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Research

Nitrogen availability in soil controls uptake of different nitrogen forms by plants

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Summary

- Nitrogen (N) uptake by plant roots from soil is the largest flux within the terrestrial N cycle. Despite its significance, a comprehensive analysis of plant uptake for inorganic and organic N forms across grasslands is lacking.
- Here we measured *in situ* plant uptake of 13 inorganic and organic N forms by dominant species along a 3000 km transect spanning temperate and alpine grasslands. To generalize our experimental findings, we synthesized data on N uptake from 60 studies encompassing 148 plant species world-wide.
- Our analysis revealed that alpine grasslands had faster NH_4^+ uptake than temperate grasslands. Most plants preferred NO_3^- (65%) over NH_4^+ (24%), followed by amino acids (11%). The uptake preferences and uptake rates were modulated by soil N availability that was defined by climate, soil properties, and intrinsic characteristics of the N form.
- These findings pave the way toward more fully understanding of N cycling in terrestrial ecosystems, provide novel insights into the N form-specific mechanisms of plant N uptake, and highlight ecological consequences of chemical niche differentiation to reduce competition between coexisting plant species.

Introduction

Nitrogen (N) is a key component of cellular biochemicals (e.g. proteins, nucleic acids, chlorophylls, enzymes, and vitamins) and limits net primary productivity in most terrestrial ecosystems globally (Vitousek & Howarth, 1991; LeBauer & Treseder, 2008b). The long-term anthropogenic disruption of the global N cycle has triggered catastrophic consequences, including species loss, eutrophication, invasion of alien plants, acidification, climate change, and adverse effects on human health (Vitousek *et al.*, 1997; Hungate *et al.*, 2003; Gruber & Galloway, 2008; Galloway *et al.*, 2013). Global grasslands are experiencing a rising level of N deposition (Yu *et al.*, 2019; Borer & Stevens, 2022). For example, the rate of wet N deposition in the Tibetan Plateau currently ranges from 0.4 to 8.0 kg N ha^{-1} yr^{-1} (Wang *et al.*, 2022). Moreover, the growing number of livestock also increases soil N availability through higher manure deposition. Meanwhile, to restore degraded grasslands effectively, sometimes N fertilizers are applied to alleviate N limitation. Under these circumstances, a comprehensive understanding of N cycling processes and their responses to global change is therefore a prerequisite to address the challenges posed by external anthropogenic N inputs into the environment and for sustainable management of N resources and processes in terrestrial ecosystems (Mason *et al.*, 2022).

Monitoring ecosystem N availability, though challenging, can be achieved through the analysis of integrative proxies of the N cycle at regional or global scales. These proxies encompass metrics of microbial soil N cycling processes, plant N status, and ecosystem N inputs and losses (Mason *et al.*, 2022). Under N-limited conditions, uptake of N by plant roots is highly and positively responsive to soil N availability (Rengel *et al.*, 2022) and thus affects net primary production (Chapin *et al.*, 1994; Lebauer & Treseder, 2008). However, under high-N availability, plant N uptake will reach the maximum and then level off, leading to low-plant N use efficiency caused by N losses via NO_3^- leaching or denitrification (Wu *et al.*, 2019). Additionally, N availability can be strongly influenced by edaphic factors such as soil pH, water content, and ratios of carbon to N in soil organic matter. Therefore, it is a prerequisite to clarifying how soil N availability influences plant N uptake for a better understanding of the N cycling. Changes in carbon uptake and storage in terrestrial ecosystems have the potential to either slow or amplify global warming resulting in a carbon-climate warming feedback. The interactions between carbon and nutrient cycles, especially N and phosphorus, are critical to the ecosystems' uptake and storage of carbon because they are the two main elements considered to be limiting plant growth and carbon uptake in most ecosystems (Elser *et al.*, 2007; Lebauer & Treseder, 2008). Plant N uptake is a critical step controlling net primary production and has potential to influence carbon uptake and storage in terrestrial ecosystems, leading to carbon-nutrient-climate feedback (Bala *et al.*, 2013; Wieder *et al.*, 2015). Thus, the assessment of plant N uptake serves as a crucial metric to track changes in the global N cycle and to deepen our comprehension of carbon-nutrient-climate feedback (Lambers *et al.*, 2008). Nevertheless, a comprehensive analysis of plant uptake metrics for inorganic and organic N forms across a breadth of plant species and ecosystems is lacking (Iversen *et al.*, 2015; Ma *et al.*, 2018; Mason *et al.*, 2022).

A long-held paradigm in N cycling theory is that inorganic N forms (such as NO_3^- and ammonium NH_4^+), originating from microbial mineralization of soil organic matter and litter, serve as the primary N source for plants (Schimel & Bennett, 2004). However, some evidence has overturned this notion, revealing that roots possess the capability to take up intact organic N forms (e.g. amino acids) from soils across all terrestrial ecosystems (von Wirén *et al.*, 1997; Jones *et al.*, 2005; Näsholm *et al.*, 2009). Using just a few amino acids, predominantly the model amino acid glycine, the contribution of soil organic N forms to total N uptake range from 0.5% for agricultural crops up to 60% for arctic and alpine plants (Chapin *et al.*, 1993; Kielland, 1994; Xu *et al.*, 2006; Zhang *et al.*, 2015). The higher organic N contribution in the latter plants is attributed to their soils with high-organic N concentrations in soil solution, derived from slow rates of organic N mineralization due to low temperatures and high-soil moisture (Chapin *et al.*, 1993; Xu *et al.*, 2006). Plants may exhibit different uptake rates for inorganic N forms and various free amino acids (FAA) due to their distinct intrinsic properties (e.g. molecular mass, N content, water solubility, net charge, isoelectric point, and hydrophobicity). These intrinsic properties can strongly affect their diffusion rate in soil solution, for example through differential sorption of these compounds to the soil matrix, the resultant differences in diffusion rates in soils being accessible to microdialysis measurements (Inselsbacher *et al.*, 2011). For instance, FAAs with low-molecular mass, high-water solubility, and low hydrophobicity are more readily soluble in water and more quickly move through diffusive transport in soil solution along concentration gradients, between FAA source regions and the root surface. By contrast, hydrophobic or (cat)ionic FAAs are more strongly sorbed and therefore move more slowly by diffusion. This makes such N forms more or less accessible for plant uptake (Fischer *et al.*, 2002). Although > 20 forms of FAA besides glycine can be typically found in soils (Persson & Näsholm, 2008), actually measuring the uptake of organic N forms and quantifying their contribution to total N uptake under field conditions is challenging (Näsholm *et al.*, 2009). This limitation results in a lack of comprehensive and robust plant N uptake metrics over larger scales, thereby impeding the incorporation of plant N uptake controlling factors into ecosystem and biogeochemical models. Addressing this challenge is crucial to raise the predictive capacity of such models by accurately representing C and N interactions (Zhu & Zhuang, 2013).

Many studies highlighted that plant species have specific preferences for available N forms, which is influenced by a combination of genetic, physiological, and environmental factors (Chapin *et al.*, 2011; Britto & Kronzucker, 2013b). For instance, some plant species have a reduced ability to utilize NO_3^- and therefore prefer NH_4^+, such as *Ericaceae*, spruce or rice (Kronzucker *et al.*, 1997; Min *et al.*, 1999; Clark *et al.*, 2003; Britto & Kronzucker, 2013; Chen *et al.*, 2022). By contrast, many forb and grass species, and fast-growing trees prefer NO_3^-, which enables them to more easily establish their cation–anion balance and meet their stoichiometric needs (Guo *et al.*, 2007; Nacry *et al.*, 2013). Genotype or species likely underlie differences in N preferences that are also plastic and responsive to environmental conditions. Conversely, other species possess adaptations facilitating the efficient utilization of organic N forms, including specialized transporters and enzymes involved in the mobilization of organic N compounds, or specific mycorrhizal associations. For instance, sedges and *Cyperaceae* thrive well on organic N forms and to prefer reduced N forms (Raab *et al.*, 1999), while many grasses prefer nitrate (Kahmen *et al.*, 2006). Moreover, coexistent plant species were demonstrated to exhibit chemical niche partitioning in N uptake, accompanied by distinguishable root traits (Liu *et al.*, 2022). These differences in N preference among plant species underpin plant community shifts in response to altered N inputs and N cycling, as well as increased ecosystem losses of N forms mineralized in excess to plant uptake. Furthermore, plant N uptake comprises a crucial root functional trait that is performed by absorptive roots and is closely linked to other root functional traits (Moreau *et al.*, 2019; Freschet *et al.*, 2021). Multiple lines of evidence have emphasized the importance of absorptive fine roots in ecosystem functioning, suggesting that root functional traits provide novel insights into global N cycling under current and future climatic conditions (Iversen *et al.*, 2015; McCormack *et al.*, 2015; Freschet *et al.*, 2021). However, *in situ* root N uptake metrics are notably limited at large scales compared to the other root functional traits, impeding our understanding of belowground processes from the perspective of root functions (Ma *et al.*, 2018; Freschet *et al.*, 2021).

Grasslands occupy > 40% of all Earth's land surface (White *et al.*, 2000) and provide important ecosystem services including food production, soil conservation, carbon storage, and climate change mitigation (O'Mara, 2012; Wilsey, 2018; Stromberg &

