



# Nitrogen Uptake in an Alpine *Kobresia* Pasture on the Tibetan Plateau: Localization by $^{15}\text{N}$ Labeling and Implications for a Vulnerable Ecosystem

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## ABSTRACT

Grasslands are very important regionally and globally because they store large amounts of carbon (C) and nitrogen (N) and provide food for grazing animals. Intensive degradation of alpine grasslands in recent decades has mainly impacted the upper root-mat/soil horizon, with severe consequences for nutrient uptake in these nutrient-limited ecosystems. We used  $^{15}\text{N}$  labeling to identify the role of individual soil layers for N-uptake by *Kobresia pygmaea*—the dominating plant in the degraded Tibetan pasture ecosystems. We hypothesized a very efficient N-uptake corresponding mainly to the vertical distribution of living roots (topsoil > subsoil). We assume that *K. pygmaea* develops a very dense root-mat, which has to be maintained by small aboveground biomass, to enable this efficient

N-uptake. Consequently, a higher N-investment into roots compared to shoots was hypothesized. The  $^{15}\text{N}$  recovery in whole plants (~70%) indicated very efficient N-uptake from the upper injection depths (0–5 cm). The highest  $^{15}\text{N}$  amounts were recovered in root biomass, whereby  $^{15}\text{N}$  recovery in roots strongly decreased with depth. In contrast,  $^{15}\text{N}$  recovery in shoots was generally low (~18%) and independent of the  $^{15}\text{N}$  injection depth. This clearly shows that the low N demand of *Kobresia* shoots can be easily covered by N-uptake from any depth. Less living root biomass in lower versus upper soil was compensated by a higher specific activity of roots for N-uptake. The  $^{15}\text{N}$  allocation into roots was on average 1.7 times higher than that into shoots, which agreed well with the very high R/S ratio. Increasing root biomass is an efficient strategy of *K. pygmaea* to compete for belowground resources at depths and periods with available resources. This implies high C-costs to maintain root biomass (~6.0 kg DM m<sup>-2</sup>), which must be covered by a very low amount of photosynthetically active shoots (0.3 kg DM m<sup>-2</sup>). It also suggests that *Kobresia* grasslands react extremely

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sensitively toward changes in climate and management that disrupt this above-/belowground trade-off mechanism.

## INTRODUCTION

The Tibetan Plateau (TP) is the world's largest alpine grassland ecosystem and is also one of the largest pastoral high altitude ecosystems, with year-round grazing of yak, sheep, and goats of the Tibetan nomads. The grazing lawns of the TP are 2–4 cm in height and are dominated by the Cyperaceae *Kobresia pygmaea* (C.B. CLARKE). The *K. pygmaea* ecosystem covers an area of approximately 450,000 km<sup>2</sup> and is thought to derive at least partly from human activity by moderate grazing pressure and partial deforestation during the Holocene (Miehe and others 2009, 2014). The *Kobresia* ecosystems are important both regionally and globally for providing grazing ground (Gao and others 2014), for their impact on the Asian climate system (Boos and Kuang 2010) and for their high storage of carbon (C) and nitrogen (N) with regard to climate change (Genxu and others 2002; Yang and others 2008; Liu and others 2012).

Plant growth in alpine ecosystems is limited by unfavorable environmental conditions: low temperature, water scarcity, high solar radiation, and nutrient deficiency (Callaway and others 2002; Körner 2003; Hermans and others 2006). Alpine plants compete under these stressful conditions by developing specific strategies to ensure survival and reproduction (Choler 2005; Onipchenko and others 2009). Livestock grazing is an additional stress factor in many alpine ecosystems, changing the vegetation and soils (Heitkamp and others 2014; Sylvester and others 2014). This requires the plants to acquire and utilize resources very efficiently.

*Kobresia pygmaea* develops very compact root mats that vary in thickness between 5 and 30 cm (Kaiser and others 2008; Miehe and others 2008). These root mats protect against mechanical degradation by trampling and also support rapid regrowth following grazing events (Miehe and others 2011). Nonetheless, large grassland areas of the TP are already degraded (Holzner and Kriechbaum 2001; Harris 2010). Grassland ecosystems on the TP react extremely sensitively to increased grazing pressure and climate change (Du 2004), but the drivers and mechanisms of grassland degradation and the impacts due to a changing environment remain unclear (Chen and others 2013). A few studies have shown that increasing temperature

**Key words:** *Kobresia pygmaea*; root activity; root biomass; plant strategy; above-belowground trade-offs; <sup>15</sup>N partitioning; pasture degradation.

and intensified livestock management accelerate C and N-mineralization (Gao and others 2008; Yang and others 2013). Therefore, both N-availability for plants and N-loss via leaching might increase (Klein and others 2004; Gao and others 2007).

Nitrogen limits net primary production in many terrestrial ecosystems (Vitousek and Howarth 1991; LeBauer and Treseder 2008), and variations in N-availability can therefore have large impacts on plant growth and ecosystem recovery, especially in ecosystems such as alpine grasslands (Xu and others 2014). Nitrogen limitation mainly occurs due to low deposition rates, a high proportion of immobilized N in the organic form, and a low N-mineralization and N-fixation rate caused by low temperature and a short vegetation period (Wang and others 2005; Xu and others 2006; Lü and Tian 2007; Liu and others 2011; Zhang and others 2012). Moreover, the dung of the main herbivore, the yak, is completely collected by nomads and serves as their only fuel source (Rhode and others 2007). As an obvious sign of N deficiency, many *K. pygmaea* grasslands are green-yellowish in color, which contrasts with the fertilized grounds around settlements and cattle resting places (Stump and others 2005; Wesche and Ronnenberg 2010). Grassland degradation has been extensive in recent decades and has destroyed mainly the upper root-mat/soil horizon (Wang and others 2005; Babel and others 2014). This makes the recapture of nutrients by roots from deeper soil layers potentially important.

