ORIGINAL ARTICLE

Effects of an actinorhizal shrub on the nitrogen status of the soil and neighboring plants in an alpine meadow of the Tibetan Plateau

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Abstract

With ongoing climate change and increasing human activities, shrub expansion has been observed worldwide. Alpine meadows are one of the ecosystem types that are sensitive to shrub expansion. This study aimed to investigate the effects of an actinorhizal shrub species on nitrogen (N) status of the soil and other plants in the alpine meadow ecosystem. We measured the ^{15}N natural abundance and N concentration of different tissues of *Hippophae tibetana*, and examined its effects on the ^{15}N natural abundance and N content of the soil and neighboring plants at different locations surrounding the shrub clumps in an alpine meadow in the Qinghai-Tibet Plateau. The results show that the δ^{15}N in branches and roots of *H. tibetana* was much lower, and N concentration much higher, than that of other plants. δ^{15}N of the plants and soil surrounding the shrub clumps was not significantly altered. Underneath the shrub canopy, due to the N-rich leaf litter of *H. tibetana*, N content of neighboring plants significantly increased compared to plants at other locations. However, the aboveground biomass of neighboring plants was marginally lower underneath the canopy than outside. These results suggest a minor N-facilitation effect of this actinorhizal shrub on the soil and neighboring plants through its N-rich leaf litter. However, the facilitation effect was not enough to offset the negative effect of the shrub on its neighboring plants due to competition of other resources.

Keywords Biotic nitrogen fixation · *Hippophae tibetana* · Natural nitrogen isotope abundance · Nitrogen facilitation · Shrub encroachment · Tibetan Plateau

Introduction

Shrub expansion is a potential issue for herbaceous ecosystems in recent decades (Naito and Cairns 2011; García Criado et al. 2020). Many abiotic and biotic factors, such as climate warming, fire disturbance, and cattle grazing, can cause shrub expansion (Van Auken 2009). Increased shrub abundance in grasslands can greatly alter ecosystem properties and functions (Eldridge et al. 2011). Due to higher photosynthetic rates and larger perennial tissues, the encroachment of shrubs can increase the productivity and standing biomass of the ecosystem (Knapp et al. 2008; Wang et al. 2016). In addition, shrub encroachment can increase soil organic carbon, because of higher litter inputs and lower litter decomposability (Aguirre et al. 2021; Liu et al. 2021). Also, these factors can lead to increase in soil nutrient availability and total N content (Ward et al. 2018; Li et al. 2019). Among the expanding shrubs, some are legumes or actinorhizal plants that form symbioses with N-fixing bacteria (Zhang et al. 2018), and they can directly take advantage of atmospheric N. The N-fixing

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capability of these shrubs can help them expand more easily in N-limited ecosystems and potentially alter ecological processes of the ecosystems.

N-fixing plants often can improve the N status of their neighboring plants (Thilakarathna et al. 2016; Zhang et al. 2018; Tang et al. 2019). This facilitation effect can be achieved by multiple pathways. First, N-fixing plants usually produce litter with high N content, which can increase soil N availability and, thus, promote N uptake by non-N-fixing plants (Roggy et al. 2004; Pillay et al. 2021). Second, most land plants live in a symbiotic association with mycorrhizal fungi and form common mycorrhizal networks belowground, through which plants can exchange materials, including signals and nutrients (Simard et al. 2012). Therefore, if a N-fixing plant is colonized by mycorrhizal fungi, it can transfer fixed N to other non-N-fixing plants in the same mycorrhizal network (Thilakarathna et al. 2016; Zhang et al. 2020). Third, N-fixing plants can secrete N-rich root exudates, which can be directly absorbed by other plants or absorbed after mineralization (Jalonen et al. 2009; Lesuffleur et al. 2013). Fourth, N-fixing plants probably uptake the soil N more slowly than non-N-fixing plants, so that the N-fixing plants have a smaller effect on depleting the communal soil N pool. Thus, the encroachment of N-fixing shrubs can also improve N status of the original herbaceous plants, despite that the shrubs may compete with them for other resources such as water and light.

