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Microbial Nitrogen Cycling Becomes Conservative and Resilient to Long-Term Warming in High-Latitude Carbon-Limited Soils

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ABSTRACT

High-latitude soils are warming rapidly due to climate change, raising concerns about long-term impacts on nitrogen (N) and carbon (C) cycling. Here, we investigate how decadal soil warming affects microbial N transformations in subarctic grasslands using natural geothermal gradients with soil temperatures ranging from ambient to +12.3°C. Seasonal measurements of N-pools and gross N transformation rates—including the production and uptake of amino acids, ammonium, and nitrate—were used to characterize microbial responses across warming intensities and time. Warming enhanced microbial turnover of amino acids by accelerating both gross amino acid production and uptake, while net depolymerization remained unchanged. In contrast, ammonium production remained stable, but its microbial uptake increased significantly with temperature. These decoupled responses suggest a microbial shift toward preferential use of organic N sources under warming, likely driven by reduced soil C availability. This strategy provides a dual source of C and N, enabling microbes to sustain high metabolic activity while limiting additional N losses. Supporting this, total soil N stocks declined early in the warming period—by 0.11 tons of nitrogen per hectare per degree Celsius over 5 years—but remained stable thereafter, indicating a transition toward more conservative microbial N cycling. Together, these findings reveal that long-term warming restructures microbial N use strategies, favoring tight organic N recycling and mineral N conservation. These physiological adjustments may buffer N losses under future warming and should be integrated into models predicting high-latitude ecosystem responses to climate change.

1 | Introduction

High-latitude soils, which store nearly half of the global soil organic carbon (SOC) pool (Tarnocai et al. 2009; Scharlemann et al. 2014; Crowther et al. 2019), are warming two to four times faster than the global average (Walsh 2014; Rantanen et al. 2022), making them particularly vulnerable to climate change. In these ecosystems, low temperatures limit microbial metabolism and soil organic matter (SOM) decomposition for most of the year (Li et al. 2020). Ongoing and projected temperature increases of 1.4°C–4.4°C, especially pronounced at northern latitudes (IPCC 2023), are therefore expected to accelerate

microbial activity and SOM breakdown, potentially triggering positive climate feedbacks through enhanced greenhouse gas emissions (Piao et al. 2008; García-Palacios et al. 2021).

Carbon (C) and nitrogen (N) biogeochemical cycles are tightly coupled at high latitudes because low temperatures also constrain microbial N mineralization, limiting the release of bioavailable N for plants (Hobbie et al. 2002). Warming is expected to increase N turnover and may modify the balance between N release and immobilization (Rustad et al. 2001; Salazar et al. 2020; Liu et al. 2016). Net increases in N mineralization rates could result in increased C inputs from vegetation to the soil, potentially offsetting C losses caused by enhanced microbial respiration (Sistla et al. 2013; Melillo et al. 2002; Dai et al. 2020). However, rapid shifts in microbial activity can also lead to soil N losses through leaching or gaseous emissions, particularly when N mineralization outpaces plant, microbial or abiotic retention mechanisms (Turner and Henry 2009; Marañón-Jiménez et al. 2019; Lacroix et al. 2022; Salmon et al. 2018), with crucial consequences for the capacity of vegetation to offset soil C losses.

These effects are not uniform throughout the year, since strong temperature and soil moisture seasonal fluctuations impose distinct constraints on microbial activity and nitrogen cycling. During long, cold winters, microbial metabolism slows dramatically, and nitrogen remains largely immobilized in organic or microbial forms (Schnecker et al. 2023). The onset of thaw triggers abrupt physical and biological changes: pulses of dissolved mineral N are commonly observed following snowmelt (Grogan et al. 2004; Edwards and Jefferies 2013), resulting from freeze–thaw–induced cell lysis, shifts in microbial community structure, and temporary reductions in microbial nutrient retention capacity (Brooks and Williams 1998; Grogan et al. 2004; Freppaz et al. 2006; Marañón-Jiménez et al. 2025). Warmer winter soils and earlier snowmelt may therefore increase net N mineralization and elevate the risk of N losses through leaching (Koller and Phoenix 2017), especially when N pulses are not synchronized with plant N uptake (Salmon et al. 2018; Lacroix et al. 2022). Investigating intra-annual patterns of microbial N transformations is therefore essential to identify critical periods of N availability and loss, and to better predict how C and N cycles will respond to ongoing climate change.

Most studies on the effects of warming on soil N cycling have focused on net N transformation rates and total soil N-pools (Liu et al. 2016; Salazar et al. 2020; Peplau et al. 2021). However, net rates represent only the balance between gross microbial N production and consumption, potentially masking the underlying physiological processes driving these changes. In contrast, gross N transformation rates provide a more complete picture of microbial N dynamics by quantifying the total fluxes of N through different pathways. Additionally, most warming studies emphasize inorganic N production, often overlooking the microbial processing of organic N compounds (Dai et al. 2020; Sorensen et al. 2018; Salazar et al. 2020), which are particularly important in high-latitude soils. In these soils, the organic N-pool is the primary source of N for microbes, and the breakdown of proteins by extracellular enzymes often acts as a critical bottleneck, driving subsequent microbial processes such as mineralization and nitrification (Näsholm and Persson 2001; Mooshammer et al. 2014; Jan et al. 2009).

Microbial and biogeochemical responses to warming vary with exposure time, reflecting a transition from transient to equilibrium states. Short- to medium-term warming can trigger rapid increases in decomposition and nutrient cycling, while longer exposure often leads to partial stabilization as soil organisms and plants adjust to new thermal conditions (Bradford et al. 2008; Walker et al. 2018). Because soil microbes adapt faster to temperature changes than vegetation or other organisms with slower turnover rates (Classen et al. 2015), this mismatch may transiently open the N cycle and cause coupled C and N losses before longer-term stabilization (Marañón-Jiménez et al. 2025). Although previous studies have examined microbial acclimation to warming (Hartley et al. 2008; Crowther and Bradford 2013; Walker et al. 2018), most have focused on growth and respiration rather than on the long-term evolution of soil N stocks and microbial N cycling. The geothermal gradient used in this study, encompassing sites warmed for approximately 5, 10, and more than 50 years, offers a unique opportunity to assess these temporal dynamics and evaluate whether microbial N cycling converges toward equilibrium under sustained warming. This study investigates how a decade of soil warming affects gross N transformation rates—including protein depolymerization, ammonification, and nitrification—in subarctic mineral soils across multiple seasons. We conducted seasonal measurements of microbial N cycling processes using natural geothermal gradients at Reykir, Iceland, which span soil temperature increases of +0°C to +12.3°C and encompass the full range of projected warming scenarios for high-latitude ecosystems and beyond (Sigurdsson et al. 2016; IPCC 2023). We also assessed cumulative soil N losses after 5, 10, and more than 50 years of continuous warming by quantifying total soil N stocks. We hypothesize that: (1) elevated soil temperatures enhance mass-specific gross N transformation rates and microbial N turnover, although it stimulates more gross N production relative to consumption, leading to higher net protein depolymerization and net N mineralization rates; (2) warming effects will be most pronounced during the snowmelt period; and (3) the acceleration of net rates of N mineralization causes current soil N losses in these C-limited subarctic soils.