Little is known about the development and future response of these sensitive grasslands against the background of climate and land-use changes. Understanding the drivers of degradation requires demonstrating the mechanisms of nutrient partitioning within the soil–plant system and their uptake in non-degraded grasslands. Here, we attempt to fill this gap in our knowledge using a <sup>15</sup>N-labeling experiment. Nitrogen uptake and N allocation were investigated within the soil–plant system in non-degraded *K. pygmaea* grasslands; <sup>15</sup>N-urea was injected into the soil to six depths and <sup>15</sup>N was analyzed in samples of shoots, roots, and soil after 45 days. We hypothesize that the <sup>15</sup>N-uptake of shoots, roots, and total plant biomass strongly depends on the vertical distribution of living root biomass (topsoil > subsoil). Consequently,

decreasing  $^{15}\text{N}$  recovery rates are expected when  $^{15}\text{N}$  tracer is injected into deeper soil (Hypothesis 1). Because alpine *Kobresia* pastures are assumed to be N-limited, we further expect that *K. pygmaea* has developed specific mechanisms for very efficient N-uptake reflected by high  $^{15}\text{N}$  recovery rates in total plant biomass (Hypothesis 2). We further hypothesize that the  $^{15}\text{N}$  incorporation into root biomass will be much higher than into shoot biomass to guarantee an efficient N-uptake. This would involve higher below- than aboveground investments and also be reflected by high R/S ratios (root-to-shoot ratio) for alpine *Kobresia* pastures (Hypothesis 3).

Due to the long-term moderate grazing history of *Kobresia* pastures, we assume that *K. pygmaea* developed a very efficient trade-off between the allocation of assimilates to roots and shoots. Nonetheless, this implies that this ecosystem might react extremely sensitively to changes in climate and management that potentially disrupt this trade-off mechanism. This can presumably explain why degradation during the last decades strongly affected this ecosystem.

## MATERIALS AND METHODS

### Site Description

The study was conducted within the vegetation period from June to September 2012 at the *Kobresia* Ecosystem Monitoring Area research station (KEMA) on a gently inclined alpine grassland pasture (Xizang, China;  $31^{\circ}16'45''\text{N}$ ,  $92^{\circ}59'37''\text{E}$ ; 4,410 m a.s.l., inclination of 2–5%, Babel and others 2014). The site is located in the core area of *K. pygmaea* distribution (Miehe and others 2008). *Carex* spp., *Festuca* spp., *K. humilis*, *Poa* spp., *Stipa purpurea*, and *Trisetum* spp. are present in addition to *K. pygmaea*, which is the dominant and eponymous species (covering up to 98% of the root-mat surface).

The mean summer precipitation (June–September) of the area (Climate station Nagqu, 4,507 m a.s.l.) is 272 mm, ranging from 21 to 442 mm (Miehe and others 2011). The pasture quality is highly dependent on the onset and duration of the summer rain. Although the vegetation period lasts from mid-May to mid-September, the greening of the pastures can be delayed until early August. Winter snowfall is comparably low. The mean maximum temperature of the warmest month (July) is  $15.1^{\circ}\text{C}$  (Miehe and others 2011, 2014). During the 45-day experiment, a mean air temperature of  $10.4^{\circ}\text{C}$  and a total precipitation of

162 mm were recorded. Forty-five percent of this precipitation fell within four days at the beginning of this experiment.

Soils are classified as Stagnic Eutric Cambisol (Humic, IUSS Working Group WRB 2006) and developed on a Holocene loess layer of variable thickness (0–30 cm), overlying a glacial moraine. The mineral soil (0–25 cm) consisted of  $50.4 \pm 1.3\%$  sand,  $33.0 \pm 0.9\%$  silt, and  $16.6 \pm 0.7\%$  clay and is therefore defined as loam. Soils were free of carbonates (0–25 cm) and showed mean pH values ( $\text{H}_2\text{O}$ ) of  $6.85 \pm 0.03$ , which marginally changed with depth. The top-soils were characterized by dense *Kobresia* root mats (up to 15-cm thick, Figure 1). These root mats consisted mainly of mineral particles, humified organic matter, and large amounts of dead and living roots. Thus, soils store high amounts of C and N (soil and roots in 0–25 cm:  $15.2 \pm 0.43 \text{ kg C m}^{-2}$  and  $1.0 \pm 0.03 \text{ kg N m}^{-2}$ , Figure 1). A detailed description of general soil and root characteristics is provided in Table S1 (supplement).

Traditionally, the site was used as a grazing ground for livestock (yaks and sheep) from January to April, and large numbers of small mammals (mainly the Plateau Pika, *Ochotona curzoniae*) compete with livestock for plant biomass resources.

### Labeling and Sampling

The  $^{15}\text{N}$  labeling experiment started on 20 July 2012 and lasted for 45 days. Plots ( $15 \times 15 \text{ cm}$ ) that were dominated by *K. pygmaea* were randomly selected and a total amount of  $2.6 \text{ mg } ^{15}\text{N}$  was added as urea to each plot (99 atom%  $^{15}\text{N}$ , Sigma Aldrich). The  $^{15}\text{N}$  urea was injected into the soil at six depths, representing treatments within this field experiment (treatments = input depths: 0.5, 2.5, 7.5, 12.5, 17.5, and 22.5, Figure 1). For each injection depth, four replicate plots were sampled, yielding a total of 24 plots. To ensure equal distribution of tracer in each depth, we used a uniform labeling grid with 16 injection points and pierced the root mats with a small metal pin. One ml of  $^{15}\text{N}$ -labeled urea solution was injected using a dispensing pipette. During the experiment, grazing was excluded by choosing plots within a fenced area.

