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

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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$_4$\textsuperscript{+}N, NO$_3$\textsuperscript{−}N) and organic (glycine-\textsuperscript{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 \textsuperscript{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$_4$\textsuperscript{+}N and NO$_3$\textsuperscript{−}N recovery by 80% and 45% on average but increased glycine-\textsuperscript{15}N recovery by 653%. Meanwhile, warming decreased the plant recovery of NH$_4$\textsuperscript{+}N and NO$_3$\textsuperscript{−}N by 75% and 45% but increased the recovery of glycine-\textsuperscript{15}N by 45% in non-degraded plots. Conversely, in degraded plots, warming markedly lowered NH$_4$\textsuperscript{+}N recovery by 40% but increased glycine-\textsuperscript{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 \textsuperscript{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 \textsuperscript{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$_4$\textsuperscript{+}N by 165% but reduced it for glycine-\textsuperscript{15}N by 66%. Conversely, in degraded plots, warming decreased the recovery ratio for NH$_4$\textsuperscript{+}N by 66% but increased it for glycine\textsuperscript{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
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$_2$O 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 *Przewalskiia 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].

![Figure 1](image-url)  
*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 $^{2}{\text{H}}^{13}$C$_2$$^{15}$N labeled glycine (99.98% atomic content) as the organic N source and ($^{15}$NH$_4$)$_2$SO$_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\(_4\)\(^{+}\)\(\text{^{15}N}\), NO\(_3\)\(^{-}\)\(\text{^{15}N}\), 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 \(\text{^{15}N}\)-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 \(\text{^{15}N}\)) 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 \(^{13}\text{C}\) and \(\text{^{15}N}\), and then rinsed again with deionized water. The plant samples were then dried at 65 \(^\circ\)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 \(^{13}\text{C}\) and \(\text{^{15}N}\) abundances. Fresh soil samples were sifted through a 2 mm sieve to remove larger debris. Parameters such as \(\text{^{15}N}\%\), \(^{13}\text{C}\%\), 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\(_2\)SO\(_4\) 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 \(\text{^{15}N}/\text{^{14}N}\) and \(^{13}\text{C}/\text{^{12}C}\) 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 \(\text{^{15}N}/\text{^{14}N}\) and \(^{13}\text{C}/\text{^{12}C}\) 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 \(\text{^{15}N}\) is calculated as the difference in atom% \(\text{^{15}N}\) 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:

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

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

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

where $^{15}$N 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. Atom%labeled represents the atomic percent excess of $^{15}$N (atom% excess $^{15}$N) in the labeled treatment, whereas Atom%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. $^{15}$N 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].

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

where $^{15}$Nrecovery (%) denotes the recovery rate of $^{15}$N in both plants and microbes. $^{15}$Nuptake (mg m$^{-2}$) is the quantity of $^{15}$N absorbed by plants per unit area, while $^{15}$Nadded (mg m$^{-2}$) represents the amount of $^{15}$N labeled on plants per unit area. The total $^{15}$N recovery (%) for plants and microbes is the aggregate recovery of $\text{NH}_4^+\cdot^{15}\text{N}$, $\text{NO}_3^-\cdot^{15}\text{N}$, and glycine-$^{15}\text{N}$.

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 ($\text{NO}_3^-\cdot^{15}\text{N}$, $\text{NH}_4\cdot^{15}\text{N}$, glycine-$^{15}\text{N}$) 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 $^{13}$N recovery were tested using linear mixed models with the plant species (K. pygmaea and A. tataricus), form of N ($\text{NO}_3^-\cdot^{15}\text{N}$, $\text{NH}_4\cdot^{15}\text{N}$, glycine-$^{15}\text{N}$), 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 ($\text{NO}_3^-\cdot^{15}\text{N}$, $\text{NH}_4\cdot^{15}\text{N}$, glycine-$^{15}\text{N}$), 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 \( ^{15} \text{N} \) in Microbes