2 | Material and Methods

2.1 | Study Site

Soil samples were collected from an unmanaged grassland located near the village of Hveragerði in southwest Iceland (64°00′01″ N, 21°11′09″ W; 83–168 m a.s.l.). The study site is part of the ForHot research infrastructure (www.forhot.is) and is described in detail by Sigurdsson et al. (2016). Between 2003 and 2015, the mean annual temperature, precipitation, and wind speed were 5.2°C, 1457 mm, and 6.6 m s^{-1}, respectively, according to records from the Eyrarbakki synoptic station, located 9 km south of the site (Icelandic Meteorological Office). The warmest month is July, with a mean temperature of 12.2°C, while the coldest month is December, averaging −0.1°C. ForHot sites receive abundant precipitation throughout the year (mean annual 1474 mm between 2006 and 2016), ranging from 70 mm in May (the driest month) to 166 mm in September (the wettest month). Soil moisture rarely approached the permanent wilting point in the top 5 cm (Leblans

et al. 2017; Verbrigghe et al. 2022), and no relationship was found between soil warming and volumetric soil water content during the study period. The growing season typically spans from late May to late August. Although snow cover is generally intermittent due to the region's mild oceanic climate, soil usually freezes for at least several weeks during mid-winter (Sigurdsson et al. 2016).

In 2008, a magnitude 6.3 earthquake altered the geothermal system in the region of Reykir, leading to the development of new geothermal bedrock channels that increased soil temperatures through radiative heating in areas that were previously unaffected (Halldórsson and Sigbjörnsson 2009). This event created a natural soil warming gradient—from ambient temperature plots to areas with elevated geothermal heating—providing a unique opportunity to investigate terrestrial ecosystem responses to medium-term soil warming (MTW). At this site, vegetation cover includes approximately 46% vascular plants and 88% moss at ambient temperatures. Dominant species include *Agrostis capillaris*, *Galium boreale*, and *Anthoxanthum odoratum*, all perennial herbs with ephemeral aboveground biomass that regenerate annually from belowground stems or rhizomes (Sigurdsson et al. 2016). The soil is classified as Silandic Andosol (IUSS Working Group WRB 2015) with a fine silt loam texture and a pH ranging from 5.43 to 6.15.

The MTW site can be compared with Grændalur, a nearby location that has experienced continuous geothermal warming for over 50 years. This long-term warming site (LTW), situated approximately 2 km away, shares the same soil type, enabling robust comparisons across different durations of warming. Importantly, no signs of geothermal contamination (e.g., elevated exchangeable sulfur) have been detected at either site (Sigurdsson et al. 2016).

2.2 | Experimental Design and Soil Sampling

Plots measuring 0.5 m × 0.5 m were established in homogeneous areas with similar topographic exposure, plant composition, and soil characteristics. These plots spanned a soil temperature gradient from ambient conditions to +12.3°C above ambient ($n = 20$ plots). The selected plots were distributed across two clusters of approximately 1000 m^2 each, centered around two main geothermal hot spots located about 700 m apart. To ensure balanced representation across the entire warming range, plots were selected based on instantaneous differences between soil temperatures at potential warmed locations and paired ambient reference plots. Soil temperature was continuously monitored in each plot at a depth of 10 cm using TidbiT v2 HOBO Data Loggers (Onset Computer Corporation, Bourne, USA), recording every 30 min. Only the surface soil layer (0–10 cm) was sampled, as soils at this site are shallow (< 30 cm) and previous studies have shown that biological activity and root biomass are almost entirely confined to this horizon (Verbrigghe et al. 2022).

To assess the medium- and long-term effects of warming on soil N stocks, two soil cores (0–10 cm depth, ø = 5.12 cm) were collected from each plot of the MTW site, in July 2013 (after 5 years of warming) and again in July 2018 (after 10 years of warming). Similarly, two 0–10 cm soil cores (corer ø = 5.12 cm) were taken within each plot LTW area (> 50 years of warming).

To assess the seasonal responses of gross N transformation rates and the dynamics of amino acid, ammonium, and nitrate pools following a decade of warming (MTW site), soil samples were collected in August 2017 ("Summer"), November 2017 ("Autumn"), during the snowmelt period in April 2018 ("Snowmelt"), and in June 2018 ("Spring"). Fresh soil samples were sieved to 2 mm and kept at 4°C for transport. Winter sampling was not conducted due to frozen soil conditions at low warming intensities, which prevented core extraction.

2.3 | Determination of Soil C and N-Pools

Upon arrival, samples were incubated at the corresponding in situ field temperatures for each sampling date. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured by extracting 2 g of fresh soil with 15 mL of 1 M KCl. Microbial biomass C and N were determined using the chloroform fumigation-extraction method (Vance et al. 1987): 2 g of fumigated soil (48 h) were extracted with 15 mL of 1 M KCl and analyzed using a TOC/TN Analyzer (TOC-V CPH E200V/TNM-122 V; Shimadzu, Austria). Microbial C and N were calculated as the difference between DOC and TDN in fumigated and non-fumigated extracts (Vance et al. 1987; Fuchslueger et al. 2019). No correction factors were applied for extraction efficiency.

Ammonium, nitrate, and total free amino acid (TFAA) concentrations were also measured in the KCl extracts. Ammonium and nitrate were determined colorimetrically using a modified indophenol blue method (Kandeler and Gerber 1988) and the VCl_3-Griess assay (Hood-Nowotny et al. 2010), respectively. TFAA concentrations were determined fluorometrically following the method of Jones (2002), as modified by Prommer et al. (2014).

To determine soil N stocks from MTW and LTW, a 2 g aliquot of oven-dried, sieved bulk soil was ground using a ball mill (Retsch MM 301 Mixer Mill, Haan, Germany) and analyzed for total N concentration (%) by dry combustion (Vario MAX CN macro elemental analyzer, Hanau, Germany). Bulk density (BD, g cm^{-3}) was measured following the method described by Verbrigghe et al. (2022), and soil N stocks (t ha^{-1}) were calculated using the equations from Bárcena et al. (2014).