Samples of shoots, roots, and soil were taken from each plot 45 days after  $^{15}\text{N}$  addition. Root and soil samples were collected at sample intervals of 0–1 cm, 1–5 cm, 5–10 cm, 10–15 cm, 15–20 cm, and 20–25-cm depth (Figure 1). Four soil pits (30-cm length  $\times$  30 cm width  $\times$  40-cm depth) were established adjacent to the plots to determine

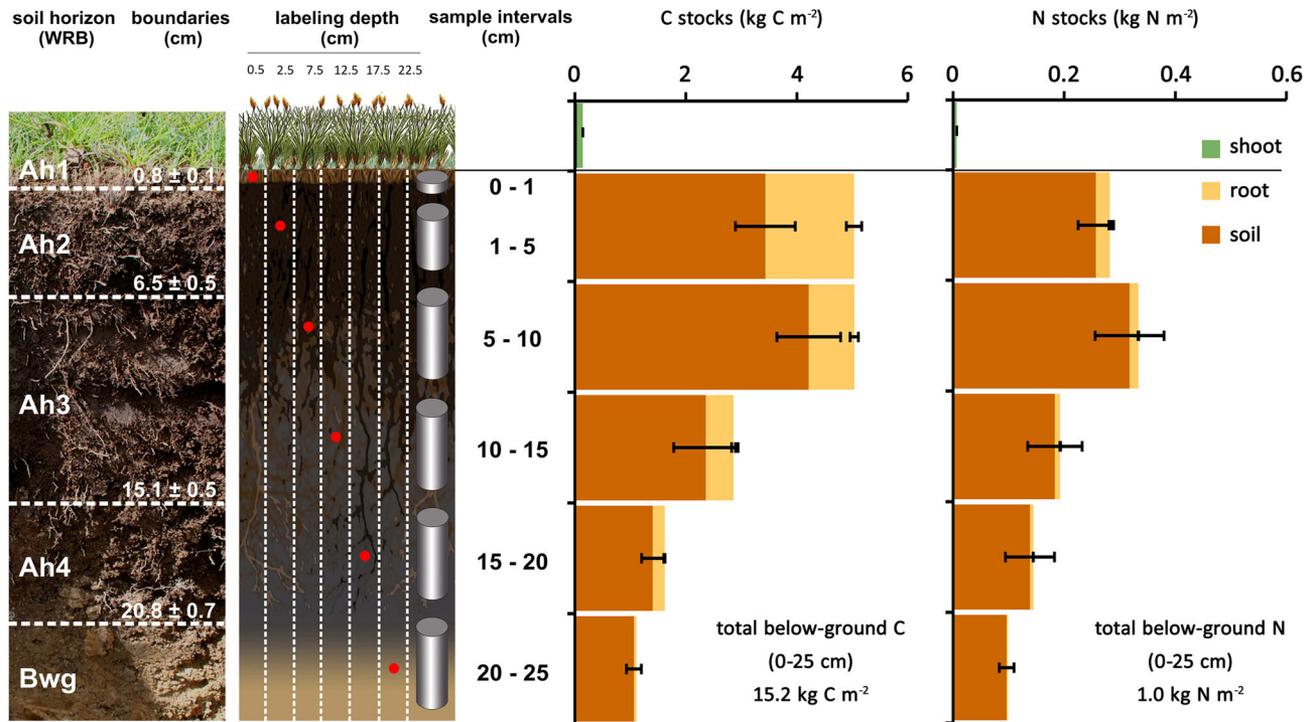


Figure 1. Characteristics of the upper 25 cm of soils and an overview of the applied study design at the KEMA study sites. The image shows the appropriate soil horizons and their boundaries ( $n = 4$ ), summarizes the applied experiment and its design, with dots marking the selected injection depths, and illustrates the C and N stocks for shoots, roots, and soil, with standard errors ( $n = 24$ ).

root and soil characteristics. Samples for  $^{15}\text{N}$  natural abundance, pH, soil texture, bulk density, living, and dead root biomass were taken at the corresponding sample depth intervals. Soil samples were sieved (2 mm), and the separated roots were washed in distilled water. The shoot biomass was cut directly on the plots in an area of  $25\text{ cm}^2$ . Eight control samples for shoot biomass and for  $^{15}\text{N}$  natural abundance were cut next to the soil pits. All samples (shoots, roots, and soil) were dried and then milled.

## Analyses and Calculations

### Basic Plant and Soil Characteristics

Bulk density was determined from dried ( $105^\circ\text{C}$ ) undisturbed soil cores (van Reeuwijk 2002). Carbon and N stocks ( $\text{kg m}^{-2}$ ) were calculated by multiplying the C and N contents with the bulk soil densities and thickness of each layer. Soil pH was measured by mixing dried sieved soil samples ( $45^\circ\text{C}$ ) with deionized water in a ratio ( $v/v$ ) of 1.0 to 2.5 (van Reeuwijk 2002). A combined sieving (particle size:  $2,000\text{--}20\ \mu\text{m}$ ) and sedimentation procedure (particle size  $< 20\ \mu\text{m}$ ) was carried out to determine particle size distribution after pre-treatment with 30%  $\text{H}_2\text{O}_2$  and 4% Na-dithionite-

citrate (van Reeuwijk 2002). Root biomass was obtained by taking volume-based soil cores, which were separated into living and dead roots. Each single root was divided by morphological criteria: the color of the root and its core, elasticity, and consistency (Hertel and Leuschner 2002). The shoot and root biomass is represented as dry matter content (DM) throughout.

### $^{15}\text{N}$ Analysis and Calculations

To determine the total C and N contents and stable isotope signatures of nitrogen ( $\delta^{15}\text{N}$ ), samples of shoots, roots, and soil were analyzed using an isotope ratio mass spectrometer (Delta plus, Conflo III, Thermo Electron Cooperation, Bremen, Germany) coupled to an elemental analyzer (NA1500, Fisons instruments, Milano, Italy). The  $\delta^{15}\text{N}$  signature was calculated using the isotope ratio of each sample ( $R_{\text{sample}} = ^{15}\text{N}/^{14}\text{N}$ ) and recalculated as  $^{15}\text{N}$  atom%. The incorporation of tracer-derived N into soil, shoots, and roots was estimated by an isotope mixing model (Robinson 2001). To avoid confusion, we used the terms tracer-N-uptake ( $N_{\text{upt}}$ ) for plant N pools and tracer-N incorporation ( $N_{\text{inc}}$ ) for the soil N pool:

