agronomy

Article

Warming Mitigates the Impacts of Degradation on Nitrogen Allocation between Soil Microbes and Plants in Alpine Meadow

Zhe Pang [2], Guoqi Wen [3], Lili Jiang [1,*], Xiaowei Nie [1,4], Zongsong Wang [5], Rui Pang [6], Wenjing Liu [2], Meirong Chen [1], Weiwai Zhao [1], Li Tang [2], Biao Zhang [2], Linfeng Li [7], Shutong Zhou [2], Xingliang Xu [8], Yanbin Hao [5,9], Xiaoyong Cui [5,9], Shiping Wang [1] and Yanfen Wang [1,2,9]

[1] State Key Laboratory of Tibetan Plateau Earth System, Resources and Environment (TPESRE), Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China
[2] College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China; pangzhe@ucas.ac.cn (Z.P.)
[3] Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, Canada
[4] Science Center of Lingshan Forum of Guangdong Province, Guangzhou 511466, China
[5] College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
[6] Key Laboratory of Agricultural Water Resources, Hebei Key Laboratory of Soil Ecology, Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang 050021, China
[7] Forestry College, Fujian Agriculture and Forestry University, Fuzhou 350002, China
[8] Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China
[9] Beijing Yanshan Earth Critical Zone National Research Station, University of Chinese Academy of Sciences, Beijing 101408, China
* Correspondence: lljiang@itpcas.ac.cn

Abstract: In alpine meadows, plants and soil microbes typically engage in competition for nitrogen (N) under N-deficient conditions. However, the acquisition and distribution of N among soil microbes and plants under alpine meadow degradation and climate warming induced by global climate change are still uncharacterized. In this study, we isotope labeled inorganic (NH_4^+-^{15}N, NO_3^--^{15}N) and organic (glycine-^{15}N) N in both degraded and non-degraded plots by using open-top chambers (OTC) to mimic increasing air temperatures. After 6 h, the ^{15}N contents in soil microbes and plants were measured to investigate the effects of degradation and rising air temperature on N allocations in the ecosystems studied. Results showed that alpine meadow degradation significantly reduced soil microbial N accumulation by 52% compared to those in non-degraded plots. In non-degraded plots, warming significantly lowered the organic N levels of soil microbes by 49%, whereas in degraded ones, it reduced both NH_4^+-^{15}N and NO_3^--^{15}N recovery by 80% and 45% on average but increased glycine-^{15}N recovery by 653%. Meanwhile, warming decreased the plant recovery of NH_4^+-^{15}N and NO_3^--^{15}N by 75% and 45% but increased the recovery of glycine-^{15}N by 45% in non-degraded plots. Conversely, in degraded plots, warming markedly lowered NH_4^+-^{15}N recovery by 40% but increased glycine-^{15}N recovery by 114%. Warming mitigates the effects of alpine meadow degradation on nitrogen allocation among soil microbes and plants. In unwarmed plots, degradation significantly elevated the total ^{15}N recovery ratio of soil microbes to plants by 60%. However, in warmed plots, the impact of degradation on this ratio was reduced. The responses of the ^{15}N recovery ratio of soil microbes and plants to rising temperatures were closely related to alpine meadow quality. In non-degraded areas, warming enhanced the recovery ratio for NH_4^+-^{15}N by 165% but reduced it for glycine-^{15}N by 66%. Conversely, in degraded plots, warming decreased the recovery ratio for NH_4^+-^{15}N by 66% but increased it for glycine-^{15}N by 232%. This indicates that warming can increase carbon limitation for soil microbes in degraded alpine meadows, and the restoration of degraded alpine meadows should prioritize restoring carbon accumulation.

Keywords: organic N; inorganic N; N partitioning; warming; degradation; Tibetan Plateau

Citation: Pang, Z.; Wen, G.; Jiang, L.; Nie, X.; Wang, Z.; Pang, R.; Liu, W.; Chen, M.; Zhao, W.; Tang, L.; et al. Warming Mitigates the Impacts of Degradation on Nitrogen Allocation between Soil Microbes and Plants in Alpine Meadow. *Agronomy* **2024**, *14*, 508. https://doi.org/10.3390/agronomy14030508

Academic Editors: Fernando Monroy and Domenico Prisa

Received: 10 January 2024
Revised: 25 February 2024
Accepted: 27 February 2024
Published: 29 February 2024

1. Introduction

Nitrogen (N), a crucial life element, significantly influences plant growth in terrestrial ecosystems and plays a central role in the competition for nutrients between plants and microbes [1–3]. Driven by soil microbial processes [4], the N cycle encompasses various stages including the synthesis of organic N, biological N fixation, ammonification, nitrification, denitrification, N runoff, and nitrate leaching [5,6]. The relationship between plants and microbes is complex, involving both competition and mutual dependence [7,8]. Soil microbes convert organic N into inorganic forms (NH_4^+ + NO_3^-) for plant utilization, while plant residues serve as a resource for microbial activity [9]. To unravel the subtleties of N cycling in terrestrial ecosystems, it is crucial to understand this dynamic interaction through the quantification and analysis of N acquisition by plants and microbes [10–14].

Grassland ecosystems have experienced substantial degradation due to various factors, including climate change [15], overgrazing, human intervention, and natural occurrences such as freeze–thaw cycles and rodent activities [16]. This degradation has resulted in significant changes to the structural stability of plant communities [17,18], a reduction in plant biomass and coverage [13,16–18], and a redistribution of photosynthetic products between above- and below-ground parts [19]. Additionally, there have been changes in the rate of soil organic matter decomposition and mineralization [20]. Furthermore, there has been a decrease in microbial biomass, N-fixing bacteria [21], and soil enzyme activities [18,22], which have impacted N storage and cycling within these grassland ecosystems along with the occurrence of grassland degradation [23].

Plants uptake available N from the soil, which includes inorganic forms [24,25] and organic N [13], such as peptides and free amino acids [26,27]. Different plant species coexisting in the same environment exhibit varied absorption of these N forms [13,25,28–30]. McKane [28] observed that the dominant plant species in the Arctic tundra ecosystem preferentially utilize the most abundant N form in the soil, which is free amino acids, while less dominant species tend to use less abundant forms like NH_4^+. The interaction between plants and microorganisms in soil N acquisition is complex, involving both competition and cooperation to prevent N loss by absorbing different N forms [31]. Research indicates a distinct partitioning of N forms between plants and microorganisms [32]. For example, dominant plants in long-enclosed alpine meadows show a preference for absorbing NO_3^- [32], whereas microorganisms favor NH_4^+-N [33]. However, the specific preferences and capabilities of plants and microorganisms in acquiring soil N following changes in grassland conditions remain unclear. Most current research focuses on how degradation affects the functional groups of plants and microorganisms [32–35], with limited understanding of its impact on the N acquisition rate of soil microorganisms and the distribution of N between plants and microorganisms. Therefore, understanding how degradation influences the allocation of different N forms between plants and microorganisms is crucial for comprehending the response of plant productivity to degradation.

In high-altitude and high-latitude regions, where soil is rich in organic matter, the decomposition of this organic matter is limited by low temperatures [36]. This makes the impact of climate warming more pronounced in these areas. Research has determined that temperature and humidity are key factors influencing the mineralization of different types of soil N [37,38], with temperature playing a more prominent role [39]. In general, an increase in soil temperature facilitates the N cycle in the soil [40,41], while a reduction in soil moisture content impedes this process [42]. Bijoor et al. [43] found that warming altered N_2O emissions, initially increasing them before causing a decrease. This change in temperature also affects the composition of plant species in grassland ecosystems. Climate warming enhances the activity of soil microorganisms, speeding up the decomposition of organic matter and boosting the release of Inorganic N in the soil [44]. Warming influences the composition and structure of microorganisms, which in turn affects N cycling processes. Jiang et al. [45] revealed that dominant plants in alpine meadows adapt their N utilization strategies under warming conditions to maintain their dominance. However, it remains unclear how the N utilization strategies of plants and microorganisms change under conditions of warming coupled with degradation, and whether their competition for N is affected.

The Tibetan Plateau, often referred to as the Earth's 'Third Pole', comprises about 30% alpine meadows [46,47]. The low temperatures and productivity levels of these meadows make them exceptionally sensitive to climate change and human activities [46]. In the plateau's grassland ecosystems, long-standing cold conditions have led plant communities and soil microbial groups to adapt to a scarcity of readily available N. As a result, unique patterns of N cycling and nutrient supply have developed, along with specific trade-offs between soil, plants, and microorganisms [32,48]. However, these ecosystems are experiencing certain degradation due to climate change and human impacts. Therefore, exploring how warming and degradation affect soil N cycling, as well as how plants and microorganisms compete for and distribute inorganic and organic N, is crucial for a full understanding of the ecosystem's responses to climate change and human disturbances [48]. Such studies are vital for improving our understanding and ability to predict changes in the productivity and sustainable development of these grasslands.