2.4 | Determination of N Transformation Rates

Gross rates of N transformation rates were quantified in fresh seasonal soil samples collected in MTW site using ^{15}N-pool dilution assays. For these measurements, specific ^{15}N tracers were applied: ^{15}N-labeled amino acids for assessing gross amino acid production and gross amino acid uptake rates; $^{15}NH_4^+$ for quantifying gross ammonium production and gross ammonium uptake rates; and $^{15}NO_3^-$ for determining gross nitrate production and gross nitrate uptake rates.

Gross amino acid production (AA_{prod}) and gross amino acid uptake rates (AA_{uptake}) were determined according to Fuchslueger

et al. (2019) and Wild et al. (2018). Briefly, ^{15}N-labelled amino acid mixture (an algal mix of 20 amino acids, >98 at% ^{15}N, Spectra and Cambridge Isotope Laboratories) was added to 2 g aliquots of fresh soil in duplicates. The tracer amount (2.5 μg per g fresh soil) was adjusted based on the estimated amino acid content of each sample, ensuring that the tracer represented no more than 20% of the native N-amino acid pool. One duplicate of each sample was incubated for 10 min at in situ field soil temperature and the other for 30 min, in order to compare changes in the ^{14}N and ^{15}N-amino acid pools over time. Following incubation, soils were extracted with 20 mL of 10 mM $CaSO_4$ containing 3.7% formaldehyde.

Extracts were centrifuged, filtered, and passed through precleaned cation exchange cartridges (OnGuard II H, 1 cc; Dionex). Amino acids were eluted using 10 mL of 3 M NH_3, then dried under N_2, re-dissolved in 20% ethanol, and dried again using a SpeedVac. Blanks and external amino acid standards were processed in parallel. After derivatization with ethyl chloroformate (Wanek et al. 2010), samples were analyzed by gas chromatography–mass spectrometry (Thermo Trace GC Ultra coupled to an ISQ MS) using an Agilent DB-5 column, splitless PTV injection at 270°C, and helium as the carrier gas (1 mL min^{-1}).

Concentrations of alanine, glycine, isoleucine, leucine, phenylalanine, proline, serine, valine, asparagine/aspartate, and glutamine/glutamate were calculated using external standards. The ^{15}N and ^{14}N isotopic composition of these amino acids was calculated based on fragment peak areas (Wanek et al. 2010). Gross rates of amino acid production and amino acid uptake were calculated following the equations of Kirkham and Bartholomew (1955) and normalized to microbial C biomass (C_{mic}).

Net mass-specific depolymerization rates (AA_{depoly}) were estimated as follows:

$$AA_{depoly} = \left(AA_{prod} / C_{mic}\right) - \left(AA_{uptake} / C_{mic}\right) \quad (1)$$

Gross ammonium production ($NH^+_{4\,prod}$) and ammonium uptake rates ($NH^+_{4\,uptake}$), as well as gross nitrate production ($NO^-_{3\,prod}$) and nitrate uptake rates ($NO^-_{3\,uptake}$) were also determined using ^{15}N-pool dilution assays. For each assay, 2 g aliquots of fresh soil were prepared in duplicate and amended with either $(^{15}NH_4)_2SO$ or $K^{15}NO_3$ (98 atom% ^{15}N) to achieve a tracer addition equivalent to 20% of the respective native NH^+_4 or NO^-_3 pool. Samples were incubated at the corresponding field soil temperature for 4 and 24 h and then extracted with 1 M KCl.

$NH^+_{4\,prod}$ and $NH^+_{4\,uptake}$ were determined through microdiffusion of NH_3 from the KCl extracts using acid traps, followed by analysis via elemental analyzer–isotope ratio mass spectrometry (EA-IRMS; EA 1110, CE Instruments, Italy, coupled to a Finnigan MAT Delta Plus IRMS, Thermo Fisher Scientific, MA, USA). For gross nitrate production and uptake, NH_3 was first removed from the KCl extracts by adding MgO. Subsequently, NO^-_3 was reduced to NH_3 using Devarda's alloy, and the resulting NH_3 was collected via microdiffusion and analyzed as above. Gross rates of ammonium production, ammonium uptake, nitrate production, and nitrate uptake were also calculated following Kirkham and Bartholomew (1955) and normalized to C_{mic}.

Net mass-specific mineralization rate (N_{min}) and net mass-specific nitrification rate (N_{nit}) were calculated as follows:

$$N_{min} = \left(NH^+_{4\,prod} / C_{mic}\right) - \left(NH^+_{4\,uptake} / C_{mic}\right) \quad (2)$$

$$N_{nit} = \left(NO^-_{3\,prod} / C_{mic}\right) - \left(NO^-_{3\,uptake} / C_{mic}\right) \quad (3)$$

Finally, turnover times were calculated for each N-pool as follows:

$$N-pool_{turnover}\,(days) = N-pool / \left(\left(N-pool_{prod} + N-pool_{uptake}\right)/2\right) \quad (4)$$

where the N-pool refers to any of the individual pools of amino acids, ammonium, or nitrates.

2.5 | Data Analysis

The warming intensity for each soil sample was calculated as the temperature difference between its corresponding plot and the average ambient soil temperature measured in the reference plots. Warming intensity remained consistent over time within the geothermal gradients (Sigurdsson et al. 2016). In addition, average soil temperatures at each plot during the 2 weeks preceding each soil sampling were considered to account for potential short-term temperature fluctuations affecting soil microbial activities.

To evaluate the effects of soil warming intensity and season on N–amino acid, NH^+_4, and NO^-_3 pools, as well as on their gross production and uptake rates, net transformation rates, and turnover rates, we used linear models implemented in the *stats* package in R v4.3.2 (Chambers et al. 1992). Soil warming was included as a continuous fixed effect, and season as a categorical factor with four levels: Snowmelt, Spring, Summer, and Autumn. Interaction terms between warming and season were also included in the models. When significant, differences among seasons were further evaluated using post hoc tests with Sidak corrections for multiple testing while adjusting for the effect of warming.

The relationships between soil ammonium concentrations and their corresponding gross microbial production and uptake rates were tested using Spearman correlations.

To assess the effects of warming intensity, warming duration, and their interaction on soil N stocks, additional linear models were fitted. In these models, warming intensity was treated as a continuous predictor, and warming duration as a categorical factor (5, 10, and > 50 years).

Model assumptions were verified by inspecting residual distributions and applying the Shapiro–Wilk test for normality and the Breusch–Pagan test for homogeneity of variance. When necessary, data were transformed using log, cube root, or Yeo–Johnson transformations to meet the assumptions of normality and homoscedasticity (Quinn and Keough 2002).

