

# Leaf and community photosynthetic carbon assimilation of alpine plants under *in-situ* warming

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## Research article

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# Abstract

**Background:** The Tibetan Plateau is highly sensitive to elevated temperatures and has experienced significant climate warming in the last decades. While climate warming is known to greatly impact alpine ecosystems, the gas exchange responses at the leaf and community levels to climate warming in alpine meadow ecosystems remain unclear.

**Results:** In this study, the alpine grass, *Elymus nutans*, and forb, *Potentilla anserina*, were grown in open-top chambers (OTCs) for three consecutive years to evaluate their response to warming. Gas exchange measurements were used to assess the effects of *in-situ* warming on leaf- and community-level photosynthetic carbon assimilation based on leaf traits and photosynthetic physiological parameters. We introduced a means of up-scaling photosynthetic measurements from the leaf level to the community level based on six easily-measurable parameters, including leaf net photosynthetic rate, fresh leaf mass per unit leaf area, fresh weight of all plant leaves, the percentage of healthy leaves, the percentage of received effective light by leaves in the community, and community coverage. The community-level photosynthetic carbon assimilation and productivity all increased with warming, and the net photosynthetic rate at the leaf level was significantly higher than at the community level. Under elevated temperature, the net photosynthetic rate of *E. nutans* decreased, while that of *P. anserina* increased.

**Conclusions:** These results indicated that climate warming may significantly influence plant carbon assimilation, which could alter alpine meadow community composition in the future.

## Background

The global average air temperature has increased continuously since the industrial revolution [1]. High-latitude and high-altitude ecosystems are exceptionally sensitive to rising temperatures and experience greater increases in amplitude [2]. Alpine meadow is a typical vegetation type in the Tibetan Plateau that is fragile and sensitive to human activities and climate change [3]. The Tibetan Plateau have experienced rapid climate warming (0.4 °C per decade for the past 50 years), exceeding the global mean value, and the warming is expected to increase by 0.6–0.9 °C per decade in the 2015–2050 period [4]. Climate warming has a significant impact on ecosystem carbon cycles, causing both positive and negative feedbacks to future climates [5]. A significant component of alpine meadow ecosystems is alpine plants, which are specially adapted to tolerate long-term low temperatures and are highly temperature-responsive [6, 7]. Climate warming leads to elevated air and soil temperatures, which can directly or indirectly affect plant photosynthesis and growth rates.

Plants are the basis of the carbon cycle. They are not only the source of photosynthetic carbon in ecosystems but are also the key regulators of carbon dioxide (CO<sub>2</sub>) release into the atmosphere from the ecosystem. A large number of simulated warming experiments have demonstrated that climate warming impacts the physiological and ecological characteristics of plants, causing changes in plant phenology, biomass, growth, and reproduction, as well as other characteristics. In cold habitats, temperature is a

limiting factor for plant photosynthesis, and species living in these colder regions benefit more from warming than those living in warmer climates [8]. Due to the temperature dependence of plants, physiological variables such as photosynthesis may be one of the variables most affected by climate change [9]. Photosynthesis is the fundamental basis for plant carbon accumulation and is also one of the vital physiological processes easily impacted by environmental changes [10]. Although leaf temperature may occasionally reach the optimal temperature for photosynthesis in alpine areas, it is typically lower than the optimal temperature throughout most of the growing season, indicating that the photosynthesis of alpine plants is often limited by low temperature [11]. Our previous study reported that air temperature is an important factor that affects the photosynthesis of alpine plants [12]. Warming can improve photosynthetic performance. *In-situ* warming experiments of alpine plants have demonstrated that under increased temperature and without water restriction, alpine plants displayed a higher photosynthetic capacity and photosynthetic nitrogen use efficiency [13]. Understanding the response of photosynthesis to temperature changes is important for predicting the carbon balance of terrestrial ecosystems and the geographical distribution of vegetation under climate change scenarios.

Photosynthesis is typically described in terms of CO<sub>2</sub> assimilation capacity, which can be described at the leaf or canopy (or community) level. Canopy photosynthesis (CAP) reflects the behavior of groups of leaves or individual plants [14]. Leaf net photosynthesis provides an overall reflection of physiological processes and is used to compare differences between individuals, while CAP is useful for evaluating competition and mutual benefits among different species. Studying CAP can provide a basis for the assessment of the carbon cycle at regional and even global scales. Synchronous observations of carbon assimilation in plants at the leaf and community levels could improve our understanding of the response and adaptability of alpine ecosystems to climate warming. The gas exchange chamber method is presently commonly used to measure gas exchange at community scales. Models that are used at large spatial scales are typically based on leaf-level gas exchange responses [15, 16]. CAP can be estimated using canopy-scale models, such as the biochemical modeling of leaf photosynthesis [17], the big-leaf model [18], the multi-layer model [19], and the two-leaf model [20]. These models are all based on a model that combines physiological and biochemical indicators (such as phloem migration rate, carboxylation rate, and transport conductance) and involves many parameters and complicated observations [21]. This led us to speculate on whether leaf photosynthesis and other easily observable parameters could be used to deduce community photosynthesis, and furthermore, the relevant factors that should be considered in the conversion process.

Most studies that have used leaf photosynthesis to evaluate community photosynthesis have focused on crops and forest ecosystems [22–24]. Ellsworth and Reich [22] reported that leaf traits such as LMA, N and  $A_{\max}$  per unit area are strongly correlated with the cumulative leaf area above the leaf position in the canopy. Hirose [25] proposed a functional model that integrates the leaf area, solar radiation, canopy structure, canopy microclimate, and photosynthesis capacity. Zhu *et al.* [26] developed a photo-acclimation model that links electron photosynthesis and leaf nitrogen concentration for the distribution of nitrogen in the main photosynthetic proteins in leaves. Song *et al.* [27] used canopy architecture, a ray

tracing algorithm, and C<sub>3</sub> photosynthetic metabolism to develop a new integrated canopy photosynthesis model. Singh and Parida [28] divided canopy into sunlit and shaded with three layers (top–mid–bottom). Based on observations on LAI and light penetration, leaf photosynthesis was then used to compute canopy photosynthesis. In conclusion, all the parameters of these models cannot be obtained by actual measurements or simple arithmetic averages. In the present study, we attempted to find a simplified method for estimating community photosynthesis, and to achieve the up-scaling studies from leaf to community photosynthesis.

The photosynthetic responses of alpine plants to climate change will determine their survival and performance and, consequently, their competitive ability. We hypothesized that the effects of climate warming on the photosynthesis of different alpine plants would differ, which would impact the photosynthesis and species composition of the community. Our objectives were to elucidate the following: (1) the influence of warming on the photosynthesis of an alpine grass and forb; and (2) the relationship between leaf and community photosynthesis using synchronous field observations. The differential responses of different species to photosynthesis under increasing temperature could alter their carbon accumulation, which in turn could impact their competitiveness, community structure, and composition. Therefore, understanding the photosynthetic response at the leaf and community level is critical for predicting the effect of future climate changes and for determining the response mechanism of alpine plants to climate change.

## Results

### Microclimatic conditions

Air and soil temperatures increased in the OTCs. The mean ground surface temperatures at 1.5 m during the vigorous growth periods were 14.2 °C and 13.3 °C in the OTCs and OAs, respectively (Table 1). The daily mean air temperature was increased by 0.9 °C in the OTCs, which is consistent with the conservative warming (possibly between 0.3–0.9 °C) predicted by the IPCC (2013) for the coming decades. The maximum mean air temperature was 2.7 °C higher in the OTCs (21.4 ± 0.4 °C in OAs and 24.1 ± 0.4 °C in OTCs). The mean soil temperatures at 5 cm depth were 17.3 °C and 16.8 °C in the OTCs and OAs, respectively, and the 5 cm T<sub>soil</sub> in the OTCs was increased by 0.8 °C and the maximum T<sub>soil</sub> in OTCs was increased by 1.6 °C.