In the Qinghai-Tibet Plateau where the ecosystems are in general N-limited, shrubs are also expanding, which is thought to be mainly caused by warming and changes in precipitation (Brandt et al. 2013; Gao et al. 2016). *Hippophae* (sea buckthorn) is a genus of actinorhizal shrubs that is widely distributed in the Qinghai-Tibet Plateau, and has also been observed to be expanding on the plateau (Liu et al. 2021; Wang et al. 2021). *Hippophae* individuals can produce large amounts of leaf litter with high N content (Li et al. 2012), and they can be colonized by arbuscular mycorrhizal fungi (Gardner et al. 1984), implying that they can have N-facilitation effects on their neighboring plants and increase soil N stock. Some studies have found that actinorhizal plants can increase N contents of the soil and neighboring plants (Kohls et al. 2003; Tang et al. 2019). However, N-facilitation effect of *Hippophae* in the Qinghai-Tibet Plateau is still uncertain (Wang et al. 2021).

In this study, we investigated the N-facilitation effect of *Hippophae tibetana* Schlecht. in an alpine meadow in the Qinghai-Tibet Plateau by measuring ^{15}N natural abundance of *H. tibetana* individuals, the soil and neighboring plants at different distances from *H. tibetana* clumps. We hypothesized that (1) *H. tibetana* would fix atmospheric N and have a higher N concentration than other plants, (2) N content of the soil and the neighboring plants would increase relative to those without *H. tibetana*, and (3) N-facilitation effect of

H. tibetana would decrease with increasing distance from the *H. tibetana* clump.

Materials and methods

Site description

Plant and soil samples were collected from Gannan Grassland Ecosystem Field Science Observation and Research Station of the Ministry of Education (101°53′ E, 33°58′ N), Gansu Province, China. The site is on the eastern edge of the Qinghai-Tibet Plateau, with an average elevation of 3550 m, mean annual air temperature of 1.8 ℃, and mean annual precipitation of 593 mm (1981–2010, National Meteorological Information Center). The soil is typical meadow soil and classified as Cambisol in FAO/UNESCO taxonomy. The vegetation of the site is typical alpine meadow, dominated by sedge *Kobresia capillifolia* (Decne.) C. B. Clarke and grasses *Elymus nutans* Griseb. and *Stipa aliena* Keng., with abundant forb species such as *Anemone rivularis* Buch.-Ham., *Aster diplostephioides* (DC.) C. B. Clarke and *Saussurea nigrescens* Maxim. Legumes are of low abundance at the site, accounting for less than 4% of the community biomass (Wang et al. 2020). Patches of *H. tibetana* are common in the vicinity of the research station, and usually occur on hill slopes.

Sample collection and measurements

On 4th and 5th August 2018, above- and belowground samples of *H. tibetana* were collected on a gentle slope near the research station. Leaf and root samples of other plants and soil samples at different positions around the *H. tibetana* clumps were also collected. Five *H. tibetana* clumps were selected, with a distance of at least 20 m between each plant. The shrub clumps had diameters of 0.8–2 m, and they had similar heights of about 0.5 m. At the center (right underneath the canopy), edge (30 cm away from the outmost branch) and outside (1 m away from the outmost branch) of each clump, a 25 cm × 25 cm quadrat was selected, with the 3 quadrats of a clump on a line and with the same elevation, and in total there were 15 sampling positions. Aboveground parts of *H. tibetana* were firstly harvested in the center quadrat and separated into branches and leaves. Afterwards, leaves of other plants in each quadrat were cut at the soil surface and separated into three life forms (graminoid, forb, and legume). A soil core (5 cm diameter and 30 cm depth) was taken at the center of each quadrat. Soil and root samples were separated by sieving the soil cores through 1 mm mesh, and then root samples were washed in tap water. In a few cores coarse roots of *H. tibetana*, which were of large size and woody texture, were also found, and they were separated

from the root samples. In a pilot excavation of *H. tibetana* roots, we found that its roots were deeply distributed at the site, with few fine roots above the depth of 60 cm. Therefore, roots of *H. tibetana* were collected by excavating the bulk soil near the shoot base of a *H. tibetana* individual of each clump. In addition, in September 2020, fully and freshly senesced leaf litter of the *H. tibetana* individuals was collected from the surface of the litter layer to measure the $\delta^{15}N$ and N concentrations.

On the same slope and more than 50 m away from the *H. tibetana* patches, four quadrats with a distance of about 3 m between each other were selected and harvested for aboveground and belowground samples as above. These samples were taken as reference samples to represent N isotopic composition and N concentration of plants and soil without the influence of *H. tibetana*.

