

RESEARCH ARTICLE

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

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Abstract

1. Low available soil nitrogen (N) limits plant productivity in alpine regions, and alpine plants thus resorb and reallocate N from senescing tissues to conserve this limited N during the non-growing season. However, the destination and extent of N redistribution during plant senescence among above- and below-ground organs, let alone other processes of translocation outside of plants and into the soil components, remain poorly understood.
2. Utilizing ¹⁵N 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 ¹⁵N partitioning among plant-soil N pools with seasonal fluctuations of plant functional traits.
3. We found a substantial depletion of ¹⁵N in fine roots ($-40\% \pm 2.8\%$) and above-ground tissues ($-51\% \pm 5.1\%$), and an enhanced ¹⁵N retention primarily in coarse roots ($+79\% \pm 27\%$) and soil organic matter ($+37\% \pm 10\%$) during plant senescence, indicating a dual role of roots with coarse roots acting as an N sink and fine roots as a source of N recycling during 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 to fine root ratio. The seasonal trait variations were highly correlated with the ¹⁵N retention in coarse roots and soil organic matter. Particularly, ¹⁵N retention in particulate and mineral-associated organic matter increased by $30\% \pm 12\%$ and $24\% \pm 9\%$, respectively, suggesting a

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potential pathway through which fine root and microbial mortality contribute 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 above-ground-below-ground N conservation strategy that may optimize the temporal coupling between plant N demand and ecosystem N supply in N-limited alpine ecosystems.

KEYWORDS

^{15}N labelling, functional traits, N resorption, N retention, plant nutrient strategies, plant–soil interactions, soil organic matter

1 | INTRODUCTION

Nutrient limitation is a major constraint of terrestrial plant performance and primary productivity worldwide (Du et al., 2020; Lambers, Chapin III, et al., 2008). Nutrient conservation thus plays a crucial role in plant adaptation to infertile environments, especially in extremely nitrogen (N) limited alpine regions (Fisher et al., 2010; Freschet et al., 2010; Parton et al., 2007). Plants have multiple strategies to conserve limiting nutrients, such as extending the lifespan of plant tissues or retranslocating nutrients from senescing parts (Chapin III & Kedrowski, 1983; Eckstein et al., 1999). In particular, perennial plants withdraw up to ~60% of N from their senescing tissues, accounting for nearly a third of their total annual N demand (Deng et al., 2018; Vergutz et al., 2012). This retranslocation of N 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 plant functional traits remain poorly understood, at the level of both shoot-root N budgets and ecosystem N cycling (Daly et al., 2021; Freschet et al., 2010).

Most studies exploring N resorption from above- or below-ground plant components have focused on responses that occur at the organ level and often neglect shoot-root and plant–soil interactions that occur at the community level. While N resorption can mediate the coexistence of plant species, vegetation regrowth and succession over time at the community level (Hayes et al., 2014; Lü et al., 2019), it also influences litter chemistry and ecosystem N retention and cycling at the ecosystem level, across different spatial and temporal scales (Deng et al., 2018; Wang et al., 2018). However, our understanding of its related processes is hindered by limited knowledge of seasonal N fluxes involving below-ground organs at the community and ecosystem levels. Although roots and other below-ground organs can comprise over 85% of total plant biomass in some alpine grasslands (Ma et al., 2021; Yang et al., 2009), 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 (Aerts, 1990; Gordon &

Jackson, 2000; Nambiar, 1987), others reported substantial N resorption in fine roots of both woody and non-woody perennials (Freschet et al., 2010; Meier et al., 1985; Woodmansee et al., 1981). This discrepancy may stem from different definitions of fine roots (McCormack et al., 2015), the sampling of non-natural phenological root senescence (Aerts et al., 1992), and/or from different estimation methods (Kunkle et al., 2009). Kunkle et al. (2009) reanalysed published results by correcting root mass loss and found an N decrease of 28% in newly senesced 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 (Cong et al., 2019; Millard & Grelet, 2010; Zadworny et al., 2015; Zhao et al., 2020), indicating the dual role of roots as N sink and source during senescence (Gordon & Jackson, 2000).

A growing body of evidence highlights how a trait-based approach can help us understand 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 nutrient strategies (Joswig et al., 2021; Keep et al., 2021; Reich, 2014; Vitra et al., 2019). In turn, changes in plant functional traits interact with seasonal and longer temporal changes in environmental stresses to affect essential ecosystem processes (Chapin III & Kedrowski, 1983; Lambers, Raven, et al., 2008). For example, plants with lower N resorption can allocate more nutrients below-ground in response to seasonal drought and species with stronger resorption can enhance above-ground investment under N enrichment (Zhao et al., 2020). In environments with low temperature and nutrient availability, the leaf N content, specific leaf area, fine root N content and specific root length decrease at the end of the growing season (Vitra et al., 2019; Zadworny et al., 2015), but the biomass of the transportive roots and its N content may increase (Cong et al., 2019; Zadworny et al., 2015). However, seasonal trait variations and their linkage with N conservation in natural ecosystems have not been extensively studied (Freschet et al., 2010). Understanding to what extent the process of nutrient redistribution, both above- and below-ground, is paralleled with temporal shifts in acquisitive versus conservative trait expression in plants will help us refine our knowledge of plant nutrient strategies and ecosystem N dynamics.