$$\text{tracer } N_{\text{upt.-N.}} \text{ or tracer } N_{\text{inc.-N.}} = N_{\text{stock}} \times \frac{\text{atom}\% \text{ } ^{15}\text{N}_{\text{labeled}} - \text{atom}\% \text{ } ^{15}\text{N}_{\text{NA}}}{\text{atom}\% \text{ } ^{15}\text{N}_{\text{Tracer}} - \text{atom}\% \text{ } ^{15}\text{N}_{\text{NA}}} \times 1000 \quad (1)$$

where  $N_{\text{stock}}$  is the total N ( $\text{g N m}^{-2}$ ),  $\text{atom}\% \text{ } ^{15}\text{N}_{\text{labeled}}$  is the content of  $^{15}\text{N}$  atoms in the sample,  $\text{atom}\% \text{ } ^{15}\text{N}_{\text{NA}}$  is the content of  $^{15}\text{N}$  atoms in the control samples, and  $\text{atom}\% \text{ } ^{15}\text{N}_{\text{Tracer}}$  is the content of  $^{15}\text{N}$  atoms in the  $^{15}\text{N}$  urea tracer. In the final step, the proportion of total input ( $^{15}\text{N}$  recovery) was calculated by relating the  $^{15}\text{N}$ -uptake of plant compartments and the  $^{15}\text{N}$  incorporation of soil to the amount of total  $^{15}\text{N}$  injected into individual soil depths.

The specific root activity (SRA in  $\text{mg } ^{15}\text{N g DM living root}^{-1}$ ) describes the relative plant N-uptake per mass unit living roots from the input layer and was calculated as follows:

$$\text{SRA} = \frac{\text{plant } N_{\text{upt.-N}}}{\text{LRB}} \times f_{\text{DC}} \quad (2)$$

where plant N-uptake ( $\text{mg } ^{15}\text{N m}^{-2}$ ) is the total amount of tracer-N incorporated in plants (roots and shoots), LRB is the amount of living root biomass ( $\text{g DM living roots m}^{-2}$ ), and  $f_{\text{DC}}$  is a depth correction factor to ensure an equal amount of tracer in each input layer (0.2 for 0–1 cm; 0.8 for 1–4 cm).

## Statistics

Statistical analyses were carried out with PASW Statistic 18 (IBM SPSS Statistics) and R version 3.0.3 (R Development Core Team). Soil and plant characteristics are illustrated by means and standard errors (mean  $\pm$  SE). In general, a significance level of  $P < 0.05$  was used. Significance of treatment effects was tested by a one-way ANOVA after testing for normality (Shapiro–Wilk-test,  $P > 0.05$ ) and for the homogeneity of variances (Levene-test,  $P > 0.05$ ). In cases of non-normal distribution, data were log- or root-transformed and retested with the Shapiro–Wilk-test. The ANOVAs were followed by post hoc tests for multiple comparisons using the Tukey-test, or LSD-test, if the homogeneity of variances was not given. In a few cases, normality was not achieved and the non-parametric Kruskal–Wallis ANOVA was performed. Subsequently, multiple comparisons coupled with the Benjamini–Yekutieli correction were performed. To analyze the relationships between various investigated plant and soil characteristics, regression analysis was used. In some cases, variables could be better explained by combining a set

of predictors and, thus, a step-wise multiple linear regression analysis (MLRA) was implemented. The MLRA results were interpreted by using the total explained variance and changes in  $R^2$ .

## RESULTS

### $^{15}\text{N}$ Partitioning in the Shoot–Root System

The total belowground biomass was  $5.98 \pm 0.23 \text{ kg DM m}^{-2}$  and was therefore 20 times higher than shoot biomass ( $0.3 \pm 0.02 \text{ kg DM m}^{-2}$ ). Total, living, and dead root biomass (TRB, LRB, and DRB, respectively) decreased linearly with depth (Figure 2A), whereas specific root activity (SRA) tended to increase with depth (Figure 2B). Dead roots dominated over living roots (ratio: 0.4–0.9) in the uppermost 15 cm, but the ratio increased and remained constant below 15 cm (ratio: 1.1).

For the  $^{15}\text{N}$  recovery in shoots and roots, we assumed a strong relationship with the vertical distribution of root biomass. Accordingly, we expected the highest  $^{15}\text{N}$  recovery from the upper layers, where living root biomass was the highest (H1).

#### $^{15}\text{N}$ Recovery in Shoot Biomass

The  $^{15}\text{N}$  recovery in shoot biomass was  $18.1 \pm 1.3\%$ . There was no significant relationship between  $^{15}\text{N}$  injection depth and  $^{15}\text{N}$  recovery in shoots (Figure 3). Linear regression between the N-uptake of shoots with the LRB ( $r^2 = 0.10$ ,  $P = 0.11$ ) and SRA ( $r^2 = 0.19$ ,  $P = 0.03$ ) showed weak relationships. However, the relationship was strongly improved by combining LRB and SRA into one model ( $r^2 = 0.44$ ,  $P < 0.001$ , Figure 4). This demonstrates that both LRB and SRA had an important role for aboveground N-acquisition from deeper soil.

#### $^{15}\text{N}$ Recovery in Root Biomass

The  $^{15}\text{N}$  recovery in roots averaged  $30.8 \pm 3.4\%$ . Total  $^{15}\text{N}$  recovery decreased with increasing injection depth. The highest  $^{15}\text{N}$  amount was recovered when tracer was injected at 0.5 cm and 2.5 cm depths, whereas the lowest  $^{15}\text{N}$ -recovery was obtained at 17.5 cm and 22.5 cm (Figure 3).

