Nitrogen redistribution and seasonal trait fluctuation facilitate plant N conservation and ecosystem N retention

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Summary

1. Low available nitrogen (N) limits plant productivity in alpine regions and alpine plants thus resorb and reallocate N from senescing tissues to conserve limiting N across the non-growing season. However, the destination and the extent of N redistribution during plant senescence among above- and belowground organs, let alone other processes of translocation outside of the plants and into the soil components, remain poorly understood.
2. Utilizing ^{15}N stable isotope as a tracer, we quantified N redistribution among above- and below-ground plant organs and different soil components during senescence in an alpine meadow ecosystem, and explored the relationship between ^{15}N partitioning among plant-soil N pools with seasonal fluctuations of plant functional traits.
3. We found substantial depletion of ^{15}N in fine roots (-40% ± 2.8%) and aboveground tissues (-51% ± 5.1%), and important ^{15}N storage primarily in coarse roots (+79% ± 27%) and soil organic matter (+37% ± 10%) during plant senescence. In parallel, we observed a temporal variation in plant functional traits, representing a shift from more acquisitive to more conservative strategies as the growing season ends, such as higher coarse root N and coarse root: fine root ratio. Particularly, ^{15}N retention in particulate and mineral-associated organic matter increased by 30% ± 12% and 24% ± 9%, respectively, suggesting a potential pathway through which fine root and microbial mortality contributes to ^{15}N redistribution into soil N pools during senescence.
4. *Synthesis.* N redistribution and seasonal plant trait fluctuation facilitate plant N conservation and ecosystem N retention in the alpine system. This study suggests a coupled aboveground-

44 belowground N conserving strategy that may optimize the temporal coupling between plant
45 N demand and ecosystem N supply in N-limited alpine ecosystems.
46
47 **Keywords:** functional traits, N resorption, N retention, ^{15}N labeling, plant nutrient strategies,
48 plant-soil interactions, soil organic matter
49

1. Introduction

Nutrient limitation is a major constraint of terrestrial plant performance and primary productivity worldwide (Lambers, Chapin III & Pons 2008; Du *et al.* 2020). Nutrient conservation thus plays a crucial role in plant adaption to infertile environments, especially in extremely nitrogen (N)-limited alpine regions (Parton *et al.* 2007; Fisher *et al.* 2010; Freschet *et al.* 2010). Plants have multiple strategies to conserve limiting nutrients, such as extending the lifespan of plant tissue or retranslocating nutrients from senescing tissues to limit losses (Chapin III & Kedrowski 1983; Eckstein, Karlsson & Weih 1999). In particular, perennial plants withdraw up to ~60% of N from their senescing tissues, accounting for over 31% of their total annual N demand (Vergutz *et al.* 2012; Deng *et al.* 2018). This N retranslocation from senescing leaves (i.e., N resorption) significantly affects plant regrowth (Lü *et al.* 2019), community composition (Lü *et al.* 2021), and ecological succession (Hayes *et al.* 2014). However, the fate of N resorbed from senescing plant organs and its relationship with the plant functional traits remains poorly understood, whether at the level of shoot-root N budget or for ecosystem N cycling (Freschet *et al.* 2010; Daly *et al.* 2021).

Most studies exploring N resorption from above- or below-ground plant components have focused on responses that occur at the organ level and often neglected shoot-root and plant-soil interactions that occur at the community level. While N resorption can mediate plant species co-existence, vegetation regrowth and succession over time at the community level (Hayes *et al.* 2014; Lü *et al.* 2019), it influences litter chemistry and ecosystem N retention and cycling at the ecosystem level (Deng *et al.* 2018; Wang *et al.* 2018). Nutrient conservation is an important aspect of plant adaptation, community succession, vegetation distribution, and

ecosystem N retention across different spatial and temporal scales. However, our understanding of these processes is limited by the lack of knowledge on seasonal N fluxes involving belowground organs at the community and ecosystem levels. Yet, roots and other belowground organs can comprise over 85% of the total plant biomass in some alpine grasslands (Yang *et al.* 2009; Ma *et al.* 2021), and fine-root production accounts for 22% of global terrestrial net primary production (McCormack *et al.* 2015). The extent to which fine-root N is resorbed during senescence largely remains unclear. While some local-scale studies showed little change in fine-root N concentration during senescence (Nambiar 1987; Aerts 1990; Gordon & Jackson 2000), others reported substantial N resorption in both woody and non-woody perennials (Woodmansee, Vallis & Mott 1981; Meier, Grier & Cole 1985; Freschet *et al.* 2010). This discrepancy may stem from different definitions of fine roots (McCormack *et al.* 2015), the sampling of non-natural phenological root senescence (Aerts, Bakker & De Caluwe 1992), or from different estimation methods (Kunkle, Walters & Kobe 2009). Recently, Kunkle et al. (2009) re-analyzed published results by correcting root mass loss and found an N decrease of 28% in recently senescing fine roots, suggesting an underestimation of N retranslocation from fine roots. On the other hand, roots and rhizomes of many deciduous woody species and herbaceous plants play an essential role in nutrient storage during winter (Millard & Grelet 2010; Zadworny *et al.* 2015; Cong *et al.* 2019; Zhao *et al.* 2020), indicating their dual role as N sink and source during senescence (Gordon & Jackson 2000).

A growing body of evidence highlights how trait-based approach can be useful for understanding the trade-offs governing plant physiological responses to environmental change. Spatial and temporal changes in climatic and soil conditions have well-known effects on plant

94 nutrient strategies (Reich 2014; Vitra *et al.* 2019; Joswig *et al.* 2021; Keep *et al.* 2021). In turn,
95 changes in plant functional traits interact with seasonal and longer temporal changes in
96 environmental stresses to affect essential ecosystem processes (Chapin III & Kedrowski 1983;
97 Lambers *et al.* 2008). For example, plants with lower N resorption can allocate more nutrient
98 belowground responding to seasonal drought and species with stronger resorption enhanced
99 aboveground investment under N enrichment (Zhao *et al.* 2020). In environments with low
100 temperature and nutrient availability, leaf N content, specific leaf area, fine-root N content and
101 specific root length decrease at the end of the growing season (Zadworny *et al.* 2015; Vitra *et*
102 *al.* 2019), but biomass and N content in transportive roots likely increase (Zadworny *et al.* 2015;
103 Cong *et al.* 2019). However, the seasonal trait variations and their linkage with N conservation
104 in natural ecosystems have not been extensively studied (Freschet *et al.* 2010). Understanding
105 to what extent the process of nutrient redistribution, both above- and below-ground, is
106 paralleled with temporal shifts in acquisitive versus conservative trait expression in plants will
107 help us refine our knowledge of plant nutrient strategies and ecosystem N dynamics.
108 At the ecosystem level, N is a major constituent of living plants, litter, soil microbial
109 biomass and soil organic matter (SOM). Differential partitioning among these N pools can
110 critically affect cumulative N retention with consequences on the cycling of other elements,
111 such as ecosystem C accumulation (Hu *et al.* 2001; Jia *et al.* 2022). For example, N in senescing
112 tissues can be redistributed into plant biomass, or transferred to SOM through rhizodeposition
113 and litterfall (Aerts, Verhoeven & Whigham 1999; Kunkle, Walters & Kobe 2009; Bernard *et*
114 *al.* 2022). Particularly, mineral N can be incorporated into and released from two generally
115 classified SOM pools, particulate organic matter (POM) and mineral-associated organic matter

(MAOM), and these forms of N have different availability for plants according to different growing seasons (Sollins, Homann & Caldwell 1996; Schimel & Bennett 2004; Jilling *et al.* 2018). During the growing season, the availability of N from POM and MAOM for plants differs because several factors such as microbial activity, temperature and moisture influence N mineralization rates (Shahzad *et al.* 2012; Bernard *et al.* 2022). For instance, during the peak growing season, high microbial activity and high temperature can lead to high N mineralization of POM. In comparison, N in MAOM is more protected by soil matrix and is less available for plants. Low temperature and microbial activities often constrain N mineralization and N availability for plants. Depending on plant nutrient demand at different growing stages, SOM may serve as a nutrient reservoir or supplier (Lambers *et al.* 2008; Chapin III, Matson & Vitousek 2011). However, we need a more holistic approach to examine N fluxes within the plant-soil-microbe system to understand their direct consequences for plant N conservation and indirect implications for ecosystem N retention and C sequestration, especially for N-limited alpine regions.