At the ecosystem level, N is a major constituent of living plant organs, litter, soil microbial biomass and soil organic matter (SOM). Differential partitioning among these N pools can critically affect cumulative N retention with consequences on the cycling of other elements, such as ecosystem C accumulation (Hu et al., 2001; Jia et al., 2022). Besides being redistributed into living plant biomass, N in senescing tissues can also be transferred to SOM through rhizodeposition and litterfall (Aerts et al., 1999; Bernard et al., 2022; Kunkle et al., 2009). Mineral N can be incorporated into and released from two generally classified SOM pools, particulate organic matter (POM) and mineral-associated organic matter (MAOM), and these forms of organic N have different availability for plants in different growing stages (Jilling et al., 2018; Schimel & Bennett, 2004; Sollins et al., 1996). From the growing season to the non-growing season, the availability of N from POM and MAOM for plants differs due to several factors, including variation in microbial activity, temperature and moisture, and N mineralization rates (Bernard et al., 2022; Shahzad et al., 2012). For instance, during the peak growing season, high microbial activity and high temperature can lead to high N mineralization in POM, while N in MAOM is more protected by the soil matrix and less available to plants. In comparison, low temperatures and microbial activities often constrain the mineralization of N and the availability of N for plants during non-growing seasons. Coupling with plant nutrient demand at different growing stages, SOM may serve as a nutrient reservoir or supplier (Chapin III et al., 2011; Lambers, Raven, et al., 2008). Therefore, 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 investigate the N redistribution among multiple N pools during plant senescence in a Tibetan alpine meadow with high N limitation. Specifically, we used a ^{15}N stable isotope to track seasonal N redistribution among plant organs and soil fractions, and to explore the link between plant-soil N fluxes and plant functional traits during plant senescence. We hypothesized that (1) at the plant community scale, alpine plants would withdraw N from above-ground tissues and fine roots, but would enhance the N storage in rhizomes and coarse roots during senescence, (2) plant senescence and its associated N redistribution would correlate with a shift in functional traits toward more resource-conservative strategies at the end of the growing season and (3) N fluxes from plants and microbes to soil during plant senescence would constitute a significant pathway of N retention in SOM. 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 the peak of vegetation growth and the end of the growing season.

2 | MATERIALS AND METHODS

2.1 | Study site and experimental design

Our study site is located on the Qinghai-Tibetan Plateau, the highest and largest plateau on the Earth, and local ecosystems are highly N-limited (Du et al., 2020). More than 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; 3538m a.s.l.) in the eastern Tibetan Plateau, Maqu County, Gansu Province, China. The climate of this ecoregion is cold-humid alpine, with a mean annual temperature of 1.2°C and a mean annual precipitation of 590mm over the last 50 years, and the precipitation occurs primarily 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 of 6.0, a total soil C of 98.2g Ckg⁻¹ and a total soil N of 6.9g Nkg⁻¹ in the upper 10cm (Zhang et al., 2020). The type of vegetation 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 above-ground 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 (23 May) of 2021, six pairs of PVC pipes (inner diameter: 19 cm, height: 22 cm) were hammered into the soil to a 20 cm depth as semi-closed systems. The tops of the PVC pipes were 2 cm above the ground to prevent 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 2 July, 2021, after 6 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 ($^{15}\text{NH}_4$)₂SO₄, 98% atom ^{15}N) was evenly injected through 24 locations at two soil depths (2 and 5 cm) within each pipe. Light rain in the subsequent days after the ^{15}N injection facilitated the homogeneity of the ^{15}N labelling. Four unlabelled 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 peak biomass stage.

2.2 | Plant-soil sampling and measurements

On 13 August, 2021, 6 weeks after ^{15}N injection, when vegetation in this region reached a peak primary productivity with its highest N assimilation, a 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. The above-ground plant communities were cut at the soil surface, separated by species, oven-dried (48°C, 48h) 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 (500g) and -20°C (100g) before laboratory analyses. Roots were thoroughly

washed, air-dried for 12h indoors (temperature: 10°C; air humidity: 50%–70%) and then weighed as the fresh root mass. A representative subsample of fresh roots (10g) 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 5g was left unseparated for morphological analyses of entire-root traits. The remaining root samples were oven-dried (48°C, 48h) and weighed. For the second mesocosm in each pair, the fresh leaf litter was timely collected twice, on 13 August and 28 August, 2021, to limit material loss through leaching and decomposition. At the end of the growing season on 10 October 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 the diversity indices of the composition of the paired plant communities and performed a principal coordinate analysis (PCoA) on the overall difference between species biomass structure (Figures S1 and S2). We observed no significant difference and therefore concluded that our temporal approach based on paired sampling was appropriate for linking the above- and below-ground N relocation during plant senescence.