Table 1

Air temperature ( $T_{\text{air}}$ ) at 1.5 m aboveground, soil temperature at the depth of - 5 cm ( $T_{\text{soil}}$ ) in OTCs and OAs in growing period

	Air temperature ( $T_{\text{air}}$ , °C)			Soil temperature ( $T_{\text{soil}}$ , °C)		
	Mean air temp.	Mean Max Air temp.	Mean Min Air temp.	Mean soil temp.	Mean Max Soil temp.	Mean Min Soil temp.
OTCs	14.2 ± 0.2	24.1 ± 0.4	6.3 ± 0.4	17.3 ± 0.3	24.9 ± 0.4	11.3 ± 0.6
OAs	13.3 ± 0.2	21.4 ± 0.4	6.6 ± 0.4	16.8 ± 0.3	23.3 ± 0.4	12.2 ± 0.6

Air and soil temperature at the studied site, recorded in open areas (OAs) and inside open top chambers (OTCs) between 1 July and 31 August. Values are means ± SE.

### Leaf physiological traits

Regarding the evaluated leaf physiological parameters, *E. nutans* and *P. anserina* responded differently to *in-situ* warming (Table 2). During the vigorous growth period (July and August), the leaf physiological parameters of *P. anserina* changed dramatically, while those of *E. nutans* changed little. In July, the TWC, RWC, LMA, and SLV in *E. nutans* were lower, while SLA was higher, in the OTCs than in the OAs, but the differences were not significant ( $P > 0.05$ ) (Table 2). Similar trends were observed in *E. nutans* in August, with RWC and LMA being lower in the OTCs, while the other parameters were increased. In contrast, LMA was significantly increased in *P. anserina* in August, while the other parameters were decreased.

Table 2

The total water content (TWC), relative water content (RWC), specific leaf area (SLA), specific leaf volume (SLV) and leaf mass area (LMA) for *E.nutans* and *P. anserina* in vigorous growth (July and August) inside warming chambers (OTCs) and in open areas (OAs)

months	species	treatments	TWC/ (%)	RWC/ (%)	LMA/ (kg m <sup>-2</sup> )	SLA / (cm <sup>2</sup> g <sup>-1</sup> )	SLV/ (cm <sup>3</sup> g <sup>-1</sup> )
July	<i>E.nutans</i>	OTCs	58.3 ± 2.1b	57.5 ± 4.8b	0.049 ± 0.001b	203.2 ± 6.6a	4.1 ± 0.4b
		OAs	60.5 ± 0.6b	66.5 ± 2.4ab	0.053 ± 0.003b	187.9 ± 8.9a	4.5 ± 0.3ab
	<i>P. anserina</i>	OTCs	59.6 ± 4.5b	58.4 ± 9.8b	0.085 ± 0.003a	118.1 ± 4.6b	3.7 ± 0.5b
		OAs	70.3 ± 3.1a	78.6 ± 1.9a	0.056 ± 0.006b	182.2 ± 21.8a	7.4 ± 1.6a
August	<i>E.nutans</i>	OTCs	63.4 ± 1.3ab	74.0 ± 0.6b	0.052 ± 0.001b	192.4 ± 4.8a	6.4 ± 0.4b
		OAs	61.1 ± 2.1b	76.9 ± 3.9b	0.053 ± 0.003b	191.0 ± 9.5a	5.8 ± 0.2b
	<i>P. anserina</i>	OTCs	62.3 ± 0.8b	74.6 ± 1.2b	0.075 ± 0.003a	135.9 ± 12.6b	6.5 ± 0.3ab
		OAs	67.9 ± 1.2a	86.8 ± 1.5a	0.059 ± 0.007b	175.3 ± 22.8ab	7.3 ± 0.2a
Values are means ± SE (n = 6). In different month, significant differences for each species were according to Duncan test, values with the same letters within columns are not significantly different at $p < 0.05$ .							

### Diurnal Variation In Leaf Photosynthesis

Each measurement day was divided into three periods, including the morning (8:00–11:00), noon (12:00–14:00), and the afternoon (15:00–18:00). In July, the diurnal changes in leaf photosynthesis of the two dominant species were similar, gradually decreasing from 8:00 to 18:00 (Fig. 2). In the OAs, the  $P_n$  of *E. nutans* was higher than that of *P. anserina*. Warming decreased the  $P_n$  of *E. nutans* but increased that of *P. anserina*, but the difference was not significant ( $P > 0.05$ ). For *E. nutans*, the daily  $P_n$  average in the OTCs and OAs was 3.7 and 4.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and the daily  $P_n$  average of *P. anserina* in the OTCs and OAs as 4.1 and 3.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. The maximum  $P_n$  for *E. nutans* and *P. anserina* occurred at 9:00 AM local time.

### Light And Co Response Characteristics Under Different Temperatures

Significant differences in photosynthesis parameters between the OAs and OTCs were detected in both species. A higher  $P_{nmax}$  and  $LSP$  were recorded in *E. nutans* (Fig. 3, Table 3). At 15 °C air temperature, the  $P_{nmax}$  of *E. nutans* and *P. anserina* were 7.39 and 7.34  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , while at 20 °C air temperature, *E. nutans* had the highest  $P_{nmax}$ ,  $LSP$ ,  $R_d$ , and  $AQY$  values. With the increase in air temperature to 25 °C, the  $P_{nmax}$ ,  $LSP$ ,  $R_d$ , and  $AQY$  decreased, while the  $LCP$  increased in comparison to 20 °C in *E. nutans*. In *P. anserina*, the increased air temperature was associated with increased  $P_{nmax}$ ,  $LCP$ ,  $LSP$ , and  $R_d$ .

Table 3

Light and  $\text{CO}_2$  response characteristics under different temperatures. Values are means  $\pm$  SE ( $n = 3$ ). Different letters in the same columns indicate significant differences ( $p < 0.05$ ).  $T_a$  – air temperature;  $P_{nmax}$  – max net photosynthetic rate;  $R_d$  – dark respiratory rate;  $AQY$  – Apparent quantum yield;  $LCP$  – Light compensation point;  $LSP$  – Light saturation point.  $V_{cmax}$  – maximum carboxylation rate of Rubisco;  $J_{max}$  – RuBP regeneration capacity.