3 | Results

3.1 | Effect of Warming Intensity and Seasons on Soil N-Pools

TDN significantly decreased with soil warming and showed modest seasonal variation (Figure 1a; Warming: $p < 0.001$; Season: $p = 0.025$; see Figure S1 for results using transformed variables). Microbial N was unaffected by either warming or season ($p > 0.05$) and represented a larger N-pool compared to TDN, TFAAs, and NH_4^+ pools (Figure 1b). TFAAs concentrations were significantly reduced by warming (Figure 1c; $p < 0.001$). Notably, the highest amino acid concentrations were observed in spring, but these also exhibited the strongest decline with increasing warming (Season: $p < 0.001$; Warming × Season interaction: $p < 0.001$). In contrast, NH_4^+ concentrations were not significantly affected by warming (Figure 1d; $p = 0.762$), although they varied significantly across seasons ($p < 0.001$), with higher values in spring and during the snowmelt period compared to autumn and summer. Nitrate concentrations remained at or below the detection limit of the colorimetric method ($< 0.01\,\mu g\ N\ g^{-1}$ soil). Consequently, the statistical analysis did not reveal significant effects of soil warming on nitrate transformation rates (Figure S2).

3.2 | Effect of Warming and Seasons on Nitrogen Transformation Rates

Both gross amino acid production and amino acid uptake were significantly affected by soil warming intensity and season, with no significant interaction between the two factors (Figure 2a,b; see Figure S3 for results using transformed variables). Warming led to an overall increase in gross amino acid production and amino acid consumption rates. As a consequence, net protein depolymerization rates were not affected by soil warming. Instead, season emerged as the most determining factor, with significantly lower net depolymerization rates observed in spring and summer compared to autumn (Figure 2c). In contrast, amino acid turnover time was exclusively influenced by soil warming, with higher temperatures resulting in faster turnover (i.e., shorter residence times; Figure 2d).

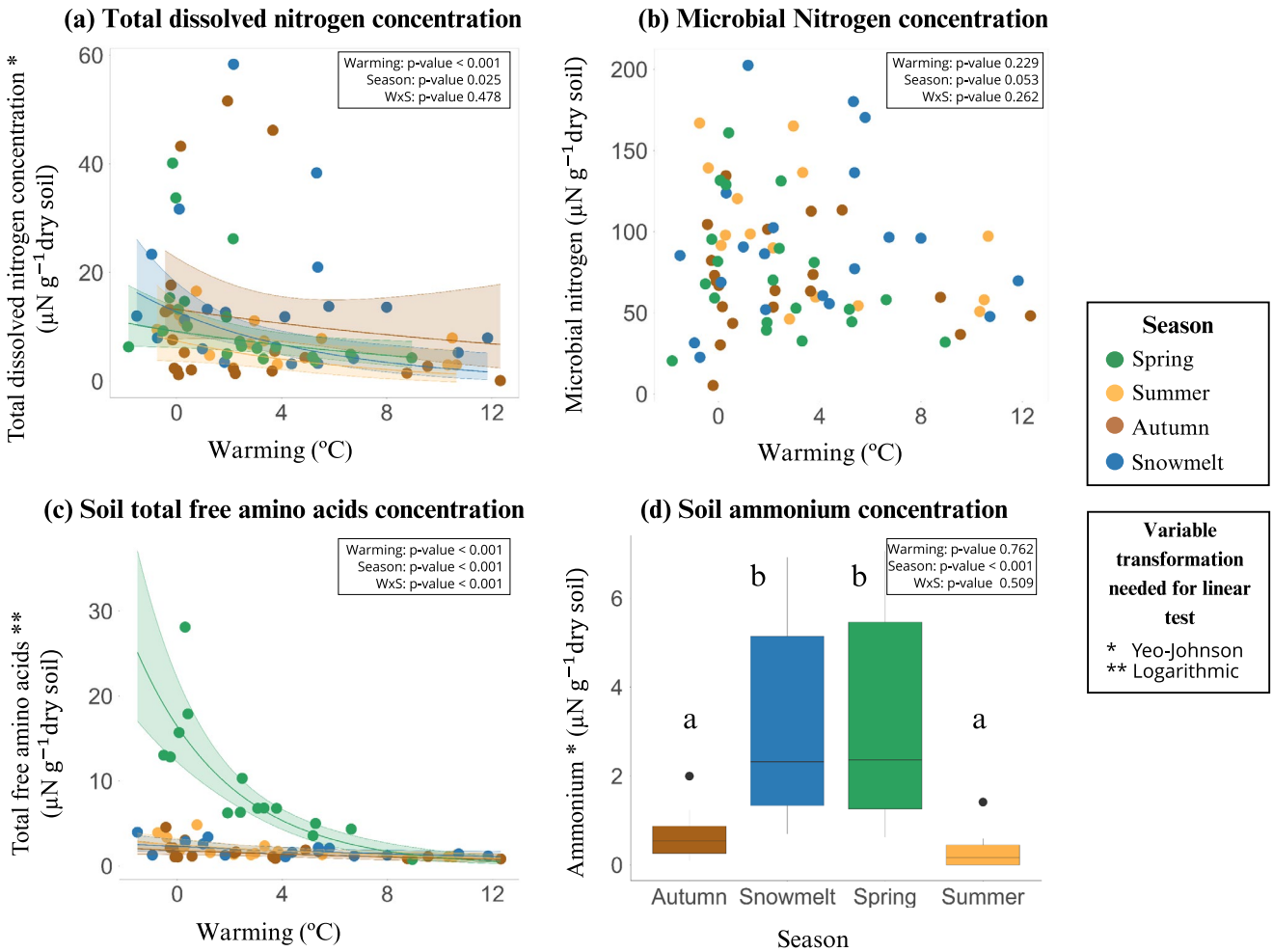

FIGURE 1 | Effect of soil warming intensity and seasonal variations on (a) total dissolved nitrogen, (b) microbial nitrogen, (c) total free amino acids, and (d) ammonium concentrations in soil. *p* values indicate the effect of warming intensity, season, and their interaction according to linear models on previously transformed variables. Lines represent significant ($p < 0.05$) effects of warming intensity for each season. Shadowed areas around lines represent the 95% confidence intervals of the regressions. Box plots indicate significant ($p < 0.05$) effects of season only. Different letters indicate significant differences among seasons according to the post hoc Sidak test for multiple comparisons.

