



Effects of Warming and Nitrogen Addition on Plant Photosynthate Partitioning in an Alpine Meadow on the Tibetan Plateau

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Abstract

Quantifying plant carbon (C) allocation among different pools is critical for understanding and predicting how C turnover responds to global climate change in terrestrial ecosystems. A field experiment with increasing warming and nitrogen (N) was established to investigate interactive effects on plant C allocation in alpine meadows. Open-top chambers (OTCs) were used to simulate warming. In OTCs, daytime air and soil temperature at 5 cm depth increased by 2.0 and 1.6 °C, respectively, compared with ambient conditions, but soil moisture at 5 cm depth decreased by 4.95% (v/v) from 2012 to 2014. Warming reduced aboveground biomass by 38, 36, and 43% in 2012, 2013, and 2014, respectively, and increased belowground biomass by 64% and 29% in 2013 and 2014, respectively, and the root-to-shoot ratio was significantly increased. Specifically, warming increased the proportion of plant roots in the deep layers (10–20 cm). Both N addition and its combination with warming substantially enhanced belowground biomass. Pulse-labeling experiments for ¹³C revealed that warming reduced the translocation of assimilated C to shoots by 8.8% (38.7% in warming, and 47.5% in the control [CK]), and increased the allocation to root by 12.2% (55.5% in warming, and 43.3% in CK) after 28 days labeling. However, N addition increased the proportion of assimilated C allocated to shoots by 6.5% (54.0% in N addition, and 47.5% in CK), whereas warming combined with N addition reduced this proportion by 10.9%. A decline in soil water content in the surface layer may be the main cause of plants allocating more newly fixed photosynthate to roots. Therefore, plants promoted root growth to draw water from deeper soil layers (10–20 cm). We concluded that climate warming will change the allocation patterns of plant photosynthates by affecting soil water availability, whereas N addition will increase plant photosynthates aboveground in alpine meadows and thus will significantly affect C turnover under future climate change scenarios.

Keywords Allocation pattern · Alpine meadow · Nitrogen addition · Newly fixed plant photosynthate · Warming

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Introduction

Knowing carbon (C) allocation over different time scales is crucial for evaluating variations in ecosystem productivity and C balance in terrestrial ecosystems (Rice and others 2004; Luo and others 2009). Changes in the proportion of plant photosynthate to different plant organs and feedback to plant growth will notably affect plant life histories, community structure, resource acquisition, and utilization strategies (Niklas and Enquist 2002). On short time scales, the change in plant photosynthate allocation to different organs affects the relative growth rate of each organ (Lacointe 2000). On long time scales, changes in plant photosynthate allocation patterns will affect plant leaf area index, nutrient and water absorption, root C turnover, and plant growth and may thereby have profound effects on community species composition (Jackson and others 2000; Malhi and others 2004).

However, studies on the allocation mechanism of plant photosynthate are far behind those on plant photosynthesis, respiration, and leaf growth (Cannell and Dewar 1994; Grechi and others 2007). Consistent conclusions on plant photosynthate allocation patterns still have not been drawn from previous studies. This severely hinders the accurate assessment of terrestrial ecosystem productivity and C balance (Friedlingstein and others 1999; Litton and others 2007).

Plant photosynthate partitioning is influenced by several environmental factors such as temperature, soil water content, and nutrient condition (Domisch and others 2001; Sigeo and others 2007). Generally, warming can lead to an increase of evapotranspiration and other environmental factors, which inevitably changes plant photosynthate distribution patterns. At an individual level, warming is reported to reduce the accumulation of plant assimilation products, increase photosynthate to shoots, and thus reduce the root-to-shoot ratio (Andrews and others 2001). The effect of photosynthate fraction is mainly determined by plant growth (Farrar and Williams 1991; Peng and Dang 2003) and nutrient conditions (Andrews and others 2001; Vogel and others 2008). At the ecosystem level, these effects are more uncertain. Previous studies have reported that elevated temperature reduced plant biomass allocation to roots in grassland ecosystems (Mokany and others 2006; Fan and others 2009). In addition, a global synthesis found that the response of plant photosynthate to temperature was different in different regions (Litton and Giardina 2008). In temperate and tropical regions, the ratio of plant biomass distribution to roots increased with annual mean temperature, whereas in northern forests, this ratio showed the opposite trend (Litton and Giardina 2008). This may be attributable to the increase of soil N mineralization and nutrient supply in response to annual temperature increase, resulting in a decrease in the ratio of plant photosynthate to belowground organs in northern forests (Vogel and others 2008). In swamp meadows and alpine meadows on the Qinghai-Tibet Plateau, both aboveground and root biomass increased under warming treatment in two meadows, and the biomass allocation pattern changed significantly from the upper soil to the deeper soil layers in warmer plots (Li and others 2011). However, in semi-arid and arid grasslands, plant production was strongly restricted by soil water availability, and the decrease of soil water content in response to warming may have different effects on plant biomass allocation compared with other ecosystem types. To date, the effects of warming on plant biomass allocation are far from clear, especially in semi-arid and arid grasslands.