Here, we investigated the N redistribution among most N pools in a Tibetan alpine meadow with high N limitation. Specifically, we used ^{15}N stable isotope to track seasonal N redistribution among plant organs and soil fractions and to explore the linkage between plant-soil N fluxes and plant functional traits during plant senescence. We hypothesized that 1) at the plant community scale, alpine plants will withdraw N from aboveground tissues and fine roots but enhance the N storage in rhizomes and coarse roots during senescence, 2) plant senescence and its associated N redistribution correlate with a shift in functional traits towards more resource-conservative strategies upon the end of the growing season, and 3) N fluxes from

plants and microbes to soil during plant senescence constitute a significant pathway of N retention in soil organic matter. We tested these hypotheses using mesocosms in the field and ^{15}N tracing *in situ* by quantifying the redistribution of ^{15}N among different N pools between vegetation growth peak and the end of the growing season.

2. Materials and Methods

2.1 Study site and experimental design

Our study site is located at the Qinghai-Tibetan Plateau, the highest and largest plateau on the Earth, and local ecosystems are highly N-limited (Du *et al.* 2020). Over 50% of its area is covered by natural alpine grasslands (Ni 2000), with little human disturbance, providing an ideal place to study seasonal N redistribution. This study was based at the Gansu Gannan Grassland Ecosystem National Observation and Research Station (33°68′ N, 101°88′ E; 3538 m a.s.l.) in the eastern Tibetan Plateau, Maqu County, Gansu Province, China. The climate of the ecoregion is a cold-humid alpine climate, with a mean annual temperature of 1.2 °C and mean annual precipitation of 590 mm over the last 50 years, and the precipitation mainly occurs during the short-cool summer (Xu *et al.* 2021). The growing season spans from early May to October. The soil type is Cambisol in FAO/UNESCO taxonomy, with a pH at 6.0, soil total C at 98.2 g C kg^{-1}, and soil total N at 6.9 g N kg^{-1} soil in the top 10 cm (Zhang *et al.* 2020). The vegetation type is an alpine meadow mainly dominated by perennial sedges (e.g., *Kobresia capillifolia*, *Kobresia tibetica*, *Carex moorcroftii*), grasses (e.g., *Elymus nutans*, *Stipa aliena*, *Festuca ovina*), compositae species (e.g., *Aster diplostephioides*, *Ligularia virgaurea*), and other forb species (e.g., *Polygonum viviparum*, *Gentiana algida*) (Wang *et al.* 2020). Based on

the species survey in this study, non-perennial herbs are rare, accounting for about 2% and 6%, and perennial plants account for about 98% and 94% of the aboveground biomass and abundance, respectively.

We established the experiment in an undisturbed area on a gentle southeastern-facing slope by setting up six pairs of mesocosms with similar plant compositions. In the early growing season (May 23rd) of 2021, six pairs of PVC pipes (inner diameter: 19 cm, height: 22 cm) were hammered into the soil to 20 cm deep as semi-closed systems. The top of the PVC pipes was 2 cm above the ground to prevent any horizontal water movement between the PVC tubes and the adjacent areas. Each pair of PVC pipes were 2 - 10 cm away from each other, and the distance between pairs was 2 - 5 m. On July 2nd, 2021, after six weeks of plant growth and soil stabilization, plant litter on the soil surface and standing dead stalks were removed. Then, 16.931 mg ^{15}N in a 120 ml solution (5 ppm, 75.698 mg $(^{15}NH_4)_2SO_4$, 98% atom ^{15}N) was evenly injected through 24 locations at two soil depths (2 cm and 5 cm) within each pipe. Light rain in the subsequent days following the ^{15}N injection facilitated the homogeneity of the ^{15}N labeling. Four unlabeled parallel mesocosms (PVC pipes) were established in the same undisturbed area following the same experimental steps as other mesocosms described above, to determine the natural ^{15}N abundance of plant and soil components at the growth peak period.

2.2 Plant-soil sampling and measurements

On August 13th, 2021, six weeks after ^{15}N injection, when vegetation in this region reaches a peak primary productivity with their highest N assimilation, one mesocosm of each pair was harvested by pulling the PVC pipes out, together with the plants and soil. The subsoil (20 - 25

cm depth, ca. 100g) was also collected below each pipe. Aboveground plant communities were cut at the soil surface, separated by species, oven-dried (48 °C, 48 hrs), and weighed as shoot biomass. The total weight of the soil in each pipe was recorded, and then manually sorted into roots and soils and sieved through a 2 mm sieve. Following this procedure, soil samples were timely stored at 4°C (500 g) and -20 °C (100g) before laboratory analyses. Roots were thoroughly washed, air-dried for 12 hours indoors (temperature: 10 °C; air humidity: 50 - 70%), and then weighed as the fresh root mass. A representative subsample of fresh roots (10 g) was manually separated into fine roots (FR, absorptive roots, root order 1 - 2) and coarse roots (CR, transportive roots and rhizomes, root order ≥ 3) for morphological analyses. Another subsample of fresh roots of 5 g was left un-separated for morphological analyses of entire-root traits. The remaining root samples were oven dried (48 °C, 48 hrs) and weighed. For the second mesocosm in each pair, the fresh leaf litter was timely collected twice, on August 13th and August 28th, 2021, to limit material loss via leaching and decomposition. At the end of the growing season on October 10th, 2021, senescent plants (including leaves that remain green at the start of winter), soil cores and subsoils of the second mesocosm of each pair were sampled and processed as described above for the peak biomass stage (the first mesocosm of each pair).

To examine whether paired plant community composition and species biomass differed across the growing stages, we compared diversity indices of paired community composition and the principal coordinates analysis (PCoA) of the overall differences between species biomass structure (Figs S1–S2). We observed no significant difference and thus concluded that our temporal approach based on paired sampling was appropriate for linking aboveground and belowground N relocation during plant senescence.

204

205 **2.3 Determination of plant, soil, and microbial properties**

206 Soil moisture, pH, ammonium (NH_4^+) and nitrate (NO_3^-), available phosphorus (AP), soil
207 dissolved organic C (DOC) and N (DON), K_2SO_4 extractable N (EN), and microbial biomass
208 C (MBC) and N (MBN) were measured as described by Xu *et al.* (2021)) for the same study
209 site. Soils were fractionated by two particle size classes: particulate organic matter (POM, > 53
210 μm) and mineral-associated organic matter (MAOM, ≤ 53 μm) using a wet sieving approach
211 (Yan, Wang & Yang 2007). Fresh samples of coarse roots and fine roots were scanned with
212 backlighting (Epson Expression 12000XL-PH, Japan), and images were processed with
213 WinRHIZO 2020a (Regent Instruments Inc., Canada) to determine root morphological
214 parameters (root diameter, length, surface area, and volume were recalculated as the sum of the
215 values provided for each diameter classes, as values provided by WinRHIZO 2020a are
216 inaccurate). Specific root length (SRL) and specific root area (SRA) were estimated by dividing
217 root length and surface area by the dry weight, respectively. Root tissue density (RTD) was
218 calculated by dividing root dry mass by fresh root volume. For coarse roots, we eliminated the
219 length, surface area, and volume of the remaining truncated base of separated fine roots,
220 identified as the following root diameter class: 0 < Diameter-Class < 0.5 mm, because it can
221 lead to severely inaccurate estimation of coarse root diameter, specific root length (SRL), and
222 specific root area (SRA), especially for grasses (Freschet *et al.* 2021a). Root mass fraction was
223 calculated by dividing root biomass by entire plant biomass. Oven-dried plant shoots (total
224 aboveground biomass including leaves that remain green and pre-collected fresh leaf litter for
225 the senescent stage), coarse roots, fine roots, soils, POM and MAOM were separately ground

into fine powder with a ball mill (Retsch MM200, Germany). C and N concentrations were determined by an elemental analyzer (Vario MICRO cube, Elementar, Germany), and ^{15}N atomic percentage (AT% [^{15}N]) was determined by EA-IRMS (DELTA V Advantage, Thermo Fisher Scientific, USA).

2.4 Estimation of ^{15}N partitioning and redistribution efficiencies in shoot, root, soil, and their sub-fractions

We quantified ^{15}N partitioning (as measured by ^{15}N recovery rate, i.e. ^{15}NRR) at two plant growing stages among eight N pools (plant shoot, fine roots, coarse roots, soil, POM, MAOM, K_2SO_4-extractable dissolved N and microbial biomass N), and estimated the percentage change in ^{15}N recovery (denoting ^{15}N redistribution efficiency) in each N pool. AT% [^{15}N] and N concentration in ^{15}N labeled shoots, coarse roots, fine roots, and soil fractions were determined on EA-IRMS as described above. Microbial biomass AT% [^{15}N] was determined after Kjeldahl digestion and diffusion (Stark & Hart 1996). Non-extractable ^{15}N content (NEOM pool, mostly organic ^{15}N) was calculated by subtracting microbial biomass ^{15}N and K_2SO_4-extractable ^{15}N (EN pool) from total soil ^{15}N. The field plots were fenced to prevent disturbance from large herbivores. Additionally, the proportion of gaseous nitrogen loss is quite low within such a brief experimental period (Zhang *et al.* 2020). The ^{15}N detected in the subsoil of the mesocosms accounted for only 1.6% of the total (Fig. 1), leading us to infer that N leaching was minimal during the experimental period. Consequently, we attributed all potential ^{15}N losses to the unrecovered ^{15}N pool.