2.3 | Determination of plant, soil and microbial properties

Soil moisture, pH, ammonium (NH_4^+) and nitrate (NO_3^-), available phosphorus (AP), soil dissolved organic C (DOC) and N (DON), K_2SO_4 extractable N (EN) and microbial biomass C (MBC) and N (MBN) were measured as described by Xu et al. (2021) for the same study site. Soils were fractionated into two particle size classes: POM ($>53\mu\text{m}$) and MAOM ($\leq 53\mu\text{m}$) using a wet sieving approach (Yan et al., 2007). Fresh samples of coarse roots and fine roots were scanned with backlighting (Epson Expression 12000XL-PH, Japan), and images were processed with WinRHIZO 2020a (Regent Instruments Inc., Canada) to determine root morphological parameters (root diameter, length, surface area and volume were recalculated as the sum of the values provided for each diameter classes, as the values provided by WinRHIZO 2020a are inaccurate). The specific root length (SRL) and specific root area (SRA) were estimated by dividing the root length and the surface area by the dry weight, respectively. Root tissue density (RTD) was calculated by dividing the root dry mass by the fresh root volume. For coarse roots, we eliminated the length, surface area and volume of the remaining truncated base of separated fine roots, identified as the following root diameter class: $0 < \text{Diameter-Class} < 0.5\text{mm}$, because it can lead to severely inaccurate estimation of coarse root diameter, SRL and SRA, especially for grasses (Freschet, Pagès, et al., 2021). Root mass fraction was calculated by dividing root biomass by entire plant biomass. Oven-dried plant shoots (total above-ground biomass including leaves

that remain green and pre-collected fresh leaf litter for the senescent stage), coarse roots, fine roots, soils, POM and MAOM were separately ground into fine powder with a ball mill (Retsch MM200, Germany). The C and N concentrations were determined using an elemental analyser (Vario MICRO cube, Elementar, Germany), and the ^{15}N atomic percentage (AT% [^{15}N]) was determined using 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 the ^{15}N recovery rate, i.e. ^{15}NRR) in 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. The AT% [^{15}N] and N concentration in the ^{15}N labelled shoots, coarse roots, fine roots and soil fractions were determined on EA-IRMS as previously describe. Microbial biomass AT% [^{15}N] was determined after Kjeldahl digestion and diffusion (Stark & Hart, 1996). The 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 the total soil ^{15}N . The field plots were fenced to prevent disturbance by 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 (Figure 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 the N concentration multiplied by the biomass or mass of each pool, then multiplied by the difference in AT% [^{15}N] between the labelled and natural samples, and finally divided by the ^{15}N tracer applied. So, the ^{15}N recovery rates in each plant and soil fractions were calculated following Equation (1):

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

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

Although changes in plant N concentrations are typically used to calculate N resorption efficiency, they are inadequate as a quantitative metric of N redistribution among shoot-root tissues because organ biomass can also change during senescence (Kunkle et al., 2009; van Heerwaarden et al., 2003). Therefore, we examined N pools instead of N concentration to assess ^{15}N redistribution in