Considering single injection depths we found most  $^{15}\text{N}$  in root biomass at the depths of tracer input. To a lesser extent,  $^{15}\text{N}$  was recovered in vertical overlying layers, but  $^{15}\text{N}$  recovery

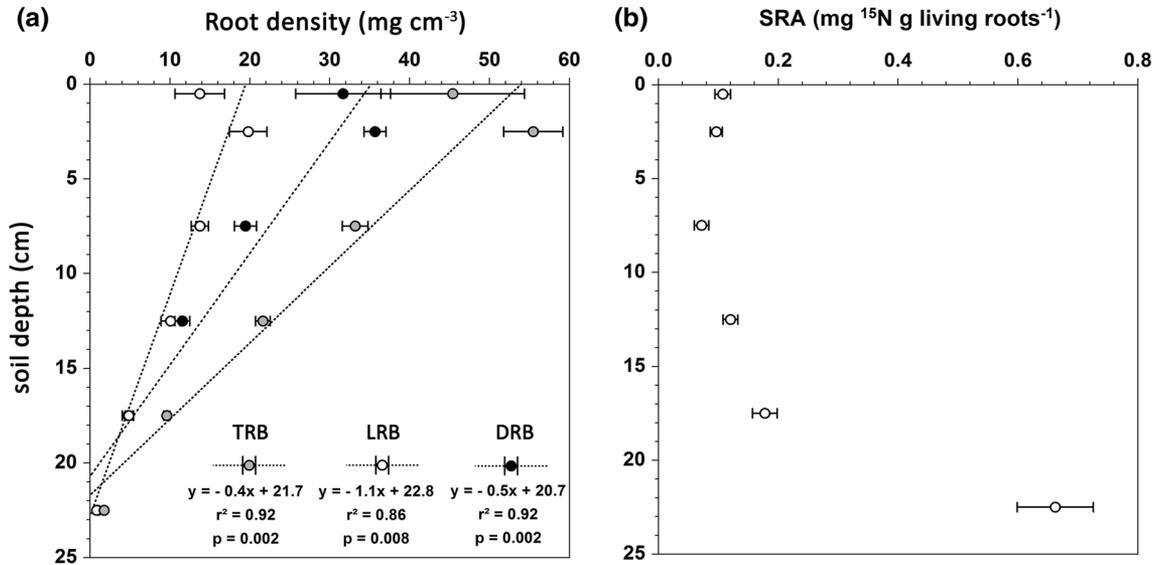


Figure 2. Relationship between soil depth and (A) total, living and dead root biomass (TRB, LRB, and DRB, respectively) and (B) specific root activity (SRA). Values are means and standard errors ( $n = 4$ ).

decreased with vertical distance from the injection depth (except for the deepest sample at 22.5 cm, Figure 5A). We further recorded a higher <sup>15</sup>N recovery for layers with a high root biomass and found the LRB to be the most important predictor to explain root N-uptake ( $r^2 = 0.63$ ,  $P < 0.001$ ).

## <sup>15</sup>N Partitioning in the Plant-Soil System

### <sup>15</sup>N Recovery in Total Plant

We used the <sup>15</sup>N recovery in total plant biomass to test the efficiency of <sup>15</sup>N-uptake of *K. pygmaea* after 45 days. We hypothesized generally high <sup>15</sup>N-uptake efficiency in this N-limited grassland (H2). A high <sup>15</sup>N recovery (~70%) occurred when <sup>15</sup>N was injected within the first 5 cm. The <sup>15</sup>N recovery in plant biomass decreased with <sup>15</sup>N injection depth, mainly due to a decreasing <sup>15</sup>N recovery in roots (Figure 3). On average,  $48.9 \pm 3.9\%$  of <sup>15</sup>N was recovered in root and shoot biomass together. This was more than the <sup>15</sup>N remaining in soil (0–25 cm) or which was lost after 45 days.

### <sup>15</sup>N Recovery in Soil

The lowest <sup>15</sup>N portion was recovered in soil, independent of the injection depth (Figure 3). The vertical distribution of <sup>15</sup>N in soil indicated that <sup>15</sup>N was also mainly present at the depths of injection (Figure 5). To a lesser extent, however, <sup>15</sup>N was also recovered in vertical overlying soil layers, reflecting mainly upward N transport.

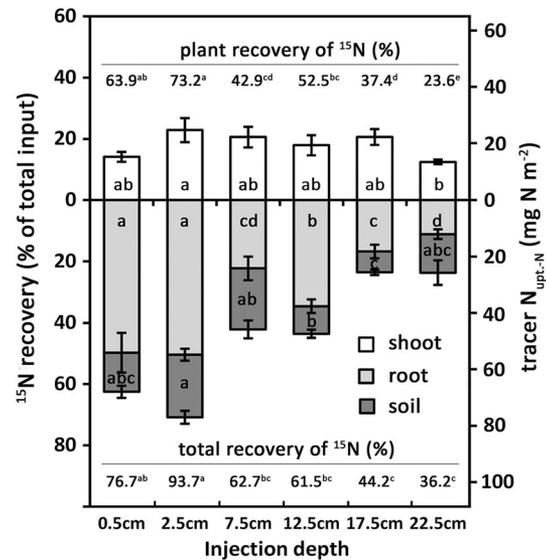


Figure 3. The <sup>15</sup>N recovery and N-uptake for shoots, roots, and soil, depending on injection depth. Values are means and standard errors ( $n = 4$ ). Lowercase letters (a–e) indicate significant differences between the injection depths ( $p < 0.05$ ).

### <sup>15</sup>N Loss

The amount of <sup>15</sup>N which was not recovered in soil or in plant biomass after 45 days was defined as short-term <sup>15</sup>N loss. It was  $37.5 \pm 4.7\%$  of the total input and increased when the tracer was injected into deeper soil layers (Figure 3). Almost no <sup>15</sup>N was lost from the 2.5-cm injection depth, whereas the highest loss occurred from 22.5 cm. A MLRA

showed that 82.2% of the variance was explained by a set of predictors including LRB (explaining 67.8% of the variance), SRA (3.3%), SOC content (4.4%), and clay content (6.7%). This showed that <sup>15</sup>N was mainly lost when the potential for root uptake and N immobilization was low. Consequently, injected <sup>15</sup>N leached deeper than 25 cm and was thus not recovered.