The percentage of ^{15}N recovery in all N pools was calculated as N concentration multiplied by the biomass or mass of each pool, then multiplied by the difference of AT% [^{15}N] between labeled and natural samples, and finally divided by ^{15}N tracer applied. So, the ^{15}N recovery rates in each plant and soil fractions were calculated following Equation 1:

$$^{15}\mathrm{NRR} = \frac{[\mathrm{N}] \times \mathrm{mass} \times (\mathrm{AT\%}\,[^{15}\mathrm{N}]_{labeled} - \mathrm{AT\%}\,[^{15}\mathrm{N}]_{natural})}{^{15}\mathrm{N}_{added} \times (\mathrm{AT\%}\,[^{15}\mathrm{N}]_{tracer} - \mathrm{AT\%}\,[^{15}\mathrm{N}]_{natural})} \times 100\% \qquad \text{(Eqn 1)}$$

Where AT%$[^{15}N]_{tracer}$, AT%$[^{15}N]_{labeled}$, AT%$[^{15}N]_{natural}$ represent the atomic percentage value ($^{15}N/(^{15}N + ^{14}N)$) of the tracer, labeled, and background samples, respectively; [N], mass, and $^{15}N_{added}$ represent N concentration in each N pool, biomass/mass of each pool at the time of sampling, and mass of ^{15}N tracer applied, respectively.

Although changes in plant N concentrations have been typically used to calculate N resorption efficiency, they are inadequate as a quantitative metric of N redistribution among shoot-root tissues because organ biomass may also change during senescence (van Heerwaarden, Toet & Aerts 2003; Kunkle, Walters & Kobe 2009). We therefore examined N pools instead of N concentration to evaluate ^{15}N redistribution in each N pool. The ^{15}N redistribution efficiency in each N pool was calculated as the percentage change in ^{15}N recovery rate between growth peak period and the end of the growing season following Equation 2:

$$\%\ \text{change in}\ ^{15}\mathrm{N}\ \text{recovery} = \frac{^{15}\mathrm{NRR}_{senescence\ stage} - {}^{15}\mathrm{NRR}_{grow\quad peak}}{^{15}\mathrm{NRR}_{growth\ peak}} \times 100\% \qquad \text{(Eqn 2)}$$

Here, $^{15}NRR_{growth\ peak}$ and $^{15}NRR_{senescence\ stage}$ represent the ^{15}N recovery rate in a given ecosystem N pool at the vegetation growth peak and the end of the growing season, respectively. For each plant or soil component, a positive value of % change in ^{15}N recovery indicates ^{15}N storage pools, while negative values denote ^{15}N depletion pools during plant senescence.

2.5 Statistical analysis

All statistical analyses were conducted using R (version 4.0.2) (R 2021). Student's paired t-tests were used for comparisons of soil properties, microbial properties, ^{15}N distribution and nutrient allocation between the two growing season stages. To investigate the temporal transition of plant economics spectrum, we conducted a principal component analysis (PCA) including sixteen root traits and two shoot traits at the community level with 95% prediction ellipses. Pearson's correlations were performed to examine the relationships of ^{15}N recovery among different plant and soil components with changes across plant growth stages, in terms of soil and microbial properties, plant nutrient concentrations and biomass allocation, and root morphology. Diversity indices of plant communities (Shannon-Wiener index, Simpson index, species richness, and species evenness) were calculated to examine any potential differences in community structure between the two plant growth stages across each pair of mesocosms using a student's paired t-test. In addition, a principal coordinate analysis (PCoA) was performed to analyze the differences in community structure (aboveground-biomass-based) between two growth stages based on Bray-Curtis distances under Robust Scaler standardization. We set the significance level at $P < 0.05$.

290 ## 3. Results

291 ### 3.1 Soil and microbial properties across the growing season

292 From August 13th to October 10th, inorganic soil N and soil available phosphorus decreased
293 (Table 1, $P < 0.01$). Similarly, DOC, DON, MBC and MBN decreased, with microbial biomass
294 being positively correlated with DOC and DON ($P < 0.05$). Soil pH increased by 0.14 units (P
295 < 0.05). Soil moisture, soil C:N ratio, and C and N concentrations in POM and MAOM showed
296 no significant change between the two sampling dates (Table 1).

297

298 ### 3.2 ^{15}N redistribution among different N pools

299 The partitioning of total ^{15}N as measured by the percentage of ^{15}N recovery from different N
300 pools in the whole root system did not differ between the peak (14.9% ± 0.97%, mean ± SE,
301 the same below) and the end (14.8% ± 0.97%) of the growing season (Fig. 1, $P = 0.94$). This
302 resulted in a neutral ^{15}N redistribution at the scale of whole-root system (% change in ^{15}N
303 recovery = 2.4% ± 11.5%, Fig. 2a). However, ^{15}N recovery in coarse roots increased from 5.38%
304 ± 0.36% (mean ± SE, the same below) at the peak biomass stage to 9.11% ± 1.36% at plant-
305 senesced stage ($P = 0.026$). Conversely, ^{15}N recovery in fine roots declined from 9.55% ± 1.00%
306 at the peak biomass stage to 5.71% ± 0.57% at the senescence stage ($P = 0.001$, Fig. 1), leading
307 to a negative % change of ^{15}N in fine roots during plant senescence (-39.9% ± 2.81%, Fig. 2a).
308 For aboveground plant tissues (including leaves, stems, flowers, seeds, and fresh litter), the ^{15}N
309 recovery substantially decreased by half from 25.5% ±1.32% to 12.3% ±0.95% after plant
310 senescence ($P = 0.001$, Fig. 1), corresponding to a negative % change of ^{15}N in aboveground
311 tissues (-50.94% ± 5.13%, Fig. 2a).

312 ^{15}N recovery in bulk soil increased from 30.1% ± 0.92% to 36.5% ± 1.86% from August
313 13 to October 10 (P = 0.027), with increases from 15.4% to 19.6% (P = 0.038) in POM and
314 from 12.7% to 15.5% (P = 0.027) in MAOM (Fig. 1), corresponding to a positicve % change
315 of ^{15}N as 29.80% ± 11.84% in POM and 24.30% ± 9.16% in MAOM (Fig. 2b). Besides, ^{15}N
316 recovery in MBN and K_2SO_4-extractable N pool (EN) significantly decreased from 5.77% to
317 4.64% (P = 0.04), and from 1.07% to 0.25% (P < 0.001), respectively (Fig. 1). As a result,
318 76.58% ± 2.92% and 17.85% ± 6.20% of ^{15}N from EN and MBN, respectively, moved into
319 other pools (Fig. 2b). On the contrary, ^{15}N recovery in non-extractable soil organic matter
320 (NEOM) increased from 23.3% to 31.6% at the end of the growing season (P < 0.05, Fig. 1),
321 corresponding to a % change of ^{15}N as 37.4% ± 10.4% in NEOM (Fig. 2b). Due to potential
322 ^{15}N loss pathways, such as gaseous N losses, herbivores consumption, seed dispersal and some
323 litter loss, there was more (6.9%) unrecovered ^{15}N with time (Fig. 1).
324
325 **3.3 Above- and below-ground plant economics traits at different growing stages**
326 A principal component analysis (PCA) suggested a temporal shift of the plant community along
327 the plant economics spectrum, moving from a more acquisitive strategy to a more conservative
328 strategy during senescence (Fig. 3). The first PCA axis accounted for 54.6% of the variance,
329 mainly defined by coarse root (CR)-N (element concentration, the same below), CR-C:N, CR
330 mass fraction, CR:FR ratio, FR-SRL, FR-SRA, FR-C, FR-N, FR-C:N, Shoot-N and Shoot C:N
331 (all traits significantly changed during senescence, Figs 4–5); and the second PCA axis was
332 mainly driven by coarse root morphological traits (i.e., CR-RTD, CR-SRL and CR-RD, Fig. 3).
333 We defined the first PCA axis as a conservation axis according to the representative plant traits.