In our study, we aim to assess the impact of warming on the ability of plants and microorganisms to compete for and utilize various forms of N in both undegraded and degraded alpine meadow plots. These plots were situated both inside and outside open-top chambers (OTC), which were used to stimulate rising temperature. Six hours after applying labels to inorganic N (NH_4^+ and NO_3^-) and organic N (glycine), we analyzed the amounts of these N forms in plants and soil microorganisms. We specifically compared the recovery rates and quantities of ^{15}N in both plants and microorganisms. Our findings are crucial for understanding how N cycling and the availability of N in alpine grassland ecosystems respond to climate change. This research provides a scientific basis for formulating effective grassland management strategies.

2. Materials and Methods

2.1. Experimental Site

Our experimental site is situated at the Nagqu Ecological and Environmental Observation Research Station in Tibet (31°17′ N, 92°06′ E; 4501 m above sea level), located in Kerma Village within the Nagqu River Basin of Nagqu County, in the Tibet Region. This region features expansive open terrain and lies at the core of the distribution area of *Kobresia pygmaea* (*K. pygmaea*) on the Tibetan Plateau, nestled between the Tanggula and Nyenchen Tanglha mountains. Characterized by a typical alpine meadow climate, the area boasts an average elevation exceeding 4450 m, intense solar radiation, and over 2790 h of annual sunshine [13]. The climate exhibits short summers and prolonged, severe winters, with an average annual temperature of -2.1 °C and an average January temperature of -14.4 °C. The area experiences windy and dry conditions with significant diurnal temperature variations and lacks a definitive frost-free period year-round. Over the past decade, the average annual precipitation has been around 406 mm, predominantly occurring from June to September. The average annual evaporation rate is 1810 mm, and the average relative humidity is 51%. The meadow's vegetation predominantly consists of perennial plants, with *K. pygmaea* and *K. humilis* being the dominant species. Other species include *Carex moorcroftii* (*C. moorcroftii*), *Gentiana straminea* (*G. straminea*), *Lancea tibetica* (*L. tibetica*), and *Poa* spp. (*P.* spp.) [49], collectively known as '*K. pygmaea* meadow'. *K. pygmaea*, a perennial, tufted, cushion-forming plant, has short stems ranging from 1 to 3 cm in height, needle-like leaves of the same length as the stems, and a simple spike-like inflorescence, ovoid to elongate, measuring 4–6 mm in length [50]. This species is distinguished by its strong ecological adaptability compared to other *K.* species in Tibet, leading to its widespread distribution [51]. The soil is predominantly alpine meadow soil, the most extensive soil type in the region, comprising 4% organic carbon, 0.34% total N, and 36% sand content [52].

2.2. Experimental Design

The non-degraded and degraded grassland were identified based on vegetation coverage and the proportion of *K. Pygmaea*. The non-degraded grassland predominantly features *K. pygmaea* with a complete turf layer and more than 90% vegetation coverage. The soil in this plot is typical of alpine meadows and is rich in organic matter. In contrast, the degraded grassland is primarily dominated by non-grass herbaceous plants, such as *Aster tataricus* (*A. tataricus*), *Chenopodium glaucum* (*C. glaucum*), and *Przewalskia tangutica* (*P. tangutica*). This degradation is marked by the displacement of the original vegetation (with 0 coverage), leading to the loss of the turf layer and a shift in the soil composition to a sandy texture.

Our experiment used a two-factor design incorporating both warming and degradation variables, leading to four distinct treatment groups (Figure 1): non-warming non-degraded (NWND), non-warming degraded (NWD), warming non-degraded (WND), and warming degraded (WD). Each treatment had four replicates, resulting in a total of 16 plots. We utilized OTC for passive warming. In May 2013, four open-top chambers (OTC) were established in degraded and non-degraded alpine grassland plots to investigate the impact of warming. These chambers were cylindrical, constructed from plastic that allowed solar radiation to pass through, and measured 0.5 m in height, with a base diameter of 1.5 m and a top diameter of 1.0 m. The warming treatment resulted in a significant increase in the seasonal mean soil temperature of approximately 1.0 °C from May to September 2014 [53–55].

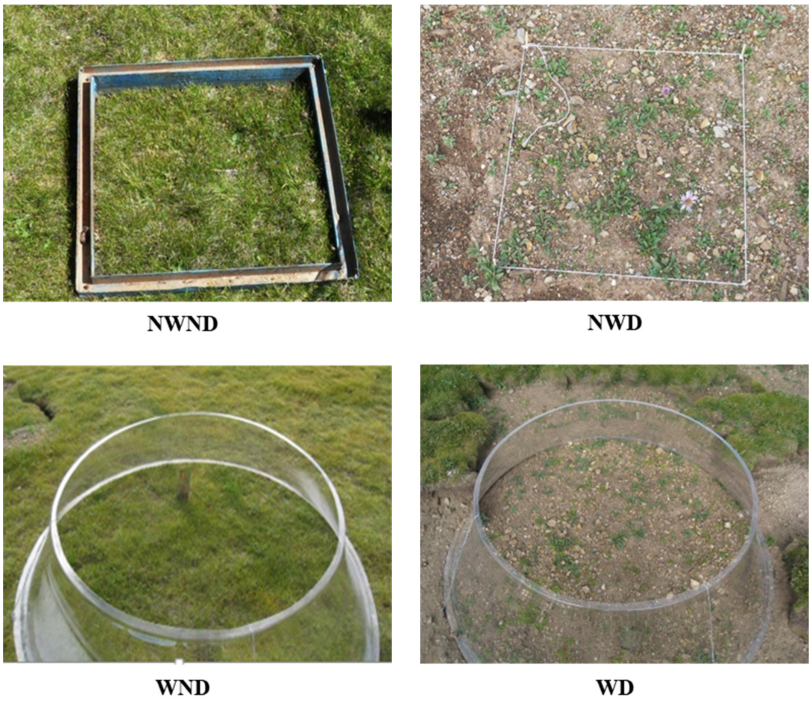
NWND
NWD
WND
WD

Figure 1. The experiment treatments in the warming plot (NMND: non-warming non-degraded; NWD: non-warming degraded; WND: warming non-degraded; WD: warming degraded).

In the warming and degradation experimental platform of the alpine meadow at Nagqu, a dual-labeling experiment was carried out. In mid-August 2014, during the peak of the growing season, we conducted the marking experiment. Four subplots were established within each experimental treatment. Each subplot measured 15 cm by 15 cm and had a depth of 10 cm, containing approximately 2700 g of soil, calculated based on a bulk density of 1.2 g cm^{-3}. We used 2-$^{13}C_2{}^{15}N$ labeled glycine (99.98% atomic content) as the organic N source and $(^{15}NH_4)_2SO_4$ and $Na^{15}NO_3$ (98.2% atomic content) as inorganic N sources. Considering the soil's dissolved organic N (DON) content [33], amino acids typically account for 5–20% of DON; we used the higher ratio for our calculations, resulting in approximately 3.6 μg g^{-1} of amino acids. Our objective was to maintain the existing levels of available N in the soil solution to prevent fertilization effects while ensuring the adequate labeling of the amino acids. An iron frame measuring 15 cm by 10 cm was placed in each plot. Solutions of NH_4^+-^{15}N, NO_3^--^{15}N, and dual-labeled glycine were injected into the soil layer between 0–10 cm depth (the main root distribution zone) using a syringe. The soil surface within each frame was divided into a grid with evenly spaced points, with 9 points per grid. At each point, 1 mL of the ^{15}N-labeled solution was injected. The syringe needle was inserted 8 cm deep into the soil, and the solution was evenly distributed through the soil layer by pushing the syringe plunger while simultaneously withdrawing the needle. The N labeling in the soil was set at 0.02 g N m^{-2}. To minimize calculation errors, water injections (lacking ^{15}N) were used as controls in each respective plot.