To consider the long-term N-loss, we used the natural abundance of δ<sup>15</sup>N in the reference soil (without <sup>15</sup>N labeling). The δ<sup>15</sup>N value increased

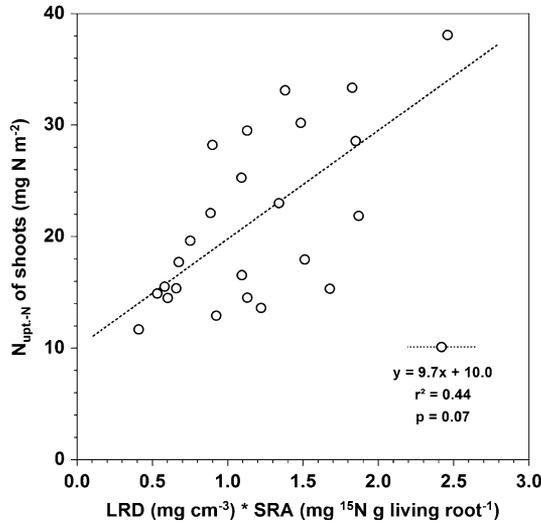


Figure 4. The product of living root density (LRD) and the specific root activity (SRA) to explain shoot N-uptake. Data were used from the <sup>15</sup>N injection depth (*n* = 24).

exponentially with increasing depth ( $r^2 = 0.93$ ,  $P < 0.001$ ), indicating greater N losses by nitrate leaching and nitrous oxide emissions from deeper soil (Amundson and others 2003; Hobbie and Quimette 2009). In addition, we demonstrate a significant relationship ( $r^2 = 0.45$ ,  $P < 0.001$ ) between <sup>15</sup>N loss after tracer application (short-term loss) and the natural-occurring δ<sup>15</sup>N in soil (long-term loss).

### Comparisons between Below- and Aboveground Investments

In line with an efficient N-uptake, we further assume higher below- than aboveground investments (H3). We therefore used the R/S ratio as a long-term indicator and the ratio of <sup>15</sup>N in root-to-shoot as a short-term indicator to compare above- and belowground investments. We observed a very high R/S ratio of 20.0. Further, the root <sup>15</sup>N-uptake was on average 1.7 times higher than shoot <sup>15</sup>N-uptake. The ratio exponentially decreased with increasing injection depth and exceeded 1.0 in the upper root mats, but was less than 1.0 if <sup>15</sup>N was injected at 17.5 cm or deeper (Figure 6).

### DISCUSSION

#### <sup>15</sup>N Partitioning in Shoots and Roots

We recorded a mean shoot biomass of 0.30 kg DM m<sup>-2</sup>, which fully agrees with the value of 0.29 kg DM m<sup>-2</sup> reported by Wang and others (2008) for

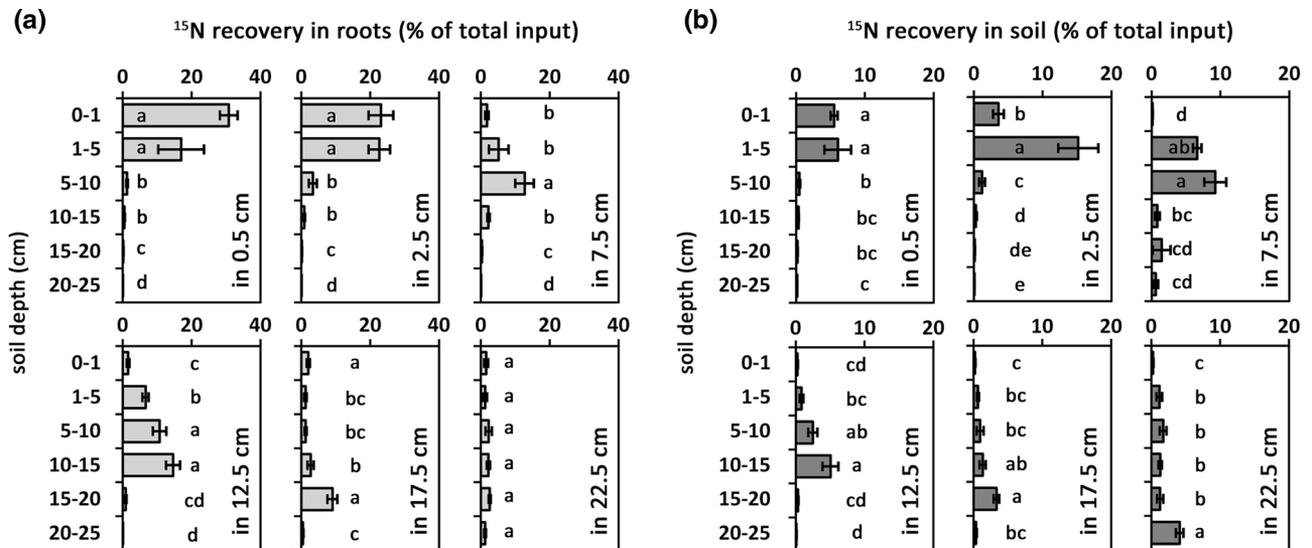
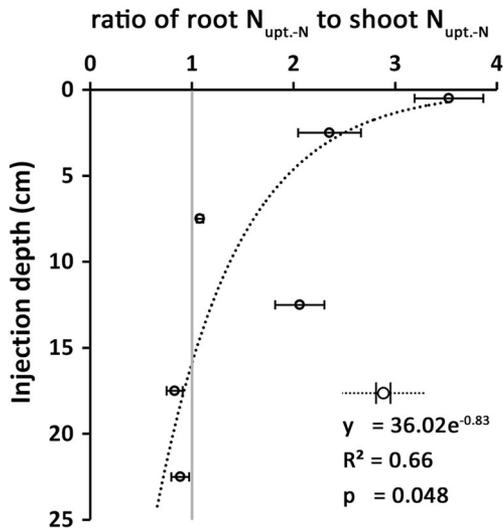


Figure 5. The <sup>15</sup>N recovery in (A) roots and (B) soil at the six sample intervals depending on injection depth (at 0.5, 2.5, 7.5, 12.5, 17.5, or 22.5), 45 days after labeling. Values are means and standard errors (*n* = 4). Lowercase letters (a–e) indicate significant differences between the sample intervals (*p* < 0.05).



