, enhancing nitrogen absorption and accumulation in the soil [31]. Furthermore, the forest environment provides favorable conditions for the proliferation of soil microorganisms. Forest soils are known to harbor richer and more active microbial communities, which play a vital role in nitrogen cycling processes. These microorganisms contribute to the decomposition of organic matter, mineralization of organic nitrogen into inorganic forms, and the conversion of nitrogen compounds through various biochemical pathways. Consequently, the increased microbial activity in forest soils enhances nitrogen availability and contributes to higher TN, AN, and SMBN content [32]. Last, forests often exhibit stronger soil conservation and water retention capabilities compared to grasslands. The forest canopy and dense vegetation cover protect the soil from erosion and reduce nutrient losses, including nitrogen leaching. This retention of nitrogen within the soil contributes to higher TN, AN, and SMBN levels [33].

Agroforestry is a land use system that integrates the cultivation of trees or woody plants with agricultural crops or livestock. In the study, the results showed that the agroforestry system displayed significantly higher levels of soil TN and AN content when compared to both the grassland and forest systems. However, in terms of SMBN content, the agroforestry system exhibited a significantly lower value. On the one hand, as an artificial agro-ecosystem, agroforestry systems may be affected by more human activities. In KRD areas, the main sources of soil nitrogen input in agroforestry systems are the application of organic fertilizers and the decomposition of crop residues [34,35]. Organic fertilizers contain abundant organic nitrogen compounds, which gradually decompose and release nitrogen elements when applied to the soil. The decomposition process of crop residues, including stems, leaves, and roots, also releases nitrogen into the soil. These processes provide available nitrogen sources for the soil, thereby increasing the nitrogen content, leading to higher levels of TN and AN. On the other hand, the lower SMBN content in the agroforestry systems may be attributed to factors such as disturbance from agricultural practices and potential changes in soil microbial community composition. The use of agrochemicals, tillage, and other agricultural practices can affect the microbial populations in the soil and potentially lead to a decrease in microbial biomass nitrogen. Additionally, the presence of woody plants and trees in the agroforestry system may create a different microenvironment compared to natural grassland and forest systems, influencing the composition and activity of soil microorganisms [36].

It is worth noting that the high supply capacity, efficiency, and low use efficiency of soil nitrogen in the agroforestry system may lead to an unbalanced soil nitrogen cycle, which may have adverse effects on the ecological environment. In KRD areas, the primary source of nitrogen pollution in water bodies is often attributed to agricultural activities [37]. The application of fertilizers and pesticides in farmland leads to the runoff and leaching of nitrogen compounds, which subsequently contaminate surface water and groundwater systems. Given the widespread and continuous nature of agricultural practices in the region, their impact on nitrogen pollution in water bodies is generally substantial [38]. Furthermore, the significant carbon content and weakened carbon sink function can lead to the accumulation of soil CO_2 [39]. This accumulation, in turn, intensifies the process of denitrification while inhibiting nitrification [40,41]. During denitrification, the process of soil respiration is enhanced [42], leading to the breakdown of excess AN. This breakdown process results in the production of N_2O, which, when not efficiently utilized, can contribute to the increased emission of greenhouse gases [43].

5.2. Soil Heavy Metals

Cr, Cu, Fe, Ni, Pb, and Zn are prevalent heavy metal elements found in the soil of KRD areas. The concentrations of these elements can indicate the degree of heavy metal pollution and mineral content in the soil. Adequate levels of Cu, Fe, and Zn are crucial for the healthy growth and development of plants. However, an excessive presence of Cr, Cu, Fe, Ni, Pb, and Zn can have adverse effects on plant growth and soil quality, potentially leading to soil acidification and plant toxicity.

Based on the analytical outcomes (Tables 2 and 3), the agroforestry system exhibited significantly higher levels of Cr, Cu, Ni and Pb compared to the grassland and forest systems, and significantly lower levels of Fe and Zn. The grassland had slightly higher Cr, Cu, Fe, Ni and Zn content than the forest, but the difference was not statistically significant. Furthermore, through the application of the single-factor pollution index method, it was found that the single-factor pollution index of Cr and Ni in the soil of the agroforestry system ranged between one and two, indicating a mild pollution risk. The Nemerow comprehensive pollution index method revealed that the agroforestry system had a higher comprehensive pollution index compared to the grassland and forest systems, approaching one, thus indicating a higher potential pollution risk [44].

Table 3. Comprehensive evaluation of soil heavy metal pollution in different ecosystem types.

Ecosystem	Pi						Pmax	Pave	P
	Cr	Cu	Fe	Ni	Pb	Zn			
Grassland	0.96	0.05	0.51	0.99	0.10	0.14	0.99	0.46	0.77
Forest	0.90	0.03	0.41	0.74	0.10	0.13	0.90	0.39	0.69
Agroforestry	1.10	0.05	0.45	1.22	0.13	0.11	1.22	0.51	0.94

Pi: single factor index; Pmax: maximum value in Pi; Pave: mean value of Pi; P: Nemerow comprehensive pollution index.