All the plant samples were dried at 60 ℃ for 72 h and weighed, and soil samples were stored at −20 ℃ and air dried at 25 ℃ for further analyses. For the analysis of N isotopic composition, plant and soil samples were finely ground with a stainless-steel ball mill (Retsch MM200, Germany). Ground plant samples were sieved through 0.2 mm mesh. Root debris in ground soil samples were firstly removed by stirring with a glass rod rubbed with silk cloth, and then the soil samples were sieved through 0.2 mm mesh. About 2 mg and 5 mg of plant and soil subsamples, respectively, were used to analyze their N concentrations and N isotopic composition with an elemental analyzer-isotope ratio spectrometer (Delta V Advantage, Thermo Fischer Scientific, US).

Calculations

Sample $\delta^{15}N$ values were calculated as

$$\delta^{15}N_{sample} = \left[\frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}N)_{standard}} - 1 \right] \times 1000‰$$

where $(^{15}N/^{14}N)_{sample}$ and $(^{15}N/^{14}N)_{standard}$ are the N isotopic composition of the collected sample and the standard material, which usually is atmospheric N_2. $\delta^{15}N$ for leaves were calculated for different plant life forms separately as well as for the community (excluding *H. tibetana*). Values for roots were only calculated for the community, as separating roots of different plant life forms was impossible.

Statistical analyses

We used one-way ANOVAs to analyze the differences in $\delta^{15}N$, N concentration and biomass of plant and soil samples between positions. The reference plant and soil samples were considered as another level of position in the analyses. We used two-way ANOVAs to analyze the differences in $\delta^{15}N$ and N concentration of plant samples between plant life-forms (graminoid, non-leguminous forb, legume) and positions (center, edge, outside), with plant life-form, position and their interaction included. Tukey-HSD tests were used for post hoc multiple comparisons when an effect was significant ($P < 0.05$).

Results

The leaves of *H. tibetana* had a positive $\delta^{15}N$ (0.65 ± 0.12), whereas $\delta^{15}N$ values of the branches and roots of *H. tibetana* were negative (Table 1). N concentration of *H. tibetana* leaves was higher than that of the branches and roots, which had similar $\delta^{15}N$ and N concentration (Table 1). The $\delta^{15}N$ (0.47) and N concentration of the leaf litter (34.75 mg g^{-1}) of *H. tibetana* leaf litter was similar to those of green leaves (Table 1).

$\delta^{15}N$ and N concentration of the soil and roots did not significantly differ among positions (Fig. 1; Table 2). For the total aboveground of plant communities surrounding the *H. tibetana* clumps, $\delta^{15}N$ did not significantly differ among positions, while N concentration was significantly higher at the clump center than the edge, outside, and the reference community (Fig. 1; Table 2).

The aboveground biomass of neighboring plant communities marginally differed among locations (Fig. 2a; Table 2), with biomass at the center marginally lower than that outside the clumps (Fig. 2a; $P = 0.066$). Root biomass did not differ significantly among positions (Fig. 2b; Table 2).

When aboveground parts of the three life forms surrounding *H. tibetana* clumps separated, $\delta^{15}N$ and N concentration significantly differed among life forms (Fig. 3a; Table 3). Non-leguminous forbs had a higher $\delta^{15}N$ than graminoids and legumes, and legumes had higher N concentration than non-leguminous forbs and graminoids (Fig. 3). Among positions, $\delta^{15}N$ of plant aboveground parts did not differ (Fig. 3a; Table 3), whereas N concentration differed marginally (Table 3), which was mainly due to higher N concentration

Table 1 $\delta^{15}N$ and N concentration of *H. tibetana* leaves, branches, roots and leaf litter

	$\delta^{15}N$ (‰)	N concentration (mg g^{-1})
Leaf	0.65 ± 0.12	39.37 ± 1.11
Branch	-1.60 ± 0.09	19.40 ± 0.65
Aboveground (leaf + branch)	-0.84 ± 0.14	26.75 ± 1.51
Root	-1.43 ± 0.17	19.15 ± 0.38
Leaf litter	0.47 ± 0.17	34.75 ± 2.00