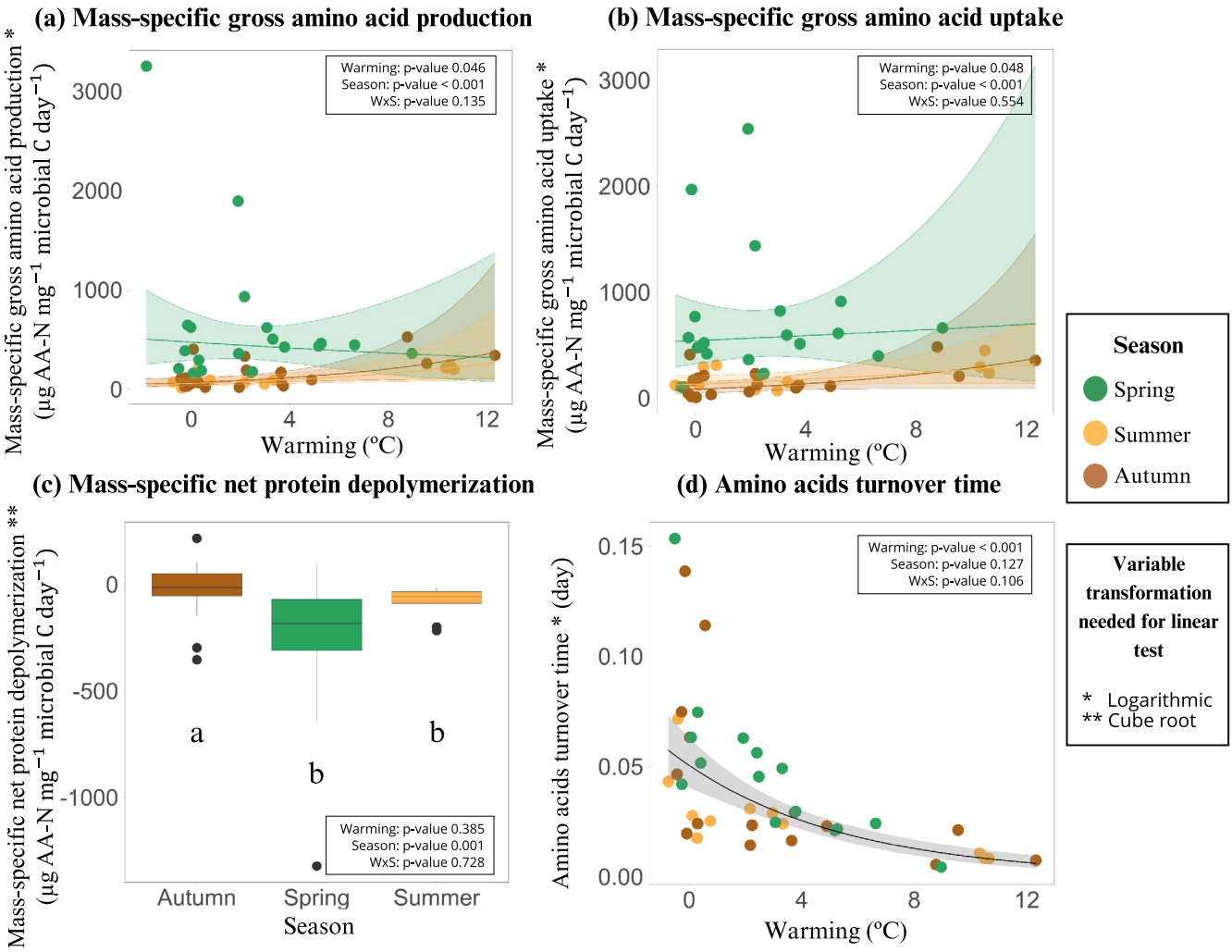

FIGURE 2 | Effect of soil warming intensity and seasonal variations on (a) mass-specific gross amino acid production, (b) mass-specific gross amino acid uptake, (c) mass-specific net protein depolymerization, and (d) amino acid turnover time. *p* values indicate the effect of warming intensity, season, and their interaction according to linear models on previously transformed variables. Lines indicate significant ($p < 0.05$) effects of warming intensity for each season, while the grey trendline indicates an overall significant ($p < 0.05$) effect of warming. Shadowed areas around lines represent the 95% confidence intervals of the regressions. Box plots indicate significant ($p < 0.05$) effects of season only. Different letters indicate significant differences among seasons according to the post hoc Sidak test for multiple comparisons. Data for the snowmelt season is unavailable due to a methodological error that occurred during sample processing.

Soil warming had no significant effect on gross ammonium production rates (Figure 3a; see Figure S4 for results using transformed variables), but seasonal variation had a strong influence ($p < 0.001$), with the highest ammonium production rates observed in summer and the lowest during spring and snowmelt. In contrast, gross ammonium uptake rates increased consistently with soil temperature across all seasons (Figure 3b), leading to a decline in net N mineralization rates under warming conditions (Figure 3c). Ammonium turnover time was not affected by warming, but it varied significantly across seasons, with slower turnover observed during spring and snowmelt compared to summer and autumn (Figure 3d).

Interestingly, a significant negative correlation was observed between mass-specific gross ammonium production or ammonium uptake and soil ammonium concentration (Figure 4a,b, $p < 0.05$).

3.3 | Effect of Warming on Total Soil N Stocks

Total soil N stocks consistently declined with increasing warming intensity, regardless of the duration of warming (Figure 5). Although soil N stocks differed significantly over years (Warming duration: $p < 0.01$), the effect of warming intensity remained consistent over time, as indicated by the lack of significant interaction between warming intensity and warming duration ($p = 0.414$).

4 | Discussion

By capturing seasonal and medium to long-term responses across a wide warming gradient, this study reveals how soil N cycling in subarctic ecosystems is reshaped under climate-relevant temperature increases. Our approach, spanning the