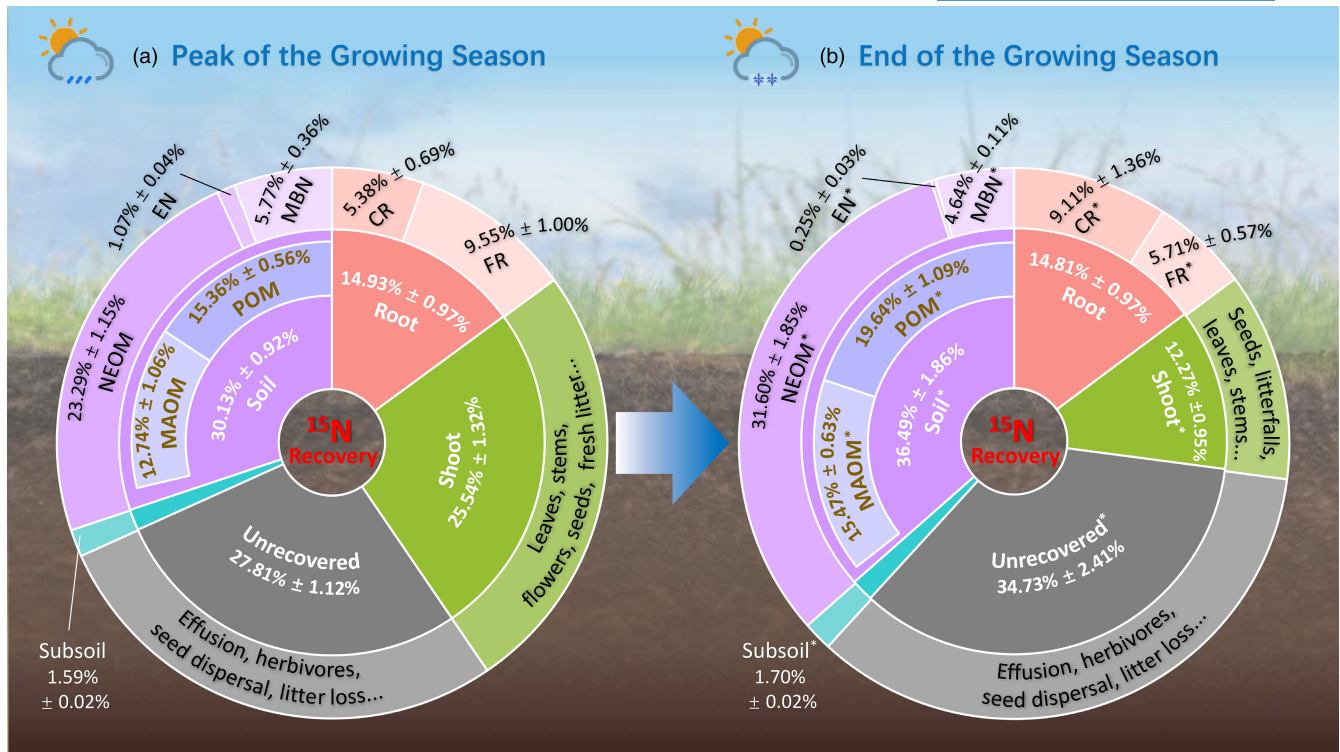


FIGURE 1 ^{15}N partitioning among plant shoots, roots, soil and their sub-fractions, (a) at the peak of vegetation growth and (b) at the end of the growing season. Total microbial biomass ^{15}N in soil was calculated from microbial biomass ^{15}N flush using an extraction efficiency of 0.45. Values are mean \pm standard error ($n=6$). The asterisk, * in (b), indicates a significant difference between growing stages ($p < 0.05$). CR, coarse root (transportive roots and rhizomes, root order ≥ 3); EN, K_2SO_4 extractable N; FR, fine roots (absorptive roots, root order < 3); MAOM, mineral-associated organic matter (particle size $\leq 53\ \mu\text{m}$); MBN, microbial biomass N; NEOM, non-extractable soil organic matter; POM, particulate organic matter (particle size $> 53\ \mu\text{m}$).

each N pool. The ^{15}N redistribution efficiency in each N pool was calculated as the percentage change in the ^{15}N recovery rate between the growth peak period and the end of the growing season following Equation (2):

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

Here, the $^{15}\text{NRR}_{\text{growth peak}}$ and the $^{15}\text{NRR}_{\text{senescence stage}}$ represent the ^{15}N recovery rate in a given ecosystem N pool at the peak of vegetation growth 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 performed with R (version 4.0.2, <https://www.r-project.org/>). Student's paired t -tests were used for comparisons of soil properties, microbial properties, ^{15}N distribution and nutrient allocation between the two growing stages. To investigate the temporal transition of the plant economics spectrum, we performed a principal component analysis (PCA) that included 16 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 in plant growth stages, in terms of soil and microbial properties, plant nutrient concentrations and biomass allocation and root morphology. The diversity indices (Shannon-Wiener index, Simpson index, species richness and species evenness) of the plant communities were calculated to examine any potential differences in the community structure between the two plant growing stages in each pair of mesocosms using a student's paired t -test. In addition, a PCoA was performed to analyse the differences in community structure (based on above-ground biomass) between the two growing stages based on Bray-Curtis distances under Robust Scaler standardization. We set the significance level at $p < 0.05$.

3 | RESULTS

3.1 | Soil and microbial properties across the growing season

From 13 August to 10 October, the inorganic soil N and available soil phosphorus significantly decreased (Table 1, $p < 0.01$). Similarly, DOC, DON, MBC and MBN decreased, with microbial biomass

Soil & microbial properties	Peak growth of vegetation	End of the growing season	<i>p</i> -value (paired <i>t</i> -test)
Soil moisture (%)	51.90 ± 1.11%	51.68 ± 1.83%	0.907
pH (H ₂ O)	5.71 ± 0.03	5.85 ± 0.04	0.032
NH ₄ ⁺ (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

Note: Values are mean ± standard error (*n* = 6). Bold values indicate significant differences between growing stages (*p* < 0.05).

Abbreviations: AP, available soil phosphorus; DOC, dissolved organic C; DON, dissolved organic N; MAOM, mineral-associated organic matter (particle size ≤ 53 μm); MBC, soil microbial biomass C; MBN, microbial biomass N; NH₄⁺, soil ammonium; NO₃⁻, soil nitrate; POM, particulate organic matter (particle size > 53 μm).

positively correlated with DOC and DON (*p* < 0.05). The soil pH increased by 0.14 units (*p* < 0.05). Soil moisture, soil C:N ratio and C and N concentrations in POM and MAOM did not show a significant change between the two sampling stages (Table 1).