Values represent mean ± 1 standard error ($n = 5$). The samples of leaf litter were collected in 2020. Other samples were collected in 2018
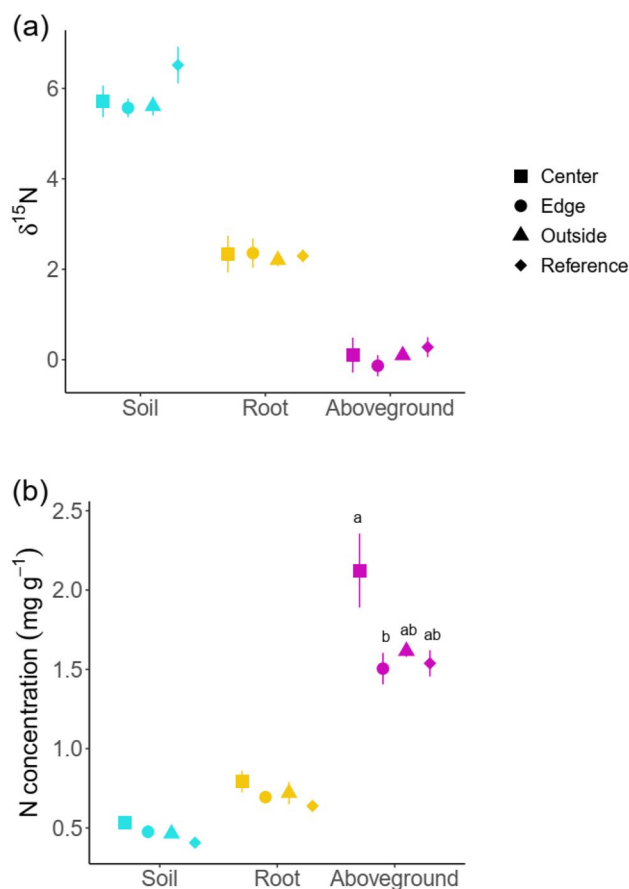
Fig. 1 $\delta^{15}N$ (**a**) and N concentration (**b**) of the 0–30 cm soil, roots and aboveground samples at the center, edge and outside of *H. tibetana* clumps and at the reference communities. Error bars denote ± standard error ($n = 5$ for shrub-clump soils and $n = 4$ for reference soil). Letters above the bars represent pairwise comparison results when the position effect was significant ($P < 0.05$), and groups without any common letter indicate they are significantly different

of forbs and graminoids at the clump centers than at the other two positions (Fig. 3b).

Discussion

Confirming our first hypothesis, $\delta^{15}N$ values of *H. tibetana* indicate a great capacity of N fixation in this actinorhizal shrub (Table 1). A rough estimation of the percentage of

nitrogen derived from atmosphere (N_{dfa}) was 57% and 78% for its aboveground parts and roots, respectively (Supplementary Appendix 1), indicating its strong dependence on N-fixation. However, the $\delta^{15}N$ of *H. tibetana* leaves was positive and similar to that of non-N-fixing plants, suggesting strong N fractionation during N transport to the leaves. It has been found that actinorhizal plants have lower $\delta^{15}N$ in roots and branches than in leaves, which is contrasted to leguminous plants (Tjepkema et al. 2000; Chaia and Myrold 2010; Gentili and Huss-Danell 2019). The reasons are still not very clear. One possible reason is that N from soil, which is of high $\delta^{15}N$, is first transported to leaves of actinorhizal plants, elevating leaf $\delta^{15}N$; during leaf senescence, the N is translocated to branches (resorption) with N fractionation, and the N in branches becomes relatively ^{15}N depleted (Boddey et al. 2000). However, in our study the green and senesced leaves had similar $\delta^{15}N$ signatures (Table 1), suggesting little fractionation occurred during resorption, and N in branches might be mainly from N fixation.

Many actinorhizal plants can alter the isotopic signature of soil N (Kohls et al. 2003; Andrews et al. 2011; Freund et al. 2018). In our study, the soils surrounding *H. tibetana* clumps showed a trend of decrease in $\delta^{15}N$ signature compared to the reference soil (Fig. 1a), and soil N content showed a trend of decrease with increasing distance from the *H. tibetana* clump (Fig. 1b), although the trends were not significant. Therefore, the results only weakly support our second hypothesis, and suggest that in the alpine meadow *H. tibetana* has limited ability to alter the isotopic signature and concentration of soil N.

Despite the trend of decrease in soil $\delta^{15}N$ surrounding the *H. tibetana* clumps, the $\delta^{15}N$ of the neighboring plants did not significantly change, either aboveground parts or roots (Fig. 1). The reason might be that the $\delta^{15}N$ of soil organic N does not necessarily represent the true isotopic signature of the N assimilated by plants, as fractionation against ^{15}N occurs with N mineralization and mycorrhizal absorption (Kramer et al. 2003; Schweiger 2016). Root $\delta^{15}N$ of the neighboring plants was not different from the reference either, implying that N transfer via mycorrhizal network and root exudation was minor. Tian et al. (2019) found that root distribution of *Hippophae* species gets deeper as they age. In our study, *H. tibetana* individuals had most of their fine roots at least deeper than 60 cm, at which depth few roots of other