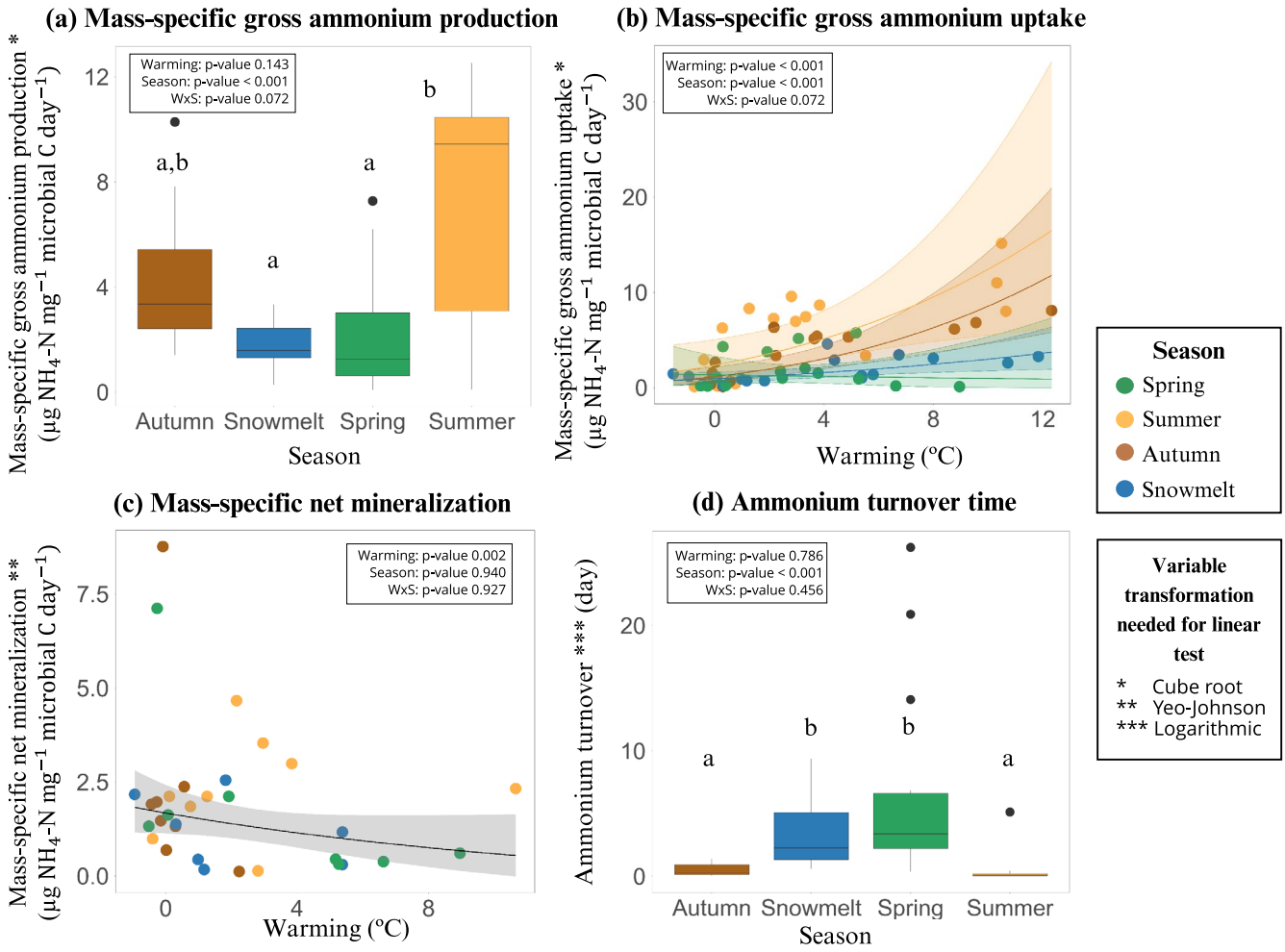

FIGURE 3 | Effect of soil warming intensity and seasonal variations on (a) mass-specific gross ammonium production, (b) mass-specific gross ammonium uptake, (c) mass-specific net N mineralization, and (d) ammonium turnover time. p values indicate the effect of warming intensity, season, and their interaction according to linear models on previously transformed variables. Lines show significant ($p < 0.05$) effects of warming intensity for each season, and the grey trendline represents an overall significant ($p < 0.05$) effect of warming. Shadowed areas around lines represent the 95% confidence intervals of the regressions. Box plots indicate significant ($p < 0.05$) effects of season only. Different letters indicate significant differences among seasons according to the post hoc Sidak test for multiple comparisons.

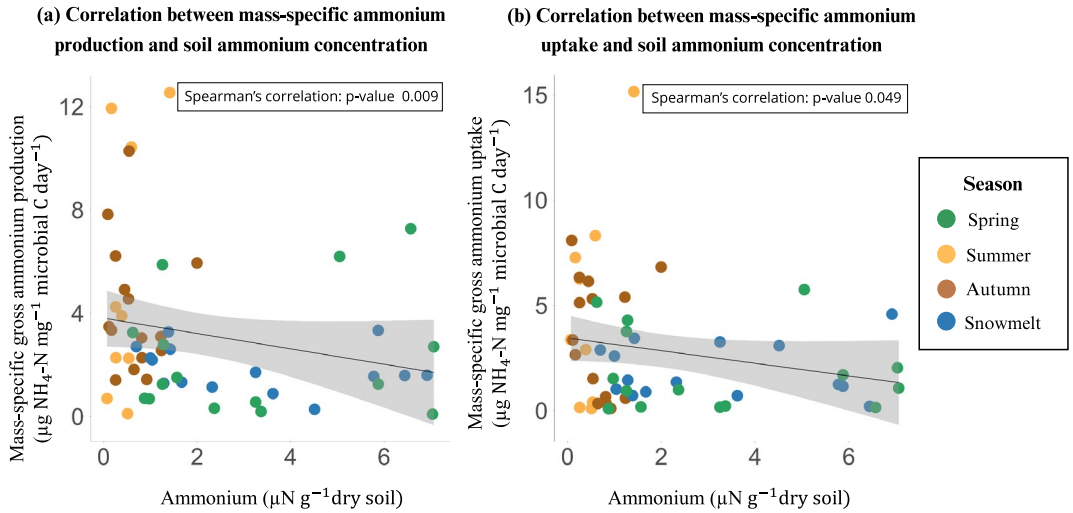

FIGURE 4 | Correlation between (a) mass-specific gross ammonium production and soil ammonium concentration and (b) mass-specific gross ammonium uptake and soil ammonium concentration. p values indicate the significance of Spearman's correlation. Grey lines indicate linear correlations, and shaded areas represent 95% confidence intervals for the fitted trend lines.

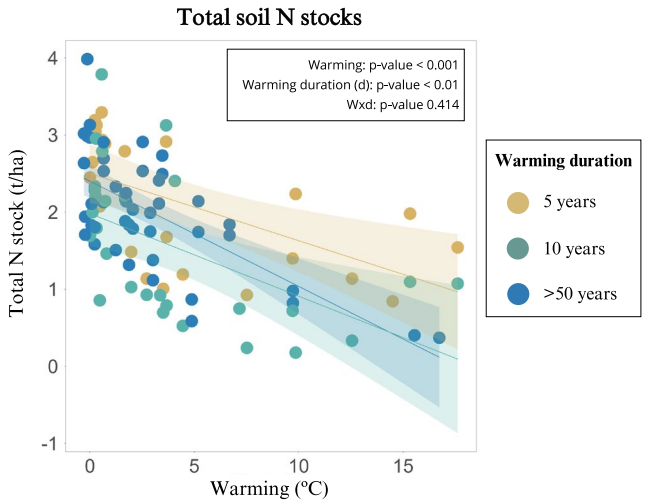

FIGURE 5 | Effect of soil warming intensity on total N stocks measured after 5 years (2013), 10 years (2018) and more than 50 years of soil warming. Lines indicate significant ($p < 0.05$) effects of warming for each sampling year. Shadowed areas around lines represent the 95% confidence intervals of the regressions.