Specifically, the root: shoot ratio ($P = 0.046$), coarse root mass fraction (CRMF, $P = 0.019$), and coarse-root: fine-root ratio (CR:FR, $P = 0.038$) increased at the end of the growing season (Fig. S3). Substantial N resorption occurred in shoots and fine roots, as their N concentration decreased at the end of the growing season ($P = 0.01$, $P = 0.007$, respectively, Fig. S3). In addition, C concentration in all belowground organs and fine roots significantly increased ($P = 0.002$, $P = 0.017$, respectively), corresponding to declines of shoot C:N ratio and FR C:N ratio ($P = 0.034$, $P = 0.003$, respectively, Fig. S3). Meanwhile, N concentration in CR significantly increased during plant senescence ($P = 0.036$), matching the reduction of the CR C:N ratio ($P = 0.038$, Fig. S3). According to root morphology, the FR-SRL and FR-SRA decreased by 29% ($P = 0.018$) and 19% ($P = 0.013$), respectively, and fine-root diameter (FR-RD) increased from 0.20 to 0.23 mm during plant senescence ($P = 0.06$, Fig. S4). In other words, fine roots became thicker and shorter during plant senescence.

3.4 Correlations of ^{15}N partitioning to plant traits and edaphic properties

^{15}N recovery in whole-plant, shoots, and extractable soil N showed positive correlations with available soil nutrients, DON, MBN, and bulk soil total N at both growing stages ($P < 0.05$, Fig. S5b). ^{15}N recovery in FR, EN and MBN pools showed similar positive relationships with soil NO_3^-, DON, and MBN ($P < 0.05$, Fig. S5b). Furthermore, ^{15}N recovery in MAOM showed positive relationships with C and N concentrations of MAOM, but negative relationships with that of POM ($P < 0.05$, Fig. S5b). Moreover, ^{15}N recovery in FR, EN and MBN pools were positively related to graminoid biomass, shoot N, FR N and CR C:N ratio ($P < 0.05$) but negatively related to CR:FR ratio, root C, FR C, CR N, shoot and FR C:N ratio ($P < 0.05$, Fig.

S5a). Interestingly, ^{15}N recovery in fine roots and coarse roots showed contrary relationships with variables representing acquisitive-conservative strategies for nutrient allocation trade-off (Fig. S5a). Specifically, ^{15}N recovery in fine roots was positively related to acquisitive-allocation traits, including graminoid biomass, shoot-N, FR-N, CR-C:N, SRL, SRA, FR-SRL, FR-SRA ($P < 0.05$; Fig. S5). In contrast, ^{15}N recovery in coarse roots was positively correlated with conservative-allocation traits, such as root-C, FR-C, CR-N, shoot-C:N, FR-C:N, RD, FR-RTD ($P < 0.05$; Fig. S5). Besides, ^{15}N recovery in coarse roots was positively related to entire-root diameter and FR-RTD but negatively related to FR-SRL and FR-SRA (Fig. S5c), with which fine-root ^{15}N recovery showed positive correlations. ^{15}N recovery in whole plants, shoots, and fine roots showed similar correlations with plant nutrient traits, including shoot-N, FR-N, CR-C:N ratio (positive correlations), and root-C, shoot-C:N ratio (negative correlations, Fig. S5a). Besides, ^{15}N recovery in bulk soil, as well as in POM and NEOM, was positively correlated with CR:FR ratio, root C, FR-C, and CR-N ($P < 0.05$, Fig. S5a), and negatively correlated with DON, NO_3^-, MBC and MBN across growing stages ($P < 0.05$, Fig. S5b).

4. Discussion

Nitrogen conservation during plant senescence benefits plant fitness and vegetation regrowth. Seasonal trait fluctuation from acquisitive to conservative may help plants to maximize nutrient use efficiency as plants move from peak growth to senescence. Yet, our knowledge of root traits and their relationship with N conservation lags far behind our understanding of aboveground parts. Through tracing ^{15}N movement, our study provided direct evidence illustrating that during the senescence process, coarse roots served as the major N storage due to increases in

both coarse root mass and N concentration, whereas fine roots and shoots showed major N resorption. More interestingly, seasonal changes in ^{15}N recovery among plant organs covaried with a temporal shift of traits, which defining the plant nutrient strategies of the grassland community, from fast acquisition at the peak of plant growth to strong conservation at the end of plant senescence.

4.1 Roots play a dual role as both sink and source in N conservation

We observed a substantial depletion of ^{15}N in fine roots and aboveground tissues, and a increased ^{15}N storage primarily in coarse roots and soil organic matter during plant senescence (Figs 1–2 and 7). The extent of N redistribution in total aboveground plant tissues (-50.9% ± 5.13%) based on changes in N pools is in line with previous estimates from N concentration in herbaceous species (Aerts 1996; Yuan & Chen 2009), but lower than global estimates of 62.1% after leaf mass loss correction (Vergutz *et al.* 2012). Although previous studies based on N concentration did not observe significant N redistribution in senescing fine roots (Nambiar 1987; Aerts 1990; Gordon & Jackson 2000), our ^{15}N-pool-based approach allowed us to observe substantial fine-root N redistribution (39.9% ± 2.8%), which is higher than a global estimation of 28% (Kunkle, Walters & Kobe 2009). Yet, the extent of N redistribution from fine roots into other N pools may still be underestimated due to the potential mismatch between root sampling and complete root senescence. Further, in contrast to leaves, not all fine roots are meant to senesce at the end of the growing season (i.e. a mixture of living and senescing fine roots), suggesting that at the level of one actively senescing root resorption might be much stronger than recorded over the entire fine root pool. Finally, the ^{15}N tracing method cannot

400 fully recover all the ^{15}N applied, owing to gaseous N losses, herbivores consumption, seed
401 dispersal, and some litter loss. These potential ^{15}N loss pathways could explain the increase in
402 the unrecovered ^{15}N pool. On the other hand, some ^{15}N in newly produced root litter may have
403 been decomposed, mineralized and even re-acquired by roots. This means changes in root ^{15}N
404 recovery are not solely due to N retranslocation during senescence. Considering this caveat,
405 the ^{15}N retranslocation in fine roots can be overestimated slightly. Importantly, we observed a
406 significant increase in the coarse root ^{15}N pool during plant senescence (+79.0% ± 27.1%),
407 supporting our first hypothesis that coarse roots and/or rhizomes act as major N storage tissues
408 during plant senescence. In other words, roots may play a dual rule in herbaceous plant N
409 conservation across the year, with coarse roots acting as an N storage and fine roots as a N
410 recycling source (Gordon & Jackson 2000; De Vries *et al.* 2012; Freschet *et al.* 2021b). Our
411 results indicate that the overall change in root N pool alone during plant senescence likely fails
412 to tell the complete story of plant internal N translocation, especially among fine and coarse
413 roots.
414
415 **4.2 Linking seasonal plant economics traits with ^{15}N partitioning in plant-soil components**
416 In line with our second hypothesis, plant N redistribution during plant senescence went along
417 with a temporal shift in plant nutrient strategies from acquisitive to conservative (Fig. 4). At
418 the peak of their growth, plants are characterized by a more acquisitive strategy to meet higher
419 plant C and N demand, resulting in higher N partitioning to N-acquisitive plant organs (shoot
420 and fine roots), also as evidenced by strong correlations between N partitioning in N-acquiring
421 fractions and acquisitive plant traits (Shoot-N; FR-N; SRL; SRA) in our study (Fig. 4).

Nevertheless, Freschet *et al.* (2010) found relative independence of plant N and P resorption from other economics traits and suggested that the dependence of nutrient resorption processes on plant water uptake capacity and resistance to early frosts could partly explain this lack of relationship. Previous works analyzing the relationships between plant economics strategy and the ability of plants to re-translocate nutrients during senescence have based their comparisons on plant functional traits, typically measured at the peak of plant growth (Weigelt *et al.* 2021). Taking a perspective from the senescence progression, plants tended to conserve more nutrients to survive for potential growth, leading to higher N retention in N-reserving organs (De Vries *et al.* 2012; Wang *et al.* 2018). Our results showed that N retention in coarse roots significantly contributed to whole-plant N conservation and was paralleled with a decrease in fine-root SRL, SRA and N concentration, as well as an increase in RD and CR: FR ratio, suggesting a shift towards longer-lived roots with lower acquisitive abilities at the end of the growing season (Luke McCormack *et al.* 2012). Similarly, Wang *et al.* (2018) reported that conservative growth strategies contributed to overall ecosystem ^{15}N retention by increasing root biomass and root N uptake in an alpine meadow. Because there was little difference in species abundance or biomass composition in plant communities between two growing stages (Figs S1–S2), the shift of nutrient strategies in plant community during senescence could be mostly explained by intraspecific trait fluctuations (Figs S1–S2). These results suggest that N partitioning may be temporally coordinated with plant nutrient utilization strategies, which may optimize the cost-benefit of plant nutrient acquisition to match seasonal fluctuations in resource availability and plant nutrient demand.