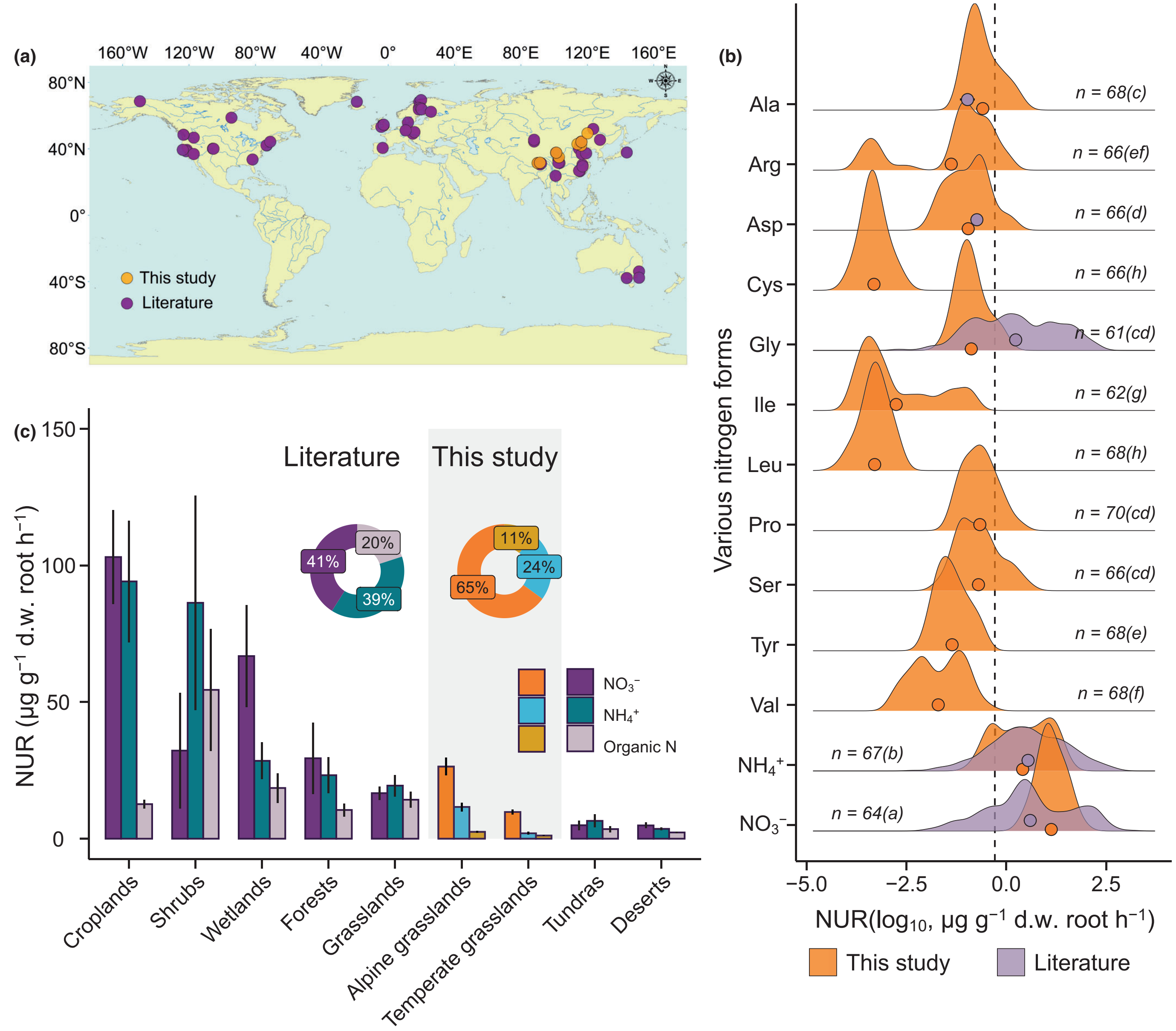

Fig. 1 Locations of sites for the meta-analysis, and of this study (a), plant uptake metrics of various nitrogen forms (b) and nitrogen uptake rates (NUR, μg g^{-1} d.w. root h^{-1}) of nitrate, ammonium, and organic nitrogen in seven ecosystem types from the literature as well as alpine and temperate grasslands in this study (c). To better fit normal distribution and a better visual effect, log$_{10}$(NUR) is plotted in this figure (b). NUR indicates N uptake rate, expressed as μg N g^{-1} d.w. root h^{-1}. Numbers identify the number of plant-specific observations. Abbreviations on the *y*-axis denote amino acid forms (in alphabetical order). Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cysteine; Gly, glycine; Ile, isoleucine; Leu, leucine; Pro, proline; Ser, serine; Tyr, tyrosine; Val, valine. Log$_{10}$(NUR) was analyzed by one-way ANOVA model by N form (11 forms of amino acids, ammonium (NH_4^+ in figure), and nitrate (NO_3^- in figure), Table 1). Small letters in brackets in (b) indicate pairwise differences between log$_{10}$(NUR) values of various N forms in the current study, analyzed by Tukey HSD test. Dotted line indicates the average NUR in both this study and the literature, as 0.517 μg N g^{-1} d.w. root h^{-1}. Mean ± SE are indicated in (c). Bars in (c) indicate NUR distribution while circle sectors in (c) indicate contribution of nitrate, ammonium, and organic N to total plant N uptake, respectively, for literature and this study. For literature, the organic N was mainly glycine, while organic N in this study was the total uptake of 11 forms of amino acids.

Staver, 2022). With their diverse plant species assemblages and broad range of environmental conditions, grasslands offer a unique opportunity to study N uptake metrics for a broad range of natural plant species across large spatial scales. Here, we provide the first comprehensive global analysis of plant uptake metrics of inorganic and organic forms of soil N in grasslands and investigate the role of N availability to regulate root N uptake. We first identified 60 studies and compiled uptake metrics from 148 plant species that simultaneously measured *in situ* uptake of amino acids (mainly as glycine or alanine), NO_3^-, and NH_4^+ by plant roots across a wide range of ecosystems including croplands, shrubs, wetlands, forests, grasslands, tundra, and deserts, while reporting soil available N (Fig. 1a). We then assessed *in situ* N uptake rates by roots using a wide

Table 1 Detailed geographic and climatic characteristics of the eight study sites.

Site name	Site ID[1]	Latitude	Longitude	Elevation (m)	MAP[2] (mm)	MAT[3] (°C)	Climate type
Baingoin County	Baingoin	31°26′N	90°02′E	4700	310	−1.1	Alpine
Nagqu County	Nagqu	31°36′N	91°29′E	4568	406	−2.1	Alpine
Hezuo Municipality Station	Hezuo	34°58′N	102°53′E	2872	630	1.1	Alpine
Haibei Station	Haibei	37°37′N	101°19′E	3400	600	−1.7	Alpine
Duolun County	Duolun	42°02′N	116°17′E	1324	385	2.1	Temperate
Sonid Left Banner Station	Sonid	42°53′N	114°04′E	1182	223	2.0	Temperate
Xilinhot Station	Xilinhot	44°11′N	116°27′E	1000	295	1.8	Temperate
Hulunbeir Station	Hulunbeier	49°21′N	120°07′E	618	308	−2.6	Temperate

[1] The distribution of study sites was displayed in map of Supporting Information Fig. S1.
[2] Mean annual precipitation.
[3] Mean annual temperature.

Table 2 Dominant plant species collected at the eight study sites.

Climate	Site ID[1]	Grass species	Species ID[2]	Family	Root : Shoot
Alpine grasslands	Baingoin	*Stipa purpurea* Griseb.	SPU1	Poaceae	0.55
		Trisetum bifidum (Thunb.) Ohwi	TBI	Poaceae	1.96
	Nagqu	*Kobresia pygmaea* (C. B. Clarke) C. B. Clarke	KPY	Cyperaceae	18.79
		Stipa purpurea Griseb.	SPU2	Poaceae	0.56
	Hezuo	*Kobresia humilis* (C. A. Mey. ex Trautv.) Serg.	KHU1	Cyperaceae	6.57
		Euphorbia fischeriana auct. non-Steud.	EFI	Euphorbiaceae	6.95
		Elymus nutans Griseb.	ENU	Poaceae	0.68
	Haibei	*Kobresia humilis* (C. A. Mey. ex Trautv.) Serg.	KHU2	Cyperaceae	3.85
		Stipa aliena Keng	SAL	Poaceae	1.02
Temperate grasslands	Duolun	*Stipa sareptana* A. K. Becker	SSA1	Poaceae	0.98
		Leymus chinensis (Trin. ex Bunge) Tzvelev	LCH1	Poaceae	0.52
	Sonid	*Stipa grandis* P. A. Smirn.	SGR	Poaceae	0.47
		Allium bidentatum Fisch. ex Prokh.	ABI	Amaryllidaceae	2.71
	Xilinhot	*Stipa sareptana* A. K. Becker	SSA2	Poaceae	1.03
		Leymus chinensis (Trin. ex Bunge) Tzvelev	LCH2	Poaceae	0.69
	Hulunbeier	*Stipa baicalensis* Roshev.	SBA	Poaceae	0.43
		Leymus chinensis (Trin. ex Bunge) Tzvelev	LCH3	Poaceae	0.19
		Carex duriuscula C. A. Mey.	CDU	Cyperaceae	2.77

[1] The distribution of study sites was displayed in map of Supporting Information Fig. S1. The detailed site name of Site ID is the same as Table 1.
[2] Short name of the corresponding grass species.

range of chemical N forms (including 11 amino acids and two inorganic N forms) by 18 combinations of plant species and study site along a 3000 km transect spanning temperate and alpine grasslands (Supporting Information Fig. S1). This transect provided a unique opportunity to explore plant N uptake metrics across large spatial scales and encompassing a broad spectrum of temperate and alpine grasslands (Shen *et al*., 2015), and gradients of soil and climate conditions.

We selected eight grasslands–four alpine and four temperate grasslands (Table 1; Fig. S1) – and quantified N uptake of the two or three most dominant plant species at each site (Table 2), using *in situ* ^{15}N and ^{13}C dual labelling (Fig. S2). Thirteen N forms (Table S1), comprising two forms of ^{15}N-labeled inorganic N and 11 forms of ^{13}C and ^{15}N dual-labeled amino acids, which collectively contributed 67% of the total pool of amino acids in soils (Fig. S3), were applied to represent the plant-available soil N pool. Cyperaceae species (e.g. sedges) exhibit a preference for organic over inorganic N forms, for the cost-effectiveness of utilizing organic N and due to adaptations to nutrient-poor environments where inorganic N forms are less available. We hypothesized that: (1) dominant grassland species have specific preferences for uptake of inorganic and organic N forms, with Cyperaceae preferring organic N forms and Poaceae and forbs preferring inorganic N forms, particularly NO_3^-. We further expected that plant uptake preferences will be plastic and phenotypically respond to N availability in soil. Therefore, we hypothesized that: (2) N uptake by plants partially reflects its availability in soil; and (3) the uptake of organic N in alpine grasslands exceeds that of inorganic N, due to the higher FAA availability in alpine than in temperate grassland soils. Overall, we postulated that root N uptake is determined by plant species, and modulated by soil N availability, which is itself influenced by climate, edaphic factors (i.e. soil pH, water content, soil organic carbon, C : N), and intrinsic factors of the N forms (e.g. molecular mass, N%, water solubility, net charge, isoelectric point, and hydrophobicity).

Materials and Methods

Study sites and plant species

Eight grassland sites in China were selected for this study (Fig. S1). These eight sites ranged from southwest China on the Tibetan plateau to northeast China in Inner Mongolia and can be classified into alpine and temperate grasslands (i.e. four alpine grasslands and four temperate grasslands) of varying distinct elevation and climatic conditions (Methods S1). Two or three dominant plant species were selected at each study site to measure the uptake of 11 amino acids and two forms of inorganic N (NO_3^- and NH_4^+). The different amino acids have distinct intrinsic properties, such as molecular mass, N%, water solubility, net charge, isoelectric point, and hydrophobicity (Table S1). Details of the selected plant species of each study site are listed in Table 2, and detailed soil background information is listed in Table S2.