3.2 | ¹⁵N redistribution among different N pools

The partitioning of the total ¹⁵N among different N pools, measured by the percentage of ¹⁵N recovery, did not differ in the entire roots between the peak (14.9% ± 0.97%, mean ± SE, the same as below) and the end (14.8% ± 0.97%) of the growing season (Figure 1, *p* = 0.94). This resulted in a neutral ¹⁵N redistribution on the scale of the entire root system (% change in ¹⁵N recovery = 2.4% ± 11.5%, Figure 2a). However, the ¹⁵N recovery in coarse roots increased from 5.38% ± 0.36% in the peak biomass stage to 9.11% ± 1.36% in the senescence stage (*p* = 0.026). In contrast, ¹⁵N recovery in fine roots decreased from 9.55% ± 1.00% in the peak biomass stage to 5.71% ± 0.57% in the senescence stage (*p* = 0.001, Figure 1), leading to a negative % change of ¹⁵N in fine roots during plant senescence (-39.9% ± 2.81%, Figure 2a). For above-ground plant tissues (including leaves, stems, flowers, seeds and fresh litter), the ¹⁵N recovery decreased substantially by half from 25.5% ± 1.32% at the peak biomass stage to 12.3% ± 0.95% after plants senesced (*p* = 0.001, Figure 1), corresponding to a negative % change of ¹⁵N in above-ground tissues (-50.94% ± 5.13%, Figure 2a).

TABLE 1 Soil and microbial properties of the topsoil (0–20 cm) in the ¹⁵N labelled mesocosms at different growing stages.

The ¹⁵N recovery in bulk soil increased from 30.1% ± 0.92% on 13 August to 36.5% ± 1.86% on 10 October (*p* = 0.027), with increases from 15.4% to 19.6% (*p* = 0.038) in POM and from 12.7% to 15.5% (*p* = 0.027) in MAOM (Figure 1), corresponding to a positive % change of ¹⁵N as +29.8% in POM and +24.3% in MAOM (Figure 2b). Furthermore, the ¹⁵N recovery in the MBN and K₂SO₄-extractable N pool (EN) significantly decreased from 5.8% to 4.6% (*p* = 0.04) and from 1.1% to 0.25% (*p* < 0.001), respectively (Figure 1). As a result, 76.6% ± 2.9% and 17.9% ± 6.2% of ¹⁵N from EN and MBN, respectively, moved to other pools (Figure 2b). On the contrary, ¹⁵N recovery in non-extractable soil organic matter (NEOM) increased from 23.3% to 31.6% at the end of the growing season (*p* < 0.05, Figure 1), corresponding to an enrichment of ¹⁵N by 37.4% ± 10.4% in NEOM (Figure 2b). Due to potential ¹⁵N losses, such as gaseous N emissions, herbivores consumption, seed dispersal and some litter movement, there were more (6.9%) unrecovered ¹⁵N by the senescence stage (Figure 1).

3.3 | Above- and below-ground plant economic traits at different growing stages

The PCA suggested a temporal shift of the plant community along the plant economics spectrum, moving from a more acquisitive strategy to a more conservative strategy during senescence (Figure 3). The first PCA axis represented 54.6% of the variance, mainly defined by the coarse root (CR)-N (element concentration, same as below),

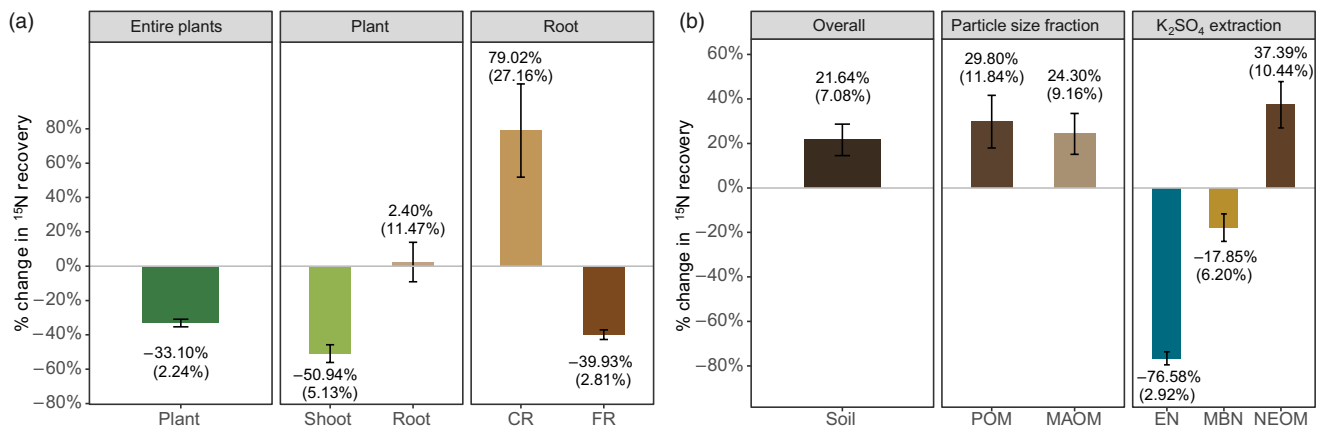


FIGURE 2 The percentage change in ¹⁵N recovery in plant–soil N pools. (a) In whole plants, shoots, roots, coarse roots and fine roots, and (b) in soil and soil sub-fractions during plant senescence from the peak growing stage to the end of the growing season. Positive values indicate ¹⁵N storage pools and negative values indicate ¹⁵N 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 ≥ 3) and fine roots (FR, absorptive roots, root order < 3). The soil was divided into several fractions, depending on particle size and K₂SO₄ extractability. Values are mean \pm standard error ($n = 6$). EN, K₂SO₄ extractable N; MAOM, mineral-associated organic matter (particle size $\leq 53 \mu\text{m}$); MBN, microbial biomass N; NEOM, non-extractable soil organic matter (particle size $> 53 \mu\text{m}$).