Plants	$T_a$	$P_{nmax}/$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$LCP/$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$LSP/$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$R_d/$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$AQY/$ ( $\text{mol mol}^{-1}$ )	$V_{cmax}/$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$J_{max}/$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
<i>E. nutans</i>	15 °C	7.39 $\pm$ 0.52d	12.73 $\pm$ 2.18e	909.3 $\pm$ 56.7c	0.68 $\pm$ 0.03c	0.057 $\pm$ 0.01a	17.14 $\pm$ 0.83d	38.33 $\pm$ 1.07d
	20 °C	10.63 $\pm$ 1.25a	15.05 $\pm$ 1.28d	1632.1 $\pm$ 83.9a	0.86 $\pm$ 0.04ab	0.061 $\pm$ 0.01a	30.84 $\pm$ 0.97b	51.36 $\pm$ 1.84b
	25 °C	9.65 $\pm$ 0.49b	26.44 $\pm$ 3.70a	1365.1 $\pm$ 33.5b	0.61 $\pm$ 0.03c	0.042 $\pm$ 0.02c	33.63 $\pm$ 1.15a	53.84 $\pm$ 1.27a
<i>P. anserina</i>	15 °C	7.34 $\pm$ 0.56d	17.37 $\pm$ 1.37c	863.7 $\pm$ 11.9c	0.84 $\pm$ 0.04ab	0.051 $\pm$ 0.01b	23.14 $\pm$ 2.18c	50.01 $\pm$ 3.29b
	20 °C	8.24 $\pm$ 0.82c	18.68 $\pm$ 1.95c	863.3 $\pm$ 10.6c	0.73 $\pm$ 0.03bc	0.041 $\pm$ 0.01c	24.83 $\pm$ 2.59c	45.82 $\pm$ 4.79c
	25 °C	9.23 $\pm$ 0.34b	24.00 $\pm$ 0.95b	905.4 $\pm$ 23.0c	1.00 $\pm$ 0.06a	0.044 $\pm$ 0.01c	34.20 $\pm$ 2.32a	49.96 $\pm$ 2.03b

Values are means  $\pm$  SE ( $n = 3$ ). In the same column, significant differences in different temperature for each species were according to Duncan test, values with the same letters are not significantly different at  $p < 0.05$ .

From 15 °C to 25 °C, the  $V_{cmax}$  and  $J_{max}$  of *E. nutans* increased significantly [Fig. 3(c), 2(d), Table 3,  $P < 0.05$ ], while in *P. anserina*,  $J_{max}$  was highest at 15 °C.

## Diurnal Variation In Alpine Meadow Community Photosynthesis

The diurnal variation in the photosynthetic rate in the alpine meadow community exhibited a decreasing trend. Inside the OTCs, the average CAP was greater than in the OAs (Fig. 4,  $2.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  in OTCs and  $1.6 \mu\text{mol m}^{-2} \text{s}^{-1}$  in OAs). The maximum values of CAP in the OTCs and OAs were observed at 10:00 AM with  $3.3$  and  $2.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. The simulated warming increased the net photosynthetic rate of the alpine meadow communities by up to 31%.

### Upscaling and correlation of the photosynthesis from the leaf to community level

Linear fitting of the net photosynthetic rate of the leaves and communities of the alpine plants indicated a significant positive correlation between the leaves and communities under *in-situ* warming. The net photosynthetic rate of the community was significantly lower than that of the leaves. The CAP in the OTCs and OAs accounted for 52.7% and 41.8% of the leaf photosynthetic rate, respectively. The overall regression equation for the net photosynthetic rate of the leaves and communities was:  $\text{CAP} = 0.329 P_n + 0.262$  ( $r = 0.79$ ) [Fig. 5(a)]. It can be seen from Fig. 5(b) and Table 4 that there was a significant correlation between the derived CAP and the measured CAP. The derived value in the OTCs was lower than the measured value, while the derived value in the OAs was greater than the measured value; however, this difference was not significant ( $P > 0.05$ ). The difference in CAP between the derived value and the measured value was generally not significant ( $0.02 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), indicating that the CAP can be obtained from the leaf photosynthesis, fresh leaf mass per unit leaf area, and the fresh weight of all plant leaves in the community.

Table 4  
Upscaling of the photosynthesis from leaf to community

Treatments	$P_n$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$A$ ( $\text{m}^2 \text{g}^{-1}$ )	$m$ ( $\text{g m}^{-2}$ )	$k$	$r$	$c$ (%)	CAP ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	CAP <sub>d</sub> ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
OTCs	3.89	0.00661	164.8	0.8	0.6	0.95	2.05	1.93
OAs	3.80	0.00636	151.1	0.8	0.6	0.95	1.59	1.67
average	3.85	0.00649	158.0	0.8	0.6	0.95	1.82	1.80

### Biomass changes in alpine meadow ecosystems under different warming years

Changes in above-ground biomass were observed under simulated warming (Fig. 6). After three consecutive years of elevated temperature, the above-ground biomass of the alpine vegetation increased, and in 2018, a more significant increase in biomass was observed in the OTCs.

## Discussion

### In-situ warming and its differential effect on the photosynthetic performance of two dominant species

Climate change is altering the structure and function of alpine ecosystems. The air temperature of the Tibetan Plateau is forecast to increase by 0.6–0.9 °C from 2015 to 2050 [4], which is within the simulated warming in our experimental setup (Table 1). Leaf traits are closely related to the resource acquisition and utilization efficiency of plants and reflect plant survival strategies to environmental changes. Our results suggested that the leaf traits of *E. nutans* and *P. anserina* have different responses to warming. Under increased temperature, the SLA of *E. nutans* increased, and the LMA of *P. anserina* increased. Our findings support the hypothesis that the phenotypic plasticity of certain traits in plants can predict the performance of communities under climate change [29, 30]. SLA and LMA are related to plant growth, resource capture, and utilization. SLA impacts light interception, photosynthesis, and plant growth, and is thus indicative of competitive ability and environmental tolerance [31, 32]. The SLA of *E. nutans* was higher than that of *P. anserina*, indicating that it possesses a higher net photosynthetic rate and higher leaf light capture area. The higher LMA value of *P. anserina* is a characteristic that allows it to flourish in alpine environments.

Under certain circumstances, warming can meet the growth requirements of plants. However, it can also change the microclimatic environment of the plant community and directly or indirectly affect plant photosynthetic physiological processes in a variety of ways. Although increased temperature in cold ecosystems, such as our study area, may promote plant growth (Fig. 6), it may also increase interspecific competition. *Elymus nutans* is a grass (Gramineae) and is the dominant species in alpine meadows, while *P. anserina* is a forb (Rosaceae) and is widespread and common. The responses of different plants to climate warming differ, and these responses determine the adaptive capacity of species to future climate warming as well as their competitive ability [33]. In our study,  $P_n$  in *E. nutans* was higher than *P. anserina* in the OAs (Fig. 2). Interestingly, the  $P_n$  of *E. nutans* decreased with increased temperature, while that of *P. anserina* increased. This is because the air temperature was high in the growing season (July), and thus the leaf temperature of *E. nutans* exceeded its optimal temperature, leading to a decrease in  $P_n$ . These results corresponded with the changes in photosynthetic parameters (Fig. 3).