Table 2 Summary of one-way ANOVAs for position effects (center, edge, outside, and reference) on $\delta^{15}N$ and N concentration of different samples. Significant effects are marked in bold

Sample	Source	Df	$\delta^{15}N$ (‰)		N concentration		Biomass	
			F value	*P* value	*F* value	*P* value	*F* value	*P* value
Soil	Position	3	2.078	0.146	1.922	0.169	–	–
Root	Position	3	0.057	0.981	1.293	0.313	0.364	0.780
Aboveground	Position	3	0.400	0.755	4.403	**0.021**	2.959	0.066

$n = 5$ for the center, edge and outside positions and $n = 4$ for the reference
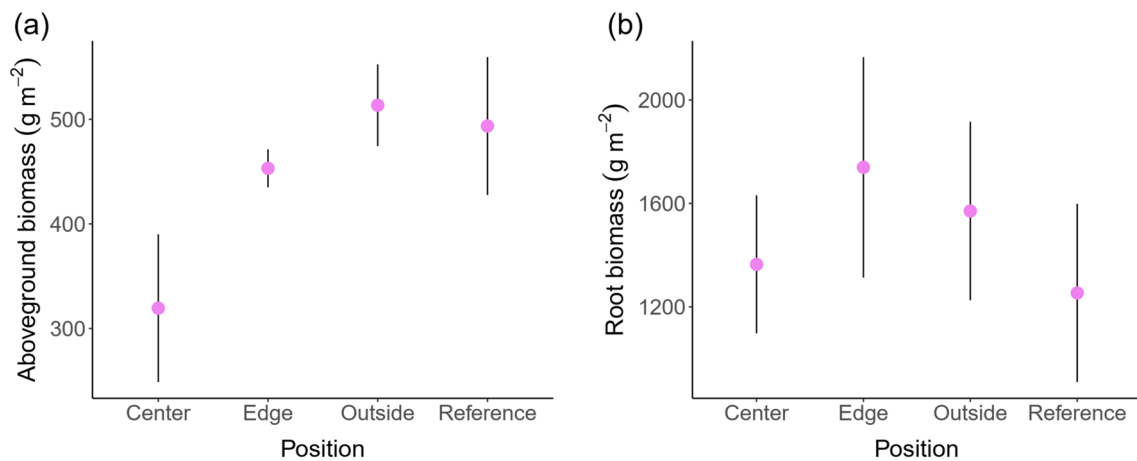
Fig. 2 Aboveground (**a**) and root biomass (**b**) of neighboring plants at different positions of the *H. tibetana* clumps and the reference plant community. The error bars denote ± standard error ($n=5$ for around-clump communities and $n=4$ for reference community)
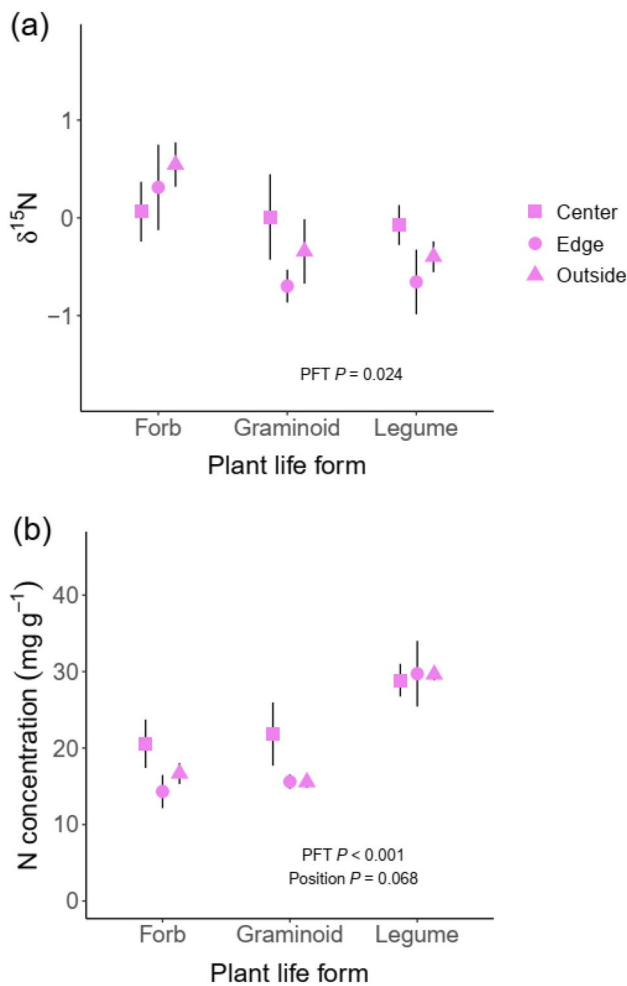