**Figure 6.** Comparison between above- and below-ground investments using the ratio of root-to-shoot N-uptake depending on injection depth. Values are means and standard errors ( $n = 4$ ). The solid gray line is the 1/1 ratio and illustrates balanced above- and below-ground investments.

alpine *Kobresia* grasslands. The  $^{15}\text{N}$  recovery in shoots was independent of injection depth (Figure 3), which indicates that aboveground N-requirements can be entirely met from any part of the soil profile. Furthermore, aboveground N-demands tend to be small (mean shoot  $^{15}\text{N}$  recovery: 18.1%) and thus agreed well with the low aboveground net primary productivity reported for alpine grasslands (Knapp and Smith 2001). This partly contradicted our hypothesis (H1), because we expected that shoot N-uptake would be higher from the upper parts of the root mats, where living root biomass was the highest. However, there was no significant relationship between living root biomass and shoot N-uptake. Nevertheless, by combining living root biomass and the specific root activity, we observed a highly significant relationship (Figure 4). This demonstrates that a higher specific root activity in deeper soil (Figure 2B) compensated for a lower living root biomass there (Figure 2A).

We assume that *K. pygmaea* prioritized aboveground N-transfer to develop and/or maintain assimilation tissue. This may be crucial for balancing the belowground C-costs and to recover from grazing events. In agreement with this, Cao and others (2004) found a strong increase in shoot biomass at the beginning of the growing season (June), with a maximum in September.

In contrast to shoot biomass, total root biomass was remarkably high ( $5.98 \text{ kg DM m}^{-2}$ ). The root

biomass was also higher than the common range of  $1.5\text{--}3.7 \text{ kg DM m}^{-2}$  reported in several earlier studies on the alpine TP (Luo and others 2005; Wang and others 2008; Xu and others 2011; Li and others 2011). We observed a trend for decreasing  $^{15}\text{N}$  recovery in roots with depth (Figure 5A). This was associated with a decrease of living root biomass ( $r^2 = 0.63$   $P < 0.001$ ). Because  $^{15}\text{N}$ -uptake of roots was mainly controlled by the vertical distribution of living root biomass, we could confirm our hypothesis (H1) in this respect.

This finding indicated that N-uptake of roots was mainly associated with the upper soil, where a high proportion of SOM serves as a nutrient sink (Table S1 and Figure 1). Soil organic matter in cool mountain environments often serves as a sink and storage for nutrients (Vitousek and others 2010). The environmental conditions in such ecosystems are highly variable and alternately favor or inhibit decomposition, nutrient release, and subsequent uptake by plants at a high temporal resolution. Higher temperatures especially favor decomposition in the topsoil (Swift and others 1979; Heitkamp and others 2012) and promote energy-intensive processes such as nutrient uptake (Basirirad 2000). The general nutrient sink may therefore also serve temporally as a source, when environmental conditions favor mineralization of SOM. Consequently, *K. pygmaea* roots preferentially grew into patches with higher nutrient availability.

### Challenges for *Kobresia pygmaea* in a N-limited Ecosystem

As a long-term consequence of moderate grazing (Miehe and others 2008), we assumed that *K. pygmaea* adopted specific mechanisms for very efficient resource uptake in this N-limited ecosystem (Xu and others 2014). To test this, we used the  $^{15}\text{N}$  recovery of total plant biomass to reflect the  $^{15}\text{N}$ -uptake efficiency (H2). The  $^{15}\text{N}$ -uptake efficiency was particularly high when tracer was injected into the upper root mats ( $^{15}\text{N}$  recovery  $\approx 70\%$ , Figure 3) and thus confirmed our hypothesis (H2) of a very efficient N-uptake. The high  $^{15}\text{N}$ -uptake efficiency was consistent with previous studies (Yuan and others 2006; Liu and others 2013), which showed that the N-use efficiency and N-uptake efficiency of grassland plants increased when N-limitation occurred.

Importantly,  $^{15}\text{N}$ -uptake efficiency decreased with injection depth and was the lowest in deeper soil ( $^{15}\text{N}$  recovery  $\approx 30\%$ , Figure 3). On average, 48.9% of  $^{15}\text{N}$  was recovered in plant biomass,

which was significantly higher than that which remained in the soil or was lost after 45 days. We suggest that the decreasing  $^{15}\text{N}$ -uptake efficiency with depth was mainly explained by high  $^{15}\text{N}$  loss from deeper soil. Most of this loss probably occurred over a short period (25–28 July 2012) directly after labeling. During this period, about 60 mm precipitation fell within four days and soil volumetric water contents showed that the soil was saturated, indicating a high probability of  $^{15}\text{N}$  leaching. Higher  $^{15}\text{N}$  loss in deeper soil was mainly explained by less root N-uptake and less N-immobilization SOM and soil minerals. The MLRA demonstrated that decreasing living root biomass, SOC, and clay contents are the main factors that explain the total variance in  $^{15}\text{N}$  losses.

We also found increasing  $\delta^{15}\text{N}$  natural abundance in non-labeled soil with increasing depth, which agreed well with trends found by Zhou and others (2014) for alpine meadows (TP) considering a depth down to 20 cm. The higher  $\delta^{15}\text{N}$  values with depth indicate a preferential removal of  $^{14}\text{N}$  over  $^{15}\text{N}$  and consequently reflect long-term N loss (Amundson and others 2003). As a consequence of decomposition and nitrification, the microbial fractionation favors  $^{15}\text{N}$ -depleted N forms (Hobbie and Quimette 2009). These, in turn, get lost via leaching and outgassing especially from deeper soil and cause an increase of the  $\delta^{15}\text{N}$  values. In contrast, the very efficient recapture and recycling of N from the upper soil by *K. pygmaea* diminishes the increasing  $\delta^{15}\text{N}$  shift in 0–5 cm. Accordingly, long-term N loss ( $^{15}\text{N}$  of natural abundance) and our short-term N loss ( $^{15}\text{N}$  loss after labeling) are in good agreement.