2.3. Sample Collection and Chemical Analysis

Six hours after labeling, both the plants and soil from the top 10 cm layer inside the frame were completely harvested and swiftly transported to the laboratory. We endeavored to separate the plant roots by species as precisely as possible, followed by a quick rinse with deionized water. Subsequently, they were treated with a 0.5 mmol L^{-1} solution of $CaCl_2$ for 2–3 min to detach any adhered ^{13}C and ^{15}N, and then rinsed again with deionized water. The plant samples were then dried at 65 °C for a minimum of 48 h until a constant weight was achieved, and the biomass was determined through weighing. Post-grinding, the samples were analyzed for their total carbon and N contents, as well as their ^{13}C and ^{15}N abundances. Fresh soil samples were sifted through a 2 mm sieve to remove larger debris. Parameters such as $^{15}N\%$, $^{13}C\%$, microbial biomass carbon (MBC), and microbial biomass N (MBN) were then measured. A portion of the air-dried samples was ground and treated with HCl to eliminate inorganic carbon, facilitating the measurement of soil organic carbon and total N.

The microbial biomass carbon and N content were determined by comparing the concentrations of extractable carbon and N in the extracts from both fumigated and non-fumigated soil samples. These samples were extracted using a 0.5 mol L^{-1} K_2SO_4 solution with a soil-to-water ratio of 1:5. The conversion factors used were KEC (0.45) and KEN (0.54). All results were derived from the dry weight of the soil. For the plant samples, after drying, they were ground into powder using a ball mill (M2, Fa. Retsch, Haan, Germany). About 2.00 mg of this powdered sample was placed into a small tin cup, sealed, and then analyzed for N (N%) and carbon (C%) content, as well as $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios. These measurements were conducted using a Flash EA1112 and the interface of Conflo III (MAT 253, Finnigan MAT, Bremen, Germany), with a testing error below 0.1 ‰. Similarly, a specific quantity of the extract obtained from potassium sulfate extraction was dried, ground into a powder, and analyzed for its N (N%) and carbon (C%) content, as well as the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios, maintaining the same level of testing accuracy.

2.4. Calculations

The soil chemical properties between the two sites—degraded and non-degraded plots—as well as the plant species biomass, and N content in each treatment, have been previously reported by our group. Detailed data can be found in Pang [13]. Plant community biomass, soil microbial biomass carbon, and soil microbial biomass nitrogen in each treatment are described in Figure S1 of the Supplementary Material. The atomic percent excess (APE) of ^{15}N is calculated as the difference in atom% ^{15}N between labeled and control samples. The contribution rate (%) for different N forms is determined by dividing the absorption amount (g m^{-2}) of each N form by the total N absorption (g m^{-2}) [56]. The formula is as follows:

$$\mathrm{APE}(\%) = \mathrm{Atom}\%_{\mathrm{labeled}} - \mathrm{Atom}\%_{\mathrm{control}} \quad (1)$$

where APE (%) is the atomic percent excess, $\text{Atom\%}_{\text{labeled}}$ is the atom% excess ^{15}N in the labeled treatment, and $\text{Atom\%}_{\text{control}}$ is the atom% excess ^{15}N under natural abundance conditions.

$$^{15}\text{N}_{\text{uptake}-\text{soil}}\left(\text{mg m}^{-2}\right) = \frac{\text{TDN} \times (\text{Atom\%}_{\text{labeled}} - \text{Atom\%}_{\text{control}}) \times 15}{\text{Atom\%}_{\text{labeled}} \times 15 + (100\% - \text{Atom\%}_{\text{labeled}}) \times 14} \quad (2)$$

$$^{15}\text{N}_{\text{uptake}-\text{MBN}}\left(\text{mg m}^{-2}\right) = \frac{\left[^{15}\text{N}_{\text{uptake}-\text{soil}}\right]_{\text{F}} - \left[^{15}\text{N}_{\text{uptake}-\text{soil}}\right]_{\text{NF}}}{0.54} \quad (3)$$

where $^{15}\text{N}_{\text{uptake–soil}}$ (mg m^{-2}) denotes the quantity of ^{15}N absorbed by soil per unit area. TDN (mg m^{-2}) indicates the total soil N content per unit area. $\text{Atom\%}_{\text{labeled}}$ represents the atomic percent excess of ^{15}N (atom% excess ^{15}N) in the labeled treatment, whereas $\text{Atom\%}_{\text{control}}$ signifies the atomic percent excess of ^{15}N (atom% excess ^{15}N) in plants under natural abundance conditions. The conversion factor KEN used here is 0.54. $^{15}\text{N}_{\text{uptake–MBN}}$ (mg m^{-2}) indicates the quantity of ^{15}N absorbed by microbial biomass N per unit area. [$^{15}\text{N}_{\text{uptake-soil}}$]F (mg m^{-2}) denotes the ^{15}N absorption in fumigated soil per unit area, while [$^{15}\text{N}_{\text{uptake-soil}}$]NF (mg m^{-2}) represents the ^{15}N absorption in non-fumigated soil, also calculated per unit area.

The ^{15}N recovery rate for both plants and microbes represents the percentage ratio of the total ^{15}N absorbed by these organisms to the total ^{15}N applied in the sample plot, as defined by Lin et al. [56].

$$^{15}\text{N}_{\text{recovery}}\ (\%) = \frac{^{15}\text{N}_{\text{uptake}}}{^{15}\text{N}_{\text{added}}} \times 100 \quad (4)$$

where $^{15}\text{N}_{\text{recovery}}$ (%) denotes the recovery rate of ^{15}N in both plants and microbes. $^{15}\text{N}_{\text{uptake}}$ (mg m^{-2}) is the quantity of ^{15}N absorbed by plants per unit area, while $^{15}\text{N}_{\text{added}}$ (mg m^{-2}) represents the amount of ^{15}N labeled on plants per unit area. The total $^{15}\text{N}_{\text{recovery}}$ (%) for plants and microbes is the aggregate recovery of NH_4^+-^{15}N, NO_3^--^{15}N, and glycine-^{15}N.

2.5. Statistical Analysis

A two-way ANOVA was used to test the differences in the effects of warming and degradation on total ^{15}N recovery by soil microbes and the plant community and the ratio of total ^{15}N recovery by microbial biomass to ^{15}N recovery by plants at the community level from ammonium-^{15}N, nitrate-^{15}N, and glycine-^{15}N six hours after ^{15}N injection at 0–10 cm soil depth. A one-way ANOVA followed by Duncan's multiple range test was used to separately examine the differences in total ^{15}N recovery by plants at the species level and the ratio of total ^{15}N recovery by microbial biomass to ^{15}N recovery by plants at the species level from ammonium-^{15}N, nitrate-^{15}N, and glycine-^{15}N in undegraded and degraded plots in alpine meadow. A multifactorial analysis of variance was performed to test the main and interactive effects of degradation, warming, and N form (NO_3^--^{15}N, NH_4^+-^{15}N, glycine-^{15}N) on ^{15}N recovery by the microbial biomass, ^{15}N recovery by plants at the community levels, and the ratio of the ^{15}N recovery by the microbial biomass to the ^{15}N recovery by the plants at the community levels.

The effects of treatment on the ^{15}N recovery were tested using linear mixed models with the plant species (*K. pygmaea* and *A. tataricus*), form of N (NO_3^--^{15}N, NH_4^+-^{15}N, glycine-^{15}N), and warming (unwarmed and warmed) as the fixed factors in the non-degraded plots and with the plant species (*C. glaucum* and *A. tataricus*), form of N (NO_3^--^{15}N, NH_4^+-^{15}N, glycine-^{15}N), and warming (unwarmed and warmed) as fixed factors in the degraded plots. Duncan's new multiple range test was used for post hoc comparisons. Post hoc tests were used to examine the differences between non-warming and warming plots.

For all the ANOVAs, normality was checked with the Kolmogorov–Smirnov test, and the assumption of homogeneity of variances was checked using Levene's test. If the assumptions of normality and homogeneity of variances were not met, the data were log- or square-root-transformed prior to analysis. Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA), and significance was considered at the $p < 0.05$ level.

3. Results

3.1. Effects of Degradation and Warming on the Recovery of ^{15}N in Microbes

The variance analysis indicates that degradation, warming, and N type significantly interact, affecting total microbial ^{15}N recovery (Table 1). Specifically, degradation reduces microbial ^{15}N recovery by 52% (Figure 2a,b). The effects of warming on ^{15}N recovery of different forms also depend on degradation. In non-degraded areas, warming significantly decreases the microbial recovery of NH_4^+-^{15}N, NO_3^--^{15}N, and particularly glycine-^{15}N by 49% (Figure 2c). In contrast, in degraded plots, warming markedly lowers microbial NH_4^+-^{15}N recovery by 80%, NO_3^--^{15}N by 45% but increases glycine-^{15}N recovery by 653% (Figure 2d).

Table 1. Effects of degradation, warming, nitrogen (N) form (NO_3^--^{15}N, NH_4^+-^{15}N, and glycine-^{15}N) and their interactions on the ^{15}N recovery of soil microbes and plant community and their ratios.

