



Full Length Article

Canopy Photosynthesis and Carbon Gains of Two Typical Alpine Plant Communities

Rui Shi^{1,2}, Peixi Su^{1*}, Zijuan Zhou¹, Tingting Xie³ and Xinjing Ding^{1,2}

¹Key Laboratory of Land Surface Process and Climate Change in Cold and Arid Regions, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, Gansu-730000, China

²University of Chinese Academy of Sciences, Beijing-100049, China

³Heihe Key Laboratory of Ecohydrology and Integrated River Basin Science, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, Gansu-730000, China

*For correspondence: supx@lzb.ac.cn

Abstract

The canopy photosynthesis of wetland and meadow vegetation communities on the Zoige Plateau, China, was measured to reveal photosynthetic characteristics and their responses to climate and soil factors and to analyze the daily carbon budgets. Results showed that the diurnal average of the canopy apparent photosynthetic rate (CAP) in late July exceeded that in late August by 13% (wetland) and 24% (meadow). The increased CAP indicated more vigorous growth in late July. In the same months, the CAP and CAW (canopy apparent water use efficiency) of meadow were higher than those in wetland. Meadow community had a higher carbon fixation rate, and exhibited water-saving strategy. Photosynthetically active radiations (PAR) were the main determining factor for the two communities in both July and August. The relative humidity in wetlands, compared with that of meadows, presented a relatively stronger directly negative effect on photosynthesis. The daily net carbon gain of wetland were $1.72 \text{ g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ in late July and $1.71 \text{ g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ in late August, and the corresponding values of meadow were $2.7 \text{ g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ and $2.2 \text{ g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. The comparatively higher CAP and leaf area index led to the larger net carbon gain in meadow. We inferred that the stronger net gain in meadow might induce more pasture yield. © 2018 Friends Science Publishers

Keywords: Canopy photosynthesis; Carbon gains; Environmental factors; Path analysis; Water use efficiency

Introduction

Alpine regions are characterized by extreme climatic conditions such as low temperature, strong solar radiation and low partial pressure. Alpine plants have developed a broad range of photosynthetic adaptation and survival strategies in these extreme conditions. Vegetation is exposed not only to overall harsh climatic conditions but also to an enormous spatial variability in environmental factors, and thus presents some remarkable responses to the different growth conditions (Zhong *et al.*, 2001; Flexas *et al.*, 2012; Henneberger *et al.*, 2015; Vieira *et al.*, 2015; Wang *et al.*, 2017). The Zoige Plateau, China, is mainly composed of wetlands and meadows (Su *et al.*, 2018). Wetland and meadow communities are under various local environments, and thus differ in functional type composition, community structure, biomass, etc. Questions to be answered include: how does photosynthesis respond to local conditions, and what are the main determinants of photosynthesis in the field?

Many studies indicate that plant photosynthesis is commonly divided into two levels: the leaves; and the canopy. Canopy photosynthesis is, by definition, equal to

the integrated sum of photosynthesis by leaves throughout the canopy volume (Baldocchi and Amthor, 2001), and can be used to indicate the dry matter production capacity of a plant or a community. CO_2 uptake can be measured by exposing whole plots of intact vegetation to a transparent gas-exchange chamber (assimilation chamber). Aerodynamic methods, which assess CO_2 fluxes without enclosures by using profile data, are an alternative approach, but the patchiness of alpine vegetation and gusty winds may produce some data deviation at high altitude (Zhang *et al.*, 2006; Wohlfahrt *et al.*, 2008). The assimilation chamber can, therefore, provide an important comparative method for data verification. Moreover, assessing productivities of vegetation communities in pastoral area exert profound effects on its development and conservation.

In this study, typical communities on Zoige Plateau were selected to measure canopy gas exchange. The aims were to: (1) make comparisons of photosynthetic characteristics between communities in wetlands and in meadows, and to explain their photosynthetic differences; (2) analyze photosynthetic parameters and phenological effects of vegetation communities at the vigorous growth and large biomass phase, respectively; (3) reveal the responses of

photosynthesis to different environmental factors, and the main determining factors in different site conditions; and (4) assess daily carbon budgets of communities in various sites at two different phases of phenology.

Materials and Methods

Selection of Site and Plant Materials

The study was carried out in two areas of the Zoige Plateau on the Eastern Qinghai-Tibetan Plateau, China. The alpine wetland (33°54' N, 102°52' E) was close to Huahu, Sichuan province; and the alpine meadow (33°55' N, 102°09' E) was located on the Hequ horse farm, Gansu province, both at approximately 3440 m elevation. The climate of the Zoige Plateau belongs to a typical alpine humid and semi-humid continental monsoon, and is in one of the abundant precipitation zones in the Yellow River Basin. The mean annual precipitation ranges from 600 mm to 800 mm, and the mean temperature from approximately 0.6°C to 1.2°C (Bai *et al.*, 2013; Zhao *et al.*, 2017). The annual average period of sunshine in the wetland was up to 2724 h, and in the meadow was up to 2621 h. In the wetland, the average photosynthetically active radiations from 08:00 to 18:00 during the growing season (May to October) were 1073 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 994 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the meadow in 2015. The average air temperature, atmospheric relative humidity and ambient CO_2 concentration were 4.8°C, 73%, 367 $\mu\text{mol}\cdot\text{mol}^{-1}$ in the wetland, and 3.5°C, 70%, and 374 $\mu\text{mol}\cdot\text{mol}^{-1}$ in the meadow, respectively, during the growing season in 2015. There was a total of 80% vegetation coverage of the wetland, and the dominant species included *Carex muliensis* and *Kobresia setchwanensis* (Table 1). Vegetation occupied as much as 90% of the meadow, which was dominated by *Elymus nutans* and *K. setchwanensis* (Table 1). The soil of the wetland was peat and that of the meadow was alpine meadow soil (Table 1). Further details of soil characteristics can be found in the study by Su *et al.* (2018).