4.3 N transfer from plants to soil organic materials during plant senescence

When ^{15}N-enriched ammonium is applied to soil, some ^{15}N may enter plant biomass or microbial biomass, while some may directly be absorbed into soil clay. Subsequently, ^{15}N in plant roots mostly ends as POM and the more labile components of N compounds such as root exudates and fine roots may be used by microbes and end up either in microbial biomass or microbial necromass which mostly associate with MAOM. Therefore, there are some tradeoffs between plant biomass N and SOM-N, or between POM-N and MAOM-N. But the numbers are not exactly matched because some N lost from the system through gaseous emissions and leaching. Since POM is largely composed of partly decomposed plant residues (Lavallee, Soong & Cotrufo 2020; Cotrufo *et al.* 2022), the substantial increase in POM-N further suggests that N uptake by plants and subsequent incorporation of plant-derived residues into POM mainly occurred via fine-root and microbial biomass turnovers (Kuzyakov & Xu 2013; Daly *et al.* 2021). Substantial ^{15}N transfer to MAOM is generally linked to soluble N forms (e.g., root N leakage) and small molecular weight compounds derived from advanced decomposition of plant and microbial residues or root exudates (Huygens *et al.* 2008; De Vries & Bardgett 2012; Daly *et al.* 2021; Cotrufo *et al.* 2022). The significant increases in bulk soil ^{15}N, as well as POM-^{15}N and MAOM-^{15}N, from the peak growing season to the plant senescence stage suggest the continuation of these processes during plant senescence. Direct displacement of MAOM-N by injected $^{15}NH_4^+$ may have also contributed to the increased MAOM-^{15}N recovery, but our method did not allow us quantifying the proportion of this displacement. Moreover, the concurrence of decreased MB^{15}N and increased ^{15}N in MAOM during plant senescence suggests that microbial mortality had contributed to ^{15}N transfer into

466 the MAOM pool (Fig. 4). In addition to changes in ^{15}N, we also observed a small, but
467 statistically significant, increase in soil pH from the peak biomass to the senescence stage
468 (Table 1). The pH increase may have occurred due to decreases in root uptake of cations, root
469 exudation of organic acids and root respirations (i.e., CO_2 release). Decreases in CO_2
470 production as a result of reduced microbial activities may also have contributed. Together, our
471 results showed that N redistribution into POM and MAOM during plant senescence constitutes
472 as an important avenue of soil N retention in the alpine meadow ecosystem.
473
474 **4.4 Conclusions and perspectives**
475 Our study with ^{15}N tracing provided direct evidence that substantial N was withdrawn from
476 senescing fine roots and aboveground tissues and the N storage was enhanced in coarse roots
477 and/or rhizomes during alpine plant senescence, thanks to an increase in both coarse root mass
478 fraction and N concentration. This work confirmed the dual role of plant roots with coarse roots
479 acting as a N storage pool and fine roots as a N depletion pool during senescence. In parallel,
480 we observed a temporal shift in plant nutrient strategies from fast acquisition during the
481 growing season to strong conservation at the non-growing season at the plant community level.
482 Overall, our findings suggest that N redistribution and seasonal plant trait fluctuation couple to
483 facilitate plant N conservation and ecosystem N retention, which may contribute to the
484 temporal coupling between plant N demand and ecosystem N supply, especially in N-limited
485 alpine ecosystems. This study clearly quantified plant shoot-root and plant-soil N redistribution
486 during senescence via a ^{15}N pool-based framework, avoiding many of the pitfalls in how N
487 resorption is typically measured, especially in roots. This work opens new perspectives for

mechanistic understandings of the plant nutrient economy, plant-driven C and N biogeochemical cycling and their responses to environmental change, which may help improve biogeochemical model predictions of plant productivity and nutrient cycling under future climate change scenarios.

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Author contributions

Qingzhou Zhao and Shuijin Hu conceived the idea and designed the methodology. Qingzhou Zhao, Peng Wang, Lingyan Hu, Xupeng Liu collected the data. Qingzhou Zhao performed the data analysis and result visualization. Qingzhou Zhao led the writing of the manuscript. Peng Wang, Shuijin Hu revised the manuscript with significant inputs from all other authors. All authors contributed critically to the drafts and gave final approval for publication.

Competing interests

The authors declare that there is no conflict of interest. Grégoire Freschet is an Associate Editor of Journal of Ecology, but took no part in the peer review and decision-making processes for

this paper.

Data availability

All data needed to evaluate the conclusions in this study are present in the paper and the supplementary materials. Additional data that support the findings of this study are available from the corresponding author upon reasonable request.

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719 *Environment,* **723,** 138106. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.138106

720

721 Table 1. Soil and microbial properties of the topsoil (0 - 20 cm) in the ^{15}N labeled mesocosms
722 at different growing stages. Values are mean ± standard error (n = 6). Bold values indicate
723 significant differences between growing stages ($P < 0.05$).

Soil & microbial properties	Peak growth of vegetation	End of the growing season	*P-value* (Paired t-test)
Soil moisture (%)	51.90% ± 1.11%	51.68% ± 1.83%	0.907
pH (H_2O)	**5.71 ± 0.03**	**5.85 ± 0.04**	**0.032**
NH_3^+ (mg kg^{-1})	1.25 ± 0.17	1.44 ± 0.18	0.560
NO_3^- (mg kg^{-1})	**24.26 ± 2.15**	**12.74 ± 1.37**	**0.005**
AP (mg kg^{-1})	**4.99 ± 0.14**	**4.10 ± 0.09**	**0.002**
DOC (mg kg^{-1})	**304.83 ± 21.90**	**195.73 ± 11.45**	**0.007**
DON (mg kg^{-1})	**79.12 ± 2.48**	**37.14 ± 2.34**	**< 0.001**
MBC (mg kg^{-1})	**1240.30 ± 65.13**	**731.32 ± 55.26**	**0.004**
MBN (mg kg^{-1})	**231.93 ± 9.56**	**163.70 ± 3.55**	**< 0.001**
MBC/MBN	5.34 ± 0.12	4.48 ± 0.34	0.054
POM-C (g kg^{-1})	21.45 ± 1.57	18.62 ± 1.63	0.106
POM-N (g kg^{-1})	1.56 ± 0.14	1.30 ± 0.13	0.099
POM-C/N	13.91 ± 0.32	14.37 ± 0.22	0.185
MAOM-C (g kg^{-1})	34.87 ± 1.48	36.08 ± 1.16	0.469
MAOM-N (g kg^{-1})	3.04 ± 0.13	3.18 ± 0.09	0.343
MAOM-C/N	11.48 ± 0.08	11.33 ± 0.08	0.147

724

725 Abbreviations: AP, soil available phosphorus; DOC, dissolved organic C; DON, dissolved
726 organic N; MBC, soil microbial biomass C; MBN, microbial biomass N; POM, particulate
727 organic matter (particle size > 53 μm); MAOM, mineral-associated organic matter (particle
728 size ≤ 53 μm).