We further hypothesized that *K. pygmaea* shows far higher belowground than aboveground investments (H3) as a mechanism for an efficient N-uptake. We demonstrated that root N-uptake was on average 1.7 times higher than shoot N-uptake, thus confirming our hypothesis (3). Nonetheless, when comparing the ratio of root-to-shoot N-uptake for a single injection depth, this ratio decreased exponentially (Figure 6). This shows that the belowground investment, in contrast to aboveground investments, strongly decreased with soil depth. The ratio was relatively high above a depth of 5 cm but then equalized in deeper soil (ratio of 1–2). It was in good agreement with the vertical pattern of  $^{15}\text{N}$  recovery in plant biomass, with the highest  $^{15}\text{N}$ -uptake efficiency within the first 5 cm (Figure 3). We suggest that *K. pygmaea* mainly concentrates root growth and/or root support in patches, where SOM decomposition releases nutrients for plant uptake.

The underlying causes for the generally higher belowground investment (especially within the first 5 cm) might be a strong resource competition of *K. pygmaea* with other plants and microbes (Song and others 2007; Xu and others 2011; Kuzyakov and Xu 2013). In these shallow-rooted grassland ecosystems, competition pressure is intensive due to naturally occurring limitations of N and other nutrients (Xu and others 2014), increasing water stress (especially in terms of global warming; Dorji and others 2013), the lack of time to acquire resources in the short growing season (Körner 2003), and the simultaneous needs of consumers (microbes and plants) (Lipson and others 1999; Song and others 2007). In a previous study, Kuzyakov and Xu (2013) showed for alpine *Kobresia* meadows that plants outcompete microbes for N-uptake when root biomass exceeds  $4.1 \text{ kg m}^{-2}$ . The root biomass in the *K. pygmaea* grassland was 1.5 times higher in our study, indicating an advantage of plants over microbes regarding N-uptake.

We therefore conclude that a crucial strategy of *K. pygmaea* is the establishment of a dense root network to improve resource uptake and storage for future needs. High belowground resource storage and N-uptake by *K. pygmaea* become particularly important when physical stress via grazing, dry spells, or short-term nutrient limitation by leaching increase. The high belowground investment prior to stress events potentially promotes rapid regrowth following the disturbance. This, in turn, provides *K. pygmaea* with a competitive advantage over other plants species and helps explain its very broad distribution and dominance on the TP.

## Implications for a Vulnerable Grassland Ecosystem

The high belowground investment is in good agreement with a high root-to-shoot ratio, which therefore can be considered as a long-term indicator for high belowground investment in *Kobresia* grasslands. The R/S ratio was distinctly higher than that of non-alpine grasslands (R/S: 0.7–4.5, (Jackson and others 1996; Mokany and others 2006) and also than that reported in most other previous studies conducted on the TP (R/S: 6–11, Wang and others 2008; Yang and others 2009; Wang and others 2010). Most studies, however, were conducted on alpine grasslands with a variable degree of degradation; the value found in this study is comparable to the mean of non-degraded *Kobresia* grasslands (R/S: 20.3, Li and others 2008). The high R/S ratios in alpine *Kobresia* grasslands were

reported to be mainly driven by environmental conditions such as a low annual mean temperature, low N-availability, and grazing pressure (Davidson 1969; Wilson 1988; Gao and others 2007; Li and others 2008). We suggest that *K. pygmaea* optimizes C-allocation to minimize resource limitation, which presumably explains the high R/S ratios of 20.0. This further indicates a very sensitive balance of resource allocation between shoot and root biomass.

To compete in this harsh environment, plants face two challenges: firstly, available belowground resources must be captured efficiently at specific depths and times when they are available. The most efficient way to do this is to increase living root biomass, resulting in a high root density especially in the upper root-mat layers (Figure 2A). Secondly, the enormous root biomass requires high C-maintenance costs, which must be covered by comparably low amounts of photosynthetically active aboveground plant parts. The latter was proven by Ingrisch and others (2015), who showed for the same study site that 70% of the  $^{13}\text{C}$  was allocated to roots 15 days after  $\text{CO}_2$  pulse labeling. From the belowground C-allocation, about 2/3 and 1/3 was recovered in 0–5 cm and 5–15 cm, respectively. This agrees well with our results because we also found the highest belowground investment and the highest  $^{15}\text{N}$  recovery in the upper layer (0–5 cm).

Plant C-economy implies that roots will only be maintained or re-supplied with C when resources are available within the growing season (Hermans and others 2006). Consequently, the R/S ratio has to be re-adjusted (for example, by root senescence) after resource exploitation to improve the C-balance between the acquisition of below- and aboveground resources (Chapin and others 2011). Accordingly, root biomass varied seasonally with the highest peaks in the growing season (July to September) in alpine lightly grazed *Kobresia* grassland on the TP (Cao and others 2004; Wu and others 2011).

The very high R/S ratio indicates that the C-balance of *Kobresia* pastures might be critical and vulnerable to changes in climate and management. For instance, a continuing removal of aboveground biomass under high grazing pressure will reduce the partitioning of assimilates to belowground biomass which, in turn, decreases the uptake of belowground resources and might trigger grassland degradation. Increasing grazing pressure, which disturbs the sensitive balance between below- and aboveground processes, might be one important driver leading to the disappearance of *Kobresia* cover and its turf-bearing root mats.

## CONCLUSIONS

We have illustrated some mechanisms for efficient nutrient acquisition by *Kobresia pygmaea* that partly explains its dominance on the TP. *Kobresia pygmaea* has developed efficient above- and belowground trade-offs to counterbalance resource limitation by N and probably some other nutrients such as P. These trade-offs include a high investment into root biomass, especially in the topsoil, to compete for resources with other plants and microbes (Song and others 2007; Xu and others 2011; Kuzyakov and Xu 2013). This requires high belowground C-maintenance costs, which must be covered by photosynthetically active shoots. To develop shoot biomass, the required N is taken up from the entire rooting zone. In particular, an increasing specific root activity with increasing depth compensated for the decreasing living root biomass for N-uptake. We therefore assume that *K. pygmaea* can recover very rapidly following moderate grazing events and can supply the high belowground C demand. The higher R/S ratio, however, also indicates that this efficient above- belowground trade-off might be extremely vulnerable to changes in climate and management. This study therefore provides the basis for further investigations and predictions concerning the future responses of *Kobresia* grasslands to changes in pasture management.

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