In situ labeling experiment

In total, 11 forms of ^{13}C : ^{15}N dual-labeled amino acids (Methods S2) and two forms of ^{15}N labeled inorganic N were used to trace the N uptake patterns by plant roots. Dual ^{13}C and ^{15}N labeled amino acids were employed to precisely evaluate their intact uptake, with the presence of both ^{13}C and ^{15}N in the plant root confirming that the amino acid itself was absorbed. This approach accounts for the rapid turnover of amino acids and the potential uptake of ^{15}N labeled breakdown products (NH_4^+ and NO_3^-) which would not be linked to concurrent uptake of ^{13}C. Isotope enrichments of the labeled amino acids and inorganic N forms were 98–99 atom% ^{15}N (and 98–99 atom% for ^{13}C in amino acids). Considering that water was used as the control treatment, there were a total of 14 treatments in this labeling experiment. For each treatment, four replicates were set up and a total of 56 (14 treatments × 4 replicates) small quadrats with a size of 15 cm × 15 cm on a uniform grassland area were established at each site using a wireframe. These small quadrats were randomly chosen for labeling for each treatment.

Sticks were used to separate the small quadrats into nine subplots, as indicated in the Fig. S2. In each small quadrat, the labeling solution was slowly injected at the center of each subplot to homogenously distribute the labeling solution and to mix the tracer well with the native soil N pools (Liu *et al.*, 2020). Nine milliliter of labeling solution was injected at each point of the subplot, with each 3 ml injected at depths of 2.5, 7.5, and 12.5 cm. To do this, the needle was first inserted to a depth of 12.5 cm and then pulled out slowly for the injections at 7.5 cm and 2.5 cm soil depth. This process was aimed at evenly distributing the labeling solution in the soil.

Short-term labeling experiments were conducted on sunny days in August 2017. The labeling solution contained on average 0.15 μg ^{15}N g^{-1} dry soil for each treatment, which comprises *c.* 10% of the soil inorganic N pool and a similarly small proportion of the soil amino acid pool (Liu *et al.*, 2020).

Sampling and analyses

Four hours after labeling, the whole quadrat was excavated to a depth of 15 cm by a spade and immediately brought to the laboratory.

Plants of the dominant species listed in the Table 2 were separated in intact form and collected. Plants were then carefully cut at the stem base to separate the shoot and root parts. Root samples were carefully washed with 50 mM KCl and water to remove soil particles and ^{15}N adsorbed onto the root surface. The soil samples remaining after collecting the roots were sieved through a 2 mm mesh and stored at −20°C before measurements. Shoot and root samples were then dried at 60°C for 48 h. After weighing the dry mass, the plant samples were ground into a fine powder using a ball mill (M200; Fa. Retsch, Haan, Germany) and weighed into tin capsules to determine their C and N contents and their ^{15}N : ^{14}N or ^{13}C : ^{12}C ratios using an Elementar Vario EL Cube or a Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced with either an Isoprime VisION continuous-flow isotope ratio mass spectrometer (IRMS, Elementar UK Ltd, Cheadle, UK) or a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK). Another group of fresh plants was collected to measure root functional traits at the same site (Methods S3).

Soil samples were thawed, extracted with 0.05 mol l^{-1} K_2SO_4 solution in a soil : solution ratio of 1 : 4 (w/v) for 60 min, and filtered through ashless cellulose filters. The extracts were used to measure soil NH_4^+ and NO_3^- concentrations using an auto-analyzer (AA3; Bran-Luebbe, Hamburg, Germany), as well as for the concentration of FAA using a high-performance liquid chromatography method (HPLC-MS/MS API3200 Q-TRAP, Foster City, CA, USA). Soil pH was analyzed using a pH analyzer at an air-dry soil : water ratio of 1 : 2. Soil electrical conductivity was analyzed using an electrical conductivity analyzer with an air-dried soil : water ratio of 1 : 2. Total soil N and carbon contents were measured using dry combustion (Vario Max; Elementar Analysensysteme GmbH, Hanau, Germany).

Calculations and statistical analysis

^{15}N atom excess (APE) was calculated as the difference in atom% ^{15}N between labeled and unlabeled plant samples, the latter collected in the water control treatments, as shown in Eqn 1.

$$\mathrm{APE}\ (\%) = \mathrm{atom\%}^{15}\mathrm{N}_{\mathrm{labeled}} - \mathrm{atom\%}^{15}\mathrm{N}_{\mathrm{unlabeled}} \qquad \text{Eqn 1}$$

Plant shoot or root ^{15}N contents (μg) were calculated by multiplying the shoot or root N content (μmol g^{-1}) by the corresponding APE and dry biomass (g), as well as the relative molecular mass of ^{15}N (Liu *et al.*, 2020) (Eqn 2).

$$^{15}\mathrm{N\ content}\ (\mu\mathrm{g}) = \mathrm{N\ content}\left(\frac{\mu\mathrm{mol}}{\mathrm{g}}\right) \times \frac{\mathrm{APE}}{100} \times \mathrm{biomass}\ (\mathrm{g}) \times 15\left(\frac{\mathrm{g}}{\mathrm{mol}}\right) \qquad \text{Eqn 2}$$

^{15}N uptake rates (^{15}NUR, $\mu g\,N\,g^{-1}$ d.w. root h^{-1}) were calculated by summing the shoot and root ^{15}N content, and then dividing by the labeling time (h) and root biomass (g), as indicated in Eqn 3.

$$^{15}\mathrm{NUR}\ \left(\mu g\ N\ g^{-1}\ \mathrm{d.w.\ root}\ h^{-1}\right) = \frac{\mathrm{shoot}\ ^{15}\mathrm{N\ content}\ (\mu g) + \mathrm{root}\ ^{15}\mathrm{N\ content}\ (\mu g)}{\mathrm{time}\ (h) \times \mathrm{root\ biomass}\ (g)} \qquad \text{Eqn 3}$$

Nitrogen uptake rates (NUR, $\mu g\,N\,g^{-1}$ d.w. root h^{-1}) were calculated by multiplying ^{15}NUR by the concentration of native NH_4^+, NO_3^-, or amino acids in soil (MN, $\mu g\ g^{-1}$), and then dividing by the concentration of added ^{15}N ($^{15}N_{added}$, $\mu g\ g^{-1}$) and ^{15}N labeling abundance (McKane *et al.*, 2002; Xu *et al.*, 2010) (i.e. 98–99%).

$$\mathrm{NUR}\left(\mu g\ N\ g^{-1}\ \mathrm{d.w.\ root}\ h^{-1}\right) = \frac{^{15}\mathrm{NUR}\left(\mu g\ N\ g^{-1}\ \mathrm{d.w.\ \ root}\ h^{-1}\right) \times \mathrm{MN}}{^{15}\mathrm{N}_{\mathrm{added}} \times 99\%} \qquad \text{Eqn 4}$$

The total N uptake was calculated by summing the amino acid and inorganic N uptake rates for each species. The uptake preference for each N form was then calculated for each specific N form divided by the total N uptake. The sum of the uptake rates of the 11 amino acids forms was calculated to represent the uptake rate of total free amino acids (TFAA). The sum of the targeted 13 forms of soil N was calculated to represent the total soil available N (SAN) concentration, and the soil N contribution (SANC) for each N form was calculated as the specific N form concentration divided by the total soil available N. Mixed-effects models were used to analyze the effects of climate (alpine or temperate grasslands), N forms, and their interactions on NUR (Methods S4). The percentage of intact ^{15}N to total ^{15}N uptake by amino acids was calculated by the $^{13}C:{}^{15}N$ excess ratio in the samples relative to the $^{13}C:{}^{15}N$ ratio of the applied amino acid, which was considered to be 100% (Näsholm *et al.*, 1998). Linear regressions between ln(NUR) and ln(soil available N), as well as ln(N uptake preferences) and ln(soil available N contribution), in both this study and data from the literature were analyzed. Data were collected from 60 published studies (Appendix A1). Structural equation modeling (SEM) was used to explore the direct and indirect relationships among plant N uptake, soil N availability, intrinsic properties, climate, soil properties, and root N content (Methods S4). Statistical analysis and figure production were performed using R (R v.3.6.1, R Core Team, Vienna, Austria). The lmerTest package was used for the mixed-effects models, and ggplot2 was used to make figures.

Results

N uptake by plants: the large scale transect and literature results

Plant species along the grassland transect displayed a clear preference for NO_3^- over NH_4^+ and FAA. All plants could take up various FAA (Fig. 1b). In addition to glycine (0.2 $\mu g\,N\,g^{-1}$ d.w. root h^{-1}), grassland plants showed comparable uptake rates for alanine, arginine, aspartic acid, proline, and serine. The uptake rates for tyrosine, valine and isoleucine were intermediate, but lower than for glycine. Conversely, uptake rates for cysteine and leucine were the lowest ($< 0.001\ \mu g\,N\,g^{-1}$ d.w. root h^{-1}, Fig. 1b; Tables S3, S4). Alpine and temperate grassland species did not show significant differences in uptake rates of the 11 specific forms of amino acids (Table S5).

The uptake rates of NH_4^+ and TFAA across the grassland transect were lower than the average rates in grasslands reported in the literature (Fig. 1c), while that for NO_3^- was 8.4% higher. Literature values indicated that NO_3^- accounted for 41%, NH_4^+ for 39%, and organic N for 20% of total plant N uptake. In comparison, our data from the grassland transect showed a much greater preference for NO_3^- uptake (65% of the total N uptake), with lower contributions of NH_4^+ (24%) and organic N (11%).

Soil N availability along the grassland transect

Based on the Köppen aridity index, expressed as MAP/(MAT+33) (Quan *et al.*, 2013), alpine sites (15 ± 4.5) had a more humid climate than the temperate sites (9.0 ± 2.0), which was reflected by higher soil water contents in alpine grasslands than in temperate grasslands, except of site Hulunbeier (Table S2). Soil NO_3^- availability differed little between all sites (range 8.3 to 15 $\mu g\,N\,g^{-1}$ d.w. soil), while soil NH_4^+ varied by a factor > 10-fold. Except for the site Sonid, soil NH_4^+ availability was higher in alpine grasslands than in temperate grasslands. Soil TFAA availability was higher in alpine than in temperate grasslands (Fig. 2) and ranged from 1.5 to 4.4 $\mu g\,N\,g^{-1}$ d.w. soil across both types. The summed concentrations of the 11 specific FAA forms used for *in situ* labelling followed a similar pattern to the 20 forms of TFAA across sites, with the 11 forms averaging 67% of the TFAA (Fig. S3). The contribution of NO_3^- to total available soil N ranged from 41% to 90% (Fig. S4) and NH_4^+ contributed more than 40% to total available N.