Soil nutrient availability is another important factor that influences plant photosynthate allocation. At an individual level, soil nutrient [mainly nitrogen (N)] shortages could increase the proportion of plant photosynthate allocated to roots, while the plant could distribute more photosynthate to leaves in nutrient-rich conditions. This pattern is consistent in

various vegetation (shrubs, herbs, or woody plants) and plant life (annual or perennial plants) types (Cronin and Lodge 2003; Vanninen and Makela 2005; Muller-Landau and others 2006; Grechi and others 2007). Under future global change scenarios, knowing how plant photosynthate allocation responds to atmospheric N deposition will be essential for understanding terrestrial ecosystem C cycle. However, studies on the effects of N addition on photosynthetic product allocation at different time scales are still lacking, especially in alpine ecosystems.

The Tibetan Plateau has an average altitude of more than 4000 m and covers about 2.5 million km², 35% of which is widely covered with alpine meadows (Zheng and others 1979). Alpine meadow ecosystems play important roles in both C uptake and storage as well as the maintenance and development of livestock husbandry (Kato and others 2006; Yang and others 2009). Over the past several decades, the Tibetan Plateau has experienced a pronounced warming (Yu and others 2010; Zhang and others 2013). Although atmospheric N deposition is relatively low on the Qinghai-Tibetan Plateau, ranging from 8.7 to 13.8 kg N ha⁻¹ year⁻¹ in the northeast (Lü and Tian 2007) and 10 kg N ha⁻¹ year⁻¹ in Damxung County (Zong and others 2016), the N deposition rate has shown an increasing trend in the past decades (Jia and others 2014). Alpine ecosystems are particularly susceptible to continued N deposition in the long term because of the thin soils and low biological buffering capacity (Williams and others 1996; Williams and Tonnessen 2000; Bowman and others 2006). Our previous study indicated that warming could result in a decline in soil water content, and soil water content was considered as one of the key factors regulating ecosystem processes in this semi-arid ecosystem (Zong and others 2013; Shen and others 2015). Thus, to examine the responses of plant photosynthate allocation to elevated temperature and N deposition, we conducted a manipulative experiment including warming and N addition using an *in situ* ¹³CO₂ pulse-labeling experiment over a diurnal time scale and biomass allocation over an annual time scale in an alpine meadow ecosystem. We hypothesized that (1) warming could increase the proportion of plant photosynthate to roots, as the effect of elevated temperature was regulated by soil water content decline under a warming treatment, (2) N addition alone could reduce the proportion of plant photosynthate allocated to roots as nutrient availability improved, and (3) the effects of warming and additional N can be complementary for plant photosynthate allocation.

Materials and Methods

Site Description and Experimental Design

This study was performed in an alpine meadow in the Damxung grassland station on the south-facing slope of the Nyainqentanglha Mountains, approximately 3 km north of

Damxung County, Tibet Autonomous Region (91°05' E, 30°29' N, 4333 m a.s.l.). Climate in this site is characterized as a semi-arid continental type, with a mean annual temperature of 1.3 °C and mean annual precipitation of 477 mm (Zong and others 2016). Precipitation mainly occurs in the growing season, with 85% falling from June to August. Annual potential evapotranspiration is 1725.7 mm (Shi and others 2006), and the aridity index is 3.6 (annual potential evapotranspiration divided by annual precipitation). The soil is classified as Mat-Gryic Cambisol, corresponding to Gelic Cambisol, with a depth of approximately 0.3–0.5 m. Detailed soil properties can be found in the study by Zong and others (2014). This alpine meadow is dominated by the sedges *Kobresia pygmaea* C.B. Clarke var. *pygmaea* and *Carex montis-everestii*, and the grass *Stipa capillacea* Keng, with total coverage of 30–50%. Total atmospheric inorganic N deposition at this study site is approximately 10.0 kg N ha⁻¹ y⁻¹ (Zong and others 2016).

This was a factorial experiment with two levels of warming (control and year-round warming) and two levels of N (control and 4 g N m⁻² year⁻¹). Four treatments (control (CK), N addition (N), Warming (W), and + Warming + N addition (W + N) treatments) were organized in a randomized block design with five replicates for each treatment. We followed the method of the International Tundra Experiment (ITEX) and applied ten open-top chambers (OTCs) (Henry and Molau 1997), a passive warming device to generate artificially warmed conditions for five controls (warming alone) and five N addition plots (combination treatment of warming and N addition). The OTCs, with a 100-cm-diameter top opening, 140-cm-diameter bottom, 40 cm in height, and a bottom area of 1.54 m², were made of 3-mm-thick polycarbonate plastic. This material has high solar transmittance in visible and ultraviolet wavelengths (about 90%) (De Frenne and others 2010). In N addition plots, we applied a pulse of aqueous ammonium nitrate (NH₄NO₃) at a rate of 2 g N m⁻² year⁻¹ at the beginning and peak growing season in early-June and early-August, respectively. This N addition rate was designed to approximate the projected increases in atmospheric deposition in this region by the year of 2050 (Galloway and others 2004).