full range of projected IPCC warming scenarios across seasons, allowed us to detect consistent shifts in microbial N transformation dynamics. Warming accelerated gross rates of amino acid production and amino acid uptake, resulting in faster amino acid turnover time (Figure 2). Interestingly, despite this increased activity in organic N pathways, net N mineralization declined with warming (Figure 3)—contrary to the sustained increases reported in previous studies (Salazar et al. 2020; Rustad et al. 2001). Total soil N stocks also declined steadily with warming across all time points, with similar rates of loss regardless of warming duration (Figure 5), suggesting that most N depletion occurred within the first 5 years after the onset of warming, after which the system came closer to a new equilibrium state. Together, these findings are crucial for understanding how prolonged soil warming affects microbial N cycling in C-limited ecosystems, especially under future climate scenarios. By revealing adaptive microbial strategies that mitigate N losses, this work contributes valuable insights into the resilience and functioning of subarctic grasslands in a warming world.

4.1 | Effects of Soil Warming and Seasonality on Amino Acid Transformation Rates

We hypothesized that increasing soil temperatures would lead to higher mass-specific gross N transformation rates. This was confirmed for amino acid metabolism: both gross amino acid production and gross amino acid uptake increased in a similar way with warming, resulting in faster amino acid turnover (Figure 2), although net depolymerization remained unchanged. Warming typically stimulates microbial enzymatic activity, enhancing the breakdown of proteins into amino acids and their subsequent assimilation (Wallenstein et al. 2010; Fuchslueger et al. 2019). This finding aligns with previous studies at the same geothermal gradient, which reported elevated microbial mass-specific activity—including respiration—associated with initial depletion of soil organic substrates (Marañón-Jiménez et al. 2018; Walker et al. 2018). As metabolic rates increase, microbial demand for readily available organic N sources, such as amino acids, also intensifies to support protein synthesis and growth (Salazar et al. 2020; Wang et al. 2022). Under reduced soil C availability, it is possible that microbes sustained their elevated metabolic rates at the expense of reducing microbial biomass, but they accelerated turnover, a process that has been also previously suggested as a potential mechanism that prevented further soil C losses (Walker et al. 2018; Söllinger et al. 2022; Verbrigghe et al. 2022). In this context, microbes may have also prioritized the uptake and rapid turnover of organic N sources (i.e., proteins and amino acids) to satisfy both their energy and N needs, explaining the increase in gross amino acid production and uptake rates. Supporting this, Séneca et al. (2021) demonstrated that prolonged warming over 8 years at the same ForHot sites led to increased transcription levels of genes encoding enzymes involved in the degradation of N-rich polymers, particularly those present in microbial necromass. A smaller, yet more active, microbial community may meet its increasing C and N demands at warmer temperatures by efficiently recycling microbial residues already present in the soil solution.

The stable net protein depolymerization rate with warming suggests a tightly coupled system where the increase in amino acid production and release to the soil is matched by an equivalent increase in microbial consumption (i.e., gross amino acid uptake), leading to a dynamic equilibrium without current changes in the net rate of depolymerized amino acids. Moreover, while the soil amino acid pool is reduced (Figure 1), amino acid turnover rates increased (Figure 2d), which also indicates that microbes are operating with greater efficiency through protein recycling. Supporting this, Söllinger et al. (2022) reported a downregulation of the bacterial protein biosynthesis machinery in warmed soils at the same study site, accompanied by higher enzyme activities that accelerated overall microbial metabolism and growth. Taken together, these ideas indicate that microbes in warmed soils become more efficient in using organic substrates, possibly due to improved protein synthesis machinery (Söllinger et al. 2022) and shifts to a smaller but more active community (Marañón-Jiménez et al. 2018) with a greater number of active bacterial taxa (Metze et al. 2023) and enhanced microbial biomass recycling (Séneca et al. 2021).

At the seasonal scale, amino acid transformation rates appear to be directly related to the size of the amino acid pool in the soil. Thus, amino acid turnover remained constant across seasons (Figure 2). Some studies have shown that microbial amino acid uptake is concentration-dependent (Vinolas et al. 2001; Wilkinson et al. 2014), which was particularly true in spring, when soil amino acid concentration was highest (Figure 1). During this season, microbial amino acid uptake rates may not immediately keep pace with the sudden influx of amino acids from organic inputs following the snowmelt period, allowing for temporarily higher amino acid concentrations (Farrell et al. 2011; Weintraub and Schimel 2005). As the growing season begins, fresh plant inputs and root exudates increase the availability of organic N compounds (Ma et al. 2022). Then, plant-derived SOM, rich in proteins, is depolymerized into amino acids, providing an initial seasonal

boost in the amino acid pool. As the subarctic short growing season progresses, a net amino acid uptake depletes the amino acid pool, which is supported by the negative values of net protein depolymerization rates during spring and summer (Figure 2c). Therefore, we conclude that microbes may dynamically adjust their metabolic activity to current substrate availability, thereby maintaining stable turnover to meet the demands for protein synthesis. This adaptation enables microbes to maximize regulation of protein synthesis according to short-term fluctuations in amino acid availability in the soil and to exploit the seasonal oscillations of this resource.

4.2 | Effects of Soil Warming and Seasonality on Ammonium Transformation Rates

Mass-specific ammonium transformation rates were 67 times lower than amino acid transformation rates, supporting the idea of the role of amino acids as the predominant N source and the preferential use of amino acids as dual C and N sources. However, contrary to our expectations, warming increased mass-specific ammonium uptake, which subsequently reduced net N mineralization rates (Figure 3). In order to meet their stoichiometric demands for C and N under the exacerbated C limitation at warmer temperatures (Meeran et al. 2023; Verbrigghe et al. 2022), microbes only immobilized N when a stoichiometrically equivalent source of C was provided (Marañón-Jiménez et al. 2019). Accordingly, C and energy obtained from efficient recycling of amino acids could have allowed microbes to maintain ammonium consumption for growth and metabolism over ammonium production. This adaptation enables microbes to sustain ecosystem function by preventing further N losses, even under continued warming. These patterns align with the notion of a shift toward a conservative N cycling regime, a conceptual framework supported by convergent lines of evidence. Although DOC, enzyme activity, and microbial stoichiometry were not measured in this study, independent data from the same sites (Marañón-Jiménez et al. 2019, 2025) show that warming-induced reductions in microbial biomass, together with strict microbial C:N stoichiometric constraints, limited microbial N retention capacity and increased soil vulnerability to coupled C and N losses. These findings support the maintenance of C:N coupling and provide further evidence for this conceptual framework.