Soil concentrations of the 20 single FAA (Fig. S5) and their proportions in TFAA (Fig. S6) varied strongly among sites. Generally, TFAA proportions of alanine (14%), arginine (8.7%), aspartic acid (7.6%), glycine (6.0%), histidine (9.0%), methionine (8.9%), proline (16%), serine (9.4%), and threonine (11%) levels were high, while asparagine, isoleucine, lysine, phenylalanine, tryptophan, tyrosine, and valine contributed less to the TFAA pool, and cysteine, glutamine, glutamic acid, and leucine were undetectable.

Traits of the plant species

The investigated grassland species were predominantly grasses (Table 2, Poaceae; *Elymus nutans*, *Leymus chinensis* (2), *Stipa aliena*, *S. baicalensis*, *S. grandis*, *S. purpurea* (2), *S. sareptana* (2), *Trisetum bifidum*), followed by sedges (Cyperaceae: *Kobresia humilis* (3), *K. pygmaea*, *Carex duriuscula*), and two forb species (*Euphorbia fischeriana*–Euphorbiaceae, *Allium bidentum*–Amaryllidaceae), with some species sampled at two or three sites

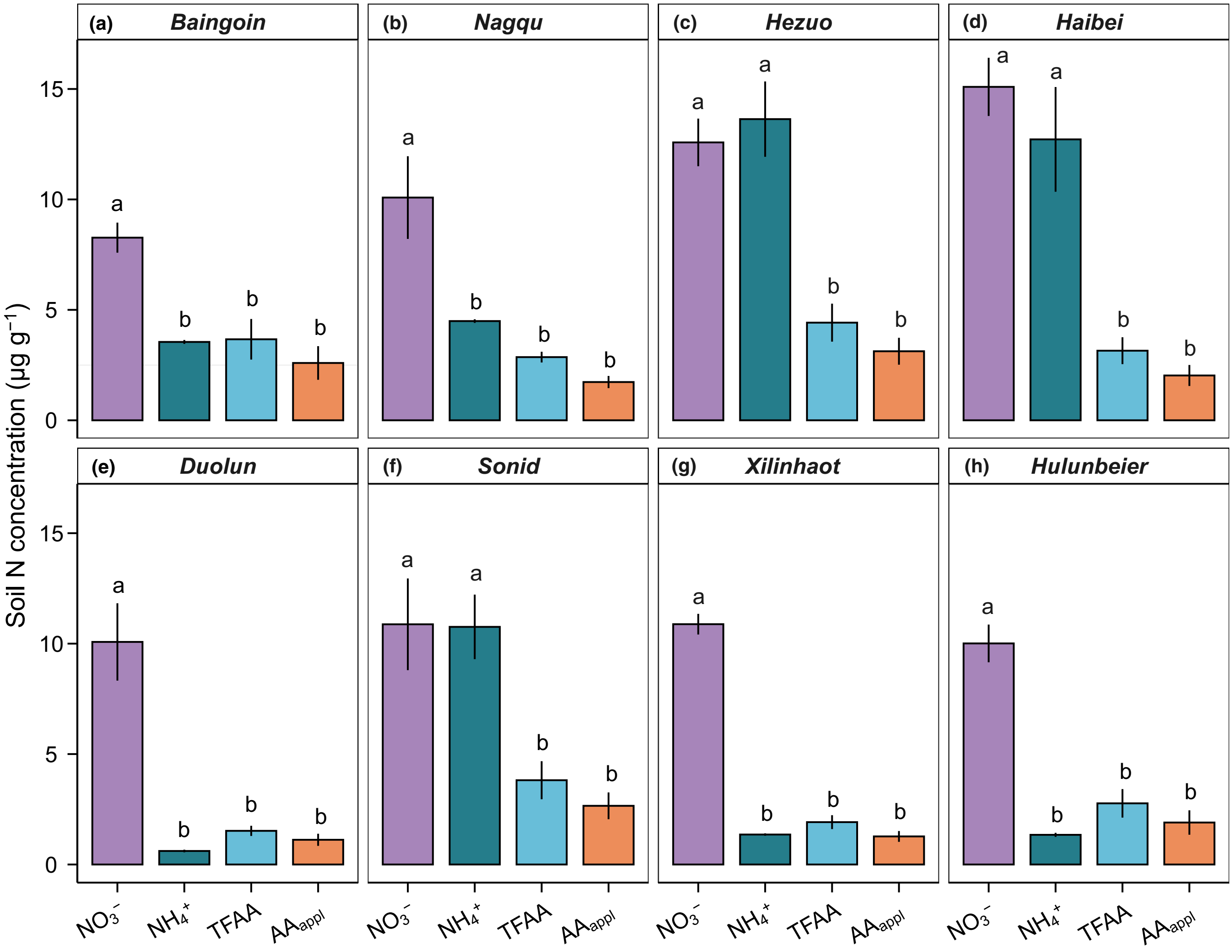

Fig. 2 Nitrogen availability in soils of the eight study sites. Nitrogen forms of NO_3^-, NH_4^+, TFAA (total free amino acids) and AA$_{appl}$ (the applied 11 forms of amino acids in this study) are displayed. The above four facets of (a–d) are alpine grasslands and the below four facets of (e–h) are temperate grasslands. Values are presented as mean ± SE of four replicates. One-way ANOVA was conducted separately for each study site to compare NUR across different nitrogen (N) forms. Lowercase letters above each bar indicate significant differences ($P < 0.05$) among the four N forms each site.

(indicated by 2 or 3 in brackets following the species name). The contribution of dominant species to total biomass in the alpine grasslands ranged from 41% to 94% (Fig. S7) while in the temperate grasslands it ranged from 82% to 100% (Fig. S8). In terms of root system development and first root diameter and length, the two forbs (the *Euphorbia fischeriana* and *Allium* species) had the thickest and longest first-order (finest) roots (Fig. S9).

Plant uptake rates of N forms by specific species

Generally, the grassland species had high-uptake rates of inorganic N, particularly of NO_3^-, followed by NH_4^+, and lower uptake rates of FAA (Fig. 3; Table S6). Most of the 18 dominant species × site combinations took up NO_3^- at the highest rate, whereas a few species (i.e. *Kobresia humilis* and *Allium bidentatum*) took up NH_4^+ at the highest rate (Fig. 3). Highest NO_3^- uptake rates were observed in three alpine Poaceae species (*S. purpurea* and *T. bifidum* in Baingoin, and *E. nutans* in Hezuo). NH_4^+ uptake rates were the second highest after NO_3^- in 15 species × site combinations and the third in these three combinations. As a result, FAA uptake was the second highest, partially shared with NH_4^+, in 3 combinations and the third in 15 combinations. Highest FAA uptake rates across species and sites were found in the alpine grass species *S. purpurea* and *T. bifidum* in Baingoin. Evaluated by the excess ^{13}C : ^{15}N ratios of FAA (Fig. S10), plant species showed a consistent preference for alanine, arginine, aspartic acid, glycine, proline, and serine (Figs S11–S14). Total N uptake rates were generally higher in alpine plants compared to temperate plants, with some variations among specific species (Fig. S15).

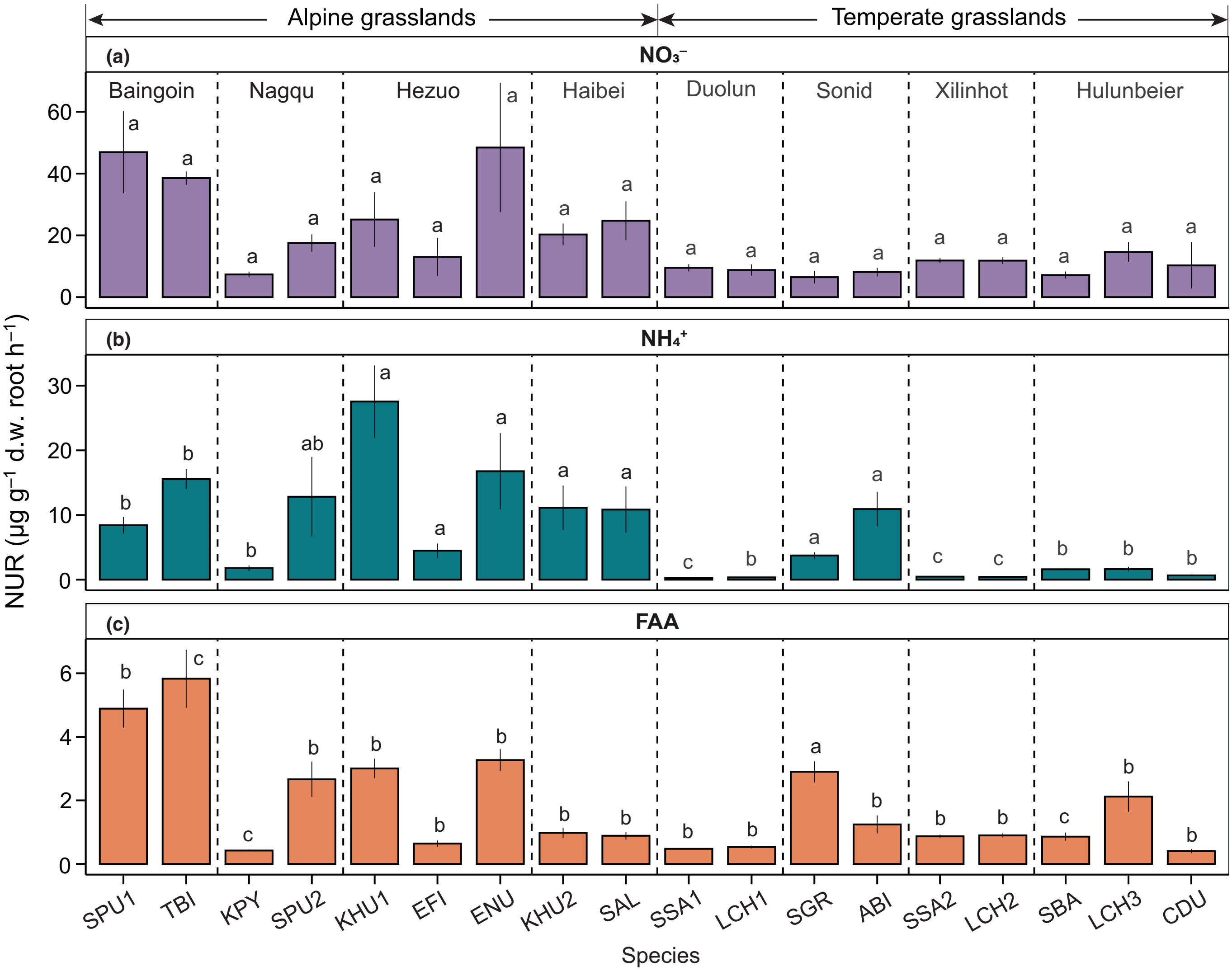

Fig. 3 Nitrogen uptake rates (NUR) of NO_3^- (a), NH_4^+ (b), FAA (free amino acids, c) between 18 species × sites combinations in eight study sites. One-way ANOVA was performed separately for each nitrogen (N) form to compare the NUR among the 18 combinations. Lowercase letters above the bar indicate significant differences of each species among N forms. Values are presented as mean ± SE of four replicates. ABI, *Allium bidentatum*; CDU, *Carex duriuscula*; EFI, *Euphorbia fischeriana*; ENU, *Elymus nutans*; KHU1, *Kobresia humilis* in Hezuo; KHU2, *Kobresia humilis* in Haibei; KPY, *Kobresia pygmaea*; LCH1, *Leymus chinensis* in Duolun; LCH2, *Leymus chinensis* in Xilinhot; LCH3, *Leymus chinensis* in Hulunbeier; SAL, *Stipa aliena*; SBA, *Stipa baicalensis*; SGR, *Stipa grandis*; SPU1, *Stipa purpurea* in Baingoin; SPU2, *Stipa purpurea* in Hezuo; SSA1, *Stipa sareptana* in Duolun; SSA2, *Stipa sareptana* in Xilinhot; TBI, *Trisetum bifidum*.