We set up the warming plots in early July 2010 and synchronously monitored the warming effects on year-round air temperature, soil moisture, and temperature at 5 cm depth using a HOBO weather station on half-hour frequency (Onset Inc., Bourne, MA, USA). The buffering distance between each replicate was at least 3 m. The warming device significantly increased air temperature and soil surface (0–5 cm) temperature, but decreased soil surface (0–5 cm) moisture (Figs. S1 and S2 in Online Appendix). From June 2012 to September 2014, the OTC warming devices increased air and soil temperature by 1.6 and 1.4 °C, respectively, and reduced soil moisture by 4.7% (v/v)

(Figs. S1A–C, S2A–C in Online Appendix). Therefore, the warming devices created warmer but dryer conditions. Precipitation levels were 312.8, 332.5, and 431.9 mm in the 2012, 2013, and 2014 growing seasons (from June to September), respectively (Fig. S2D in Online Appendix).

¹³C Pulse-Labeling

The ¹³CO₂ labeling experiment was conducted at 11:00 in the morning on 14 August 2014: three replicate plots were labeled in each treatment. The ¹³CO₂ pulse was applied simultaneously (within 2–3 min) into each chamber. The chambers were 50 cm long, 50 cm wide, and 10 cm high and consisted of four timber bars covered with transparent polyethylene film with more than 90% transmittance of photosynthetically active radiation. In the center of each chamber, a 15-cm-high timber bar was inserted into the soil. To avoid gas losses, the film was buried into the soil and additionally sealed with wet soil afterwards (Hafner and others 2012).

The ¹³CO₂ gas was produced by injecting 8 mL of 4 M sulphuric acid (H₂SO₄) into a solution of distilled water containing 0.8 g sodium carbonate (Na₂¹³CO₃) enriched with ¹³C to 99 at.%. Plastic tubes containing the Na₂¹³CO₃ solution were fixed to timber bars in the center of each chamber. The chamber was then closed and H₂SO₄ was carefully injected into the Na₂¹³CO₃ solution using syringes, to ensure complete evolution of ¹³CO₂ into the chamber atmosphere. The tiny hole left by the injection was covered with scotch tape to avoid gas leakage. To guarantee a uniform distribution of ¹³CO₂, a 5-volt fan was used inside the chamber. The labeling chambers were kept closed for 5 h (Wu and others 2010).

Plant Tissue and Soil Sample Collection and Analysis

Shoot, root, and soil samples were collected on the labeling day (coded as 0 day) and the following 2, 7, 14, and 28 days after labeling. Plant aboveground parts of all species were harvested and pooled as shoot samples by clipping at the soil surface. Soil cores (5 cm in diameter) were taken to 15 cm depth. All roots and soil in the cores were carefully extracted and sieved with a 2-mm sieve. Soil samples passed through the sieve were air-dried for total C and ¹³C analysis. The sampled roots were carefully washed by wet sieving through a 0.5-mm sieve to remove attached soil and debris. Shoot and root samples were oven-dried at 65 °C for 48 h.

Annual plant aboveground biomass was estimated by a non-destructive sampling method (Lin and others 2011; Wang and others 2012). Briefly, the average height and cover of vegetation canopy were measured using a 50-cm × 50-cm quadrat divided into twenty-five 5 cm × 5 cm sub-squares in each plot on August 15 in each year from 2012 to 2014. In 2012, we also carried out

this process in a nearby alpine meadow by measuring the mean height and cover of the vegetation canopy, harvesting, oven-drying, and weighing plant materials. The equation that was used to simulate the relationship between aboveground biomass (AGB) and vegetation height (H) and cover (C) was $AGB = 0.269 + 3.466 C + 0.752 H$ ($R^2 = 0.658$, $P < 0.001$, $N = 80$). Plant community investigation was conducted every year and we modified the equation every year according to the investigation. We also used a soil drill sampler (5 cm in diameter) to take 0–10-cm and 10–20-cm soil samples in mid-August and these root samples were immediately washed, separated, oven-dried at 65 °C for 48 h, and weighed.

Plant tissues and soil samples were ground to a homogeneously fine powder manually using a MM 200 steel ball mill (Retsch GmbH, Haan, Germany), packed in tin cups, and combusted in an elemental analyzer. Carbon content and $^{13}\text{C}/^{12}\text{C}$ ratio were measured with a MAT 253 stable isotope ratio mass spectrometer system (Mat 253, Finnigan MAT, Bremen, Germany).