Effect	df	^{15}N Recovered in Microbial Biomass		Plant ^{15}N Recovery		Ratio of Microbial ^{15}N Recovery to Plant ^{15}N Recovery	
		F Value	*p* Value	F Value	*p* Value	F Value	*p* Value
Degradation	1	56.41	**<0.001**	85.15	**<0.001**	2.03	0.17
Warming	1	11.73	**0.002**	26.89	**<0.001**	4.38	**0.05**
N type	2	2.92	0.07	35.64	**<0.001**	17.19	**<0.001**
Degradation × Warming	1	3.93	0.06	28.62	**<0.001**	0.45	0.51
Degradation × N type	2	1.35	0.28	13.68	**<0.001**	13.26	**<0.001**
Warming × N type	2	3.97	**0.03**	17.75	**<0.001**	0.92	0.41
Degradation × Warming × N type	2	13.94	**<0.001**	7.90	**0.002**	39.24	**<0.001**

Data were collected six hours post ^{15}N injection, focusing on the 0–10 cm soil depth. *p* values for significant effects are presented in bold.

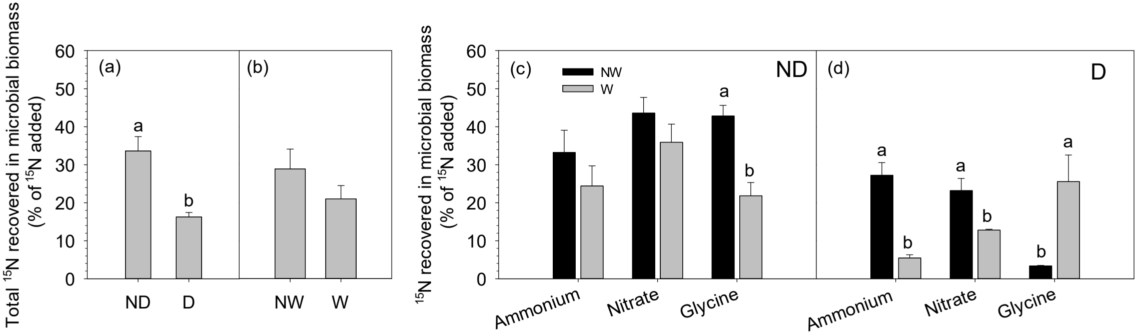

Figure 2. Total ^{15}N recovered in microbial biomass (% of added ^{15}N) for both overall (**a**,**b**) and specific N types (NO_3^--^{15}N, NH_4^+-^{15}N, and glycine-^{15}N) (**c**,**d**) measured six hours post-^{15}N-injection at 0–10 cm soil depth in alpine meadow. Significant differences ($p < 0.05$) are marked with different letters above bars, comparing no degradation (ND) to degradation (D) in panels a, and no warming (NW) to warming (W) in panels (**b**–**d**).

3.2. Effects of Degradation and Warming on the Recovery of ^{15}N in Plants

On a community scale, warming, degradation, and N type significantly interact, affecting the plant community's ^{15}N recovery (Table 1). Specifically, degradation reduced the community's ^{15}N recovery by 61%, while warming lowered it by 40%. In non-degraded plots, warming decreased the plant community's ^{15}N recovery by 52% (Figure 3a). In terms of different N forms, in non-degraded areas, warming notably decreased the plant recovery of NH_4^+-^{15}N and NO_3^--^{15}N by 75% and 45%, respectively, but it significantly boosted the recovery of glycine-^{15}N by 45%. Conversely, in degraded plots, warming markedly lowered NH_4^+-^{15}N recovery by 40% but increased glycine-^{15}N recovery by 114% (Figure 3c).

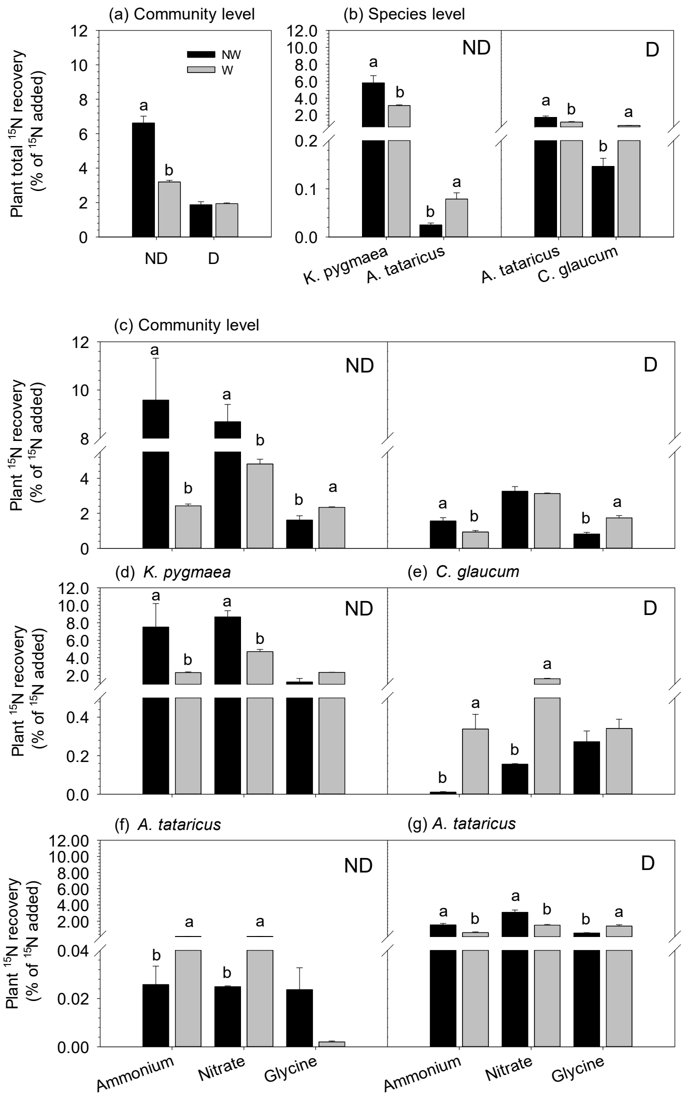

Figure 3. Total ^{15}N recovery by plants (% of added 15N) at both community (**a**) and species level (**b**) and the recovery from NO_3^--^{15}N, NH_4^+-^{15}N, and glycine-^{15}N at community (**c**) and species level (**d**–**g**), measured six hours post-^{15}N-injection at 0–10 cm soil depth in alpine meadow. Significant differences ($p < 0.05$) are marked with different letters above bars, comparing no warming (NW) to warming (W). D and ND represent degradation and no degradation, respectively.

On a species-specific basis, warming had a significant impact on the total ^{15}N recovery of plants in both non-degraded and degraded grasslands. The effects of warming on dominant species and non-dominant species are consistent in undegraded and degraded plots. Specifically, in non-degraded areas, warming notably decreased the total ^{15}N recovery of the dominant plant, *K. pygmaea*, by 46%, while simultaneously enhancing the total ^{15}N recovery of the non-dominant plant, *A. tataricus*, by 218% (Figure 3b). Conversely, in degraded grassland, warming reduced the total ^{15}N recovery of the dominant plant, *A. tataricus*, by 32%, but dramatically increased the total ^{15}N recovery of the non-dominant plant, *C. glaucum*, by 419% (Figure 3b). In terms of different N forms, in non-degraded areas, warming notably lowered the dominant plant *K. pygmaea*'s recovery of NH_4^+-^{15}N and NO_3^--^{15}N by 69% and 46%, respectively. In contrast, for the non-dominant plant *A. tataricus*, warming markedly enhanced its recovery of NH_4^+-^{15}N and NO_3^--^{15}N by 352% and 373%, respectively (Figure 3d,f). In degraded sites, warming enhanced the non-dominant plant *C. glaucum*'s recovery of NH_4^+-^{15}N and NO_3^--^{15}N by 2845% and 931%, respectively (Figure 3e). For the dominant *A. tataricus* in these degraded plots, warming decreased its recovery of NH_4^+-^{15}N and NO_3^--^{15}N by 62% and 51%, respectively, but increased its recovery of glycine-^{15}N by 160% (Figure 3g).