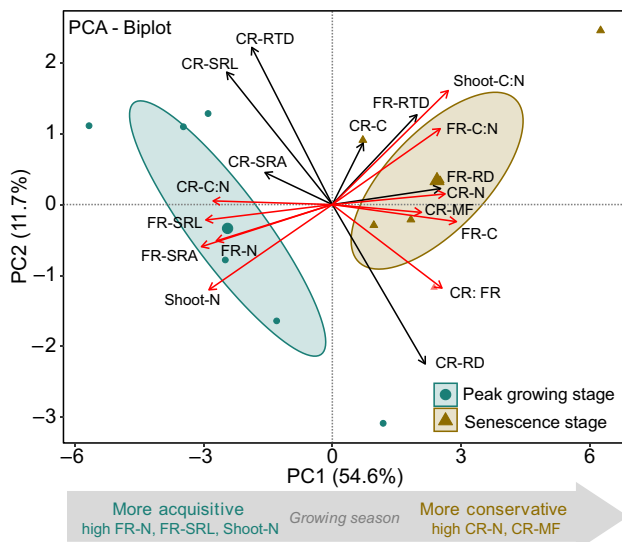


FIGURE 3 Results of principal component analysis (PCA) coded by different growth stages for plant economics spectrum, including 16 root traits and two shoot traits with 95% prediction ellipses. Red arrows represent significantly-changed traits during senescence ($p < 0.05$). Below-ground traits are differentiated between those of coarse below-ground organs (CR, transport roots and rhizomes, root order ≥ 3) and fine roots (FR, absorptive roots, root order < 3). C, carbon concentration; C:N, ratio of C to N concentrations; CR:FR, ratio of CR to FR biomass; N, nitrogen concentration; RD, root diameter; RTD, root tissue density; SRA, specific root area; SRL, specific root length.

CR-C:N, CR mass fraction, CR:FR ratio, FR-SRL, FR-SRA, FR-C, FR-N, FR-C:N, Shoot-N and Shoot C:N (all traits significantly changed during senescence, Figures S3 and S4); and the second PCA axis

was mainly driven by coarse root morphological traits (i.e. CR-RTD, CR-SRL and CR-RD). We define the first PCA axis as an acquisition-conservation axis according to the representative plant traits.

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

3.4 | Relationships between ¹⁵N recovery and plant or edaphic properties

The ¹⁵N recovery in whole-plant, shoots and extractable soil N showed positive correlations with available soil nutrients, DON, MBN and total bulk soil N in both growing stages ($p < 0.05$, Figure S5b). The ¹⁵N recovery in the FR, EN and MBN pools showed similar positive relationships with soil NO₃⁻, DON and MBN ($p < 0.05$, Figure S5b). Furthermore, the ¹⁵N recovery in MAOM showed positive relationships with C and