Calculations and Data Analysis

The natural abundance of ^{13}C in samples was expressed as $\delta^{13}\text{C}$ ‰ units relative to Pee Dee Belemnite. To facilitate comparisons with other studies, we also calculated the enrichment values as ^{13}C at.% excess, the increase in ^{13}C atoms due to pulse-labeling expressed as the percentage of total C atoms in the sample using the following equations (Wu and others 2010; Hafner and others 2012):

$$R_{\text{sample}} = \left(\frac{\delta^{13}\text{C}}{1000} + 1 \right) \times 0.011237,$$

$$^{13}\text{C} \text{ atom\%} = \left(\frac{R_{\text{sample}}}{R_{\text{sample}} + 1} \right) \times 100,$$

^{13}C at.% excess = ^{13}C at.% of samples – ^{13}C at.% of natural abundance, where R_{sample} is the isotope ratio of sample $^{13}\text{C}/^{12}\text{C}$, and 0.011237 is the ratio of $^{13}\text{C}/^{12}\text{C}$ in Pee Dee Belemnite. ^{13}C at.% represents the percent of ^{13}C atoms in total C atoms.

To estimate the amount of ^{13}C incorporated into various plant and soil pools, the following equation was used (Hafner and others 2012; Wu and others 2010):

^{13}C amount (mg m^{-2}) = ^{13}C at.% excess \times C pool (g m^{-2}) \times 10, where C pool size is the C content in shoots, roots, and soil, and was assumed to be constant during the whole tracing period. Atmospheric background was corrected using ^{13}C at.% excess instead of $\delta^{13}\text{C}$ as in the above equation.

Statistical Analysis

Statistical analysis was performed using the SPSS 16.0 software package (SPSS, Chicago, IL, USA). A two-factor analysis of variance (ANOVA) followed by Duncan's multiple comparisons was used to detect the effects of warming and N addition on aboveground and belowground plant biomass as well as the proportion of deep-layer parts to total root biomass. Linear regression was used to analyze the relationship between the change in $\delta^{13}\text{C}$ in plant root and the differences in soil temperature and water content under warming and ambient conditions. Statistical significance was $P < 0.05$. All the figures were produced using Origin Pro 8.0 (OriginLab Corporation, Northampton, MA, USA).

Results

Allocation Patterns of Plant Production between Aboveground and Belowground

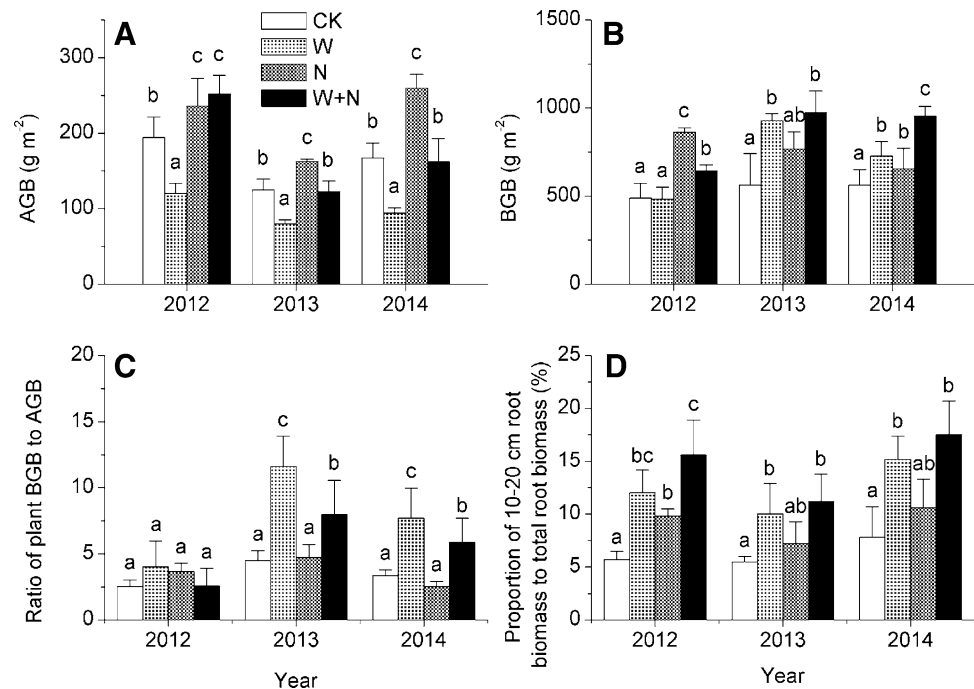
Compared with the control, warming significantly reduced aboveground biomass by 38, 36, and 43%, whereas N addition significantly increased aboveground biomass 22, 30, and 55% from 2012 to 2014, respectively (Fig. 1A). Aboveground biomass was increased by the coupling treatment with N addition and warming in 2012, but not significantly affected in the following 2 years (Fig. 1A). Belowground biomass was not affected by warming in 2012, but significantly increased with treatment time (Fig. 1B). Both N addition and its interaction with warming substantially enhanced belowground biomass (Fig. 1B).