Photosynthetic parameters are very important for estimating the alpine C budget. Previous studies showed that warming would increase plant C uptake by providing optimal temperature conditions [34, 35]. In our study, *P. anserina* had a higher photosynthetic rate than *E. nutans* under warming (Figs. 2 and 3). Shi *et al.* [36] suggested that forbs (*Vicia unijuga* and *Allium atosanguineum*) would adapt better to future climate warming than grasses (*E. nutans* and *Koeleria macrantha*) in alpine meadows, which is consistent with our findings. In cold climates or in areas with no water restrictions, species will change their optimal photosynthetic temperature to increase photosynthesis under warming [37, 38]. A temperature increase from 15 °C to 20 °C resulted in increased  $P_{nmax}$ ,  $LSP$ ,  $R_d$ , and  $AQY$  in *E. nutans*, while a decrease was observed at 25 °C. This suggests that 20 °C is the approximate optimum growth temperature for *E. nutans*. Conversely,  $P_{nmax}$ ,  $LCP$ ,  $LSP$ ,  $R_d$ , and  $V_{cmax}$  all increased with increased temperature in *P. anserina*, which implies that *P. anserina* can survive at a higher temperature. Similar responses have also been reported by Shi *et al.* [39], who found that elevated temperature increased the photoinhibition of *E. nutans* but reduced the photoinhibition of *P. anserina*. Elmendorf *et al.* [33]

discovered that the response to long-term warming was opposite by grasses, sedges, and rushes. In the present study, *E. nutans* demonstrated the highest  $P_{nmax}$  at 20 °C, which thereafter decreased at 25 °C, but was still higher than in *P. anserina*. A higher  $P_{nmax}$  is associated with higher photosynthetic gain, suggesting that *E. nutans* had a greater photosynthetic gain. *E. nutans* demonstrated have greater photosynthetic gain, while *P. anserina* can survive at a higher temperature, suggesting that the community structure of alpine meadow may change from grass to forb with climate warming.  $CO_2$  utilization during photosynthesis is indicative of photosynthetic efficiency, and a higher  $R_d$  is indicative of greater consumption of photosynthetic products.  $V_{cmax}$  and  $J_{max}$  increased with increased temperature in both species, which might be related to the changes in nitrogen distribution and photosynthetic enzyme activity in the leaves [40].

In alpine regions, lower temperatures and a short growing season are the main limiting factors for plant growth and ecosystem productivity. Studies have shown that in temperature-limited ecosystems, the extension of the growing season under long-term warming will significantly improve net primary productivity by increasing photosynthetic capacity [41, 42]. Peng *et al.* [43] concluded that alpine ecosystems with low temperature and relatively high soil moisture tend to absorb more C in a warmer climate. In our study, the above-ground biomass of the alpine vegetation increased under three consecutive years of warming, which is consistent with the increase in community photosynthesis under warming conditions (Fig. 4). Climate warming increased the aboveground biomass of the arctic willow *Salix arctica*, which had a positive feedback on its photosynthetic activity [44]. Under warming, the tested alpine meadow plants increased their above-ground biomass due to the increased community photosynthetic rate, which is consistent with Chapin *et al.* [45]. Liang *et al.* [46] used a meta-analysis to estimate the effects of warming on leaf photosynthesis of terrestrial plants, and found that the effect of warming on grass was greater than that of forbs, indicating that forbs may accumulate lower biomass and have less competitive than grasses under climate warming.

### **Community photosynthetic carbon assimilation and its relationship with leaf-level carbon assimilation**

The leaf is the smallest unit of a plant community, and a plant community is the basic component of an ecosystem. CAP represents the photosynthesis of both the top and bottom leaf layers. In the present study, we offer a simplified approach for estimating community photosynthesis. We used six easily-measurable parameters to calculate the community photosynthesis and compared these with the observed results (Eq. 3, Fig. 5). Ellsworth and Reich [22] suggested that LMA is an effective means of combining the effects of canopy structure and light environment on leaf photosynthetic performance. Li *et al.* [47] found that under experimental warming, leaf area could determine the response of ecosystem productivity in an Alpine Steppe. In our study, we used fresh leaf mass per unit leaf area ( $A$ ) and fresh weight of all plant leaves ( $m$ ) in the scale conversion. Using our equation, the result agrees with the observations under natural conditions (Fig. 5).

Leaf position, leaf age, and different leaf orientations or growth angles (horizontal and vertical) also influence the photosynthetic rate of the leaves [48]. Alpine meadow plants have obvious stratified

structures. Grasses (such as *E. nutans*) largely grow in full-sun conditions, while sedges and forbs (such as *P. anserina*) mostly grow in shaded environments. In the canopy, the light absorbed by the upper leaf layer is usually more than its saturation level, and the excess light energy is dissipated primarily by heat dissipation, while the lower layer leaves are usually limited by available light [49]. Considering the degree of shading between the plants and the angle of the leaves, the parameter  $r$  (the percentage of received effective light by leaves in the community) was used in the estimation of community photosynthetic capacity. As for the different maturity of leaf maturities in the community, we used the parameter  $k$  to represent the percentage of healthy leaves, and these parameters were successfully incorporated in the model. Using these parameters, the leaf-level carbon assimilation can be accurately estimated to the community level. Comparing with the canopy model, all of the parameters were more simplified and easier to obtain.

## Conclusions

This study linked leaf- and canopy-level photosynthetic parameters and revealed the effects of warming on the photosynthesis and productivity of alpine plants. The responses of different plants to climate warming differ, which is associated with differential adaptability and competitiveness. Under a temperature increase from 15 °C to 25 °C, the net photosynthetic rate of *E. nutans* increased at 20 °C and then decreased, suggesting that 20 °C is the optimum growth temperature for *E. nutans*. For *P. anserina*, an increase in air temperature was associated with increased photosynthetic capacity, suggesting that *P. anserina* could survive at a higher temperature. The net photosynthetic rate of the community was significantly lower than that of the leaves. Under climate warming, the photosynthetic capability and productivity of the alpine meadow communities all increased, suggesting that an increase in temperature under climate warming may have a significant influence on net plant C uptake. Furthermore, we used six easily-measurable parameters to scale up from leaf-level to community-level photosynthesis, and the difference in CAP between the derived value and the measured value was not significant. In addition, the response of photosynthesis to environmental factors are different at different scales. In the further study, it is necessary to strengthen the research on the response and differences between photosynthesis and environmental factors at different scales, and these factors should be also considered in the canopy model.

## Methods

### Study site

The study site was located at the Zoige Alpine Wetland Ecosystem Research Station (33°51'52"N, 102°08'46"E, 3440 m) on the eastern Tibetan Plateau. The region has a plateau continental semi-humid climate, with no absolute frost-free period throughout the year. From 1967 to 2010, the annual mean air temperature was 1.7 °C and the annual mean precipitation was 600 mm, with 80% of precipitation falling between June and September. The soil is classified as silt clay loam, which is composed of 31.2% sand, 56.0% silt, and 12.8% clay in the top 30 cm soil layer based on the classification of the US Department of

Agriculture. Soil organic carbon content, total carbon, and total nitrogen in the top 30 cm layer are 44.5, 46.2, and 4.3 g kg<sup>-1</sup>, respectively. The pH value of the 0–10 cm soil layer is 7.7 [50]. The area is dominated by the perennial plant *Elymus nutans* Griseb., which is an important forage species with high yield and good reproductive capacity. Other common plants include *Potentilla anserina* L., *Roegneria nutans* (Keng) Keng & S.L.Chen, *Poa pratensis* L., *Kobresia setschwanensis* Hand.-Mazz., *Leymus secalinus* (Georgi) Tzvel, *Plantago depressa* Willd., and *Ajania tenuifolia* (Jacq.) Tzvel. These species together account for ~ 80% of the aboveground biomass (g m<sup>-2</sup>). In this study, two dominant herbaceous species (*E. nutans* and *P. anserina*) were selected for leaf physiological trait and leaf photosynthesis analysis. *Potentilla anserina* is a cosmopolitan species and miscellaneous forb in alpine meadows that is able to reproduce asexually and also exhibits great colonization ability and morphological plasticity. We have permissions to collect such samples, and the permission was obtained from the Zoige Plateau wetlands ecosystem research station. All the plants were identified by plant taxonomists from Northwest Normal University, and confirmed by consulting the "Flora of China". The voucher specimen of *E. nutans* and *P. anserina* were deposited in the key laboratory of land surface process and climate change in cold and arid regions of the Chinese Academy of Sciences.