Plant N uptake rates by climate condition and plant family

Based on the analysis of mixed-effects models, climate (alpine vs temperate grassland) did not affect the N uptake rates of the 11 forms of FAA tested but it affected the uptake rates of NO_3^-, NH_4^+, and TFAA (Tables S3, S7; Fig. S16). Generally, plants in all grasslands showed an uptake pattern of $NO_3^- > NH_4^+ >$ TFAA (Fig. 4a). Particularly, the uptake rates of NH_4^+ were higher in alpine grasslands than in temperate grasslands (Table S8; Fig. S17). Uptake rates of NO_3^- and TFAA did not differ between plant species in alpine and temperate grasslands (Fig. S17).

Plant species belonging to Poaceae, Cyperaceae, and other families all had high-uptake rates for NO_3^- (Fig. 4b). For Poaceae and Cyperaceae species, the uptake of NO_3^- was higher than that of NH_4^+. While uptake rates of NH_4^+ were similar to that of TFAA in Poaceae, uptake rates of NH_4^+ were greater than for TFAA in Cyperaceae and in other families (Table S9).

Plant N uptake preference regulation

The uptake of alanine, proline, and serine contributed the most to the total uptake of N (the sum of both, organic and inorganic forms) (Fig. 4c). Individually these contributions ranged between 2.1 and 2.3%, followed by arginine, aspartic acid, and glycine (0.7–1.4%). Overall, FAA uptake contributed 10.5% (calculated by ^{15}N) and 4.6% (evaluated by excess $^{13}C : ^{15}N$ ratio) to total plant N uptake.

Climate differences had only a minimal effect on N uptake preferences at the species level (Tables S3, S10), but N form and its

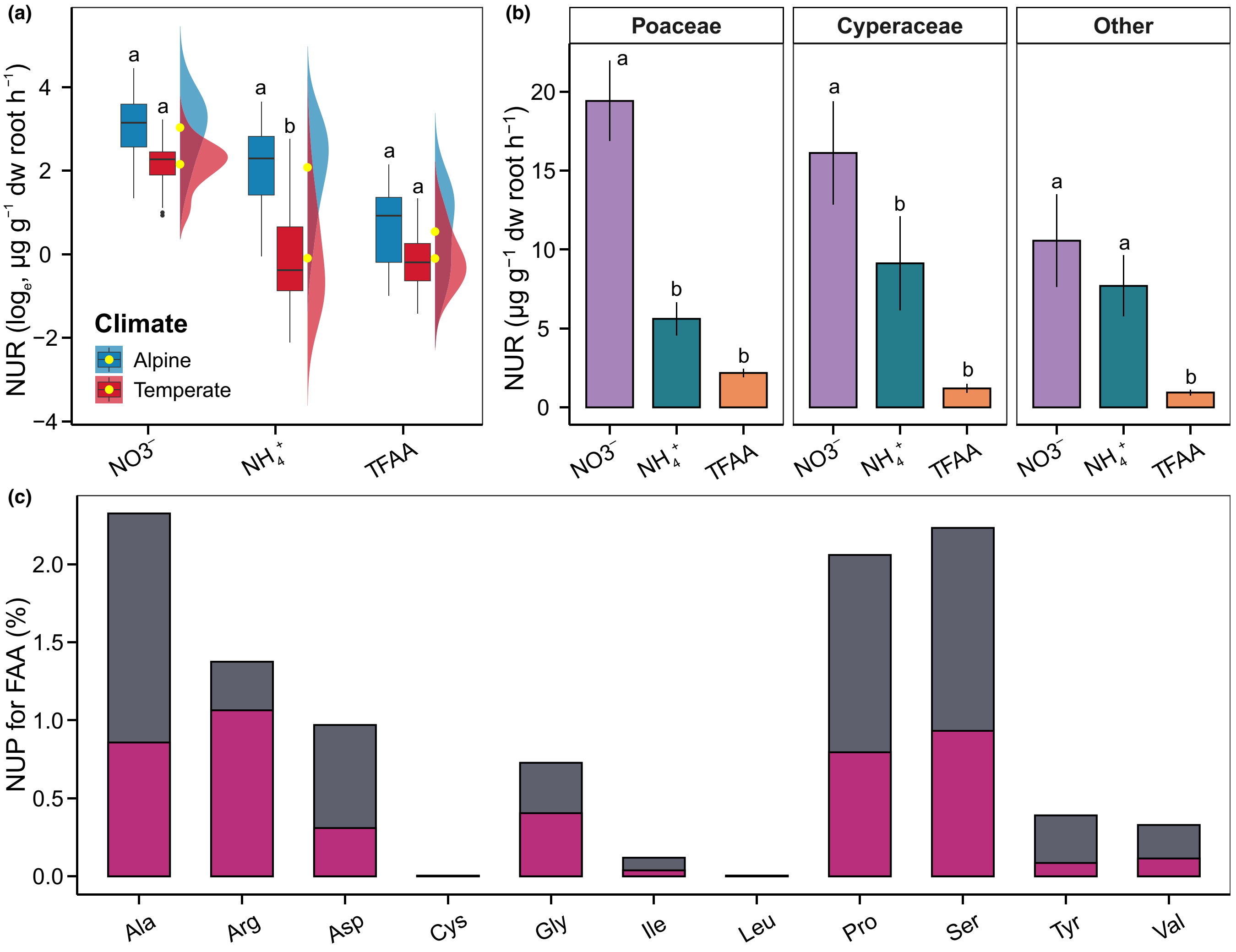

Fig. 4 Pairwise comparisons of nitrogen uptake rates (NUR) between alpine and temperate grasslands for NO_3^-, NH_4^+ and total free amino acids (TFAA, a), and between plant families (b). Bar plots of nitrogen uptake preferences (NUP) for specific forms of FAA to total N uptake (c). Alpine plants exhibited higher NUR for NH_4^+ than temperate plants (a). Log$_e$-transformed data are shown in (a). Pairwise comparisons between alpine and temperate grasslands are based on the Tukey test of mixed-effects models with ecosystem and N form as fixed factors, and site and plant species as random factors (model 2 in Supporting Information Table S3). Boxplots of NUR show the 25th to 75th percentiles and median, with whiskers indicating 10th and 90th percentiles and circles marking outliers (a). Yellow dots indicate group means and the half-violin plots show the data density for each group (a). Lowercase letters above the boxes indicate significant differences of each species between the two climates. Values are presented as mean ± SE in (b). 'Other' in (b) includes Euphorbiaceae and Amaryllidaceae. Pairwise comparisons between each N form by the same family are shown by uppercase letters based on the Tukey test of mixed-effects model 3 in Table S3. NO_3^- contributed most to N uptake by grassland species, and total free amino acids (TFAA) contributed 10.5% (4.6% in an intact form) to total organic and inorganic NUR (c). *x*-axis codes denote amino acid forms. Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cysteine; Gly, glycine; Ile, isoleucine; Leu, leucine; Pro, proline; Ser, serine; Tyr, tyrosine; Val, valine. Ala, Arg, Asp, Pro, and Ser contributed more than Gly to total NUR. Purple colored parts in c indicate the adjusted preference based on the excess of $^{13}C : {}^{15}N$ ratios (Fig. S5).

interaction with climate had significant effects (Table S3). At each site, plant N uptake preferences showed specific patterns among coexisting grassland species (Fig. 5a; Table S11). For the specific contributions of the studied 11 forms of FAA, N uptake preferences by the 18 species × site combinations showed similar patterns. Across the sites, grassland species consistently preferred to take up alanine, arginine, aspartic acid, glycine, proline, and serine, while other FAA forms were less preferred (Figs S18–S21).

The N uptake preferences of NO_3^-, NH_4^+ and TFAA of coexisting species in alpine grasslands of Baingoin and Haibei, as well as temperate grasslands of Duolun, Xilinhot, and Hulunbeier all showed a strong preference for NO_3^- (Fig. S22). Large species-specific differences in N uptake preferences in coexisting species at each site were based on alanine, arginine, aspartic acid, glycine, proline, and serine (Fig. S23).