Fig. 3 δ^{15}N (**a**) and N concentration (**b**) of aboveground parts of different plant life forms at the three locations of *H. tibetana* clumps. Error bars denote ± standard error ($n=5$). ($P<0.05$)

Table 3 Summary of two-way ANOVAs for position (center, edge, and outside) and life form (forb, graminoid, and legume) effects on δ^{15}N and N concentration of aboveground samples surrounding *H. tibetana* clumps ($n=5$). Significant effects are marked in bold

Source	df	δ^{15}N (‰)		N concentration	
		F value	*P* value	*F* value	*P* value
Position	3	0.973	0.390	2.943	0.068
Life form	3	4.253	**0.024**	36.009	**< 0.001**
Position × life form	3	0.772	0.552	1.078	0.385

herbaceous plants existed. Roggy et al. (2004) suggested that N transfers between N-fixing plants and non-N-fixing plants are mainly affected by the extent of root contact. As mycorrhizal colonization and actinorhizal nodulation mostly occur in fine roots (Gardner et al. 1984; Andrews et al. 2011), the deep distribution of fine roots of *H. tibetana* means that the mycorrhizal network with *H. tibetana* may be weak, and root exudation of *H. tibetana* cannot be directly used by other plants. In addition, due to the deep fine-root distribution of *H. tibetana*, soil N pool for *H. tibetana* is likely quite different than that of other plants. Deep soils usually have higher δ^{15}N than shallow soils (Nadelhoffer et al. 1996; Zhou et al. 2018; Drollinger et al. 2019), so that the N *H. tibetana* individuals take up from deep soils probably has higher δ^{15}N than that of other plants. This can counteract the effect of N fractionation during N fixation on *H. tibetana* δ^{15}N signature and, thus, reduce the ability of *H. tibetana* individuals to alter the N isotopic signature of the soil and other plants.

Nevertheless, N concentrations of surrounding plants were slightly higher in both aboveground parts and roots at the clump center (Figs. 1, 3b), supporting our second hypothesis. This facilitation effect for plant aboveground parts was not found at the edge and outside of the clumps

(Figs. 1, 3b), which is partly inconsistent with our third hypothesis. This may be caused by the distribution of N-rich *H. tibetana* leaf litter, which was even higher than the green-leaf N content of other plants (Table 1), and thus higher N inputs than their leaf litter. The narrow and lanceolate leaves of *H. tibetana* may not be able to spread far away from the clump, and thus the N-rich litter input from *H. tibetana* was probably limited to the shrub canopy, which lead to N facilitation for other plants there. In addition, shrubs are known to increase soil fertility beneath their canopy (Ward et al. 2018; Turpin-Jelfs et al. 2019), which can also benefit the N status of the plants growing under the shrub cover.

Although N concentration of clump-center plants was slightly facilitated, their growth was not promoted. On the contrary, plant biomass underneath the shrub canopy decreased (Fig. 2a). In the alpine meadow, light competition is also intense, and shading can lead to significant reduction in plant biomass and diversity (Li et al. 2022). Our results suggest that the N-facilitation effect of *H. tibetana* may be not large enough to offset the negative effect on its neighboring plants due to the competition for light and nutrients other than N (Chapin et al. 2016; Wang et al. 2021), which has also been found in other N-fixing plants (Taylor et al. 2017; Staccone et al. 2021).

Taken together, our study shows that *H. tibetana* has great capability of N-fixing, and that the presence of *H. tibetana* can slightly increase the N concentration of the plants underneath its canopy, mainly through its N-rich leaf litter. However, the N-facilitation effect of this actinorhizal shrub in the alpine meadow is minor, and cannot negate its negative effect on surrounding plants through shading. In addition, the N-fixing ability of the shrub can help it grow well in N-poor environments, and the deep root distribution can help it endure drought. As the Qinghai-Tibet Plateau is N- limited, and precipitation on the plateau is predicted to reduce with climate change (Zhao et al. 2019), this shrub will probably expand in the Qinghai-Tibet Plateau.

Declarations

Conflict of interest We declare no competing interest.

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