## **Experimental Design**

In April 2015, three open-top chambers (OTCs) were built to evaluate the effects of warming on the alpine meadow ecosystem. The OTCs possessed underground anti-lateral seepage treatment, and warming can be adjusted by the ancillary facilities. Each OTC comprised an aluminum frame fitted with 8-mm-thick transparent plexiglass boards with a light transmittance > 92%. Each unit covered an area of 6.4 m<sup>2</sup>, was 2 m in height with a bottom side length of 1.15 m, and was shaped as a regular octagon with an outer diameter of 3 m. Open areas (OAs) were established as control areas with similar characteristics to those where the OTC was placed. The layout of the experimental design was shown in Fig. 1. We installed microclimate data loggers (HOBO U23-002, RH/Temp Onset, Pocasset, MA, USA) in the center of the OTCs and OAs at 1.5 m height and recorded the relative humidity (%) and air temperature (°C) at 30 min intervals. An ECH<sub>2</sub>O-TE sensor and EM50 data acquisition system were used to monitor the soil temperature and moisture composition. Individuals of both species growing in OTCs and OAs were randomly selected for growth measurements and photosynthesis during the growing seasons.

## **Leaf Physiological Traits**

### **Plant biomass**

At the end of the growing seasons in 2016–2018 (late September), plant above-ground biomass (AGB) was investigated in the OTCs and OAs. The above-ground plants growing within a 50 × 50 cm quadrat were cut and weighed. Three replicates were tested.

### **Relative water content, specific leaf area, specific leaf volume, and leaf mass area**

Fresh leaf samples from three to five plants measured for each species were weighed immediately after collection (fresh weight, FW), and then completely immersed in distilled water in the dark until saturated.

The saturated fresh weight (SW) was determined, following which the leaves were oven-dried at 70 °C for 48 h to assess the dry weight (DW). The leaf relative water content (RWC, %) was calculated using the following calculation:

$$\text{RWC (\%)} = \frac{[(\text{FW}-\text{DW}) (\text{SW}-\text{DW})]}{(\text{SW}-\text{DW})} \times 100. \quad (1)$$

The leaf area of the fresh leaves was analyzed using image analysis software (ImageJ) [51]. Specific leaf area (SLA) was calculated as the ratio of the area of one side of the leaf in each group of leaves to its dry mass [52]. Specific leaf volume (SLV) was determined using the drainage method and was calculated as the ratio of the leaf volume to its dry mass [50]. Leaf mass area (LMA) was calculated as the ratio of DW to leaf area. For all of these measurements, three to five replicates were measured for each species.

## **Determination Of Photosynthetic Characteristics**

### **Leaf gas exchange**

Due to the late start of the growing season, plants in the study area began to turn green in May and turned yellow and went dormant by late September, and thus the photosynthetic parameters of the plants were usually relatively stable from July to August. The leaf gas exchange parameters of *E. nutans* and *P. anserina* were measured three times per month in July 2016 and 2017. We used a LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA) to measure the leaf net photosynthetic rate ( $P_n$ ). For *E. nutans*, three leaves were fastened side by side to a flat surface with adhesive tape at both ends in order to cover the entire leaf chamber of the infrared gas analyzer and to avoid overlap of the leaves. The leaves were then collected, and their areas were measured with ImageJ for accurate calculations.

The measurement days were bright and clear with no wind, with hourly measurements from 08:00 to 18:00 h. Measurements were repeated three times. As the local time delay is 72 min from Beijing time, local time was used in the analyses.

### **Light And Co Response Of Photosynthesis**

To determine the effect of photosynthetic photon flux density (PPFD) levels on photosynthesis, standard light response curves were constructed using a LI-6400 portable photosynthesis system with a LI-6400-02 LED source. An automatic procedure was used to measure  $P_n$  at leaf temperatures of 15°C, 20°C, and 25°C, respectively. The ambient CO<sub>2</sub> concentration (380 μmol mol<sup>-1</sup>) was controlled by the LI-6400 CO<sub>2</sub> injecting system. The PPFD started at 2,000 and decreased to 0 μmol m<sup>-2</sup> s<sup>-1</sup>. The response of net photosynthesis CO<sub>2</sub> uptake ( $P_n$ ) to varying substomatal CO<sub>2</sub> concentration ( $C_i$ ) was determined from  $P_n - C_i$  curves in the same way as reported in Sáez *et al.* [53].

Light and CO<sub>2</sub> responses were taken from 08:00 to 13:30 h during mid-July. Each species was measured in triplicate. Afterwards, all the leaves were collected, and the areas were measured with ImageJ to re-compute the photosynthesis data.

Analysis of light and CO<sub>2</sub> response curves involved calculations of the following parameters:  $P_{nmax}$  (max net photosynthetic rate),  $R_d$  (dark respiratory rate),  $AQY$  (apparent quantum yield),  $LCP$  (light compensation point),  $LSP$  (light saturation point),  $V_{cmax}$  (maximum carboxylation rate of Rubisco), and  $J_{max}$  (RuBP regeneration capacity). The light response curve used the modified model of non-rectangular hyperbola [54], and the CO<sub>2</sub> response curve used Photosynthesis Assistant (Dundee Scientific, Dundee, UK), which implements a biochemical model describing photosynthetic rate [17].

### **Community Gas Exchange Measurements**

A LI-8100 carbon flux (LI-COR) measurement system and a modified assimilation chamber (0.5 × 0.5 × 0.5 m) (Beijing Ecotek Ltd. Co., Beijing, China) were used to measure canopy gas exchange (mixed community). In order to seal the canopy chamber to the soil surface, we installed a 0.5 × 0.5 m square aluminum frame into the soil at a depth of 3 cm, which provided a plane interface between them. During measurements, two small fans were installed diagonally inside the chamber and fanned continuously to mix the atmosphere. The measurements were synchronized with the leaf gas exchange measurements. The measurements were taken once-hourly between 08:00–18:00 h, using a 5-min measurement that was repeated three times to obtain average values. The CAP rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was calculated as follows based on the measurement principle of Gao *et al.* [14]:

$$CAP = \frac{-V_A \times P \times \left( \frac{\partial C}{\partial t} - n \frac{\partial C_s}{\partial t} \right)}{A \times (T + 273.15) \times R}, \quad (2),$$

where  $A$  is the total leaf area of the plant canopy ( $m^2$ ),  $V_A$  is the total volume of the canopy photosynthesis measurement system, which is the product of the height of the assimilation chamber (0.5 m) and the soil surface ( $0.25 m^3$ ) equal to  $0.125 m^3$ ,  $\frac{\partial C}{\partial t}$  is the rate of chamber  $CO_2$  change ( $\mu mol CO_2 mol^{-1} s^{-1}$ ),  $\frac{\partial C_s}{\partial t}$  is the rate of chamber  $CO_2$  change in soil respiration measurements ( $\mu mol CO_2 mol^{-1} s^{-1}$ ),  $P$  is the atmospheric pressure (Pa),  $T$  is the air temperature inside the chamber ( $^{\circ}C$ ), and  $R$  is the gas constant ( $8.314 Pa m^3 mol^{-1} K^{-1}$ ).