3.3. Effects of Degradation and Warming on the Ratio of Microbial to Plant ^{15}N Recovery

On a community scale, warming, degradation, and the type of N interaction significantly influenced the ratio of total microbial ^{15}N recovery to total plant ^{15}N recovery (Table 1). Warming mitigates the effects of alpine meadow degradation on nitrogen allocation among soil microbes and plants. In unwarmed plots, degradation significantly elevated the total ^{15}N recovery ratio of soil microbes to plants by 60%. However, in warmed plots, the impact of degradation on this ratio was reduced (Figure 4a). For different N forms, in non-degraded areas, warming boosted the recovery ratios for NH_4^+-^{15}N by microbes and plants by 165%, but markedly reduced their glycine-^{15}N recovery ratio by 66%. Conversely, in degraded plots, warming led to a reduction in NH_4^+-^{15}N recovery ratios by 66%, while significantly enhancing the glycine-^{15}N recovery ratio by 232% (Figure 4c).

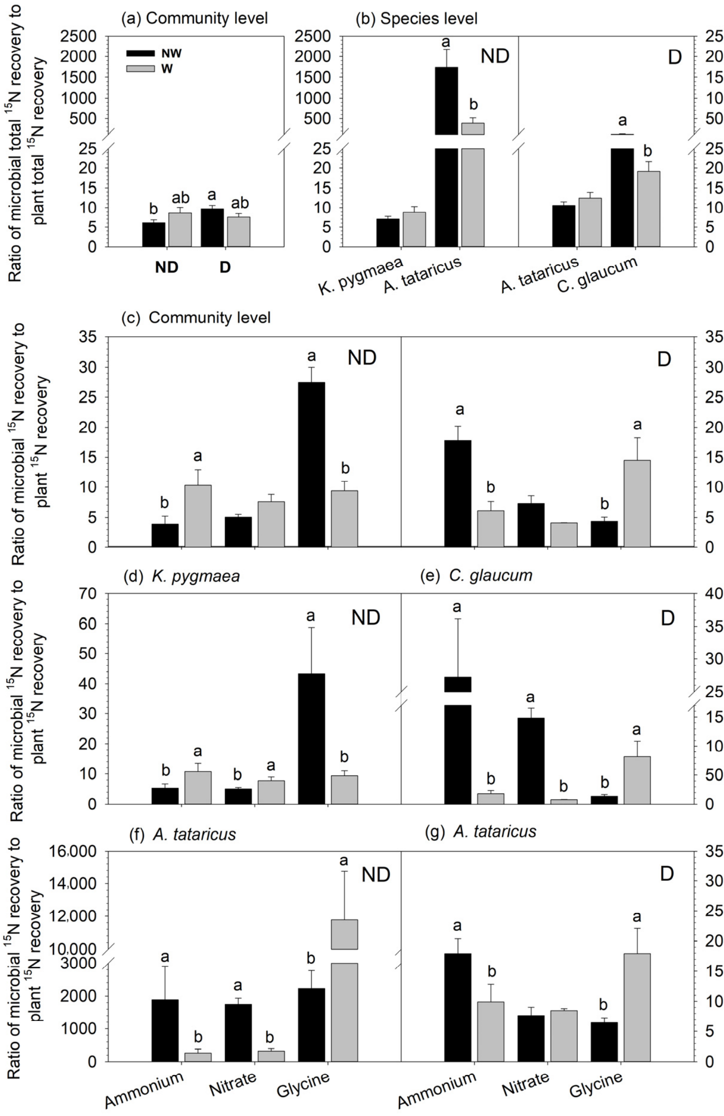

Figure 4. Ratios of total ^{15}N recovery in soil microbials to plants at the community (**a**) and species level (**b**) and ratios of total ^{15}N recovery in soil microbials to plants from NO_3^--^{15}N, NH_4^+-^{15}N, and glycine-^{15}N at community (**c**) and species level (**d**–**g**) six hours post-^{15}N-injection at 0–10 cm soil depth in alpine meadow. Significant differences ($p < 0.05$) are marked with different letters above bars, comparing no warming (NW) to warming (W). D and ND represent degradation and no degradation, respectively.

On a species level, warming significantly altered the ratios of total ^{15}N recovery between soil microbes and non-dominant plants in both non-degraded and degraded plots. In non-degraded plots, the ratio decreased by 78% for *A. tataricus*, while in degraded plots, it decreased by 85% for *C. glaucum* (Figure 4b). In terms of different N forms, in non-degraded plots, warming markedly raised the NH_4^+-^{15}N and NO_3^--^{15}N recovery ratios for microbes and dominant plant *K. pygmaea* by 101% and 53%, respectively, while it reduced their glycine-^{15}N recovery ratio by 78% (Figure 4d). Regarding microbes and non-dominant *A. tataricus*, warming significantly decreased their recovery ratios for NH_4^+-^{15}N and NO_3^--^{15}N by 86% and 82%, respectively, but it boosted glycine-^{15}N recovery ratio by 429% (Figure 4f). In degraded plots, warming reduced the NH_4^+-^{15}N and NO_3^--^{15}N recovery ratios for microbes and non-dominant *C. glaucum* by 99% and 95%, respectively, but it increased the glycine-^{15}N recovery ratio by 495% (Figure 4e). For microbes and dominant *A. tataricus* in degraded areas, warming notably decreased the NH_4^+-^{15}N recovery ratio by 45% while increasing glycine-^{15}N recovery ratio by 178% (Figure 4g).

4. Discussion

4.1. N Allocation between Community-Level Plants and Microbes

Our study indicated that degradation has significantly diminished soil microbial ^{15}N recovery, consistent with the results reported by Lai [32], where the recovery of ^{15}N by microorganisms decreased in degraded plots due to a reduction in available soil N to a low level. Warming reduced microbial inorganic N recovery but enhanced organic N recovery in degraded plots, aligning with plant communities' N recovery responses to warming. Jiang et al. [45] posit that plants' rapid adjustment of N strategies under warming is a key factor in sustaining community equilibrium.

Warming mitigates the impact of alpine meadow degradation on nitrogen allocation among soil microbes and plants. Specifically, in unwarmed plots, degradation resulted in a significant increase of 60% in the total ^{15}N recovery ratio of soil microbes compared to plants. However, in warmed plots, the effect of degradation on this ratio was reduced (Figure 4a). Our findings revealed that, in both degraded and non-degraded plots, the ratio of microbial ^{15}N recovery to plant ^{15}N recovery surpasses 1, regardless of warming, indicating microbial dominance in N utilization. This could stem from microbes' larger surface area, rapid turnover, and movement with soil moisture, which boost their competitiveness in short-term labeling experiments [57,58], and represent a key adaptation against N loss [9,59]. This mirrors the results of Zogg et al. [60] in northern broadleaf forests, where plants retained less N than soil microbes, highlighting microbes' pivotal role in N fixation in short-term studies. This dominance in inorganic N competition is a common theme in many short-term ^{15}N labeling studies [31]. However, Han's [61] temperate forest study suggested that plants can outcompete microbes for N in short-term labeling experiments. Over time, N released by microbes can be absorbed by plants, giving them a long-term competitive edge due to their longer lifespans [9]. Xu's [62] research on the Qinghai-Tibetan Plateau alpine meadow also found that soil microbes and plants exhibit varying N-fixing capabilities over different experimental phases. Variations in findings might be attributed to differences in N turnover between plant roots and soil microbes, factors like soil N availability, microbial distribution, timing mechanisms in microbes, plant root turnover, ecosystem types, seasonal changes, plant and microbial species, and the duration of the experiment [9,63,64].

Elevated soil temperature triggers alterations in nutrient cycling and microbial activity, with varying impacts on different vegetation types. Zheng et al. [65] and Guan et al. [66] found that short-term warming decreases carbon constraints for soil microorganisms in tropical forests, while long-term warming mitigates carbon limitations in alpine shrublands, with limited influence on grasslands [67]. However, our study revealed distinct responses to warming in terms of carbon limitation in non-degraded and degraded alpine meadows. In non-degraded areas, warming significantly increased the recovery ratios of NH_4^+-^{15}N by both microorganisms and plants by 165%, but concurrently decreased their glycine-^{15}N recovery ratio by 66%. Conversely, in degraded plots, warming decreased NH_4^+-^{15}N

recovery ratios by 66% but unexpectedly increased the glycine-^{15}N recovery ratio by a substantial 232% (Figure 4c). This indicates that in undegraded sites, the competition for inorganic nitrogen by microorganisms increases, while the competition for organic nitrogen decreases, indicating that warming has alleviated carbon limitations. This alleviation may be related to the accumulation of soil available carbon with increasing temperature, thereby reducing the microbial demand for carbon [68]. Conversely, in degraded sites, the competition for organic nitrogen by microorganisms increases, while the competition for inorganic nitrogen decreases, suggesting intensified carbon limitations. This intensification may be due to the reduction in soil carbon stocks caused by soil degradation, coupled with warming accelerating the decomposition of organic carbon and changes in plant growth, further affecting the distribution and accumulation of soil carbon. Overall, warming has a mitigating effect on carbon limitations in undegraded meadows but intensifies carbon limitations in degraded meadows, which has a significant impact on the survival and activity of soil microorganisms.