Experiment Design and Sampling

We measured the diurnal course of canopy gas exchange and soil respiration in late July and August 2015 in the selected enclosed alpine wetland and meadow. The plant species composition, coverage, aboveground biomass, underground biomass and leaf area index of each community were monitored for 2 months. We then gathered all the living plants from five quadrats (50 cm \times 50 cm) in each community, including their underground (0–30 cm) parts (using the soil column method). All leaves were taken to the laboratory to measure the leaf areas, using a leaf area meter (LI-3000; Li-Cor, Lincoln, NE, USA). The aboveground and underground biomasses were then determined gravimetrically by oven drying to constant weight (Table 1). Soil samples were collected with a shovel

from soil profiles in each plot at depths of 0–20, 20–40 and 40–60 cm, each repeated three times, to measure the soil water content after drying at 105°C for 6 h (Table 2).

Measurement and Calculation of Canopy Photosynthesis

An assimilation chamber with an infrared gas analyzer (IRGA) can measure directly the CO_2 and H_2O fluxes of plant canopies (Boelman *et al.*, 2003; Burkart *et al.*, 2007; Shaver *et al.*, 2007). The assimilation chamber here, compared to previous one, had been improved: (1) installing the cooling components inside the chamber, automatically control air temperature in order not to increase the air temperature and vapor pressure deficit (VPD) in microclimate. (2) The assimilation chamber was made of acrylic, possessed transmissivity of over 95%, high abrasion resistance and hardness. (3) The canopy photosynthetic measurement system was auto-measuring all parameters continuously.

The canopy photosynthetic measurement system consisted of an LI-8100 automated soil CO_2 flux system (LI-COR, Lincoln, NE, USA) and an improved assimilation chamber (Beijing Ecotek Ltd. Co., Beijing, China). The LI-8100 measured the changes in CO_2 and H_2O concentrations in the chamber by an infrared gas analyzer. The 50 cm \times 50 cm \times 50 cm acrylic assimilation chamber, which had >95% transmissivity, was connected with the LI-8100, and placed on a pedestal fixed to the ground. The pedestal was a stainless steel square frame (49 cm internal side length, 51 cm external side length and 3 cm in height). There were no gaps between the pedestal and the assimilation chamber. The gas in the assimilation chamber passed into the infrared gas analyzer through the outlet within the LI-8100 at the start of the measurement, and then back into the assimilation chamber through the inlet after measurement of CO_2 and H_2O concentrations, forming a closed-circuit system.

The pedestal was placed on the ground 1 day before measurements were made. The measurements were taken on sunny days, for 4 min every 1 h between 8:00 and 18:00; they were repeated three times to obtain average values. Atmospheric pressure and temperature in the assimilation chamber were automatically recorded. Cleared all live aboveground plants in chamber after measurement, and then determined the leaf areas using a leaf area meter (LI-3000; Li-Cor, Lincoln, NE, USA). The “photosynthetic leaf area” refers to all green areas of plants, including green leaves, stems and pedicels (plant litter and non-green flowers and panicles are removed). The formula of Gao *et al.* (2010) for canopy photosynthetic rate calculation is as follows:

$$CAP = -\frac{V_A \cdot P \cdot \left(\frac{\partial C}{\partial t} - \frac{\partial C_S}{\partial t} \right)}{A \cdot (T + 273.15) \cdot R} \quad (\text{Eq. 1})$$

Where CAP is the canopy apparent photosynthetic rate ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); V_A is the total volume of the canopy photosynthetic measuring system (m^3), recorded

Table 1: General condition of the alpine wetland and e meadow plant communities

Type	Elevation (m)	Plant species composition		Soil type	Total vegetation coverage (%)	Aboveground biomass/g·m ²	Underground biomass/g·m ²	Leaf Area Index
Alpine wetland	3440	Dominant species	Common species	Peat soil	80	167±8 July 243±7 August	1755±43 July 2148±39 August	2.3 July 2.8 August
		<i>Carex muliensis</i> , <i>Kobresia setchwanensis</i>	<i>Blasmusino compressus</i> , <i>Deschampsia caespitosa</i> , <i>Koeleria cristata</i> , <i>Scirpus distigmaticus</i>					
Alpine meadow	3440	<i>Elymus nutans</i> , <i>Kobresia setchwanensis</i>	<i>Poa pratensis</i> , <i>Roegneria nutans</i> , <i>Carex moorcroftii</i> , <i>Pedicularis kansuensis</i> , <i>Roegneria nutans</i>	Alpine meadow soil	90	267±9 July 356±6 August	869±26 July 1276±32 August	2.8 July 3.3 August