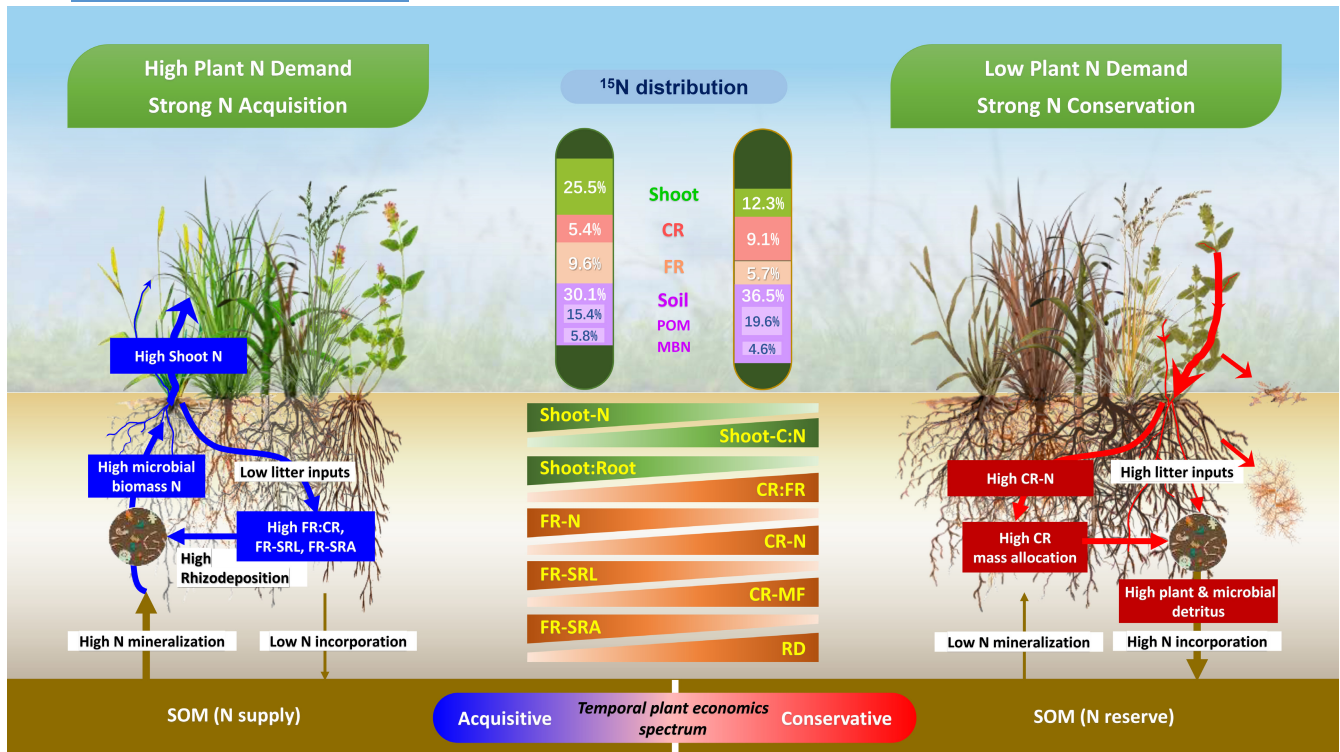


FIGURE 4 Conceptual summary of temporal plant–soil N dynamics during plant senescence, and shift in plant economics traits, as a potential mechanism for minimizing N losses and improving the coupling between plant N demand and bioavailable N supply in a N-limiting alpine ecosystem. CR, coarse roots (transportive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

N concentrations of MAOM, but negative relationships with that of POM ($p < 0.05$, Figure S5b). Moreover, ^{15}N recovery in the pools of FR, EN and MBN was positively related to graminoid biomass, shoot N, FR N and CR C:N ratio ($p < 0.05$) but negatively related to the CR:FR ratio, root C, FR C, CR N, shoot and FR C:N ratio ($p < 0.05$, Figure S5a). Interestingly, ^{15}N recovery in fine roots and coarse roots showed contrary relationships with variables representing acquisitive or conservative strategies for the trade-off of nutrient allocation (Figure S5a). Specifically, the ^{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 ($p < 0.05$; Figure 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 ($p < 0.05$; Figure S5). Besides, the ^{15}N recovery in coarse roots was positively related to the diameter of the entire roots and the FR-RTD but negatively related to the FR-SRL and FR-SRA (Figure S5c), with which the 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, Figure S5a). Furthermore, ^{15}N recovery in bulk soil, as well as in POM and NEOM, was positively correlated with the CR:FR ratio, root C, FR-C and CR-N ($p < 0.05$, Figure S5a), and negatively correlated with DON, NO_3^- , MBC and MBN in two growth stages ($p < 0.05$, Figure S5b).

4 | DISCUSSION

Nitrogen conservation during plant senescence benefits plant fitness and vegetation regrowth. Seasonal trait fluctuation from more acquisitive to more conservative can help plants optimize nutrient use efficiency as plants move from peak growth to senescence. However, our knowledge of root traits and their relationship with N conservation lags far behind our understanding of the above-ground parts. By tracing ^{15}N movement, we found that during the senescence procession, the coarse roots served as the major N sink due to increases in both the biomass of coarse roots and its N concentration, while the fine roots and shoots showed substantial N resorption. More interestingly, seasonal changes in ^{15}N recovery among plant organs were correlated with a temporal shift of traits, which defines the plant nutrient strategy of the grassland community, from more acquisitive at the peak of plant growth to more conservative 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 above-ground tissues, and a significant increase of ^{15}N primarily in coarse roots and SOM during plant senescence (Figures 1, 2 and 4). The

degree of N redistribution in total above-ground plant tissues ($-50.9\% \pm 5.1\%$) based on changes in N pools is in line with previous estimates derived 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 (Aerts, 1990; Gordon & Jackson, 2000; Nambiar, 1987), our ^{15}N -pool-based approach allowed us to observe substantial fine-root N resorption ($39.9\% \pm 2.8\%$), which is higher than a global estimate of 28% (Kunkle et al., 2009). However, 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. Furthermore, in contrast to leaves, not all fine roots senesce at the end of the growing season (i.e. there is a mixture of living and senescing fine roots), suggesting that nutrient resorption might be much stronger in completely senescing roots than would be recorded over the entire fine root pool. Finally, the ^{15}N tracing method cannot fully recover all the ^{15}N applied, owing to gaseous N emissions, herbivores consumption, seed dispersal and some litter loss. These potential ^{15}N loss pathways could explain the increase in the unrecovered ^{15}N pool. On the other hand, some ^{15}N in newly produced root litter may have been decomposed, mineralized and even reacquired by roots. This means that the changes in root ^{15}N recovery were not solely due to N retranslocation during senescence. Considering this caveat, the ^{15}N redistribution from fine roots and storage in coarse roots may be slightly overestimated. Importantly, we observed a significant increase in the coarse root ^{15}N pool during plant senescence ($+79.0\% \pm 27.1\%$), supporting our first hypothesis that coarse roots and/or rhizomes act as major N storage tissues during plant senescence. In other words, roots can play a dual role in herbaceous plant N conservation across the year, with coarse roots acting as an N sink and fine roots as a source of N recycling (De Vries et al., 2012; Freschet, Roumet, et al., 2021; Gordon & Jackson, 2000). Our results indicate that the overall change in the entire root N pool alone during plant senescence likely fails to tell the complete story of internal plant N translocation, especially between fine roots and coarse roots.