The sources of Cr, Cu, Fe, Ni, Pb and Zn in soil in KRD areas mainly include natural factors and human activities. On the one hand, these heavy metal elements are ubiquitous in the earth's crust, and the soil will also contain a certain natural content. They can come from rock weathering, the release of minerals during soil formation, and the dissolution of rainfall and groundwater. On the other hand, pesticides and fertilizers widely used in agricultural production contain a certain amount of heavy metals, and long-term use may lead to the accumulation of heavy metals in the soil. As an artificial agricultural ecosystem, the higher content of heavy metals in the soil of agroforestry systems compared to grassland and forest systems may be attributed to the following factors: (1) Agricultural activities: Agroforestry systems often consist of a combination of farmland and woodland. Agricultural activities, such as fertilizer application, pesticide use, and irrigation, can lead to the accumulation of heavy metals in the soil [45]; (2) Anthropogenic disturbances: Agroforestry systems are commonly subject to anthropogenic disturbances, including artificial cultivation, changes in land use, and human activities. These disturbances can contribute to an increase in the concentration of heavy metals in the soil [46]; (3) Geological background: Agroforestry systems in KRD areas are typically found at lower altitudes, which increases their susceptibility to the accumulation and concentration of heavy metals [47]; (4) Vegetation type: Agroforestry systems in KRD areas established often involve plant species that possess stronger adaptability to the environment and greater capacity for nutrient absorption. Consequently, these plants may also exhibit enhanced abilities in adsorbing heavy metal elements.

It is worth noting that according to the analysis of the Bray–Curtis distance (Figure 4), the changing trend of the included angle of the heavy metal elements was as follows: forest > agroforestry > grassland, indicating that the sources of heavy metal elements in forest soil are more diverse, while the sources of heavy metal elements in grassland soil tend to be more singular. The main reason for this may be that forest system has a more complex ecological structure, allowing for more complex and diverse biological, physical, and chemical interactions [48].

5.3. Soil Nitrogen and Heavy Metals

Soil nitrogen and heavy metals are two distinct components that can interact and influence each other in various ways. The relationship between soil nitrogen content, form, and heavy metals is indeed complex and influenced by various factors, including soil characteristics, environmental conditions, and management practices. Different soil types and environmental conditions can lead to variations in this relationship. Additionally, human activities, such as the use of chemical fertilizers, pesticide application, and industrial emissions, can significantly impact the input of heavy metals and nitrogen into the soil, thereby affecting their relationship.

Based on the Spearman correlations of soil nitrogen and heavy metals (Figures 3 and 4), the correlation order of soil nitrogen and heavy metals was as follows: grassland > agroforestry > forest. This suggested that in the grassland system, there is a stronger correlation between soil nitrogen and heavy metals compared to the agroforestry and forest systems. In the grassland system, there was a significant negative correlation between soil TN and Cr, Cu, Ni, Pb, and Zn. And there was a significant negative correlation between soil AN, SMBN and Cr, Cu, Ni, and Zn. In the forest system, there was a significant negative correlation between soil SMBN and Cu. In the agroforestry system, there was a significant negative correlation between SMBN and Cr, Pb, and a significant positive correlation between AN, SMBN, and Zn.

Ecosystem stability refers to the capacity of an ecosystem to maintain a relatively constant state of its structure, function, and ecological processes in the presence of internal and external disturbances or changes [49–51]. A stable ecosystem is characterized by a balanced state of biodiversity, energy flow, material cycling, and ecological interactions, while also exhibiting resistance and resilience to external disturbances. To some extent, a more complex ecosystem structure can enhance ecosystem stability. This is because a complex structure typically involves a greater number of species, functional groups, and interactions, providing a wider range of ecological niches, resource utilization strategies, and ecological processes. This diversity and functional variety can increase the resilience and adaptability of the ecosystem, enabling it to better cope with disturbances and changes [52–59].