The belowground/aboveground biomass ratio increased in warming treatments including warming alone and its interaction with N addition from 2013 on (Fig. 1C). Further analysis indicated a distinct increase in deeper layer root biomass ratio at the depth of 10–20 cm compared with the total profile in warming and its interaction with N addition treatments (Fig. 1D). The addition of N alone only increased the ratio of deeper layer root biomass (10–20 cm) in 2012, but had no significant effects on root biomass distribution (Fig. 1D).

Dynamics of Plant Assimilate Partitioning in Different Pools

The $\delta^{13}\text{C}$ values in shoot samples under warming were markedly enriched after labeling (314‰) compared with unlabeled controls (–27‰). In the following 2 days, these values decreased by 54.8% and stayed between 50 and 100‰ (Fig. 2A). The addition of N tended to decrease the proportion of newly fixed C allocation to shoots, whereas warming tended to slightly increase $\delta^{13}\text{C}$ in shoots (Fig. 2A).

Fig. 1 Effects of warming and N addition on plant above-ground (AGB, **A**), belowground biomass (BGB, **B**), the ratio of BGB to AGB (**C**), and the proportion of deep-layer (10–20 cm) to total root biomass (**D**). Different lowercase letters in the same year represent significant differences among treatments. Mean \pm SE are shown in the figure ($N=4$). Abbreviations of CK, N, W, and W+N represent the control, N addition, warming, and combination of N addition with warming, respectively, and below is the same



¹³C pulse-labeling also increased the values of $\delta^{13}\text{C}$ in plant roots and these values exhibited a gradual increase in labeling. Warming delayed the time of newly fixed C allocation to plant roots (Fig. 2B). The maximum $\delta^{13}\text{C}$ value in roots under N addition occurred in 7 days after labeling, whereas under warming and W+N treatments the peak of $\delta^{13}\text{C}$ was postponed to 14 days. At the end of the labeling period, the $\delta^{13}\text{C}$ values in plant roots under control conditions were higher than those under other treatments 28 days after labeling (Fig. 2B).

¹³C Allocation within the Plant–Soil System during the Labeling Period

¹³C contents in plant–soil system were obtained by calculating $\delta^{13}\text{C}$ allocation to each pool. The proportion of plant newly fixed photosynthate allocated to leaves decreased gradually, but increased in allocation to plant roots. The amount of ¹³C allocated to soil increased at the beginning and then declined (Fig. 3).

In the control, the proportion of plant photosynthate allocated to plant leaves decreased from 87.1 to 47.5% during the tracing period, and those in warming and W+N treatments were significantly lower than the control (Fig. 3A). The proportion of plant photosynthate to roots increased gradually from 11.0 to 49.4% at the beginning of labeling, with significantly higher values in warming and W+N treatments than in the control, and N addition alone (Fig. 3B). The distribution ratio of photosynthate to soil increased at the beginning and then decreased, but the proportion under warming was the highest in the tracing

period (mean value was 4.0%). The proportions for the control, N addition, W+N treatments were 3.0, 2.8, and 2.8%, respectively (Fig. 3C).

The dynamic curve of $\delta^{13}\text{C}$ in plant tissues showed a sharp decreasing trend in the first 48 h of labeling and then tended to be stable (Fig. 3). Therefore, the period of 28 days after labeling was enough to reach a state of equilibrium of plant photosynthetic C distribution in this experiment. During the tracing period, 37.2% of the newly fixed C was allocated to plant shoots, 38.6% to plant roots, and only 2.4% to the soil C pool (Table 1). Compared with the control, the W+N treatment significantly reduced newly fixed C loss to only 9.3%. Treatments of warming and W+N significantly increased newly fixed C to roots, whereas N addition alone allocated less C to roots (Table 1).

Factors Regulating Plant Assimilate and Production Allocation

From analysis of the relationship between $\delta^{13}\text{C}$ and environmental factors, the change of $\delta^{13}\text{C}$ was not correlated with soil temperature (Fig. 4A), but significantly decreased with soil water content in warming and ambient condition (Fig. 4B). A greater difference of soil moisture inside and outside the warming device led to more plant photosynthetic C being allocated to the root system, indicating that the decrease in soil water was the main reason for more C allocation to plant roots.