Table 2: Soil mass water content of the alpine wetland and meadow plant communities

Month	Communities	Soil mass water content (%)			
		0-20 cm	20-40 cm	40-60 cm	Mean value
Late July	Alpine wetland	74.1 ± 0.9	52.9 ± 0.8	49.6 ± 1.2	58.9 ± 7.7
	Alpine meadow	16.1 ± 0.6	11.4 ± 0.2	10.0 ± 0.9	12.5 ± 1.9
Late August	Alpine wetland	74.2 ± 1.4	81.6 ± 0.5	84.2 ± 1.2	80.0 ± 3.0
	Alpine meadow	24.2 ± 0.3	13.4 ± 0.3	11.2 ± 0.0	16.3 ± 4.0

automatically; A is the total leaf area of the plant canopy in the assimilation chamber (m²); P is the atmospheric pressure (Pa); and T is the air temperature (°C) in the assimilation chamber automatically recorded by the LI-8100. R is the gas constant (8.314 Pa m³ mol⁻¹ K⁻¹); $\partial C/\partial t$ is the CO₂ changing rate in the canopy photosynthetic measurement (μmol·mol⁻¹·s⁻¹); and $\partial C_s/\partial t$ is the CO₂ changing rate in the soil respiration measurement (μmol·mol⁻¹·s⁻¹), all calculated by the accessory software FV8100 of the LI-8100.

CAT is the canopy apparent transpiration rate (mmol H₂O·m⁻²·s⁻¹); its formula is as follows:

$$CAT = \frac{V_A \cdot P \cdot (\frac{\partial W}{\partial t} - \frac{\partial W_s}{\partial t})}{A \cdot (T + 273.15) \cdot R} \quad (\text{Eq. 2})$$

Where $\partial W/\partial t$ is the H₂O changing rate in the canopy photosynthetic measurement (mmol·mol⁻¹·s⁻¹) and $\partial W_s/\partial t$ is the H₂O changing rate in the soil respiration measurement (mmol·mol⁻¹·s⁻¹), all calculated through linear regression of the H₂O concentration in the chamber to its corresponding time t. V_A, P, A, T and R are the same as Eq. 1.

In the following equations, canopy water use efficiency is CAW (mmol CO₂·mol⁻¹ H₂O) (Eq. 3); and light use efficiency is LUE (mmol CO₂·mol⁻¹ photons) (Eq. 4), where photosynthetic active radiation is PAR (μmol·m⁻²·s⁻¹):

$$CAW = \frac{CAP}{CAT} \quad (\text{Eq. 3})$$

$$LUE = \frac{CAP}{PAR} \quad (\text{Eq. 4})$$

Calculation of Daily Carbon Gains

The net ecosystem CO₂ uptake rate (NEC) is calculated from the change in CO₂ fluxes in the assimilation chamber, as determined by the canopy photosynthetic measurement system throughout day. The details of measurement could

be found in 2.3. The formula is:

$$NEC = - \frac{V_A \cdot P \cdot \frac{\partial C}{\partial t}}{S_A \cdot (T + 273.15) \cdot R} \quad (\text{Eq. 5})$$

Where the unit of NEC is μmol CO₂·m⁻²·s⁻¹; S_A is the soil area of the assimilation chamber (0.25 m²); V_A, P, $\partial C/\partial t$, T, R are the same as Eq. 1.

$$C_{\text{net gain}} = 12 \times 3600 \times 10^{-6} \times \sum_{i=1}^n [(NEC_{i+1} + NEC_i) \div 2 \times (T_{i+1} - T_i)] \quad (\text{Eq. 6})$$

Simplified to:

$$C_{\text{net gain}} = 0.0432 \times \sum_{i=1}^n [(NEC_{i+1} + NEC_i) \div 2 \times (T_{i+1} - T_i)] \quad (\text{Eq. 7})$$

Where C_{net gain} is the daily carbon net gains (g·m⁻²·d⁻¹), NEC_i is the instantaneous net ecosystem CO₂ uptake rate of the initial measurement point, NEC_{i+1} is the instantaneous net ecosystem CO₂ uptake rate of the next measurement point (μmol CO₂·m⁻²·s⁻¹), T_i is the instantaneous time of the initial measurement point, T_{i+1} is the instantaneous time of the next measurement point (h), n is measuring times throughout the day, 3600 is 1 h = 3600 s, 10⁻⁶ is 1 μmol = 10⁻⁶ mol and 12 is the molar mass of carbon (g·mol⁻¹).

Measurement and Calculation of Soil Respiration

Soil respiration includes microbial respiration, root respiration, faunal respiration and chemical oxidation of soil organic matter (Singh and Gupta, 1997). Soil respiration was measured with the LI-8100 and an improved assimilation chamber. After the canopy photosynthesis measurement, cleared all aboveground plants in the chamber. The soil respiration rate was restored to the level it had been before the plants had been removed after 24 h. We selected similar weather conditions to those used for canopy measurement and determined the day and night soil

respiration. The methods of installing and preparing the LI-8100 to measure soil respiration were the same as those used for the canopy photosynthesis measurement. The volume of the assimilation chamber was relatively large, and gas mixing took a much longer time, so 120 s was set as the invalid measurement time. Soil temperature and volumetric soil water content at 5 cm depth were monitored with a soil temperature and humidity monitoring system (ECH2O+EM50, Decagon, Pullman, Washington, USA).