Factors determining plant N uptake

Across the grassland transect, N uptake rates increased with increasing soil N availability, explaining 92.2% of the variance in N uptake rates by the 18 species × sites combinations (Fig. 5b). Preferences for particular N forms increased with an increasing

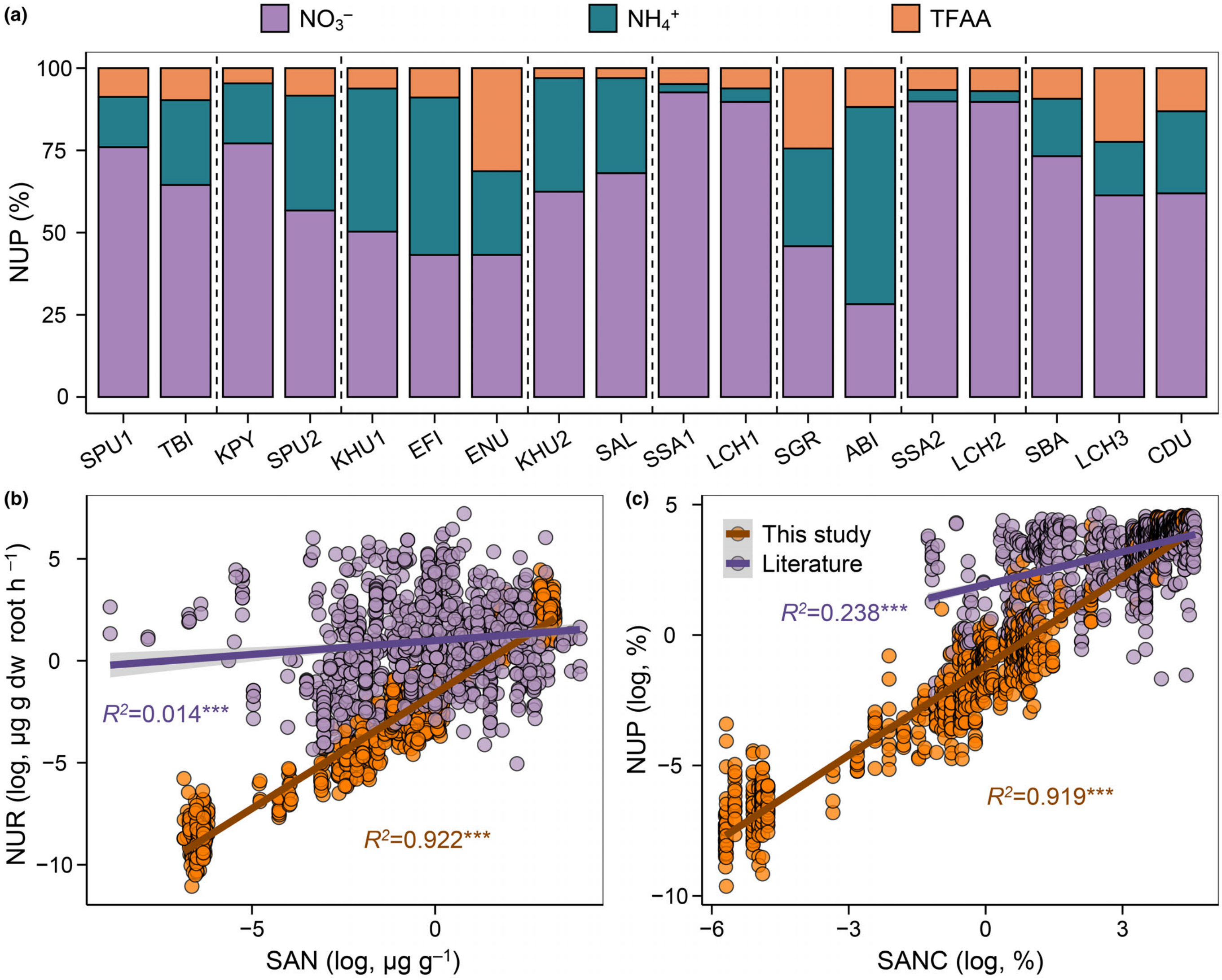

Fig. 5 Nitrogen uptake preference (NUP) for NO_3^-, NH_4^+ and total free amino acids (TFAA, a) between 18 plant species in eight study sites. Pairwise comparisons between nitrogen form and the 18 combinations are based on the Tukey test of mixed-effects models for NUP (a). Linear regressions between soil available nitrogen (SAN) concentration in soil (μg g^{-1}), and nitrogen uptake rates (b, NUR, μg g^{-1} d.w. root h^{-1}), and soil available nitrogen contribution in soil (c, SANC, %) and nitrogen uptake preference (NUP, %). To fit to normality, log_{10}-transformation was used in these analyses. The data from this study are in orange, and values from the literature are in purple. *** indicates significant results at $P < 0.001$. For data from this study, the df was 858, and for data from the literature, the df was 1002. ABI, *Allium bidentatum*; CDU, *Carex duriuscula*; EFI, *Euphorbia fischeriana*; ENU, *Elymus nutans*; KHU1, *Kobresia humilis* in Hezuo; KHU2, *Kobresia humilis* in Haibei; KPY, *Kobresia pygmaea*; LCH1, *Leymus chinensis* in Duolun; LCH2, *Leymus chinensis* in Xilinhot; LCH3, *Leymus chinensis* in Hulunbeier; SAL, *Stipa aliena*; SBA, *Stipa baicalensis*; SGR, *Stipa grandis*; SPU1, *Stipa purpurea* in Baingoin; SPU2, *Stipa purpurea* in Hezuo; SSA1, *Stipa sareptana* in Duolun; SSA2, *Stipa sareptana* in Xilinhot; TBI, *Trisetum bifidum*.

contribution of that N form to the total available soil N pool, explaining 91.9% of the variance in uptake preferences of the 18 species × sites combinations (Fig. 5c). For data derived from literature, the relationships were much weaker, explaining only 1.4% in uptake rates (Fig. 5b) and 23.8% in uptake preferences (Fig. 5c). These patterns were also discovered by separating N forms of NH_4^+, NO_3^-, and FAA in the regression (Fig. S24). In our data set, the strong control of plant N uptake rates by soil available N concentrations and of plant N uptake preferences by the contributions of soil available N, respectively, were further highlighted by PCA (Fig. 6). In the PCA analysis, N uptake rates and N uptake preferences both scaled positively with principal component 2 (Dim2, explaining 15.3% of overall variance), which co-varied positively with respective soil N concentration, MAP, soil pH, fine root length and strongest root diameter, and negatively with shoot biomass, shoot C : N and molecular mass of the respective N form (Fig. S25; Table S12).

To better constrain direct and indirect effects of soil properties, of climate and plant root properties on root N uptake rates, a structural equation model approach (SEM) was applied (Fig. 7). This approach also clearly showed that N uptake rates across different plant species and N forms was driven by their

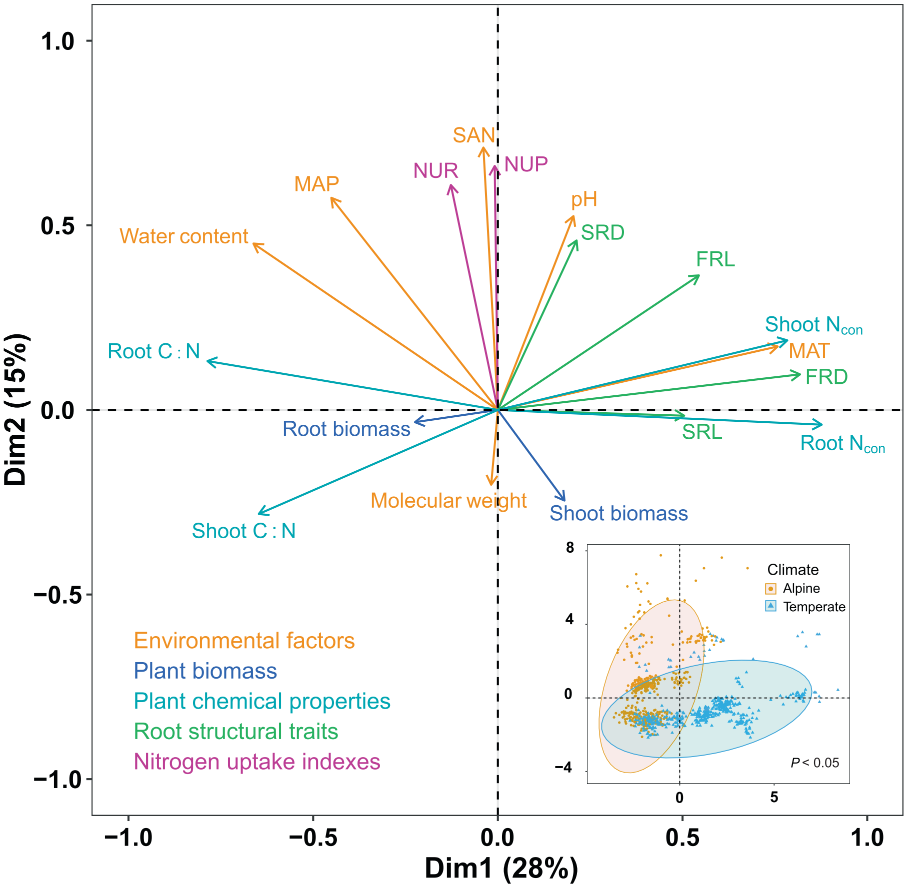

Fig. 6 Principal component analysis (PCA) of plant nitrogen uptake rates (NUR), nitrogen uptake preferences (NUP) and a range of plant and environmental traits. Parameters are classified by environmental factors, plant biomass, plant chemical properties, root structural traits, and nitrogen uptake indexes. Environmental factors include site elevation (Elevation), soil water content (Water content), mean annual precipitation (MAP), soil available nitrogen (SAN), soil pH (pH), mean annual temperature (MAT), and the molecular weight of the corresponding N form (Molecular weight). Plant biomass include shoot biomass and root biomass. Plant chemical properties include shoot N content (Shoot N$_{con}$), root N content (Root N$_{con}$), C : N ratio in root (root C : N), and C : N ratio in shoot (shoot C : N). Root structural traits include the first-order root diameter (FRD), the first-order root length (FRL), the strongest root diameter (SRD), and the strongest root length (SRL). Nitrogen uptake indices include plant N uptake rates (NUR) and plant N uptake preference (NUP). Plant N uptake rates and preferences were positively related to the corresponding soil N concentrations and were negatively related with the molecular weight of the N forms.

concentrations in soils (standardized path coefficient, spc 0.94), and to a much lower proportion affected by edaphic properties (spc −0.03; e.g. soil pH, soil water content, soil total C and soil C : N), and N form intrinsic properties (spc −0.04; e.g. molecular mass, element composition, hydrophobicity, solubility, isoelectric point). Climate (MAT, MAP) had no direct effect and root properties (here only root N content, sdc 0.03) only a small effect on N uptake rates.

Discussion

Plant N uptake metrics of amino acids

Our experimental results illustrate that dominant plant species in grasslands take up a diverse range of amino acids in their intact form at rates comparable to those reported in other studies (Xu *et al*., 2006; Zhang *et al*., 2015) (Fig. 1b,c). N uptake by grasslands, as reported in both literature and this study, is generally comparable to other ecosystems such as forests, tundra, and deserts (Fig. 1c). Plants in croplands exhibit particularly high uptake of inorganic N, primarily due to selection of high-yield crop varieties with high-N requirements (Salvagiotti *et al*., 2008). Shrubs demonstrate similar uptake rates of NH_4^+, NO_3^- and glycine (Fig. 1c). By contrast, wetlands exhibit especially high uptake of NO_3^-, attributed to its efficient diffusion in water-saturated soils (Haynes & Goh, 1978). Among the 11 amino acids, alanine, aspartic acid, proline, and serine have the highest uptake rates, which were comparable to glycine (Fig. 1b). Glycine, serine, alanine, and arginine are among the most abundant bioavailable organic N forms in agricultural and forest soils, and play an important role in soil N cycling (Inselsbacher & Näsholm, 2012). This largely conforms with our results on plant N uptake (Fig. 1b) and soil amino acid concentrations (Fig. S6) across alpine and temperate grasslands. Amino acids displaying high-uptake rates belong to the category of hydrophilic, highly soluble, or low-molecular weight compounds, whereas those with comparatively low-uptake rates (such as cysteine, isoleucine, and leucine) are characterized by high hydrophobicity, low solubility or high-molecular weight (Table S1). We found that uptake rates of amino acids increase with lower molecular weight and with decreasing hydrophobicity (Fig. S26), besides arginine and aspartic acid. Hydrophilicity and water solubility are contingent upon an organic and inorganic N ability to form hydrogen bonds with water, a property that declines with increasing molecular size. These characteristics facilitate solute diffusivity within the soil solution, thereby allowing rapid transport to the root surface.