4.2 | Linking seasonal plant economics traits with ^{15}N redistribution in plant–soil components

In line with our second hypothesis, plant N redistribution during plant senescence coincided with a temporal shift in plant nutrient strategies from acquisitive to conservative (Figure 4). At the peak of their growth, plants are characterized by a more acquisitive strategy to meet higher plant C and N demand, resulting in greater N partitioning to plant organs with more acquisitive traits, also as evidenced by strong correlations between N partitioning in N-absorbing fractions and acquisitive plant traits (Shoot-N; FR-N; SRL; SRA) in our study (Figure 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 analysing the relationships between plant economics strategy and the ability of plants to retranslocate 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 greater 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 toward 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 ^{15}N retention in the ecosystem by increasing root biomass and root N uptake in an alpine meadow. Because there were only minor differences in species abundance or biomass composition in plant communities between the two growing stages (Figures S1 and S2), the shift of nutrient strategies at the plant community level could be primarily explained by the intraspecific shift in plant traits as the growing season ends (Figures S1 and S2). These results suggest that N partitioning may be temporally coordinated with plant nutrient utilization strategies, which may optimize the cost–benefit trade-offs 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 the soil, some ^{15}N may enter plant biomass or microbial biomass, while some can be absorbed directly 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, can be used by microbes, ending up either in microbial biomass or in microbial necromass that is mostly associated with MAOM. Therefore, there are some trade-offs between plant biomass N and SOM-N, or between POM-N and MAOM-N. However, the numbers do not exactly match because some N was lost from the system through gaseous emissions and leaching. Since POM is largely composed of partially decomposed plant residues (Cotrufo et al., 2022; Lavalley et al., 2020), the substantial increase in POM- ^{15}N further suggests that N uptake by plants and subsequent incorporation of plant-derived residues into POM occurred mainly via fine-root and microbial biomass turnovers (Daly et al., 2021; Kuzyakov & Xu, 2013). Substantial ^{15}N transfer to MAOM is generally related to soluble forms of N (e.g. root N leakage) and small molecular weight compounds derived from the advanced decomposition of plant and microbial residues or root exudates (Cotrufo et al., 2022; Daly et al., 2021; De Vries & Bardgett, 2012;

Huygens et al., 2008). 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. The direct displacement of MAOM-N by injected $^{15}\text{NH}_4^+$ may also have contributed to the increased MAOM- ^{15}N recovery, but our method did not allow us to quantify 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 the transfer of ^{15}N into the MAOM pool (Figure 4). In addition to the changes in ^{15}N , we also observed a small, but statistically significant increase in soil pH from the peak biomass to the senescence stage (Table 1). The pH increase may have occurred due to decreases in root uptake of cations, root exudation of organic acids and root respirations (i.e. CO_2 release). The decrease in CO_2 production as a result of reduced microbial activities may also have contributed to the increase in soil pH. Together, our results showed that the N redistribution into POM and MAOM during plant senescence constitutes an important avenue of soil N retention in the alpine meadow ecosystem.

5 | CONCLUSIONS AND PERSPECTIVES

Our study with ^{15}N trace provided direct evidence that a substantial proportion of N was withdrawn from senescing fine roots and above-ground tissues, and redistributed into coarse roots and/or rhizomes during alpine plant senescence, due to an increase in both coarse root biomass and its N concentration. This work confirmed the dual role of plant roots, with coarse roots acting as a N sink and fine roots as a source of N recycling during senescence. In parallel, we observed a temporal shift in plant nutrient strategies from more acquisitive during the growing season to more conservative during the non-growing season at the plant community level. Overall, our findings suggest that N redistribution and seasonal fluctuations of plant traits are coupled to minimize N losses and facilitate plant N conservation and ecosystem N retention, which may contribute to the temporal coupling between plant N demand and ecosystem N supply, especially in N-limited alpine ecosystems. Our study clearly quantified the whole-plant and plant-soil N redistribution during senescence using a ^{15}N pool-based framework, avoiding many of the pitfalls in the way N resorption is typically measured, especially in roots. This work opens new perspectives for mechanistic understanding 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.

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 and Shuijin Hu revised the manuscript with significant input from all other authors. All authors contributed to this work.

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CONFLICT OF INTEREST STATEMENT

The authors declare 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.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14246>.

DATA AVAILABILITY STATEMENT

The data is archived with Dryad Digital Repository: <https://doi.org/10.5061/dryad.jh9w0vtj1> (Zhao et al., 2023).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1: Paired plant communities' diversity between different growing stages.

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

Figure S3: Above- and below-ground plant traits and nutrient allocation in different growing stages.

Figure S4: Morphological traits of fine roots (FR), coarse roots (CR: transport roots and rhizomes) and entire at different growing stages.

Figure 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.

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