Grassland systems typically exhibit relatively simple ecological structures and are dominated by herbaceous plants. Due to the short life cycle and low height of grassland plants, their ecological structure remains relatively simple. In contrast, natural forest systems often possess more complex and diverse ecological structures. Through extended periods of natural succession, natural forests develop multi-level and multi-species vegetation compositions, resulting in rich biological diversity. This complexity and diversity contribute to higher stability in the ecological structure of natural forest systems [60–63]. And agroforestry systems are characterized by a combination of different habitat types, such as farmland and forest. This system shares similarities with both grassland and forest ecosystems and exhibits a higher degree of complexity in its ecological structure [64]. However, the stability of the ecological structure may vary across different regions or components of the system. Factors such as land management practices, local environmental conditions, and specific species composition can influence the stability of the ecological structure within different parts of the system. Based on this, we can infer that the grassland system may show higher sensitivity to heavy metals when compared to forest and agroforestry systems. Heavy metal elements were more likely to inhibit microbial activity and functionality in the soil, leading to reduced rates of organic matter decomposition and, consequently, a decrease in the release of organic nitrogen and nitrogen transformation. Additionally, certain heavy metal elements can form complexes or precipitates with nitrogen in the soil, resulting in nitrogen fixation and reduced nitrogen availability. Cr, Cu, Ni, and excessive Zn can inhibit the activity of nitrogen-fixing bacteria and nitrifying bacteria, which may lead to a decrease in AN and indirectly affect soil TN content. Furthermore, these heavy metals can form complexes or chelates with nitrogen in the soil, affecting nitrogen transformation and availability, resulting in nitrogen fixation and reduced accessibility. On the contrary, the forest system exhibited the lowest sensitivity to heavy metals. There was no significant correlation observed between soil TN, AN, SMBN, and Cr, Cu, Fe, Ni, Pb, Zn, indicating that the interactions between these indicators are not significant. This suggested that the influence of heavy metal elements on soil nitrogen content and forms is relatively weak or that there was a weak interaction between the two. It implied the existence of a relatively stable balance between soil nitrogen cycling and heavy metal accumulation in the forest system. This stability can be attributed to the complexity, diversity, and regulatory capacity of ecological processes in forest system. In the agroforestry system, there was no significant correlation observed between soil TN, AN, and Cr, Cu, Fe, Ni, Pb, Zn. However, SMBN showed a significant negative correlation with Cr and Pb, and a significant positive correlation with Zn. Due to human factors, higher levels of TN and AN can form complexes or chelates with Cr, Cu, Ni, Pb, and other elements, promoting the accumulation of heavy metals in the soil and inhibiting microbial activity and functionality. Additionally, human intervention can lead to soil nutrient imbalance. In this study, a significant positive correlation was observed between SMBN and Zn, indicating a limitation of Zn on soil nutrient status [65].

It is important to note that these relationships are based on correlations and further research is needed to fully understand the underlying mechanisms. Other factors such as soil properties, microbial activity, and environmental conditions may also influence the observed relationships between soil nutrients and heavy metals in the three systems.

6. Conclusions

The variation in soil nitrogen in the three ecosystems is as follows: TN: agroforestry * > forest > grassland; AN: agroforestry * > forest > grassland; SMBN: forest * > grassland * >> agroforestry. The agroforestry system exhibited significantly higher TN and AN, as well as significantly lower SMBN, compared to the grassland and forest systems. This suggests that while agroforestry systems have a higher nitrogen supply capacity, their nitrogen cycling and nitrogen utilization efficiencies are relatively low. The excessive nitrogen content may lead to the accumulation of heavy metals in the soil, posing a risk of nitrogen pollution to the surrounding ecological environment.

The changes in soil heavy metals in the three ecosystems are as follows: Cr: agroforestry * > grassland > forest; Cu: agroforestry > grassland > forest; Fe: agroforestry * > grassland > forest; Ni: agroforestry * > grassland > forest; Pb: agroforestry * > grassland > forest; Zn: grassland > forest * >> agroforestry. The Nemerow comprehensive pollution index for the three ecosystems were as follows: 0.77 for grassland, 0.69 for forest, and 0.94 for agroforestry. The agroforestry ecosystem, being influenced by human activities, exhibits a higher risk of heavy metal pollution.

The sensitivity of soil nitrogen and heavy metals in the three ecosystems can be ranked as follows: grassland > agroforestry > forest. In the grassland system, there was a significant negative correlation between soil TN and Cr, Cu, Ni, Pb, and a significant negative correlation between soil AN, SMBN and Cr, Cu, Ni, and Zn. These correlations indicated a significant inhibitory effect of heavy metals on soil nitrogen supply capacity, nitrogen cycling efficiency, and nitrogen utilization efficiency. It suggested that heavy metals have a negative impact on the grassland system's ability to retain nitrogen and efficiently cycle and utilize it. In the agroforestry system, there was a significant negative correlation between SMBN and Cr, Pb, and a significant positive correlation between AN, SMBN, and Zn. These correlations indicated a significant inhibitory effect of heavy metals on soil nitrogen cycling efficiency and nitrogen utilization efficiency. Additionally, the positive correlation with Zn suggested a significant nutrient imbalance in the soil, particularly with Zn limitation. In the forest system, there was a significant negative correlation between soil SMBN and Cu. This implied that the interaction between soil nitrogen and heavy metals in the forest system is in a more stable and balanced state. Unlike the grassland and agroforestry systems, the forest system appeared to be less affected by heavy metals, indicating a more resilient and well-regulated nitrogen–metal interaction. Overall, these findings suggest that heavy metals can have significant impacts on soil nitrogen dynamics and availability in different ecosystems, with grassland and agroforestry systems being more sensitive to such effects when compared to the forest system.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14071497/s1, Table S1: Raw experimental data.

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