The soil respiration measurement (R_s) is calculated as follows:

$$R_s = \frac{V_A \cdot P \cdot \frac{\partial C_s}{\partial t}}{S_A \cdot (T + 273.15) \cdot R} \quad (\text{Eq. 8})$$

Where S_A is the soil area of the assimilation chamber (0.25 m²), V_A , P , T , R and $\partial C_s / \partial t$ are the same as Eq. 1.

Measurement of Meteorological Factors

The daily dynamics of PAR, air temperature (T_a), atmospheric relative humidity (RH) and ambient CO₂ concentrations (C_a) were measured by the LI-6400 Portable Photosynthetic System (LI-COR, Lincoln, NE, USA) at the same time as the canopy photosynthetic measurements were made.

Statistical Analysis

One-way ANOVA was applied to analyze the differences among canopy photosynthetic parameters. Stepwise regression and path analysis were used to find the main factors affecting canopy photosynthesis with SPSS17.0. The software Origin 8.6 was used for mapping.

Results

Diurnal Changes in Micro-meteorological Factors

Fig. 1a indicates that, for the wetland, PAR reached its maximum at 12:00 with 1998 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in late July and 1684 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in late August. The daily mean PAR (08:00–18:00) in late July was $1204 \pm 111 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, significantly higher than that in August ($1026 \pm 68 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). The air temperature (T_a) largely followed PAR and peaked at 29°C in late July and 27°C in late August (Fig. 1b). The average temperature in late July was about 2°C above that in late August. The atmospheric RH in the wetland was high in the morning, then declined to its lowest value at 14:00, increasing slowly thereafter (Fig. 1c). The daily mean RH was $44 \pm 2\%$ in late July and $46 \pm 3\%$ in late August. There was a similar variation trend in ambient CO₂ (C_a) of the wetland over the 2 months; this ranged between 355 $\mu\text{mol} \cdot \text{mol}^{-1}$ and 384 $\mu\text{mol} \cdot \text{mol}^{-1}$ (Fig. 1d).

In the meadow, PAR peaked at 1890 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at 13:00 in late July and at 1795 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in late August (Fig. 1e). There was a marked decrease in daily mean PAR

in late August compared with that in late July. The daily average T_a in late July was 4°C higher than in late August (Fig. 1f). During the day, the RH values ranged between 24% and 63%, and the daily averages of $41 \pm 3\%$ in late July and $44 \pm 2\%$ in late August (Fig. 1g) show there was no significant difference. There was no significant difference in the daily mean C_a over the 2 months, which ranged between 343 $\mu\text{mol} \cdot \text{mol}^{-1}$ and 402 $\mu\text{mol} \cdot \text{mol}^{-1}$ (Fig. 1h). By contrast, the mean PAR, T_a and C_a of the meadow were higher than those of the wetland; whereas RH was opposite.

Canopy Photosynthesis and Response to Micro-meteorological Factors

In the alpine wetland, the diurnal changes in CAP showed a single-peak curve in both months (Fig. 2a). The CAP in late July reached the maximum at 12:00 with 2.8 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, and the maximum was 2.5 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at 13:00 in late August. The daily average value (08:00–18:00) shows there was no significant difference between the 2 months: $1.8 \pm 0.1 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in late July and $1.6 \pm 0.1 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in late August. The diurnal changes in CAT showed basically the same trend, and the daily peak values were 0.6 mmol H₂O · m⁻² · s⁻¹ in both months (Fig. 2b). There was no difference in the daily mean values of CAT between the 2 months. The daily CAW decreased from 8:00 and reached the minimum at 12:00, and then gradually increased in late July and August (Fig. 2c). The average CAW was $7.8 \pm 0.3 \text{ mmol CO}_2 \cdot \text{mol}^{-1} \text{ H}_2\text{O}$ in late July and $7.3 \pm 0.2 \text{ mmol CO}_2 \cdot \text{mol}^{-1} \text{ H}_2\text{O}$ in late August. LUE during the day in July and August decreased first and then increased, and the maximum appeared at 18:00 (Fig. 2d). LUE varied between 1.4 mmol CO₂ · mol⁻¹ photons and 1.7 mmol CO₂ · mol⁻¹ photons in late July, and between 1.2 mmol CO₂ · mol⁻¹ photons and 2.2 mmol CO₂ · mol⁻¹ photons in late July, but there was no difference in the daily average value between the 2 months.

In the alpine meadow, from Fig. 3a, the daily changes in CAP over the 2 months were all unimodal curves. The maximum CAP was 3.4 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at 14:00 in late July and 2.6 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at 13:00 in late August. The daily average values of CAP showed no differences between the 2 months, being $2.1 \pm 0.1 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in late July and $1.7 \pm 0.1 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in late August. Fig. 3b shows that the maximum CAT all appeared at 14:00 and then then began a slow decline. The daily average value in late July was higher than that in August, at 0.25 ± 0.01 and 0.22 ± 0.01 , respectively. The diurnal changes in CAW presented a downward trend from 08:00; the minimum appeared at 13:00 in late July and at 14:00 in late August (Fig. 3c). The daily changes in LUE were down at first and then gradually increased; its average values were almost the same (Fig. 3d). By contrast, the average CAP and CAW values were higher in the alpine meadow than in the alpine wetland, and the CAT value was higher in the alpine wetland than in the alpine meadow.