Molecular weight influences amino acid uptake (Figs 6, S24, S25) as smaller molecular weight amino acids have higher diffusion coefficients in soils and more easily pass through the specific transporters located in the plasma membranes of root epidermal cells (Tegeder & Rentsch, 2010). The net charge of an amino acid is determined by its isoelectric point relative to soil pH, which reinforces soil sorption to cation exchange sites, thus impeding diffusion and uptake processes. Notably,

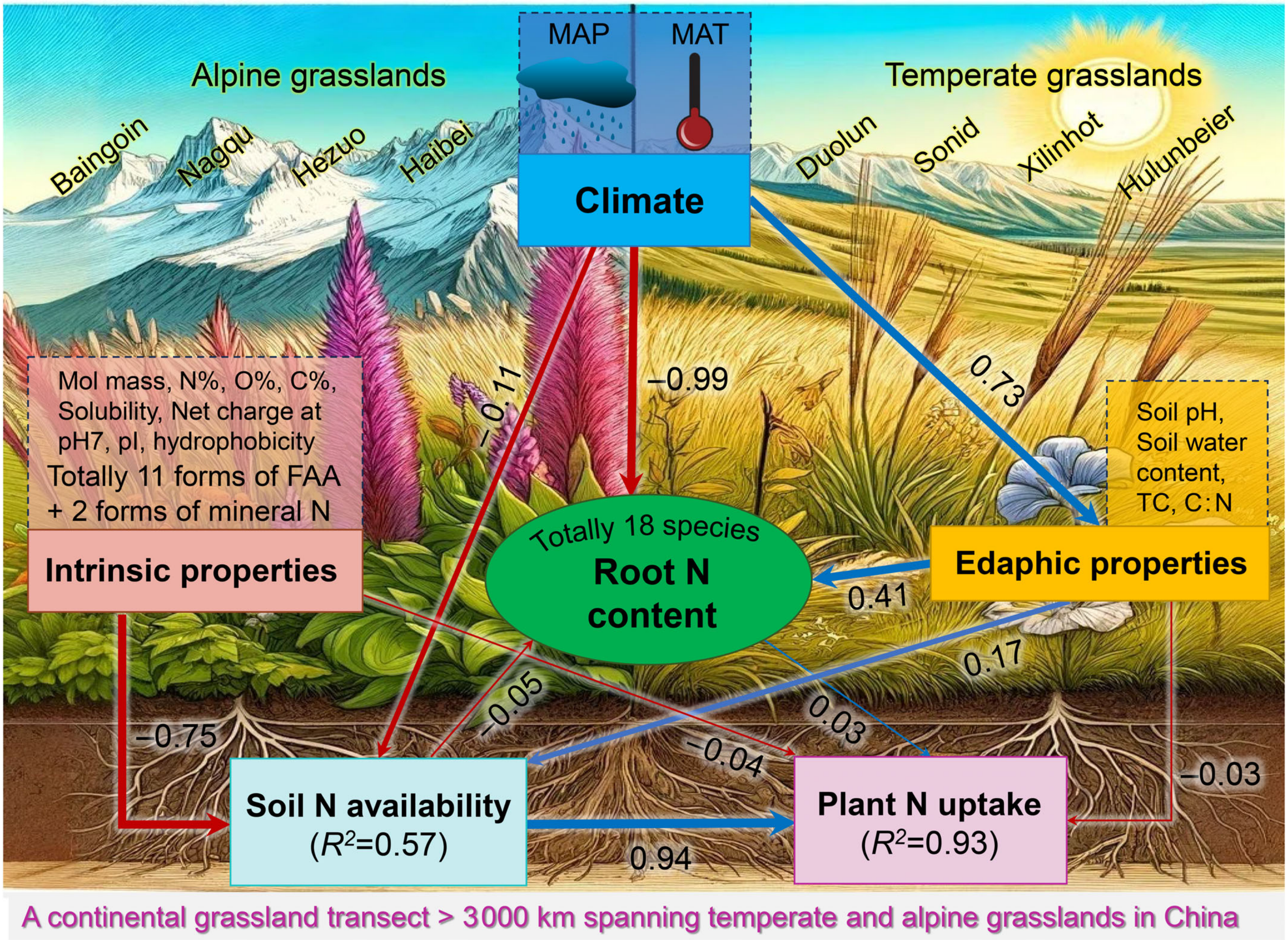

Fig. 7 Piecewise structural equation model (SEM) showing the direct and indirect relationships among plant nitrogen (N) uptake, soil N availability, intrinsic properties of N forms, climate, edaphic properties, and root N content. The background image was generated by an AI-based assistant (OpenAI's ChatGPT-4 model), on April 3, 2024. The intrinsic properties and edaphic properties were represented by the PC1 of principal coordinate analysis. Indices included into intrinsic properties were molecular mass (mol mass), N%, O%, C%, water solubility, net charge at pH 7, isoelectric point (pI), and hydrophobicity of the targeted N forms. Edaphic properties included soil pH, soil water content, soil organic carbon (SOC), and soil C : N. Climate was represented by MAP/(MAT+5). Red and blue paths refer to the nature of the relationships (negative and positive, respectively). The numbers given adjacent to the arrows are the standardized path coefficients, and the thickness of the paths is scaled based on this. The R^2 values associated with soil N availability and plant N uptake are presented within the boxes.

although soil pH was similar across sites, the interplay between net charge and soil pH can influence the kinetics of amino acid uptake. Clearly, the widespread nature of amino acid uptake by plants, albeit at rates differing by > 100-fold among amino acid species (e.g. leucine vs alanine) and across plant species, underscores the need for investigations into plant uptake metrics of a broad array of amino acids relative to inorganic N in soils to fully understand plant N acquisition strategies and their role in terrestrial N cycling.

Nitrogen uptake preference

Throughout the grassland transect, plant species displayed a clear preference in uptake hierarchy, with $NO_3^- > NH_4^+ >$ TFAA, mirroring the overall mean pattern of *in situ* observations across the 8 sampling sites (Figs 1c, 8). These preferences are moderated by the availability of N forms, supporting our second hypothesis that N uptake by plants partially reflects its availability in soil. This N uptake pattern was attributed to the greater NO_3^- mobility in soils (Haynes & Goh, 1978) and its greater availability relative to other N forms (Fig. S3), which is likely caused by fast nitrification rates across grasslands. Uptake of NO_3^- by plants is generally induced by high-NO_3^- concentrations (Devienne-Barret *et al.*, 2000). However, the specific uptake preference along the grassland transects diverged from the mean in grasslands from prior reports, which demonstrated a similar preference for NO_3^- and NH_4^+ but lower preference for FAA (Fig. 1c). Such disparities primarily arise from differences in

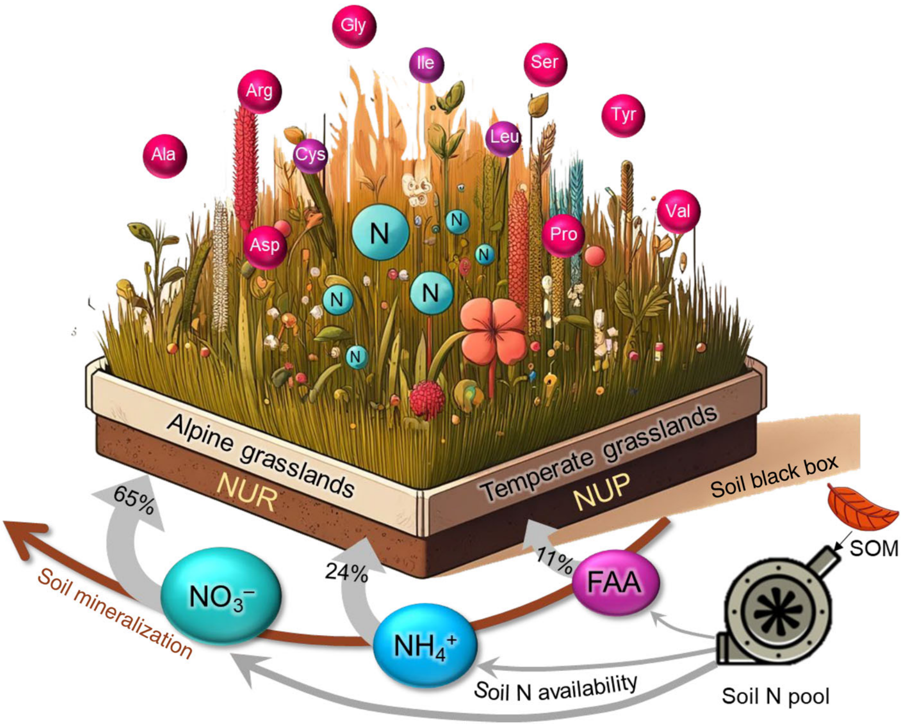

Fig. 8 Conceptual figure of nitrogen (N) uptake strategies by 18 species × site combinations in grasslands. Plants in grasslands largely prefer to take up NO_3^- over NH_4^+ and amino acids. Soil N availability and soil N form composition is strongly affected by microbial transformation processes which controls plant N uptake strategies (NUR, N uptake rates, and NUP, N uptake preferences) by plants. A continental grassland transect > 3000 km spanning temperate and alpine grasslands in China was studied, by *in situ* ^{15}N labelling experiments based on 11 forms of amino acids and two forms of inorganic N.

applied concentrations of N forms across the studies and encompasses study-wise differences in other parameters related to plant physiology, mycorrhizal symbiosis, and the supply of available N forms in soils as well as their delivery to the root surface (Moreau *et al.*, 2019), reflecting the complexity and intricate nature of plant N uptake and preference.