### Upscaling the photosynthesis measurements from the leaf to the community

Considering the degree of shading between the plants and the angle of the leaves, the parameter  $r$  (the percentage of received effective light by leaves in the community) was used in the estimation of community photosynthetic capacity. As for the different leaf maturities in the community, we used the parameter  $k$  to represent the percentage of healthy leaves.

$$CAP_d = \bar{P}_n \times \bar{A} \times m \times k \times r \times c \quad (3)$$

where  $CAP_d$  is the deduced value of the community photosynthetic rate from leaf level,  $\bar{P}_n$  is the average value of the leaf photosynthetic rate with different plants in the community ( $\mu mol m^{-2} s^{-1}$ ),  $\bar{A}$  is the average value of fresh leaf mass per unit leaf area with different plant species in the community ( $m^2 g^{-1}$ ),  $m$  is the fresh weight of all plant leaves in the community ( $g m^{-2}$ ) (for the community area of  $0.25 m^2$ ,  $m$  is the biomass of all fresh leaves within  $0.25 m^2$ ),  $k$  is the percentage of healthy leaves,  $r$  is the percentage of received effective light by leaves in the community (considering the degree of shading between the species and the angle of the leaves, the correction parameters are used in the estimation of community photosynthetic capacity), and  $c$  is the community coverage (%).

## Statistical analysis

All data were analyzed using SPSS 20.0 (Armonk, NY, USA) and the means and standard error ( $\pm SE$ ) were computed. The parameters were analyzed using one-way analysis of variance (ANOVA) and compared using Duncan's multiple range tests to determine if they differed significantly at the 0.05 probability level. Correlation analysis was used to evaluate the relationship between  $P_n$  and CAP.

## Abbreviations

A: Leaf mass per unit leaf area; AGB: Above-ground biomass; AQY: Apparent quantum yield; c: the community coverage; CAP: Canopy photosynthesis;  $CAP_d$ : The deduced value of the community photosynthetic rate;  $J_{max}$ : RuBP regeneration capacity;  $k$ : The percentage of healthy leaves; LCP: Light

compensation point); LMA: Leaf mass area; *LSP*: Light saturation point); *m*: Fresh weight of all plant leaves; OAs: Open areas; OTCs: Open-top chambers;  $P_n$ : Photosynthetic rate;  $P_{nmax}$ : Max net photosynthetic rate); *r*: The percentage of received effective light;  $R_d$ : Dark respiratory rate; RWC: Relative water content; SLA: Specific leaf area; SLV: Specific leaf volume; TWC: Total water content;  $V_{cmax}$ : Maximum carboxylation rate of Rubisco

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no conflict of interest.

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### Author's Contributions

ZJ Zhou and PX Su designed the experiments and measured the leaf and canopy photosynthesis, XK Wu made data analysis, R Shi and XJ Ding collected the plant samples and measured the leaf traits. All the authors read and approved the final manuscript.

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## Figures

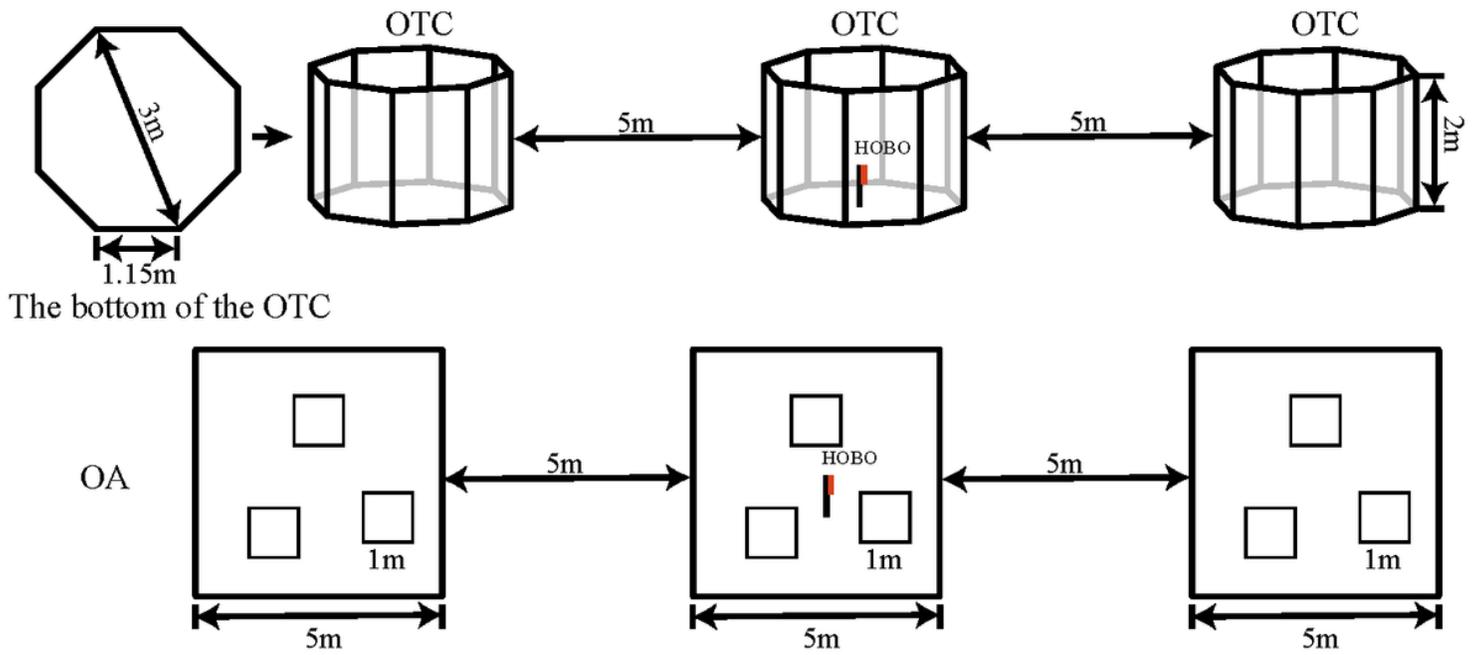
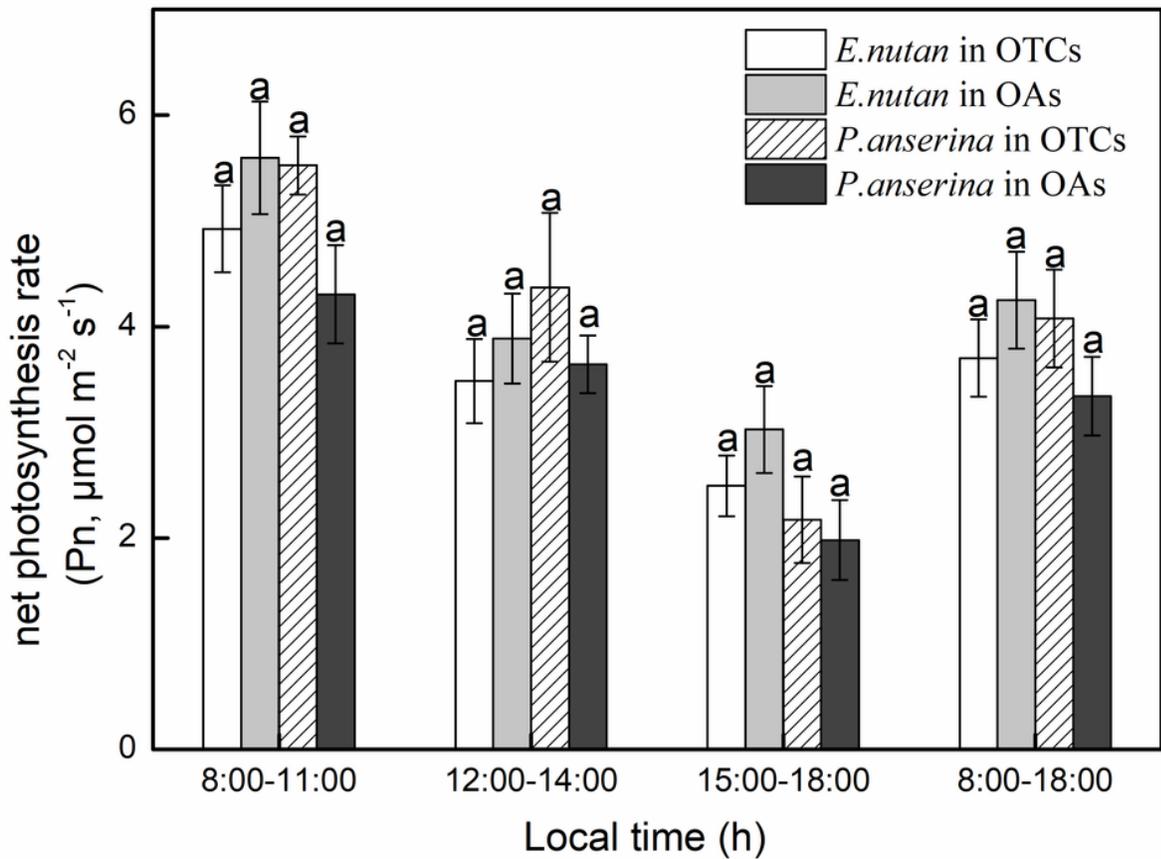