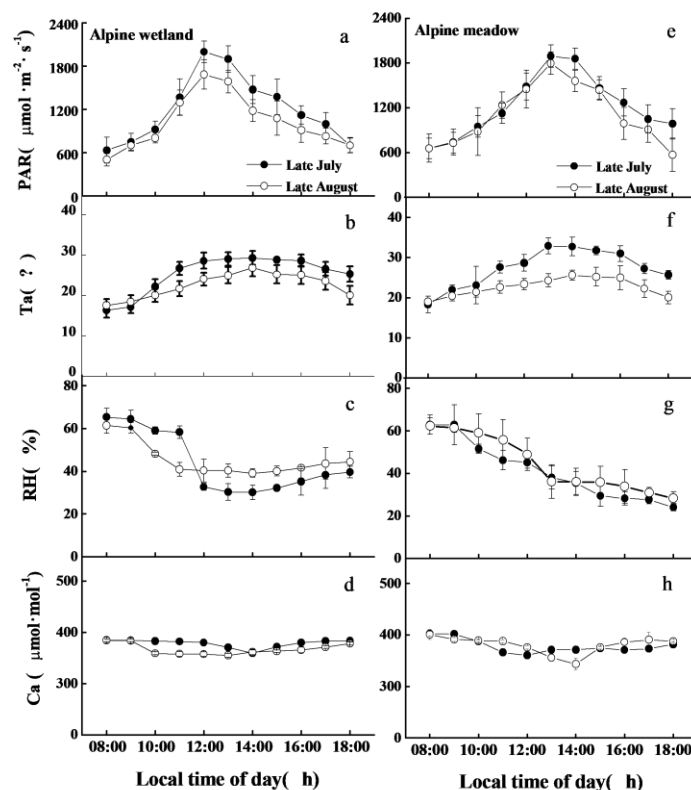


Fig. 1: Diurnal changes in meteorological factors of the alpine wetland and alpine meadow communities (mean \pm SD, N = 11) in late July and August PAR = photosynthetically active radiation, Ta = air temperature, RH = atmospheric relative humidity, Ca = ambient CO₂ concentration

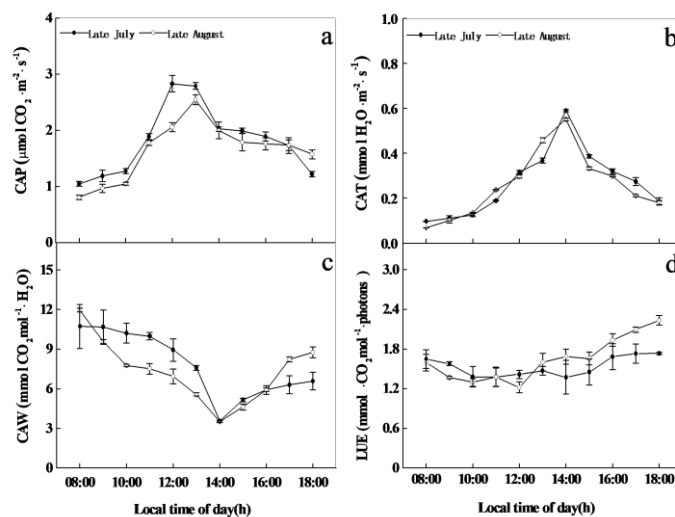


Fig. 2: Diurnal changes in CAP, CAT, CAW and LUE in the alpine meadow CAP = canopy apparent photosynthetic rate, CAT = canopy apparent transpiration rate, CAW = canopy apparent water use efficiency, LUE = light use efficiency

Descriptive and correlation analyses were done between CAP and environmental factors, PAR, Ta, RH, Ca, STa (soil temperature) and SVWC (soil volumetric water content), and their correlation coefficients are not shown

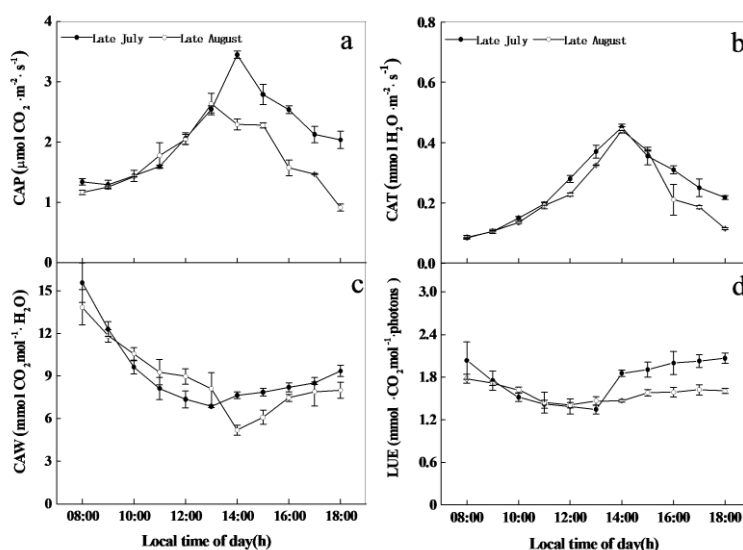
here. The equation of multiple linear regressions between the CAP and those environment factors was established (Table 3) by stepwise regression analyses. Some factors did not meet the $r = 0.05$ significance level of entry into the