The variance analysis indicates that degradation, warming, and N type significantly interact, affecting total microbial \( ^{15} \text{N} \) recovery (Table 1). Specifically, degradation reduces microbial \( ^{15} \text{N} \) recovery by 52% (Figure 2a,b). The effects of warming on \( ^{15} \text{N} \) recovery of different forms also depend on degradation. In non-degraded areas, warming significantly decreases the microbial recovery of \( \text{NH}_4^{+}-^{15} \text{N}, \text{NO}_3^{-}-^{15} \text{N}, \) and particularly glycine-\( ^{15} \text{N} \) by 49% (Figure 2c). In contrast, in degraded plots, warming markedly lowers microbial \( \text{NH}_4^{+}-^{15} \text{N} \) recovery by 80%, \( \text{NO}_3^{-}-^{15} \text{N} \) by 45% but increases glycine-\( ^{15} \text{N} \) recovery by 653% (Figure 2d).

Table 1. Effects of degradation, warming, nitrogen (N) form (\( \text{NO}_3^{-}, \text{NH}_4^{+}, \) and glycine-\( ^{15} \text{N} \)) and their interactions on the \( ^{15} \text{N} \) recovery of soil microbes and plant community and their ratios.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>( ^{15} \text{N} ) Recovered in Microbial Biomass</th>
<th>Plant ( ^{15} \text{N} ) Recovery</th>
<th>Ratio of Microbial ( ^{15} \text{N} ) Recovery to Plant ( ^{15} \text{N} ) Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F Value  ( p ) Value</td>
<td>F Value  ( p ) Value</td>
<td>F Value  ( p ) Value</td>
</tr>
<tr>
<td>Degradation</td>
<td>1</td>
<td>56.41 &lt;0.001</td>
<td>85.15 &lt;0.001</td>
<td>2.03 0.17</td>
</tr>
<tr>
<td>Warming</td>
<td>1</td>
<td>11.73 0.002</td>
<td>26.89 &lt;0.001</td>
<td>4.38 0.05</td>
</tr>
<tr>
<td>N type</td>
<td>2</td>
<td>2.92 0.07</td>
<td>35.64 &lt;0.001</td>
<td>17.19 &lt;0.001</td>
</tr>
<tr>
<td>Degradation × Warming</td>
<td>1</td>
<td>3.93 0.06</td>
<td>28.62 &lt;0.001</td>
<td>0.45 0.51</td>
</tr>
<tr>
<td>Degradation × N type</td>
<td>2</td>
<td>1.35 0.28</td>
<td>13.68 &lt;0.001</td>
<td>13.26 &lt;0.001</td>
</tr>
<tr>
<td>Warming × N type</td>
<td>2</td>
<td>3.97 0.03</td>
<td>17.75 &lt;0.001</td>
<td>0.92 0.41</td>
</tr>
<tr>
<td>Degradation × Warming × N type</td>
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<td>13.94 &lt;0.001</td>
<td>7.90 0.002</td>
<td>39.24 &lt;0.001</td>
</tr>
</tbody>
</table>

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

Figure 2. Total \( ^{15} \text{N} \) recovered in microbial biomass (% of added \( ^{15} \text{N} \)) for both overall (a,b) and specific N types (\( \text{NO}_3^{-}, \text{NH}_4^{+}, \) and glycine-\( ^{15} \text{N} \)) (c,d) measured six hours post-\( ^{15} \text{N} \)-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 \( ^{15} \text{N} \) in Plants

On a community scale, warming, degradation, and N type significantly interact, affecting the plant community’s \( ^{15} \text{N} \) recovery (Table 1). Specifically, degradation reduced the community’s \( ^{15} \text{N} \) recovery by 61%, while warming lowered it by 40%. In non-degraded plots, warming decreased the plant community’s \( ^{15} \text{N} \) recovery by 52% (Figure 3a). In terms of different N forms, in non-degraded areas, warming notably decreased the plant recovery of \( \text{NH}_4^{+}-^{15} \text{N} \) and \( \text{NO}_3^{-}-^{15} \text{N} \) by 75% and 45%, respectively, but it significantly boosted the recovery of glycine-\( ^{15} \text{N} \) by 45%. Conversely, in degraded plots, warming markedly lowered \( \text{NH}_4^{+}-^{15} \text{N} \) recovery by 40% but increased glycine-\( ^{15} \text{N} \) recovery by 114% (Figure 3c).
A. tataricus and C. glaucum plots. Specifically, in non-degraded areas, warming notably decreased the total of plant species and non-dominant species are consistent in undegraded and degraded grasslands. The effects of warming on 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.