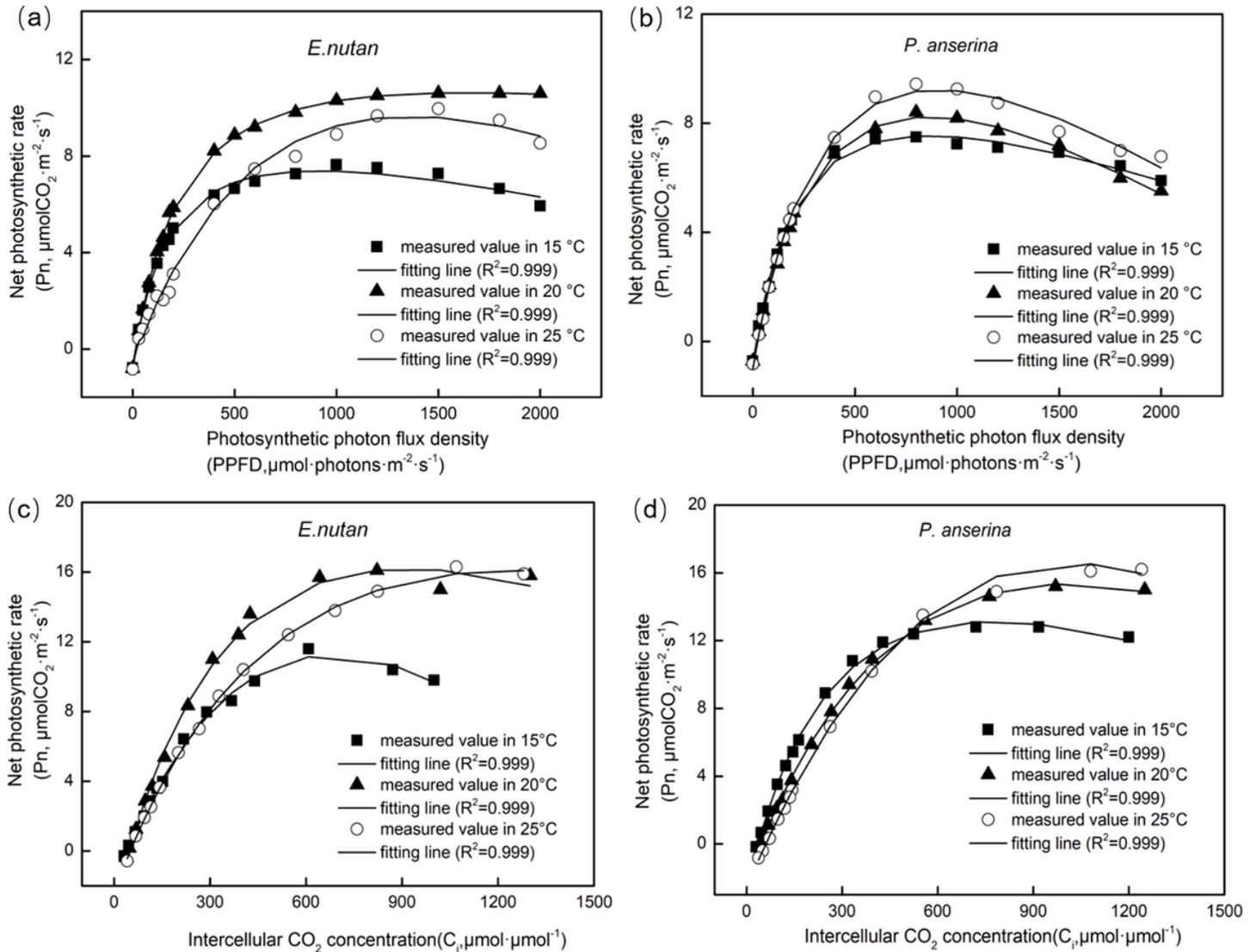
Figure 1

layout of the experiment design.



**Figure 2**

Diurnal changes of net photosynthetic rate (Pn) in alpine meadow at simulated warming. The values are the means  $\pm$  SE, and are the average of two years.



**Figure 3**

Net photosynthetic rate (PN) responses to different photosynthetic photon flux density (PPFD) and intercellular  $\text{CO}_2$  concentration ( $C_i$ ) in *E.nutan* and *P.anserina* under different temperatures. The values are the means  $\pm$  SE ( $n = 3$ ).