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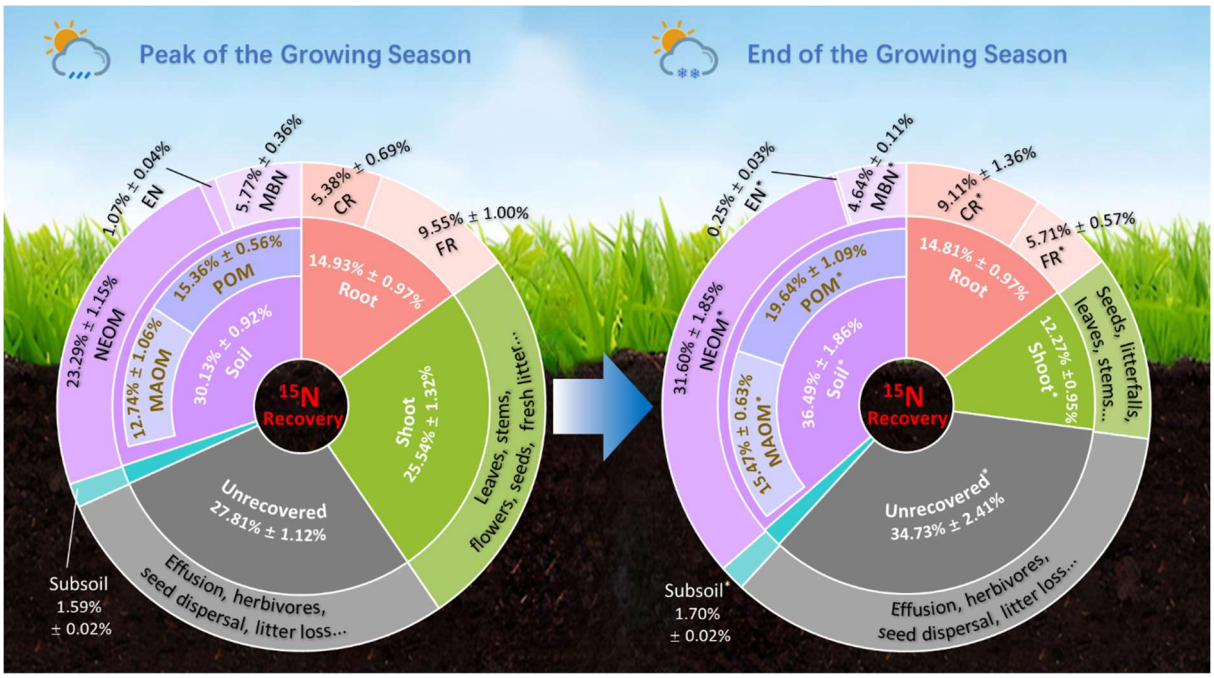


730

731 Fig. 1. ^{15}N partitioning among plant shoots, roots, soil, and their sub-fractions, a. at the peak of
732 vegetation growth, and b. at the end of the growing season. Total microbial biomass ^{15}N in soil
733 was calculated from microbial biomass ^{15}N flush using an extraction efficiency of 0.45. Values
734 are mean ± standard error (n = 6). The asterisk, * in b, indicates a significant difference between
735 growing stages ($P < 0.05$). Abbreviations: NEOM, non-extractable soil organic matter; EN,
736 K_2SO_4 extractable N; MBN, microbial biomass N; CR, coarse root (transportive roots and
737 rhizomes, root order ≥ 3); FR, fine roots (absorptive roots, root order < 3); POM, particulate
738 organic matter (particle size > 53 μm); MAOM, mineral-associated organic matter (particle
739 size ≤ 53 μm).

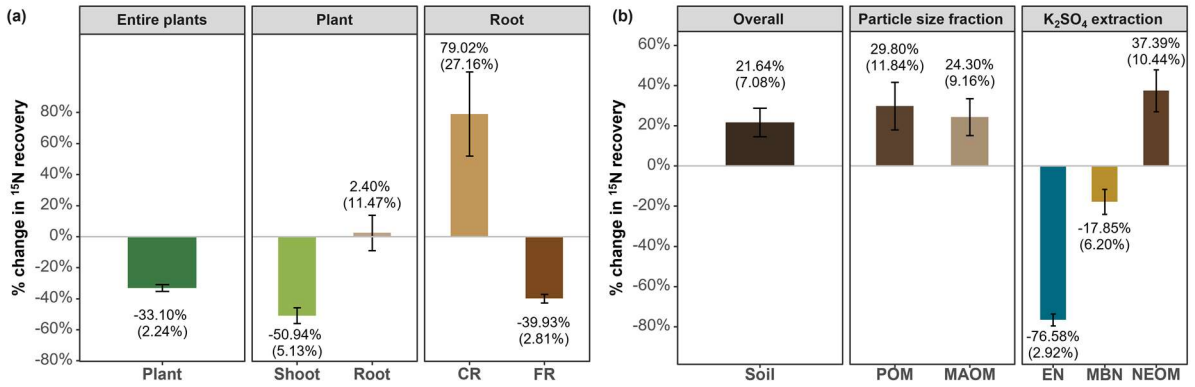
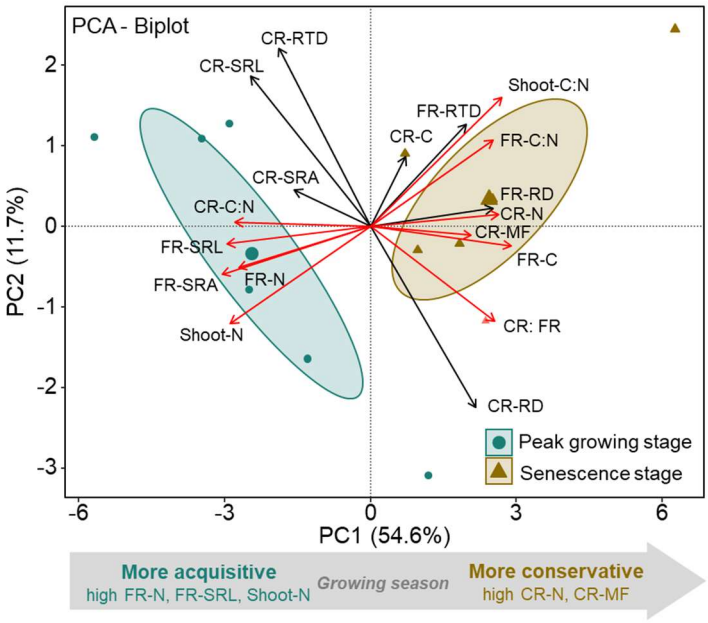


Fig. 2. The percentage change in ^{15}N recovery in plant-soil N pools. (a), In whole plants, shoots, roots, coarse roots, and fine roots, and (b), in soil and soil sub-fractions during plant senescence from peak growth stage to the end of the growing season. Positive values indicate ^{15}N storage pools and negative values indicate ^{15}N depletion pools during plant senescence. Plants were separated into shoots and roots (all above- and below-ground biomass, respectively), and roots were further classified between coarse organs (CR, transport roots and rhizomes, root order $\geqslant$ 3) and fine roots (FR, absorptive roots, root order < 3). The soil was divided into several fractions, depending on particle size and K_2SO_4 extractability. Values are mean (± standard error) (n = 6). Abbreviations: POM, particulate organic matter (particle size > 53 μm); MAOM, mineral-associated organic matter (particle size ≤ 53 μm); EN, K_2SO_4 extractable N; MBN, microbial biomass N; NEOM, non-extractable soil organic matter.



752

753 Fig. 3. Results of principal component analysis (PCA) coded by different growth stages for
754 plant economics spectrum, including 16 root traits and two shoot traits with 95% prediction
755 ellipses. Red arrows represent significantly-changed traits during senescence ($P < 0.05$).
756 Belowground traits are differentiated between those of coarse belowground organs (CR,
757 transport roots and rhizomes, root order $\geqslant$ 3) and fine roots (FR, absorptive roots, root order
758 < 3). Abbreviations: RD, root diameter; SRL, specific root length; SRA, specific root area;
759 RTD, root tissue density; C, carbon concentration; N, nitrogen concentration; C:N, ratio of C
760 to N concentrations; CR:FR, ratio of CR to FR biomass.