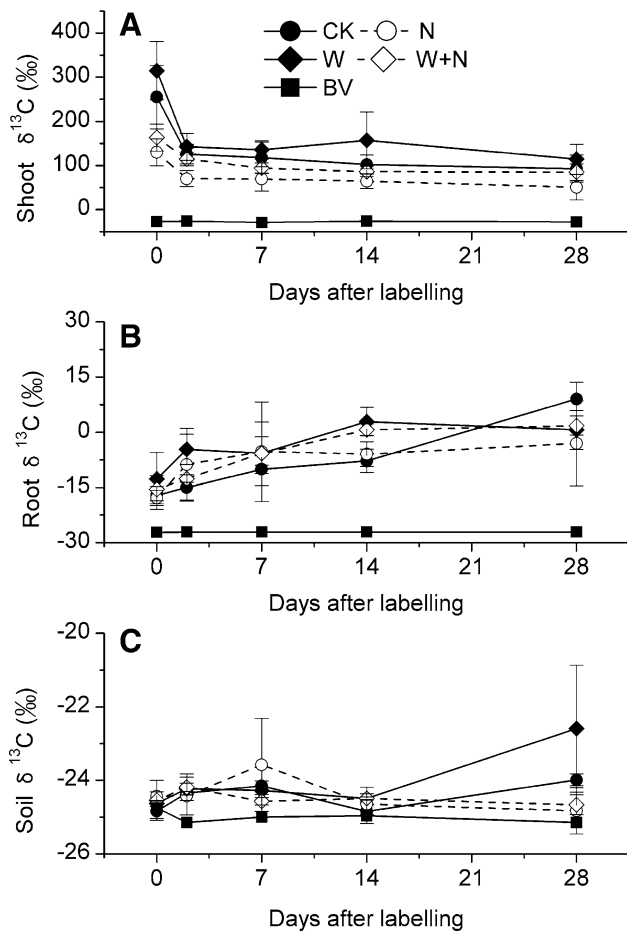


Fig. 2 Temporal variations of C isotope composition soil C during the 28-day trace period. Mean values ($N=3$) and standard deviations are given. BV represents the background values of $\delta^{13}\text{C}$ in soil. Abbreviations of treatments can be seen from this figure

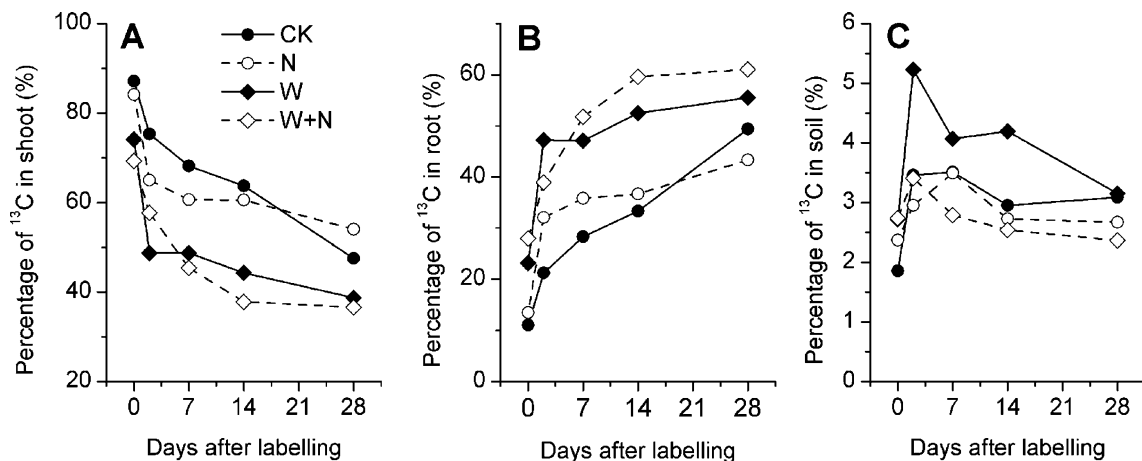


Fig. 3 Temporal changes of ^{13}C distribution among different treatments after labeling in plant shoot (A), root (B), and soil (C) C pool (%). Abbreviations of treatments are the same with Fig. 2

Discussion

The OTCs in our study site created a warmer but drier microclimate. Warming significantly reduced aboveground biomass, but increased belowground biomass. This was consistent with our first hypothesis that the effects of warming were regulated by the decline of soil water content. The addition of N significantly increased aboveground biomass, but did not decrease belowground biomass, which was not consistent with our second hypothesis. The third hypothesis was partly supported by the results, as the complementary effects of warming and N addition were only verified in aboveground biomass. Over short time scales, warming reduced the allocation of plant newly fixed carbohydrates to shoots, but increased the translocation of assimilated C to roots. So the allocation patterns of plant biomass under warming and N addition were verified at diurnal time scales. This result indicated that the greater the difference in soil moisture between the inside and outside of the warming device, the more plant photosynthetic C would be allocated to the root system, suggesting that the decrease of soil water was the main reason for more C allocation to plant roots.