4.2. N Distribution between Plant Species and Microbes

In non-degraded alpine meadows, N recovery in dominant plants mirrored that of the entire plant community. Our research revealed that warming notably diminished inorganic N recovery while boosting organic N uptake in dominant plants in non-degraded areas, aligning with similar findings in alpine meadows [63]. This indicated that dominant plants swiftly adapted their N strategies under warming, favoring the absorption of prevalent organic N to maintain their dominant status [11,45]. Interestingly, our study suggested that in non-degraded ecosystems, non-dominant plants responded to warming inversely to dominant plants regarding N absorption, implying a complementary N utilization strategy between these plant groups to ensure their coexistence in the community. However, degradation disrupted this equilibrium. Typically, non-dominant species in non-degraded plots may become dominant in severely degraded alpine meadows. In contrast, severely degraded plots often featured annual or sporadic species. Consequently, in degraded areas, the responses of dominant and non-dominant plants to warming might not exhibit such complementarity. Our findings indicated that warming in degraded sites enhanced both inorganic and organic N uptake in dominant plants, while non-dominant plants showed increased organic N absorption but reduced inorganic N uptake.

Different plant species and microorganisms exhibited varied responses to N competition based on the degradation status of alpine meadows when subjected to warming. In undegraded areas, warming intensified the competition for organic N between microorganisms and the plant *K. pygmaea*, while reducing their competition for inorganic N. This aligns with the findings of Jiang et al. [63], who observed that microorganisms' ability to compete for organic N diminished when temperatures were higher in the peak growing season compared to the early season. Jiang et al. [45] argued that in alpine meadows, higher temperatures favored plant biomass accumulation, thereby increasing competition for N between plants and microorganisms. Our study indicated that in undegraded plots, the distribution of total ^{15}N recovery between microorganisms and the non-dominant species *A. tataricus* significantly decreased under warming conditions. This suggested that warming strengthened the competition between the non-dominant species *A. tataricus* and the dominant species *K. pygmaea*, as well as with soil microorganisms, potentially enhancing biodiversity in alpine meadows. The competitive dynamics for different N forms between microorganisms and plant species are crucial for maintaining plant diversity [32,69]. Similarly, in degraded plots, warming markedly reduced the allocation of inorganic N absorption between microorganisms and plant species but increased it for organic N absorption. This implies that under degradation, competition intensifies between the non-dominant species *C glaucum.* and the dominant species *A. tataricus*, as well as with microorganisms, favoring the growth of non-dominant species at the expense of dominant ones. Consequently, warming tends to decrease the dominance of certain species within the community, further exacerbating the degradation of *K. pygmaea* alpine meadows.

The trends of total ^{15}N recovery by dominant and non-dominant species remain consistent in both non-degraded and degraded sites. Regardless of degradation status, warming enhances the competitiveness of non-dominant plants by increasing their ability to acquire total ^{15}N. This is advantageous for the growth of non-dominant species. On the other hand, from the perspective of competition between plants and microorganisms, although microorganisms still maintain a dominant position, warming also improves the competitiveness of non-dominant plants. This shift in the competitive balance favors the non-dominant species, but it is disadvantageous to the dominant species. Consequently, this process increases the biodiversity of alpine meadows, yet it may also hasten the degradation of alpine meadows.

5. Conclusions

Alpine meadow degradation significantly reduced soil microbial N accumulation compared to non-degraded areas. In non-degraded alpine meadows, warming had a marked impact on soil microbial organic N levels, resulting in their decline. Conversely, in degraded alpine meadows, warming led to a reduction in both NH_4^+-^{15}N and NO_3^--^{15}N recovery. Simultaneously, plant recovery of NH_4^+-^{15}N and NO_3^--^{15}N decreased, while glycine-^{15}N recovery increased in non-degraded plots. The responsiveness of soil microbial N recovery to plants under rising temperatures was strongly influenced by alpine meadow quality. Specifically, in non-degraded alpine meadows, warming favored NH_4^+-^{15}N recovery but suppressed glycine-^{15}N recovery. However, in degraded alpine meadows, warming had the opposite effect, decreasing NH_4^+-^{15}N recovery and increasing glycine-^{15}N recovery. These findings suggest that warming exacerbates carbon limitations for soil microbes in degraded alpine meadows, emphasizing the need to prioritize carbon accumulation during restoration efforts to mitigate the negative impacts of warming.

Regardless of degradation status, the trend of total ^{15}N recovery remains consistent among dominant and non-dominant species in alpine meadows. Warming enhances the non-dominant plants' capacity to acquire total ^{15}N, subsequently boosting their competitiveness. This shift results in a boost to biodiversity, yet it may also hasten the degradation of alpine meadows. This underscores the urgency of closely monitoring species interactions and their influence on ecosystem stability amidst climate change.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14030508/s1, Figure S1: Separate effects of warming on plant community biomass (a–c), MBC and MBN content (d–f) in plots with and without degradation in alpine meadow.

Author Contributions: Conceptualization, Z.P., L.J., S.W. and Y.W.; data curation, Z.P. and L.J.; formal analysis, Z.P., L.J. and Z.W.; funding acquisition, Z.P., L.J., S.W. and Y.W.; investigation, Z.P., L.J., Z.W., R.P., W.L., M.C., W.Z., L.T., B.Z., L.L. and S.Z.; methodology, Z.P., L.J. and X.X.; project administration, L.J., S.W. and Y.W.; resources, L.J., S.W. and Y.W.; writing—original draft, Z.P. and G.W.; writing—review and editing, L.J., X.N., X.X., Y.H., X.C., S.W. and Y.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Lhasa Key Science and Technology Plan (LSKJ202425); the Second Tibetan Plateau Scientific Expedition and Research Program (2019QZKK0302-02, 2019QZKK0304-02), Project of the Naqu Science and Technology Bureau of Xizang Autonomous Region of China (NQKJ-2023-05), the Joint Research on Ecological Conservation and High-Quality Development of the Yellow River Basin program (2022-YRUC-01-0102), and the Fundamental Research Funds for the Central Universities.

Data Availability Statement: The data that support this study are available upon reasonable request from the corresponding author.