Taken together, all 11 amino acids collectively contributed 11% (solely from ^{15}N incorporation) and 4.6% (i.e. 41% in the intact form) to total plant N uptake. The pool of amino acid is extremely transient (turning over *c.* 20 times per d) and amino acid diffusive fluxes are comparatively fast as demonstrated by measurements of dissolved organic N dynamics (Jones & Kielland, 2002) and microdialysis approaches (Inselsbacher *et al.*, 2011b; Inselsbacher & Näsholm, 2012). Despite lower concentrations, amino acids can reach plant roots faster than inorganic N, resulting in the soil N supply to roots to be dominated by organic N (Inselsbacher & Näsholm, 2012). Consequently, without considering these higher fluxes of amino acids, our estimates could underestimate the contribution of plant-available amino acids. Generally, organic N plays an important role in plant nutrition and biomass production, especially under N-limited conditions, though in the studied grasslands organic N uptake was limited by low-amino acid availability in soils. Among the 11 amino acids investigated, plants preferred alanine, aspartic acid, proline, and serine over other amino acids such as leucine, isoleucine, and cysteine (Fig. 1b). Notably, despite being a model amino acid, intact glycine contributed only 0.7% to total N uptake and 8.8% to TFAA uptake (via intact forms), indicating that the use of glycine as the representative of organic N uptake strongly underestimates the actual uptake of TFAA by plant roots.

Alpine grasslands are distinguished from temperate grasslands by their high altitude, low temperature and short vegetation period (Ma *et al.*, 2012). Alpine plants are capable of fast uptake of amino acids due to the high-soil organic N concentrations caused by slow rates of mineralization in alpine soils (Näsholm *et al.*, 1998; Xu *et al.*, 2004; Persson & Näsholm, 2008). Contrary to our expectation, alpine plants did not absorb more organic N than temperate plants (Fig. S17), even though mean values of TFAA concentrations were higher in alpine grasslands than in temperate grasslands (Fig. 2). By contrast, alpine plants had greater uptake preferences for NH_4^+ than temperate plants (Fig. 4a), due to larger NH_4^+ concentrations in alpine grassland soils. Additionally, the ability of alpine plants, particularly the dominant species *Kobresia pygmaea*, to develop very dense fine root systems contributes to high-NH_4^+ uptake (Miehe *et al.*, 2019). Although NH_4^+ is much more conservatively cycled than NO_3^- due to its adsorption to soil particles with a negative electric charge (Haynes & Goh, 1978), the extensively developed root systems of alpine plants may facilitate NH_4^+ foraging to a greater extent than observed in temperate plants.

Nitrogen availability in soil controls plant N uptake

Along the grassland transect, we found a strong positive correlation between plant uptake of specific N forms and their respective soil concentrations ($R^2 = 0.922$, $P < 0.001$; Figs 6, 7), confirming our second hypothesis. Notably, the strong effect of N form properties, encompassing both organic as well as inorganic N compounds, on soil N availability and consequently on plant uptake rates was largely attributed to the microbial decomposition trajectory. The microbial decomposition trajectory followed the decreasing energy content and slower turnover rates across the various N forms, spanning from organic to inorganic N forms. In line with this, the N availability increased with the decrease of C content, increasing oxygen content (inversely related to the isoelectric point and net positive charge), increased water solubility, and with lower molecular size and hydrophobicity (Fig. 7; Table S1). In addition to supplying plant-available N, microorganisms also compete with plants for this N (Kuzyakov & Xu, 2013). Thus, plant N uptake and uptake preferences are influenced by rhizosphere microbes. Despite alterations of root traits induced by climate and soil properties, their direct impact on plant N uptake remained minimal.

Analysis of data from 60 studies revealed that plant root N uptake rates were weakly but significantly influenced by available N concentrations in soil across N forms ($R^2 = 0.011$, $P < 0.001$; see Fig. 5b). Only the plant uptake preferences for specific N forms increased with their contribution to soil available N ($R^2 = 0.238$, $P < 0.001$; see Fig. 5c). The weaker correlations between N uptake preferences (and uptake as rates) and soil N contributions (and N concentrations) in the global data synthesis suggests that these relationships weaken across diverse studies, due to uncertainties in ^{15}N tracing methodology (tracer exposure time and concentration) and soil inorganic and FAA forms recoveries differing between N extraction techniques. For instance, extraction methods inadequately reflect the *in situ* bioavailability of N as roots may encounter them in soils (Inselsbacher *et al.*, 2011b). Moreover, across wide management and climatic gradients soil N availability may not match plant root N uptake due to constraints by other factors such as water availability, the availability of phosphorus and micronutrients, and/or short-term fertilizer amendment.

While the data synthesis only partially confirmed our hypothesis that plant N uptake reflects its availability in soil, our field results strongly supported it, by consolidating the previous contention that root N uptake follows a linear relationship with soil N availability (von Wirén *et al.*, 1997b; McKane *et al.*, 2002; Ashton *et al.*, 2010; Chapin III *et al.*, 2011). This underscores the idea that dominant plant species preferentially absorb the dominant N forms. Such availability-dependent uptake rates of N result from diverse strategies that plants have evolved to maximize N uptake, particularly through root system adaptations to fluctuating N availability. Specifically, roots have evolved low- and high-affinity transport systems to regulate N uptake across varying levels of available N (Crawford & Glass, 1998; Fan *et al.*, 2017). Typically, in natural ecosystems, available N concentrations in soil solution span between 0.1 and 1000 μM, with absorptive roots mainly employing high-affinity transport systems (Tsay *et al.*, 2007). Given the generally low-N soil concentrations (Fig. 2) (Tsay *et al.*, 2007), it is likely that the expression of high-affinity uptake systems in roots is induced, facilitating the thorough exploitation of the soil NO_3^- pool and obscuring any disparities in NO_3^- uptake preferences between alpine and temperate grasslands.

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Competing interests

None declared.

Author contributions

ML, XX, WW, YK and YW designed the conceptual of the research. ML, JS, YT, XC, LJ and ZM performed the experiment and collected the metadata. ML, XX, WW and YK analyzed the data. ML, XX, HO, YW, WW, RDB and YK wrote, review and edit the manuscript.

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Data availability

The data that support the findings of this study are openly available in figshare at https://figshare.com/s/95aafd00f2e5202e8b39, reference number [doi: 10.6084/m9.figshare.27851463].

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Appendix A1: Meta data publication list

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Distribution of study sites in map.

Fig. S2 Diagrammatic sketch of the nine points labeling.

Fig. S3 Proportions of the using of 11 forms of amino acids to the TFAA (total free amino acids) in soil by the eight study sites.

Fig. S4 Contributions of NO_3^-, NH_4^+, TFAA (total free amino acids) contents in eight grasslands in China.

Fig. S5 Soil amino acids content of the eight study sites.

Fig. S6 Contribution of 20 forms to TFAA in each study site.

Fig. S7 Above- or belowground biomass of the dominant grass species at the four studying sites in alpine grasslands.

Fig. S8 Above- or belowground biomass of the dominant grass species at the four studying sites in temperate grasslands.

Fig. S9 Root structural traits by the 18 combinations of species and sites in alpine and temperate grasslands.

Fig. S10 Excess of ^{13}C : ^{15}N ratios and the percentage of real ratio to theory ratio by the 11 targeted amino acid forms.

Fig. S11 Uptake rates of 11 forms of free amino acids by dominant plant species in Baingoin and Nagqu.

Fig. S12 Uptake rates of 11 forms of free amino acids by dominant plant species in Hezuo and Haibei.

Fig. S13 Uptake rates of 11 forms of free amino acids by dominant plant species in Duolun and Sonid.

Fig. S14 Uptake rates of 11 forms of free amino acids by dominant plant species in Xilinhot and Hulunbeier.

Fig. S15 Total nitrogen uptake rates (NUR) by 18 combinations of plant species and study sites.

Fig. S16 Pairwise comparisons for equal variance in nitrogen uptake rates (NUR) of the interaction of climate and amino acids form using Tukey test.

Fig. S17 Pairwise comparisons for equal variance in nitrogen uptake rates (NUR) of the interactions of climate and N form (NH_4^+, NO_3^-, and FAA) using Tukey test.

Fig. S18 Uptake contributions of 11 forms of amino acids by dominant plant species in Baingoin and Nagqu counties.

Fig. S19 Uptake preference of 11 forms of amino acids by dominant plant species in Hezuo and Haibei counties.

Fig. S20 Uptake preference of 11 forms of amino acids by dominant plant species in Duolun and Sonid counties.

Fig. S21 Uptake preference of 11 forms of amino acids by dominant plant species in Xilinhot and Hulunbeier counties.

Fig. S22 Uptake preference among NO_3^-, NH_4^+ and total free amino acids (TFAA) between the dominant plant species in eight study sites.

Fig. S23 Uptake preference among 11 forms of free amino acids between dominant plant species in eight study sites.

Fig. S24 Linear regressions between soil available nitrogen contribution (SANC, %) and nitrogen uptake preferences (NUP, %) of ammonium (NH_4^+), nitrate (NO_3^-), and free amino acids (FAA).

Fig. S25 Correlation between ^{15}NUR (nitrogen uptake rates, μg g^{-1} d.w. root h^{-1}) and the corresponding molecular weight (mol).

Fig. S26 Bubble figure of the responses of nitrogen uptake rates based on the relationship between hydrophobicity and molecular weight of the amino acids.

Methods S1 Study sites and plant species information.

Methods S2 Reasons of employing 11 forms of amino acids.

Methods S3 Measurements of root traits.

Methods S4 Details of mixed-effects models and structural equation modeling.

Table S1 Details of the 11 forms of amino acids and two forms of inorganic nitrogen used in this study.

Table S2 Background values of soils at eight sites.

Table S3 Mixed-effects models for the effects of climate or plant species family, nitrogen form, and their interactions on nitrogen uptake rates (NUR, model 1–3) or nitrogen uptake preferences (NUP, model 4).

Table S4 Pairwise comparisons for equal variance in nitrogen uptake rates (NUR) by each N form. Tukey was used by emmeans as the *post hoc* tests of mixed-effects model 1 in Table S3.

Table S5 Pairwise comparisons for equal variance in nitrogen uptake rates (NUR) between alpine and temperate climate using Tukey test.

Table S6 One-way ANOVA analysis results of N forms on plant NUR between NO_3^-, NH_4^+, TFAA, and the 11 forms of amino acids.

Table S7 Pairwise comparisons for equal variance in nitrogen uptake rates (NUR) between alpine and temperate climate using Tukey test.

Table S8 Pairwise comparisons for equal variance in nitrogen uptake rates (NUR) among N forms using Tukey test.

Table S9 Pairwise comparisons for equal variance in nitrogen uptake rates (NUR) of different nitrogen form by each family.

Table S10 Pairwise comparisons for equal variance in nitrogen uptake preference between alpine and temperate ecosystem using Tukey test.

Table S11 One-way ANOVA analysis results of N forms on NUP between the NO_3^-, NH_4^+, TFAA, and 11 forms of amino acids.

Table S12 Mixed-effects model for effects of molecular, climate, and their interactions ^{15}NUR (nitrogen uptake rates).