Allocation Patterns of Plant Newly Fixed C in Alpine Meadow

Generally, plants have three ways to allocate newly synthesized C, including assimilation into plant shoots, release into the atmosphere as CO_2 , and transfer belowground as plant roots and soil organic matter (Wu and others 2010). The plant shoot $\delta^{13}\text{C}$ value tended to be stable after 48 h, which confirmed that a part of the new photosynthesis C was stored in plant shoots and 28 days after labeling was enough to reach a state of equilibrium of plant photosynthetic C distribution in this experiment. The $\delta^{13}\text{C}$ value in plant shoots

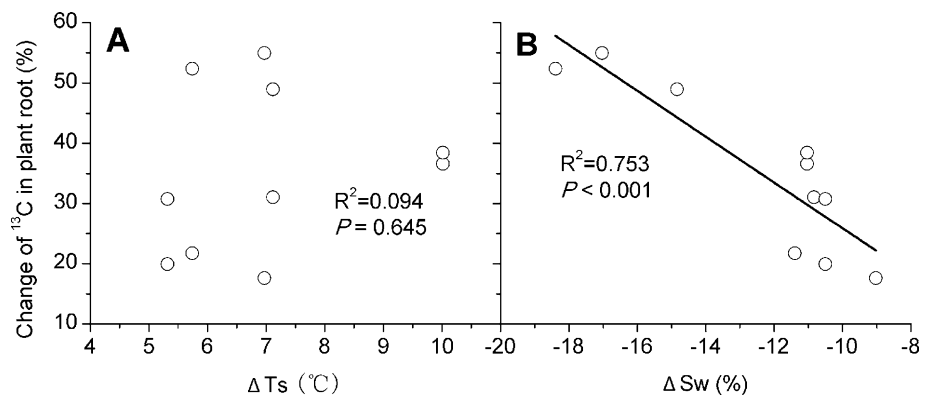
Table 1 Allocation of newly fixed C to plant shoots, roots, and soil pool in the plant–soil system after labeling for 28 days

Destination of C	CK		N		W		W+N	
	Labeled C ^a	Partitioning rate (%)	Labeled C	Partitioning rate (%)	Labeled C	Partitioning rate (%)	Labeled C	Partitioning rate (%)
Total amount	245.8	100	220.2	100	192.5	100	197.0	100
Total loss ^b	53.5	21.8	48.7	22.1	40.1	20.8	18.3	9.3
Shoots	91.4	37.2	92.6	42.0	59.0	30.7	65.4	33.2
Roots	94.9	38.6	74.3	33.8	84.6	43.9	109.1	55.4
Soil	5.9	2.4	4.6	2.1	8.7	4.5	4.3	2.2

^aThe unit of labeled C was mg m⁻². Abbreviations of treatments can be seen in Fig. 2

^bCalculated as the difference between total assimilated C and total C remaining in the system on the 28th day

Fig. 4 Dependence of change of $\delta^{13}\text{C}$ in plant root on the differences of soil temperature (A) and water content (B) between warming treatment and ambient condition



decreased rapidly by 54.8% within 48 h after labeling in the control. Ostle and others (2000) showed that $\delta^{13}\text{C}$ of plant tissue decreased by 77.4% in 48 h in a field experiment in hilly grassland, whereas over the 28-day tracing period of this study, 63.6% of the photosynthetic C was exported from the shoots in the control treatment, in the range of 30–90% in grasslands (Baxter and Farrar 1999; Dilkes and others 2004; Wu and others 2010). In the first 48 h, the plant assimilate output from plant shoots accounted for 50.7% of total output during the whole tracing period (Fig. 2), indicating that the transport of the newly fixed photosynthetic C was very rapid, and mostly completed within 1 or 2 days.

Effects of Warming on Allocations of Plant Assimilate C

Compared with the control, warming significantly reduced aboveground biomass, but increased belowground biomass, which was consistent with the first hypothesis. This was similar to other studies. A transect study in temperate grasslands reported that the proportion of belowground biomass increased as temperature decreased (Fan and others 2009), whereas in swamp meadow and alpine meadow on the Qinghai-Tibet Plateau, both plant aboveground and root

biomass increased under warming treatment in these two meadows (Li and others 2011). These different responses may be attributed to ecosystem types and soil water availability, as soil moisture is a key limiting factor in grassland ecosystems, especially in semi-arid and arid types (Niu and others 2008). However, in semi-arid and arid grasslands, plant production is strongly restricted by soil water availability and warming effects on plant biomass allocation may be different in other ecosystem types. The decrease of soil water content under warming treatments could lead plant production to be more severely limited by soil water availability, and plants may allocate more biomass to roots to obtain enough water to maintain growth. Previous studies also found that warming could enhance soil nutrient availability (Rustad and others 2001; Turner and Henry 2010), and plants generally allocated less biomass to belowground in fertile soil (Reynold and Thornley 1982). However, the change of biomass allocation patterns in this study was not caused by an increase in soil nutrient availability, because our previous study found that warming did not significantly change soil N content (Zong and others 2013). Thus, this change should be mainly attributed to the decline of soil water content. This was also verified by the short time of photosynthetic product allocation.