Table 3: Multiple linear regression equations for canopy apparent photosynthetic rate of the alpine wetland and alpine meadow communities

Community	Month	Regression equations	R ²	F	Sig.
Alpine wetland	Late July	$P_n = -3.526 + 0.001PAR - 0.009RH + 0.011Ca$	0.993	159.741	0.000
	Late August	$P_n = 2.554 - 0.035RH + 0.001PAR$	0.926	24.016	0.000
Alpine meadow	Late July	$P_n = -8.288 + 0.002PAR - 0.024RH + 0.024Ca$	0.983	67.082	0.000
	Late August	$P_n = 0.256 + 0.001PAR$	0.991	473.619	0.000

Table 4: Path analysis of impact factors on canopy apparent photosynthetic rate

Community	Month	Variables	Direct effect	Σ	By PAR	By RH	By Ca
Alpine wetland	Late July	PAR	0.917	0.062			
		RH	-0.224	-0.500	-0.595	0.145	-0.083
		CO ₂	0.147	-0.662	-0.518	-0.144	
	Late August	PAR	0.467	0.376		0.376	
		RH	-0.436	-0.328	-0.328		
Alpine meadow	Late July	PAR	1.025	-0.126		0.249	-0.375
		RH	-0.18	-0.219	-0.532		0.313
		CO ₂	0.193	-1.085	-0.780	-0.305	
	Late August	PAR	0.991				

**Fig. 3:** Diurnal changes in CAP, CAT, CAW and LUE in the alpine wetland CAP = canopy apparent photosynthetic rate, CAT = canopy apparent transpiration rate, CAW = canopy apparent water use efficiency, LUE = light use efficiency

model, and the remaining factors strongly correlated with CAP. The F test showed that the value P of regression models was less than 0.01 in both months in the communities. On this basis, path analysis was carried out to learn the direct and indirect effect of environmental factors on the CAP. Table 4 shows that, in different months and communities, PAR had the highest positive direct effect on CAP. The RH of the wetland and meadow had a negative effect on the CAP in late August.

Daily Carbon Gains

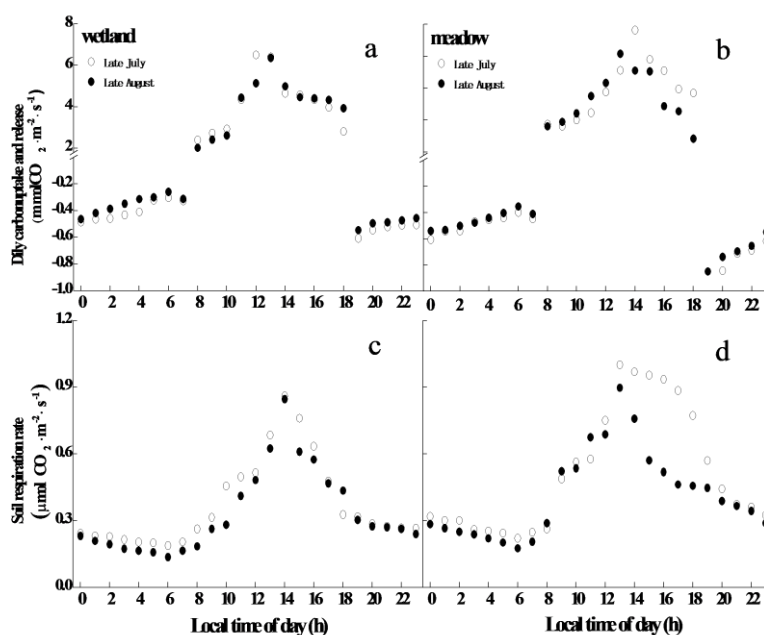
In the wetland, the course of the carbon uptake (8:00-18:00) as showed in Fig. 4a, had the same tendency in both months. In late July, it reached upwards of $6.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 12:00

and averaged $4.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whereas peaked at 13:00 with $6.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and averaged $4.1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in late August. The carbon release at night ranged between $0.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $0.6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and averaged at $0.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in late July, $0.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in late August. The total carbon uptake in the daytime was $2.0 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in late July, $1.9 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in late August. However, the total carbon release in the nighttime was $0.3 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in late July, $0.2 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in late August. Therefore, the total daily net carbon gains was $1.72 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in late July, $1.71 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in late August.

From Fig. 4b, in the meadow, the daytime mean values of carbon uptake and nighttime release in late July were $6.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $0.6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, $5.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $0.6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in late August, respectively.