761

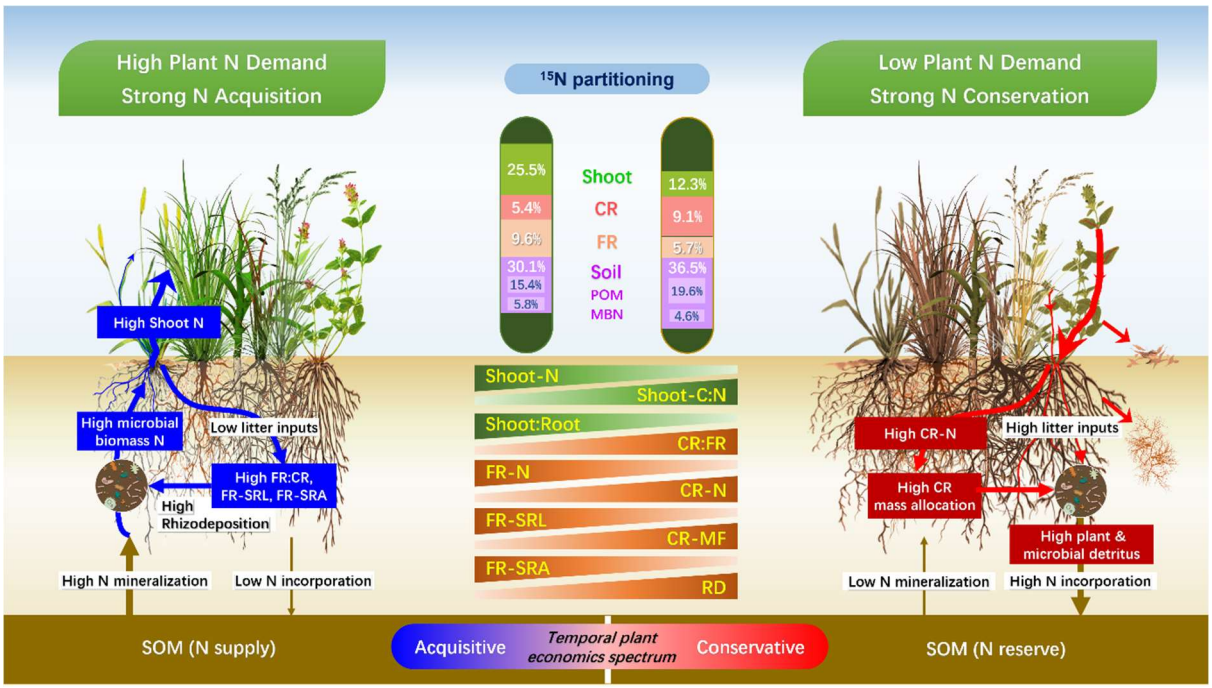
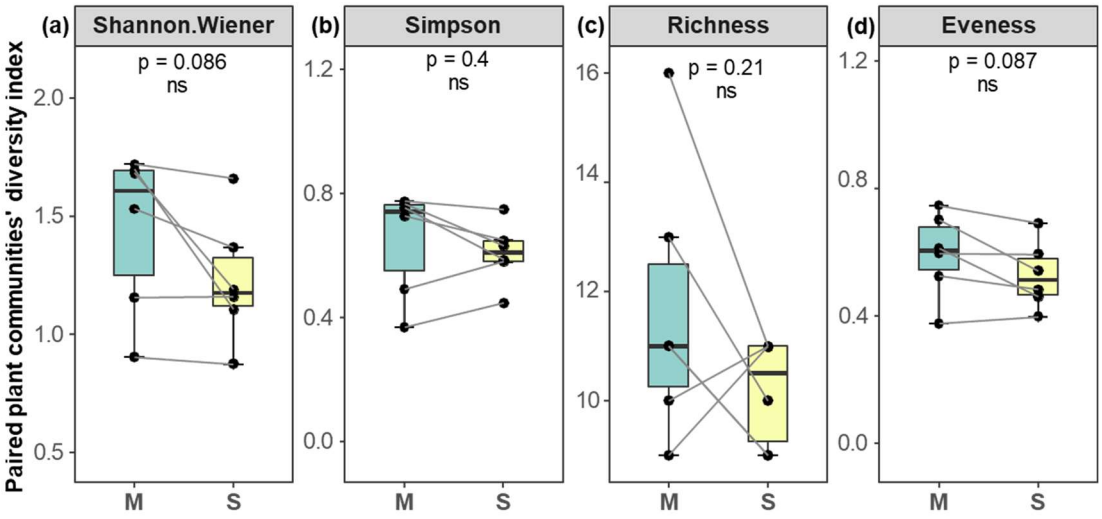


Fig. 4. Conceptual summary of temporal plant-soil N dynamics during plant senescence, and shift in plant economics traits, as a potential mechanism for minimizing N losses and improving the coupling between plant N demand and bioavailable N supply in a N-limiting alpine ecosystem. Abbreviations: Shoot:Root represents here the biomass ratio of all above- to below-ground organs; CR, coarse roots (transportive roots, root order ≥ 3) and rhizomes; FR, fine roots (absorptive roots, root order < 3); POM, particulate organic matter (particle size > 53 μm); MBN, microbial biomass N; N, nitrogen concentration; RD, root diameter; SRL, specific root length; SRA, specific root area.



773

774 Figure S1. Paired plant communities' diversity indices between different growing stages. M,

775 mature stage at the peak of vegetation growth; S, senescence stage at the end of the growing

776 season. Black dots and lines are paired observations. The *P*-values and significance levels of

777 student's paired t-tests are displayed.

778

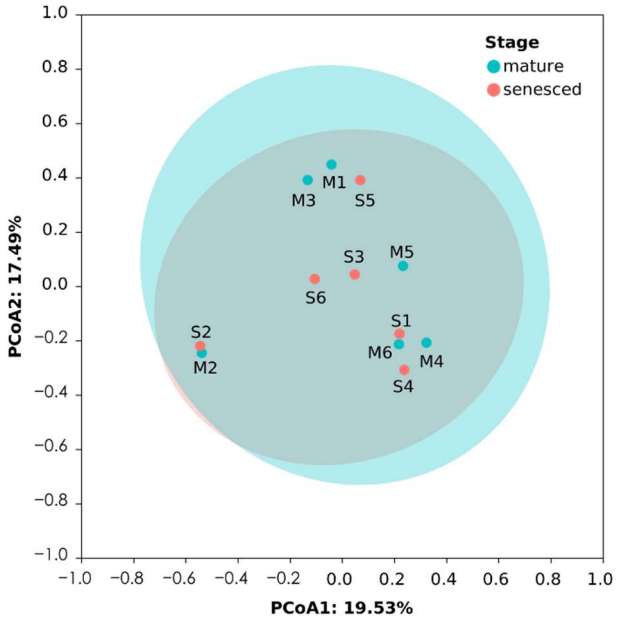


Figure S2. Principal coordinates analysis (PCoA) of the overall differences between species aboveground biomass of the plant communities at two growing stages based on Bray-Curtis distances under Robust Scaler standardization.

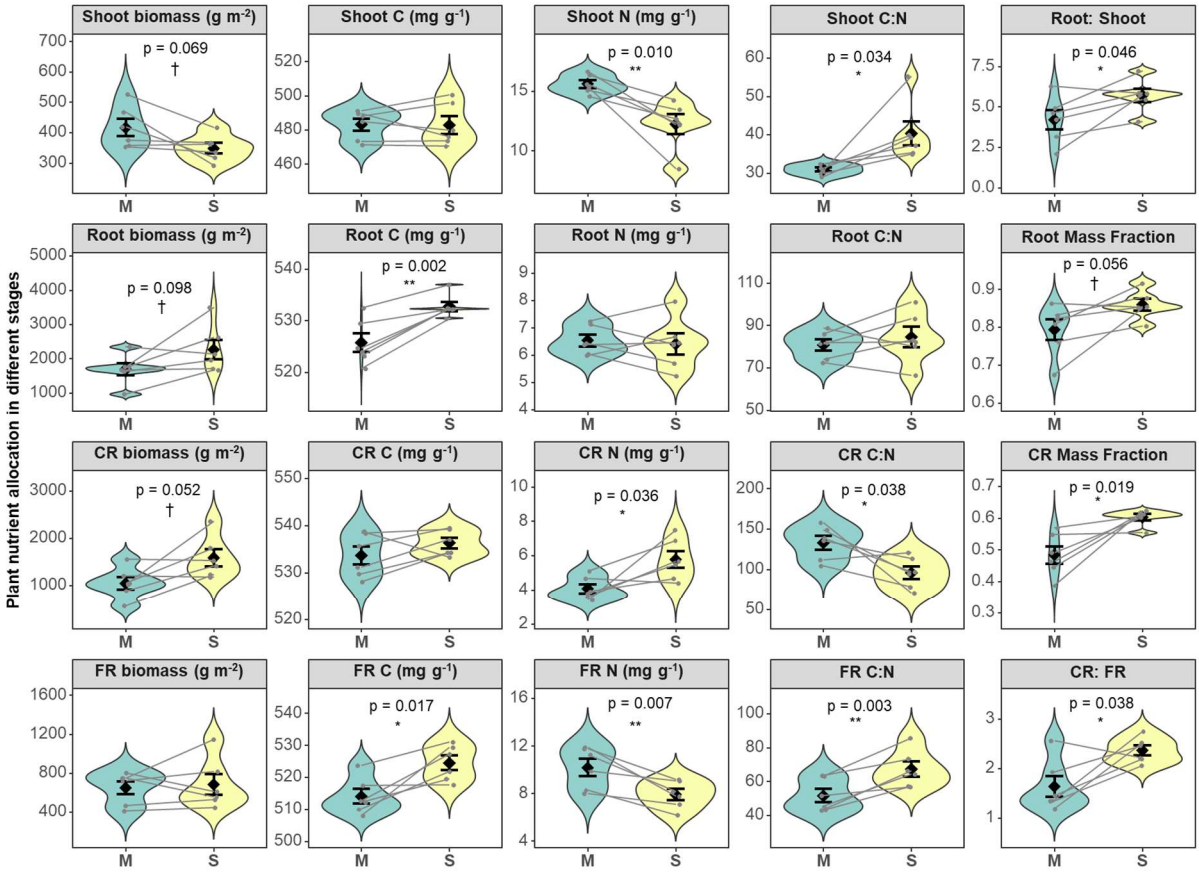


Fig. S3. Above- and below-ground plant nutrient allocation in different growing stages. Abbreviations: M, mature stage at the peak of vegetation growth; S, senescence stage at the end of the growing season. CR, coarse roots (transportive roots, root order ⩾ 3) and rhizomes; FR, fine roots (absorptive roots, root order < 3); C: N, ratio of C to N concentration; Root: Shoot, biomass ratio of all below- to above-ground organs. Grey dots and lines are paired observations. Mean ± SE (n = 6) is shown as black point and dumbbell. The *P*-values and significance levels are labeled with † $0.05 < P < 0.10$; * $0.01 < P \leqslant 0.05$; ** $0.001 < P \leqslant 0.01$.