Acknowledgments: Thank you to all those who helped with this study and to the research projects that sponsored it.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Vitousek, P.M.; Howarth, R.W. Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* **1991**, *13*, 87–115. [CrossRef]
2. Wang, Z. *Plant Physiology*; Scientific and Technical Documentation Press: Beijing, China, 2006.
3. Zayed, O.; Hewedy, O.A.; Abdelmoteleb, A.; Ali, M.; Youssef, M.S.; Roumia, A.F.; Seymour, D.; Yuan, Z.-C. Nitrogen Journey in Plants: From Uptake to Metabolism, Stress Response, and Microbe Interaction. *Biomolecules* **2023**, *13*, 1443. [CrossRef] [PubMed]
4. Robson, T.M.; Baptist, F.; Clément, J.-C.; Lavorel, S. Land use in subalpine grasslands affects nitrogen cycling via changes in plant community and soil microbial uptake dynamics. *J. Ecol.* **2010**, *98*, 62–73. [CrossRef]
5. Holst, J.; Liu, C.; Brüggemann, N.; Butterbach-Bahl, K.; Zheng, X.; Wang, Y.; Han, S.; Yao, Z.; Yue, J.; Han, X. Microbial N Turnover and N-Oxide (N_2O/NO/NO_2) Fluxes in Semi-arid Grassland of Inner Mongolia. *Ecosystems* **2007**, *10*, 623–634. [CrossRef]
6. Müller, C.; Rütting, T.; Kattge, J.; Laughlin, R.J.; Stevens, R.J. Estimation of parameters in complex 15N tracing models by Monte Carlo sampling. *Soil Biol. Biochem.* **2007**, *39*, 715–726. [CrossRef]
7. Chen, J.; Carrillo, Y.; Pendall, E.; Dijkstra, F.A.; Dave Evans, R.; Morgan, J.A.; Williams, D.G. Soil Microbes Compete Strongly with Plants for Soil Inorganic and Amino Acid Nitrogen in a Semiarid Grassland Exposed to Elevated CO_2 and Warming. *Ecosystems* **2015**, *18*, 867–880. [CrossRef]
8. Afkhami, M.E.; Almeida, B.K.; Hernandez, D.J.; Kiesewetter, K.N.; Revillini, D.P. Tripartite mutualisms as models for understanding plant–microbial interactions. *Curr. Opin. Plant Biol.* **2020**, *56*, 28–36. [CrossRef] [PubMed]
9. Kuzyakov, Y.; Xu, X. Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytol.* **2013**, *198*, 656–669. [CrossRef]
10. Albano, L.J.; Turetsky, M.R.; Mack, M.C.; Kane, E.S. Deep roots of Carex aquatilis have greater ammonium uptake capacity than shallow roots in peatlands following permafrost thaw. *Plant Soil* **2021**, *465*, 261–272. [CrossRef]
11. Alexandre, A.; Quintã, R.; Hill, P.W.; Jones, D.L.; Santos, R. Ocean warming increases the nitrogen demand and the uptake of organic nitrogen of the globally distributed seagrass Zostera marina. *Funct. Ecol.* **2020**, *34*, 1325–1335. [CrossRef]
12. Kuster, T.M.; Wilkinson, A.; Hill, P.W.; Jones, D.L.; Bardgett, R.D. Warming alters competition for organic and inorganic nitrogen between co-existing grassland plant species. *Plant Soil* **2016**, *406*, 117–129. [CrossRef]
13. Pang, Z.; Jiang, L.; Wang, S.; Xu, X.; Rui, Y.; Zhang, Z.; Luo, C.; Wang, Y. Differential response to warming of the uptake of nitrogen by plant species in non-degraded and degraded alpine grasslands. *J. Soils Sediments* **2019**, *19*, 2212–2221. [CrossRef]
14. Reay, M.K.; Marsden, K.A.; Powell, S.; Chadwick, D.R.; Jones, D.L.; Evershed, R.P. Combining field and laboratory approaches to quantify N assimilation in a soil microbe-plant-animal grazing land system. *Agric. Ecosyst. Environ.* **2023**, *346*, 108338. [CrossRef]
15. Liu, S.; Li, T.; Liu, B.; Xu, C.; Zhu, Y.; Xiao, L. Grassland vegetation decline is exacerbated by drought and can be mitigated by soil improvement in Inner Mongolia, China. *Sci. Total Environ.* **2024**, *908*, 168464. [CrossRef]
16. Wang, X.; Dong, S.; Gao, Q.; Zhang, Y.; Hu, G.; Luo, W. The rate of soil nitrogen transformation decreased by the degradation of alpine grasslands in the Qinghai Tibet Plateau. *Acta Pratacult. Sin.* **2018**, *27*, 1–9.
17. Chen, L.; Shi, J.; Wang, Y.; Ma, Y.; Dong, Q.; Hou, X. Study on Different Degraded Degrees Grassland Community Structure Characteristics of the Alpine Area. *Acta Agrestia Sin.* **2016**, *24*, 210–213.
18. Lu, H.; Yao, T.; Li, J.; Ma, W.; Chai, X. Vegetation and soil microorganism characteristics of degraded grasslands. *Acta Pratacult. Sin.* **2015**, *24*, 34–43.
19. Wang, J.; Zhang, D.; Cao, G.; Tian, Q. Regional characteristics of the alpine meadow degradation succession on the Qinghai-Tibetan Plateau. *Acta Pratacult. Sin.* **2013**, *22*, 1–10.
20. Wen, L.; Dong, S.; Li, Y.; Wang, X.; Li, X.; Shi, J.; Dong, Q. The impact of land degradation on the C pools in alpine grasslands of the Qinghai-Tibet Plateau. *Plant Soil* **2013**, *368*, 329–340. [CrossRef]
21. Li, J.; Li, X.; Lu, H.; Yao, T.; Wang, L.; Guo, C.; Shi, S. Characteristics of, and the correlation between, vegetation and N-fixing soil bacteria in alpine grassland showing various degrees of degradation. *Acta Ecol. Sin.* **2017**, *37*, 3647–3654.
22. He, F.; Zeng, W.; Wang, Z.; Zeng, H.; Wang, W. Effect of temperate grassland deterioration on soil microbiological characteristics at different depths. *Microbiol. China* **2016**, *43*, 702–711.
23. Bai, Y.; Li, X.; Wang, H.; Wen, W.; Li, R.; Li, G.; Wang, H. Nitrogen storage variations in typical steppe during grassland degradation progress—A case study of typical steppe in Xilin Hot City, lnner Mongolia. *Pratacult. Sci.* **2015**, *32*, 311–321.
24. Månsson, K.F.; Olsson, M.O.; Falkengren-Grerup, U.; Bengtsson, G. Soil moisture variations affect short-term plant-microbial competition for ammonium, glycine, and glutamate. *Ecol. Evol.* **2014**, *4*, 1061–1072. [CrossRef]
25. Xiang, X.; De, K.; Lin, W.; Feng, T.; Li, F.; Wei, X.; Wang, W. Different fates and retention of deposited NH_4^+ and NO_3^- in the alpine grasslands of the Qinghai-Tibet plateau. *Ecol. Indic.* **2024**, *158*, 111415. [CrossRef]
26. Hill, P.W.; Farrar, J.; Roberts, P.; Farrell, M.; Grant, H.; Newsham, K.K.; Hopkins, D.W.; Bardgett, R.D.; Jones, D.L. Vascular plant success in a warming Antarctic may be due to efficient nitrogen acquisition. *Nat. Clim. Chang.* **2011**, *1*, 50–53. [CrossRef]
27. Cort, C.E.; Stricker, E.; Crain-Wright, G.M.; Darrouzet-Nardi, A. Rapid foliar uptake of inorganic and amino acid nitrogen in three dryland plant species. *Res. Sq.* 2024; *preprint*. [CrossRef]
28. McKane, R.B.; Johnson, L.C.; Shaver, G.R.; Nadelhoffer, K.J.; Rastetter, E.B.; Fry, B.; Giblin, A.E.; Kielland, K.; Kwiatkowski, B.L.; Laundre, J.A.; et al. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature* **2002**, *415*, 68–71. [CrossRef]