In the control, the proportion of photosynthate distributed to plant aboveground parts decreased from 87.1 to 47.5% during the whole tracing time, significantly higher than that in the warming treatment (Fig. 3), indicating that warming was not conducive to C distribution to plant shoots. This result was contrary to other studies (Farrar and Williams 1991; Andrews and others 2001), because the effects were confounded by the decline of soil water content under warming treatments. Studies have shown that the effect of warming on ecosystem processes was regulated by soil moisture (Niu and others 2008; Hutchison and Henry 2010). Hutchison and Henry (2010) reported that the effect of warming on plant production was closely related to inter-annual variability of rainfall. Warming in arid years had no effect on plant production, whereas in wet years both winter warming and annual warming and N addition doubled plant aboveground productivity (Hutchison and Henry 2010). When plant growth is restricted by water availability, plants always allocate more photosynthate to roots to promote water absorption (McConnaughay and Coleman 1999; Ngugi and others 2003). In addition, we found that $\delta^{13}\text{C}$ in plant roots gradually increased from 11.0 to 49.4% in the control, whereas the $\delta^{13}\text{C}$ value in roots under warming treatment was significantly higher than that in the control and N addition treatments (Fig. 3B). Warming, especially under N addition, facilitated more photosynthetic C allocation to plant roots (Table 1). This result corresponded to the allocation pattern of plant biomass at an annual time scale. In the semi-arid alpine meadow, soil moisture is an important factor limiting plant production, and warming will lead to a decrease in the soil water content in the surface soil. To absorb enough soil water to maintain plant production and growth, plants allocated more C to deep soil layers. Thus, we concluded that under warming treatments, the effects of soil water content decline on plant photosynthate allocation were more significant than temperature increases.

Effects of N Addition on Plant Assimilate C Allocation

The addition of N significantly increased aboveground biomass, which was consistent with the second hypothesis and verified by the ^{13}C labeling experiment (Fig. 3B). Previous studies have shown that plants generally distributed more photosynthate to leaves in nutrient-rich conditions (Reynold and Thornley 1982), whereas soil nutrient (mainly N) shortages could increase the proportion of plant photosynthate to roots in different vegetation types (shrubs, herbs, or woody plants) (Cronin and Lodge 2003; Vanninen and Makela 2005; Grechi and others 2007). Generally, N shortages can reduce cytokinin transportation from plant roots to shoots and slow down the cell division rate, and result in the decrease of sucrose delivery from phloem to stem (Ping

and others 2010). This delivery block causes the accumulation of sucrose around the phloem and an increase in leaf pressure. Cell division in plant roots continues, while cell swelling does not change, which results in a swelling gradient between the source (leaf) and the sink (root) and the allocation of relatively more photosynthetic products to roots (Ping and others 2010).

In addition to the direct effect, we also found that N addition and warming have complementary effects on plant biomass allocation at different time scales, especially for aboveground biomass. At a short-term scale, the ^{13}C labeling experiment showed that N addition could promote the effects of warming on the allocation of plant newly fixed photosynthate to roots (Fig. 3B). At inter-annual time scales, plants allocated more biomass to belowground under the combination treatment of N addition with warming than warming treatment alone (Fig. 1). Further analysis showed that warming significantly decreased soil inorganic N after the winter warming period (Zong 2015), and this indicated that the winter warming process would lead to soil N loss, consistent with other studies (Hutchison and Henry 2010; Turner and Henry 2010). Increased N mineralization in cold seasons at a time when plant roots are largely inactive, coupled with a frequency increase of soil freeze–thaw cycles, may increase soil N losses by leaching or gas emission (Hobbie and Chapin 1996; Matzner and Borken 2008; Turner and Henry 2010). The addition of N compensated for soil N loss due to warming, so N addition and warming have complementary effects on plant biomass allocation.

Conclusions

In this study, the investigation of plant carbon allocation at different time scales indicated that warming significantly changed the allocation patterns of newly fixed assimilates between aboveground and belowground. Warming reduced the allocation of carbohydrates to shoots, but increased the translocation of assimilated C to plant roots, and specifically increased the proportion of plant roots in the deep layers to draw water from the deeper soil. However, warming under N addition can increase the proportion of assimilated C allocated to stems and leaves. A decline in the soil water content at the surface might be the main cause for more plant newly fixed photosynthate allocation to roots. Climate warming in future global change scenarios will lead plants to allocate more biomass to belowground and thus affect C turnover and storage in these alpine meadows; however, this pattern also depends on atmospheric N deposition and the seasonality and amounts of precipitation.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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