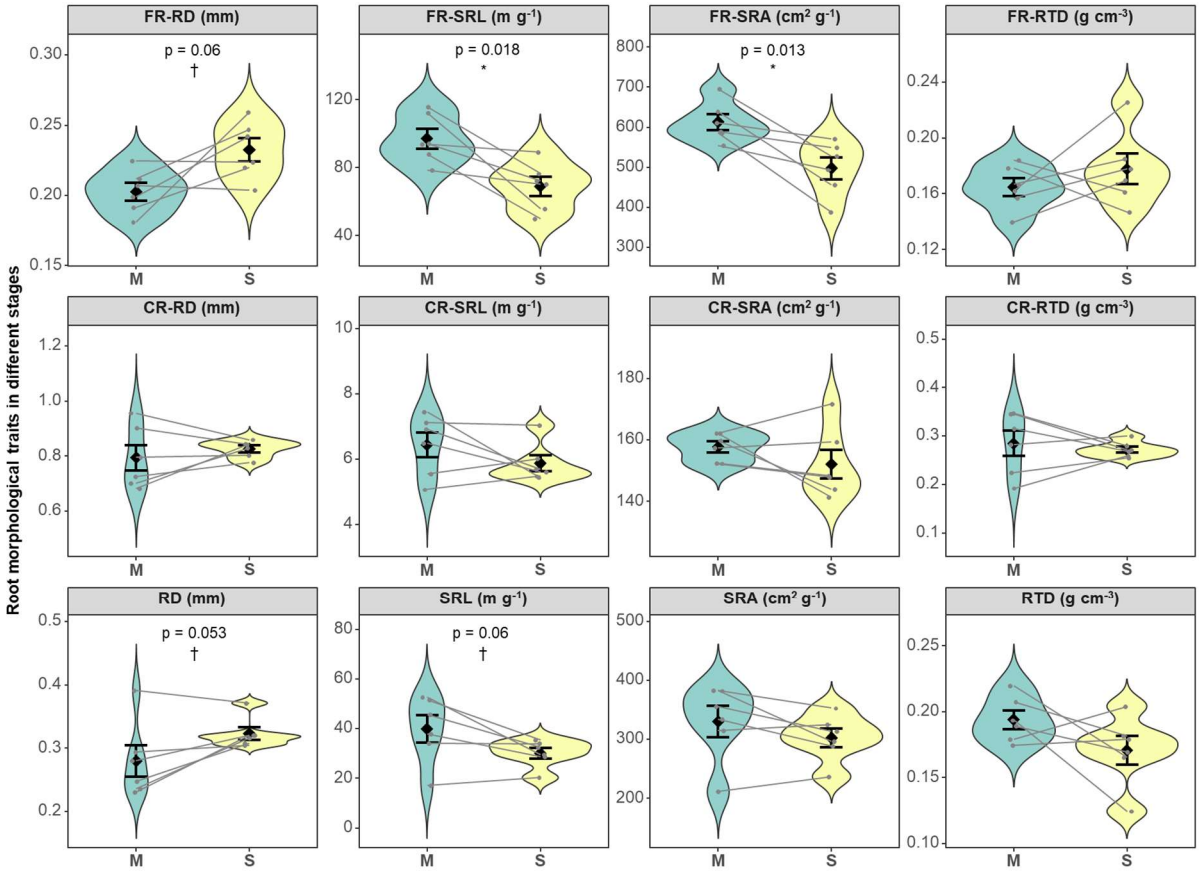


Fig. S4. Morphological traits of fine roots (FR), coarse belowground organs (CR: transport roots and rhizomes) and all belowground organs at different growing stages. Abbreviations: M, mature stage at the peak of vegetation growth; S, senescence stage at the end of the growing season. RD, root diameter; SRL, specific root length; SRA, specific root area; RTD, root tissue density. Grey dots and lines are paired observations. Mean ± standard error (n = 6) is shown as black point and dumbbell. The P-values and significance levels are labeled with † $0.05 < P < 0.10$; * $0.01 < P \leqslant 0.05$.

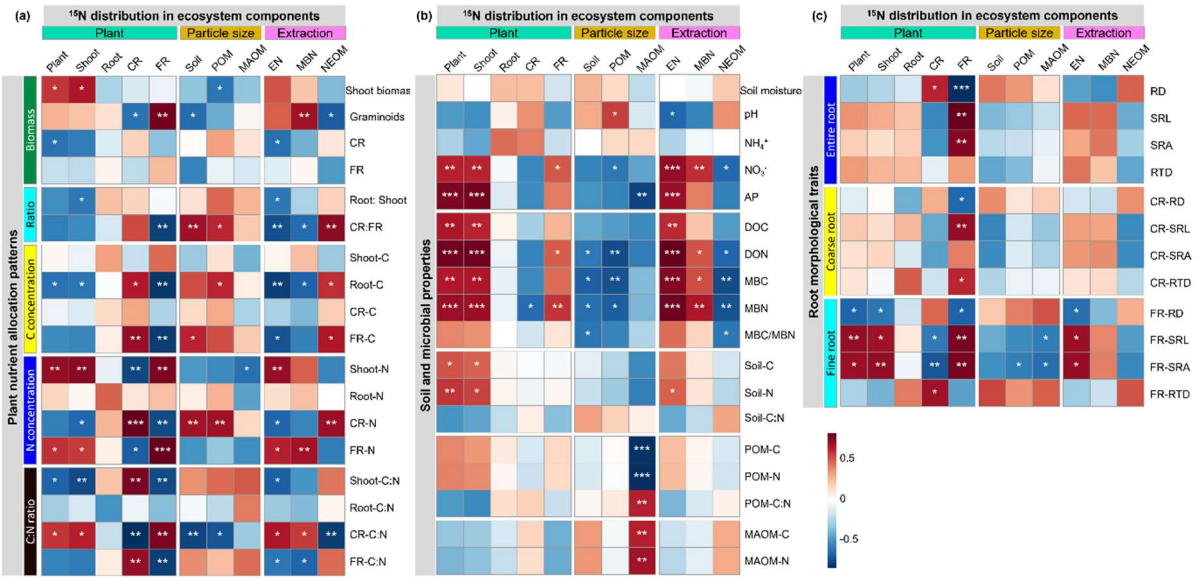


Fig. S5. Pearson correlation coefficients between ^{15}N recovery rate and plant nutrient allocation (a), soil and microbial properties (b), and root morphological traits (c) across the growing season. Red indicates positive correlations, and blue indicates negative correlations. Darker colors are associated with stronger correlation coefficients. The significance levels are labeled with * $0.01 < P \leq 0.05$; ** $0.001 < P \leq 0.01$; *** $P \leq 0.001$. Abbreviations: Root, all belowground organs; CR, coarse roots (transportive roots, root order ≥ 3) and rhizomes; FR, fine roots (absorptive roots, root order < 3); Root:Shoot, biomass ratio of all below- to above-ground organs; POM, particulate organic matter (particle size > 53 μm); MAOM, mineral-associated organic matter (particle size ≤ 53 μm); EN, K_2SO_4 extractable N; MBC, microbial biomass C; MBN, microbial biomass N; NEOM, non-extractable soil organic matter; C, carbon concentration; N, nitrogen concentration; NH_4^+, soil ammonium concentration; NO_3^-, soil nitrate concentration; AP, available soil phosphorus; DOC, dissolved organic C; DON, dissolved organic N; RD, root diameter; SRL, specific root length; SRA, specific root area; RTD, root tissue density.