29. Chen, X.; Zhang, T.; Guo, R.; Li, H.; Zhang, R.; Allan Degen, A.; Huang, K.; Wang, X.; Bai, Y.; Shang, Z. Fencing enclosure alters nitrogen distribution patterns and tradeoff strategies in an alpine meadow on the Qinghai-Tibetan Plateau. *CATENA* **2021**, *197*, 104948. [CrossRef]
30. Yao, B.; Shi, G.; Zhou, H.; Zhao, X.; Peñuelas, J.; Sardans, J.; Wang, F.; Wang, Z. Uneven distributions of unique species promoting N niche complementarity explain the stability of degraded alpine meadow. *Sci. Total Environ.* **2024**, *911*, 168487. [CrossRef]
31. Yu, C.; Liu, M.; Song, M.; Xu, X.; Zong, N.; Zhu, J.; Shi, P. Nitrogen enrichment enhances the competition for nitrogen uptake between Stipa purpurea and microorganisms in a tibetan alpine steppe. *Plant Soil* **2023**, *488*, 503–516. [CrossRef]
32. Lai, C.; Peng, F.; Sun, J.; Zhou, J.; Li, C.; Xu, X.; Chen, X.; You, Q.; Sun, H.; Sun, J.; et al. Niche differentiation and higher uptake of available nitrogen maintained the productivity of alpine meadow at early degradation. *Biol. Fertil. Soils* **2023**, *59*, 35–49. [CrossRef]
33. Xu, X.; Ouyang, H.; Cao, G.; Richter, A.; Wanek, W.; Kuzyakov, Y. Dominant plant species shift their nitrogen uptake patterns in response to nutrient enrichment caused by a fungal fairy in an alpine meadow. *Plant Soil* **2011**, *341*, 495–504. [CrossRef]
34. Wu, B.; Ding, M.; Zhang, H.; Devlin, A.T.; Wang, P.; Chen, L.; Zhang, Y.; Xia, Y.; Wen, J.; Liu, L.; et al. Reduced soil multifunctionality and microbial network complexity in degraded and revegetated alpine meadows. *J. Environ. Manag.* **2023**, *343*, 118182. [CrossRef]
35. Zhang, L.; Wang, X.; Wang, J.; Liao, L.; Lei, S.; Liu, G.; Zhang, C. Alpine meadow degradation depresses soil nitrogen fixation by regulating plant functional groups and diazotrophic community composition. *Plant Soil* **2022**, *473*, 319–335. [CrossRef]
36. Zhang, C.; He, J.; Shen, J. Global change field manipulative experiments and their applications in soil microbial ecology. *Chin. J. Appl. Ecol.* **2016**, *27*, 1663–1673.
37. Plymale, A.E.; Boerner, R.E.J.; Logan, T.J. Relative nitrogen mineralization and nitrification in soils of two contrasting hardwood forests: Effects of site microclimate and initial soil chemistry. *For. Ecol. Manag.* **1987**, *21*, 21–36. [CrossRef]
38. Hu, X.; Zhang, Y.; Wang, D.; Ma, J.; Xue, K.; An, Z.; Luo, W.; Sheng, Y. Effects of Temperature and Humidity on Soil Gross Nitrogen Transformation in a Typical Shrub Ecosystem in Yanshan Mountain and Hilly Region. *Life* **2023**, *13*, 643. [CrossRef]
39. Puri, G.; Ashman, M.R. Relationship between soil microbial biomass and gross N mineralisation. *Soil Biol. Biochem.* **1998**, *30*, 251–256. [CrossRef]
40. Loiseau, P.; Soussana, J.F. Effects of elevated CO2, temperature and N fertilization on nitrogen fluxes in a temperate grassland ecosystem. *Glob. Chang. Biol.* **2000**, *6*, 953–965. [CrossRef]
41. Mu, X.; Fan, X. A review on ecological models of soil N mineralization. *Chin. J. Appl. Ecol.* **1999**, *10*, 114–118.
42. Calderón, F.J.; Jackson, L.E.; Scow, K.M.; Rolston, D.E. Microbial responses to simulated tillage in cultivated and uncultivated soils. *Soil Biol. Biochem.* **2000**, *32*, 1547–1559. [CrossRef]
43. Bijoor, N.S.; Czimczik, C.I.; Pataki, D.E.; Billings, S.A. Effects of temperature and fertilization on nitrogen cycling and community composition of an urban lawn. *Glob. Chang. Biol.* **2008**, *14*, 2119–2131. [CrossRef]
44. Liu, B.; Wang, C.; Huang, J.; He, N.; Wang, Q.; Dong, K. Applications of ^{15}N Pool Dilution and ^{15}N Tracer Techniques in the Quantifying N Transformations of Grasslands: Methodology and Advances. *Acta Agrestia Sin.* **2014**, *22*, 1153–1162.
45. Jiang, L.; Wang, S.; Pang, Z.; Xu, X.; Kardol, P.; Li, Y.; Zhang, L.; Wang, Y.; Lei, Z.; Lan, Z.; et al. Plant organic N uptake maintains species dominance under long-term warming. *Plant Soil* **2018**, *433*, 243–255. [CrossRef]
46. Piao, S.; Fang, J.; He, J. Variations in Vegetation Net Primary Production in the Qinghai-Xizang Plateau, China, from 1982 to 1999. *Clim. Chang.* **2006**, *74*, 253–267. [CrossRef]
47. Shen, M.; Tang, Y.; Chen, J.; Zhu, X.; Zheng, Y. Influences of temperature and precipitation before the growing season on spring phenology in grasslands of the central and eastern Qinghai-Tibetan Plateau. *Agric. For. Meteorol.* **2011**, *151*, 1711–1722. [CrossRef]
48. Rui, Y. Effects of Warming and Grazing on Soil Nitrogen Transformation and the Associated Functional Microorganisms in an Alpine Meadow of the Qinghai-Tibet Plateau. Ph.D. Thesis, Graduate University of Chinese Academy of Sciences, Beijing, China, 2012.
49. Meng, F. Effects of Changing Temperature and Moisture on Phenological Sequences of Plant and Plant Community on the Alpine Meadow. Ph.D. Thesis, Institute of Tibetan Plateau Research Chinese Academy of Sciences, Beijing, China, 2016.
50. Wu, Z. *Flora of Tibet*; Science Press: Beijing, China, 1987.
51. Zhou, L. Process and Reasons of Rangeland Degeneration In Naqu Perfecture of Tibet Autonomous Region. *Mt. Res.* **1998**, *16*, 239–243.
52. Li, Y.; Wang, S.; Jiang, L.; Zhang, L.; Cui, S.; Meng, F.; Wang, Q.; Li, X.; Zhou, Y. Changes of soil microbial community under different degraded gradients of alpine meadow. *Agric. Ecosyst. Environ.* **2016**, *222*, 213–222. [CrossRef]
53. Cui, S.; Meng, F.; Suonan, J.; Wang, Q.; Li, B.; Liu, P.; Renzeng, W.; Lv, W.; Jiang, L.; Zhang, L.; et al. Responses of phenology and seed production of annual Koenigia islandica to warming in a desertified alpine meadow. *Agric. Meteorol.* **2017**, *247*, 376–384. [CrossRef]
54. Pan, X.; Lin, B.; Liu, Q. Effects of elevated temperature on soil organic carbon and soil respiration under subalpine co-niferous forest in western Sichuan Province, China. *Chin. J. Appl. Ecol.* **2008**, *19*, 1637–1643.
55. Li, N.; Wang, G.; Gao, Y.; Wang, J.; Liu, L. Effects of simulated warming on soil nutrients and biological characteristics of alpine meadow soil in the head waters region of the yangtze river. *Acta Pedol. Sin.* **2010**, *47*, 1214–1224.

56. Lin, X.; Zhang, Z.; Wang, S.; Hu, Y.; Xu, G.; Luo, C.; Chang, X.; Duan, J.; Lin, Q.; Xu, B.; et al. Response of ecosystem respiration to warming and grazing during the growing seasons in the alpine meadow on the Tibetan plateau. *Agric. For. Meteorol.* **2011**, *151*, 792–802. [CrossRef]
57. Owen, A.G.; Jones, D.L. Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. *Soil Biol. Biochem.* **2001**, *33*, 651–657. [CrossRef]
58. Wilkinson, A.; Hill, P.W.; Farrar, J.F.; Jones, D.L.; Bardgett, R.D. Rapid microbial uptake and mineralization of amino acids and peptides along a grassland productivity gradient. *Soil Biol. Biochem.* **2014**, *72*, 75–83. [CrossRef]
59. Bardgett, R.D.; Streeter, T.C.; Bol, R. Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. *Ecology* **2003**, *84*, 1277–1287. [CrossRef]
60. Zogg, G.P.; Zak, D.R.; Pregitzer, K.S.; Burton, A.J. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. *Ecology* **2000**, *81*, 1858–1866. [CrossRef]
61. Han, X. Effects of Soil Nitrogen to Plant-Microbial on Nitrogen Competition in Temperate Forest. Master's Thesis, Beijing Forestry University, Beijing, China, 2015.
62. Xu, X.; Bai, J.; Ouyang, H. Advances in Studies on Organic Nitrogen Uptake by Terrestrial Plants. *J. Nat. Resour.* **2011**, *26*, 715–724.
63. Jiang, L.; Wang, S.; Pang, Z.; Wang, C.; Kardol, P.; Zhong, L.; Yu, Q.; Lan, Z.; Wang, Y.; Xu, X.; et al. Effects of grazing on the acquisition of nitrogen by plants and microorganisms in an alpine grassland on the Tibetan plateau. *Plant Soil* **2017**, *416*, 297–308. [CrossRef]
64. Näsholm, T.; Huss-Danell, K.; Högberg, P. Uptake of organic nitrogen in the field by four agriculturally important plant species. *Ecology* **2000**, *81*, 1155–1161. [CrossRef]
65. Zheng, H.; Liu, Y.; Chen, Y.; Zhang, J.; Li, H.; Wang, L.; Chen, Q. Short-term warming shifts microbial nutrient limitation without changing the bacterial community structure in an alpine timberline of the eastern Tibetan Plateau. *Geoderma* **2020**, *360*, 113985. [CrossRef]
66. Guan, P.; Yang, J.; Yang, Y.; Wang, W.; Zhang, P.; Wu, D. Land conversion from cropland to grassland alleviates climate warming effects on nutrient limitation: Evidence from soil enzymatic activity and stoichiometry. *Glob. Ecol. Conserv.* **2020**, *24*, e01328. [CrossRef]
67. Qiao, L. Effects of Long-Term Warming and Fertilization on Soil Aggregate Stability and Nutrient Accumulation Mechanism in Alpine Meadow. Master's Thesis, Northwest A&F University, Xianyang, China, 2020.
68. Wang, Q.; Chen, L.; Xu, H.; Ren, K.; Xu, Z.; Tang, Y.; Xiao, J. The effects of warming on root exudation and associated soil N transformation depend on soil nutrient availability. *Rhizosphere* **2021**, *17*, 100263. [CrossRef]
69. Øien, D.-I.; Pedersen, B.; Kozub, Ł.; Goldstein, K.; Wilk, M. Long-term effects of nutrient enrichment controlling plant species and functional composition in a boreal rich fen. *J. Veg. Sci.* **2018**, *29*, 907–920. [CrossRef]