Moreover, the increase in net ammonium consumption, coupled with unchanged ammonium concentrations with warming (Figures 1 and 3), suggests that current ammonium losses from the system are not occurring after a decade of warming. This has two implications: first, this raises the possibility of additional ammonium sources contributing to the system. One potential source could be N-fixing cyanobacteria associated with mosses, which can substantially enhance N inputs, particularly in nutrient-poor ecosystems like boreal and subarctic regions (Rousk and Michelsen 2016). An increase in the moss-to-plant ratio was observed along the geothermal gradient in the same study sites (Fang et al. 2023), which could theoretically support higher N fixation. However, as no direct measurements of N-fixation activity have been conducted in the study sites, this remains a hypothesis that requires further verification. Secondly, the absence of current ammonium losses, along with stable net protein depolymerization rates, suggests that N losses primarily occurred during the initial years of warming exposure. Notably, the effect of warming on total N stocks remained unchanged along the warming duration (Figure 5). This indicates that soil N depletion was confined to the first 5 years, as has also been observed for soil C (Verbrigghe et al. 2022), after which microbial communities adapted N transformation strategies to minimize further N losses from the ecosystem. In line with this, Radujković et al. (2018) found that microbial community composition in these study sites stabilized after 5–7 years of warming, supporting the emergence of a new steady-state condition under chronic temperature increase.

On a seasonal scale, ammonium concentrations were higher in spring and during the snowmelt period (Figure 1). Several factors could explain this seasonal increase in ammonium concentration: first, nutrient release resulting from snowmelt (Koller and Phoenix 2017); second, the contribution of freeze–thaw cycles releasing previously unavailable NH_4^+–N from inorganic and organic colloids (Freppaz et al. 2006); and third, a reduction in ammonium uptake by dormant plants during winter (Maslov and Maslova 2021; Xie et al. 2020). In contrast to amino acids, ammonium transformation rates were inversely related to ammonium pool size (Figure 4), indicating slower ammonium turnover in seasons with higher ammonium concentrations. This further supports the idea of microbial C limitation over N limitation and on the preferential use of organic over mineral N sources. It also suggests a seasonal regulatory mechanism under limited conditions that may operate at both physiological and community levels. At the physiological level, microbial communities may downregulate N assimilation pathways and inhibit the activity of key enzymes involved in ammonia transformations (Verhamme et al. 2011; Ouyang et al. 2017). At the community level, persistently high ammonium levels may select for microbial taxa with reduced efficiency in ammonium transformation, such as fungi and actinomycetes (Xu et al. 2018; Waring et al. 2013). Together, these results suggest that microbial communities can also actively modulate ammonium use under C-limited conditions, helping to stabilize N cycling across seasons.

4.3 | Study Limitations and Perspectives for Future Research

While the geothermal warming approach provides a powerful natural laboratory to investigate soil responses over time, we acknowledge several limitations that should be considered when interpreting our results. First, this study focused exclusively on the surface soil layer (0–10 cm), although given the shallow profile of these soils (< 30 cm, Verbrigghe et al. 2022), this layer represents the most biologically active and relevant zone for microbial N transformations. Second, our study did not include measurements of gaseous or leaching N losses, nor direct assessments of denitrification processes, which limits our capacity to close the N balance. Nevertheless, complementary work at the same sites currently includes lysimeter sampling, in situ gas flux measurements, and laboratory incubations aimed at quantifying these loss pathways. Third, geothermal warming primarily heats the soil, without directly affecting aboveground temperatures, which may alter aboveground–belowground interactions in ways that differ from those observed under climatic warming. Nonetheless, soil temperature strongly controls microbial activity, nutrient cycling, and carbon stabilization in cold ecosystems,

making the focus on soil processes especially pertinent (Ferrari et al. 2018). Finally, geothermal gradients represent an abrupt and sustained temperature increase, as most warming experiments rather than the gradual warming expected under climate change scenarios; however, they provide a valuable analog for understanding long-term equilibration processes after chronic warming. Despite these considerations, the absence of physical disturbance, the stability of geothermal gradients, and the long-term, continuous nature of the warming exposure make this system exceptionally well suited for assessing the persistence and adaptation of soil N cycling processes. Ongoing and future research at the ForHot sites will integrate these missing components to build a more complete mechanistic understanding of N cycling responses to sustained soil warming.

5 | Conclusions

Our findings demonstrate that medium-to-long-term soil warming fundamentally alters microbial N cycling in subarctic grasslands by differentially affecting key transformation pathways. While gross amino acid production and uptake accelerated with warming—indicating faster turnover of organic N—gross ammonification did not increase accordingly. Instead, warming consistently enhanced ammonium uptake. This decoupling suggests a shift in microbial N strategy: as warming progresses, microbes maintain high metabolic activity and organic N turnover, but may also become increasingly constrained by N availability, promoting tighter internal cycling and more conservative use of mineral N. These patterns highlight the central role of organic N pathways in shaping microbial responses to warming and suggest that future N dynamics in high-latitude soils will be governed less by increased mineralization and more by microbial regulation of resource allocation. Understanding this balance between N acquisition and conservation is essential for predicting long-term N availability and ecosystem feedbacks under climate change. Moreover, our results point to a previously unrecognized resilience mechanism in subarctic soils to warming, driven by microbial adaptation to C limitation. These insights call for a reassessment of current predictive models of N cycling in a warming world.

Author Contributions

Ana Leticia Zevenhuizen: conceptualization, data curation, formal analysis, visualization, writing – original draft, writing – review and editing. **Andreas Richter:** conceptualization, methodology, resources, validation, writing – review and editing. **Lucia Fuchslueger:** methodology, resources, writing – review and editing. **Judith Prommer:** investigation, methodology. **Ivan A. Janssens:** conceptualization, resources, writing – review and editing. **Niel Verbrigghe:** data curation, investigation, methodology, writing – review and editing. **Josep Peñuelas:** funding acquisition, resources, writing – review and editing. **Bjarni D. Sigurdsson:** project administration, resources, writing – review and editing. **Sara Marañón-Jiménez:** conceptualization, funding acquisition, investigation, methodology, project administration, supervision, validation, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in ZENODO at DOI 10.5281/zenodo.17467240.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** The effect of soil warming and seasonal variations on transformed variables (a) total dissolved. **Figure S2:** The effect of soil warming and seasonal variations on (a) mass-specific gross nitrate production. **Figure S3:** The effect of soil warming and seasonal variations on transformed variables (a) mass-specific gross. **Figure S4:** The effect of soil warming and seasonal variations on transformed variables (a) mass-specific gross.