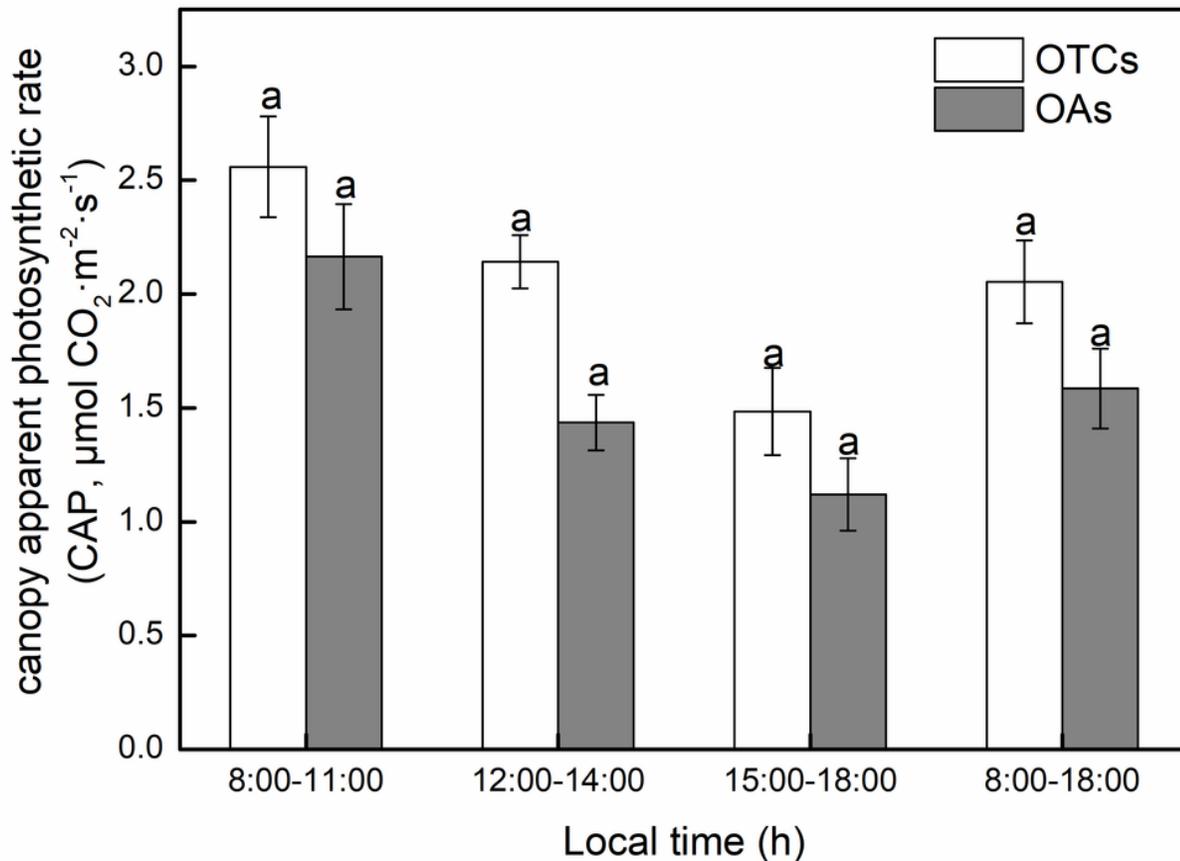


Figure 4

Diurnal changes of community apparent photosynthetic rate (CAP) in alpine meadow at simulated warming. The values are the means  $\pm$  SE, and are the average of two years.

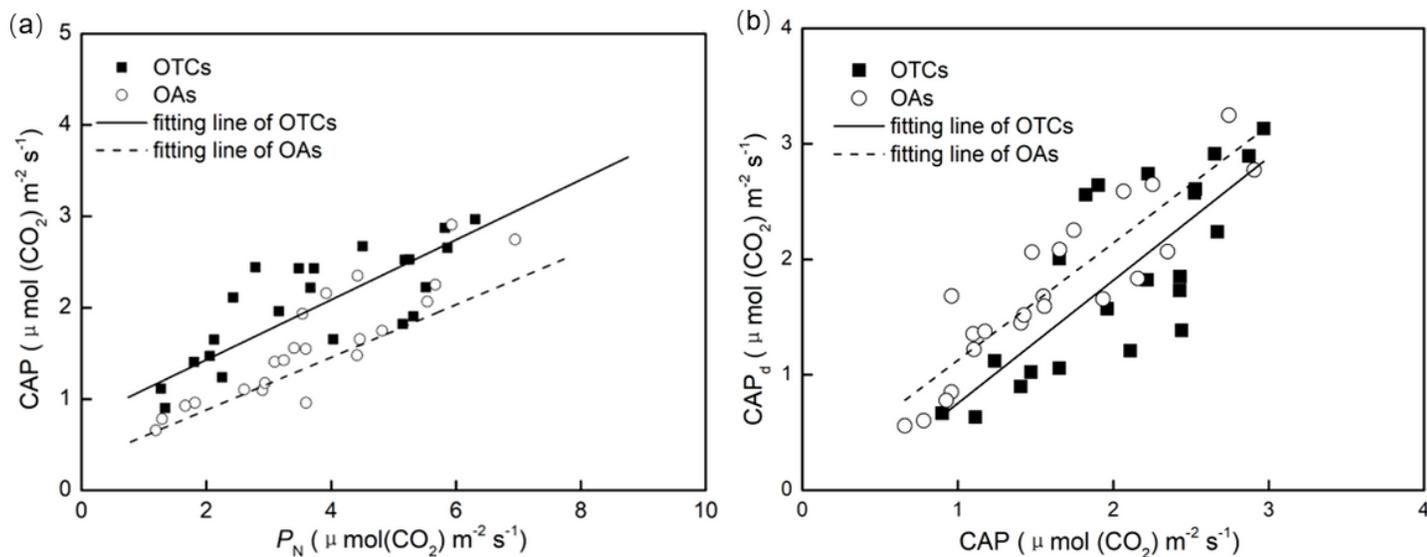


Figure 5

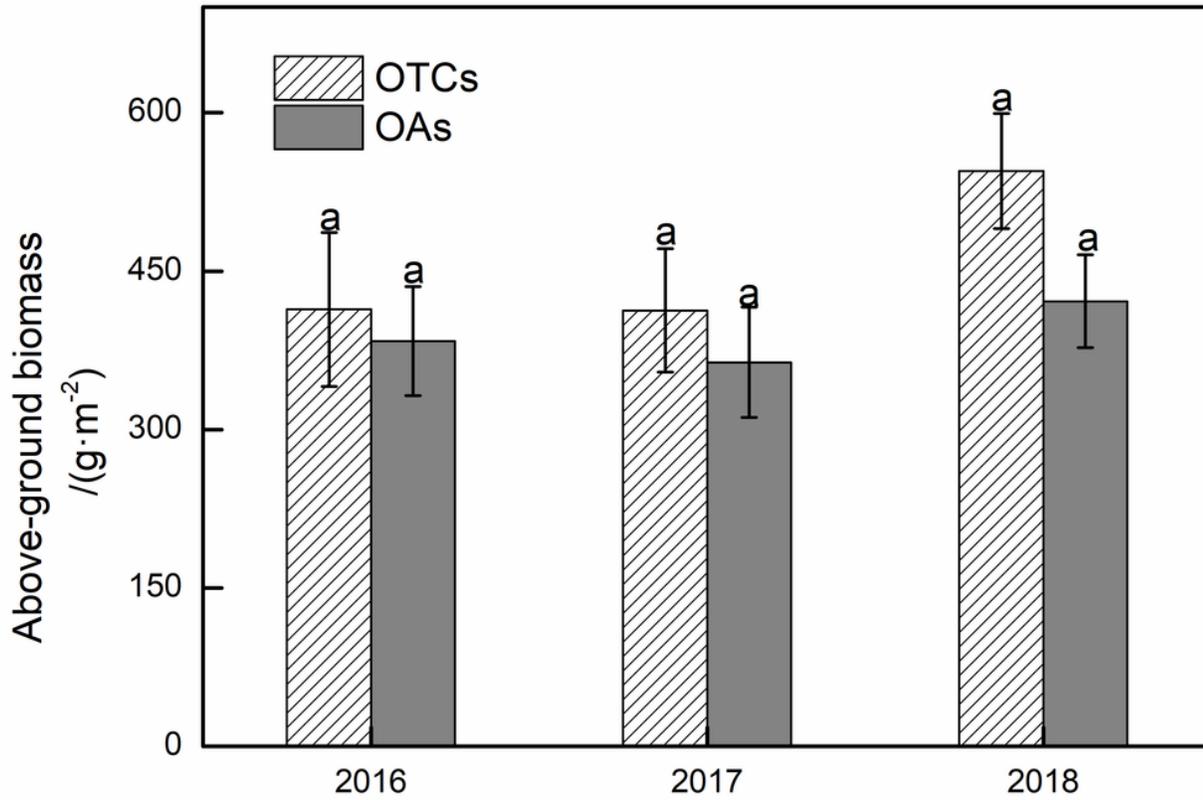


Figure 6

Above-ground biomass changes in alpine grassland under simulated warming in 2016-2018