Figure 3. Total \(^{15}\)N recovery by plants (% of added \(^{15}\)N) 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\textsubscript{4}^{+}-\textsuperscript{15}N and NO\textsubscript{3}^{-}-\textsuperscript{15}N by 62% and 51%, respectively, but increased its recovery of glycine-\textsuperscript{15}N by 160% (Figure 3g).

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

On a community scale, warming, degradation, and the type of N interaction significantly influenced the ratio of total microbial \textsuperscript{15}N recovery to total plant \textsuperscript{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 \textsuperscript{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\textsubscript{4}^{+}-\textsuperscript{15}N by microbes and plants by 165%, but markedly reduced their glycine-\textsuperscript{15}N recovery ratio by 66%. Conversely, in degraded plots, warming led to a reduction in NH\textsubscript{4}^{+}-\textsuperscript{15}N recovery ratios by 66%, while significantly enhancing the glycine-\textsuperscript{15}N recovery ratio by 232% (Figure 4c).

**Figure 4.** Ratios of total \textsuperscript{15}N recovery in soil microbials to plants at the community (a) and species level (b) and ratios of total \textsuperscript{15}N recovery in soil microbials to plants from NO\textsubscript{3}^{-}-\textsuperscript{15}N, NH\textsubscript{4}^{+}-\textsuperscript{15}N, and glycine-\textsuperscript{15}N at community (c) and species level (d–g) six hours post-\textsuperscript{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 $\text{NH}_4^{+} - ^{15}\text{N}$ and $\text{NO}_3^{-} - ^{15}\text{N}$ recovery ratios for microbes and dominant plant K. pygmaea by 101% and 53%, respectively, while it reduced their glycine-$^{15}\text{N}$ recovery ratio by 78% (Figure 4d). Regarding microbes and non-dominant A. tataricus, warming significantly decreased their recovery ratios for $\text{NH}_4^{+} - ^{15}\text{N}$ and $\text{NO}_3^{-} - ^{15}\text{N}$ by 86% and 82%, respectively, but it boosted glycine-$^{15}\text{N}$ recovery ratio by 429% (Figure 4f). In degraded plots, warming reduced the $\text{NH}_4^{+} - ^{15}\text{N}$ and $\text{NO}_3^{-} - ^{15}\text{N}$ recovery ratios for microbes and non-dominant C. glaucum by 99% and 95%, respectively, but it increased the glycine-$^{15}\text{N}$ recovery ratio by 495% (Figure 4e). For microbes and dominant A. tataricus in degraded areas, warming notably decreased the $\text{NH}_4^{+} - ^{15}\text{N}$ recovery ratio by 45% while increasing glycine-$^{15}\text{N}$ 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 $^{15}\text{N}$ recovery, consistent with the results reported by Lai (2023) [32], where the recovery of $^{15}\text{N}$ 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 $^{15}\text{N}$ 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 $^{15}\text{N}$ recovery to plant $^{15}\text{N}$ 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 $^{15}\text{N}$ 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. (2020) [65] and Guan et al. (2020) [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 $\text{NH}_4^{+} - ^{15}\text{N}$ by both microorganisms and plants by 165%, but concurrently decreased their glycine-$^{15}\text{N}$ recovery ratio by 66%. Conversely, in degraded plots, warming
decreased $\text{NH}_4^+$-$^{15}\text{N}$ recovery ratios by 66% but unexpectedly increased the glycine-$^{15}\text{N}$ 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 intensifies 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 $^{15}\text{N}$ 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. glaucom* 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.


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