Table 5: Comparisons of NEC_{max} in alpine wetland and alpine meadow communities

Type of community	Month	Altitude /m	LAI	$NEC_{max}/\mu\text{mol}\cdot\text{ground m}^{-2}\cdot\text{s}^{-1}$	$NEC_{max}/LAI/\mu\text{mol}\cdot\text{leaf m}^{-2}\cdot\text{s}^{-1}$
Alpine Zone (Alps) ¹					
<i>Carex</i> community	I	2470	1.8	9.5	5.3
Upper montane zone(Caucasus) ²					
<i>Hordeum</i> dom. community	II	1850	5.4	19	3.5
<i>Deschampsia</i> dom. community	II	1750	5.8	22	3.8
Short and long grass Prairie (US) ²					
Low density short and tall grass	II	ca 2000	1.5	ca 6	ca 4
High density tall grass	II	ca 1500	ca 3	ca 15	ca 5
Alpine wetland (ZoigePlateau)	III	3400	2.3	6.5	2.8
<i>Carex</i> dom. community					
Alpine meadow(ZoigePlateau)	III	3400	2.8	9.6	3.4
<i>Elymus</i> dom. community					

References: ¹Diemer (1994), ²Tappeiner and Cernusca (2006). I, July; II, Late July and early August; III Late July**Fig. 4:** Carbon budget and soil respiration rate of the alpine wetland and alpine meadow throughout the day

The daytime carbon uptake was far above the night carbon release in both months. The total daily net carbon gains was $2.5 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in late July, which is greater than that $2.2 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in late August. By contrast, the whole-day carbon gains of the meadow are higher than that of the wetland in Late July and August.

It can be seen from Fig. 4c and 4d, the diurnal changes of the soil respiration (R_s) had the similar variation trend in two months and communities. The daily average R_s had no significant differences between two months in the wetland, while there were marked differences between two months in the meadow. The daytime average values in wetland and meadow were $0.5 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $0.7 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively, with remarkable difference. The nighttime average of R_s in wetland was much higher than that in meadow, $0.3 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $0.2 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Discussion

The results regarding canopy photosynthesis showed that, in late July and late August, the diurnal changes in CAP between the two communities were unimodal type. The "midday depression" phenomenon (Flexas and Medrano, 2002; Haldimann and Feller 2004; Salvucci and Crafts-Brandner, 2004; Good and Izawa, 2017), in leaf scale, may be induced by high light, high temperature, drought, etc. In our study, canopy photosynthesis of wetland and meadow communities on the Zoige Plateau did not present this phenomenon. It might be due to the "non-depression" of leaves themselves or offset effects of leaves group. They reached their maximum at about 12:00–14:00, because solar radiation was strongest during this period, and other environmental conditions were relatively favorable to the photosynthesis of communities. The daily average of CAP in

late July exceeded that in late August, being 13% in the wetland and 24% in the meadow. The enhanced CAP indicated more vigorous growth in late July. From late July to late August, the CAP decreased, and aboveground and underground biomass increased (Table 1). We identified late July and late August as the vigorous growth phase and the large biomass phase, respectively. There were notable differences in PAR and Ta between July and August ($P < 0.01$), while other factors remained relatively stable. Consequently, the increases in PAR and Ta might account for the enhanced photosynthesis in July. In the same month but various sites, the daily CAP average in the meadow was slightly higher than that in the wetland, being 10% in late July and 6% in late August, respectively.

At the same sites but in different months, the variations in canopy photosynthesis parameters mainly came from the changes of micro-meteorological factors. The high PAR and Ta promoted the canopy photosynthesis. In the same month but different plots, since two plots were not far from each other, the micro-meteorological remained stable. The diversities in photosynthetic characteristics of two communities came from the difference of community composition, community structure, soil factor, and so on. Liu *et al.* (2015) concluded that, in leaf scale, gramineae plants had higher photosynthetic rate than cyperaceae plants. Here, dominant species in both communities were cyperaceae plants, but gramineae plants had a greater percentage in meadow community. The larger percentage might be one of reasons for enhanced CAP in meadow.

Transpiration is an essential physiological process related to plant water status. CAT increased with light intensity and temperature from 08:00 to 14:00, reached its maximum at about 14:00 and then decreased as light intensity and temperature declined, which was similar to CAP. Under different conditions and in different months, all CAWs began to decrease at 08:00, and reached its lowest point at noon, then began to rise slowly. Due to variations in soil mass water content, wetland transpiration was higher than that in the meadow in both July and August, but not remarkably. The relatively high CAP and low transpiration rates determined the comparatively high WUE in the meadow community. The LUE, the efficiency of carbon assimilation using solar energy, is the key to assessing productivity. Despite the difference of community composition and structure, the diurnal average values of LUE in both wetland and meadow communities were about $1.5 \text{ mmol CO}_2 \cdot \text{mol}^{-1} \text{ photons}$. Moreover, we noticed that soil mass water content in the wetland was more than four times that in the meadow and had obvious variations in two months (Table 2). We concluded, in our study, that the addition of water seemed to have no significant effects on the LUE. Similar results were reported by Bowman *et al.* (1995), who observed no effects of water addition on LUE in Rocky Mountains species, in both wet and drained locations. However, in similar time and places, the LUE of the leaves of dominant species in the wetland and meadow

communities was about $12.3 \text{ mmol CO}_2 \cdot \text{mol}^{-1} \text{ photons}$ and $11.7 \text{ mmol CO}_2 \cdot \text{mol}^{-1} \text{ photons}$, respectively (Zhou *et al.*, 2017). We think that the huge difference of LUE between leaf and canopy mainly comes from optimal measurement conditions in leaf scale (non-shading, optimum shooting angle, mature leaves, etc.).

The major limitation of alpine plant photosynthesis response to environment confirmed many times is PAR (Körner and Diemer, 1987; Tian *et al.*, 2009). In both months and locations, PAR presented the largest direct path coefficients, indicating the strongest direct impact on CAP among all the parameters included in the model. As it had no significant association with CAP, Ta was excluded from the process of stepwise regression. In this study it appeared to give us a “counterintuitive” conclusion: Ta was not one of main factors that influenced photosynthesis. The explanation for this is the physiological thermal acclimation of alpine plants: the temperature optimum of photosynthesis shifts with PAR so that the optimum is at low temperatures (when PAR is low) and at high temperatures (when PAR is high); readjustments of the temperature optimum to prevailing temperatures are relatively fast (Körner, 1982; Rada, 1998). Atmospheric humidity may indirectly influence photosynthesis. RH influences the VPD, and then impacts on stomata. At the community level, however, dense leaf canopies with high aerodynamic resistance (Lösch and Mülders, 1994) buffer many alpine plants against such influences. Apart from the meadow in August, RH had a directly restrictive role on photosynthesis. RH of the wetland produced a greater effect on CAP than that of meadow. In addition, RH had indirectly restrictive effects on photosynthesis, mainly by its negative correlation with PAR. Ca, in our study, had a non-significant direct influence on photosynthesis. Combining the above analysis, we conclude that PAR is the main determining factor in the diurnal variation of photosynthesis; RH has a directly restrictive role on photosynthesis, and had stronger negative effects on photosynthesis in the wetland than that in the meadow.

As regards daily net gains, the CO_2 uptake of alpine vegetation has previously been measured on only a few occasions. In Table 5, we limited the discussion to a range of low-stature alpine vegetation communities. LAI (leaf area index), which is strongly associated with the NEC of plant communities (Yao *et al.*, 2016; Badgley *et al.*, 2017), was included as a reference index, and latitude was treated as a comprehensive environmental parameter (latitude change can induce variations in air temperature, CO_2 concentration and solar radiation intensity). Here we used the parameter NEC/LAI (Table 5). It translates the NEC from per unit ground area to per unit leaf area, and thus reflects net ecosystem CO_2 uptake rate in unit leaf area. We noticed that the expression may be not reasonable, because it has no practical implication if soil respiration rate were translated to per unit leaf area. Soil respiration has a small proportion of total productivity (Fig. 4), so the NEC/LAI here can be used for numeric comparison. We can see from Table 5 that the

maximum NEC/LAI (NEC_{max}/LAI) from other results fluctuated in the range 3.5–5.3, mainly concentrating on values of about 4. Despite the complex variations in living conditions, community composition and structure, the values of (NEC_{max}/LAI) from others (Diemer, 1994; Tappeiner and Cernusca, 2006) remained generally stable. Street *et al.* (2007) came to a very similar conclusion: canopy leaf area is an excellent predictor of productivity in diverse low arctic tundra, across a wide range of plant functional types and can explain 80% of the variation in productivity by leaf area alone. Our results— NEC_{max}/LAI of wetland $2.8 \mu\text{mol}\cdot\text{leaf m}^{-2}\cdot\text{s}^{-1}$ and meadow $3.4 \mu\text{mol}\cdot\text{leaf m}^{-2}\cdot\text{s}^{-1}$ —were roughly consistent with the data in Table 5, which confirmed our measurements to some extent.

The CO_2 release includes the respiration of the aboveground parts of plants and soil respiration. Soil respiration (microbial respiration, root respiration, faunal respiration and the chemical oxidation of soil organic matter), represents the sum total of all soil metabolic processes in which CO_2 is produced. Temperature is the main external driver for respiration (Carbone *et al.*, 2015; Thomey *et al.*, 2015; Carey *et al.*, 2016). Soil respiration in July was higher than in August because of higher soil temperatures in the same site. Soil moisture also had an obvious effect on soil respiration (Bao *et al.*, 2016; Hawkes *et al.*, 2017). The soil respiration of the meadow was higher than that of the wetland. The restrictions imposed by excessive soil moisture in the wetland might be one of the reasons. We also noticed that the underground biomass in the wetland was almost twice that in the meadow (Table 1), thus the relatively low soil respiration in the wetland might be explained by limitation of excessive soil moisture and the small ratio of roots respiration to soil respiration. Soil respiration during the daytime accounted for about 65% of soil respiration throughout the day in both sites, and the percentage had no obvious variations between the 2 months.

The CO_2 uptake through photosynthesis and losses through respiration determine net carbon gains in plants and entire communities. The CO_2 release at night (Fig. 4) was about 10% of net carbon gain in the day. By subtracting soil respiration from total CO_2 release, we were able to obtain the aboveground plant respiration at night. Aboveground plant respiration was about 50% of total CO_2 release, and thus CO_2 releases at night by the aboveground parts of plants and soil respiration were at a ratio of roughly 1:1. The daily net carbon gain in the wetland was $1.72 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in late July and $1.71 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in late August, and the corresponding values in the meadow were $2.7 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ and $2.2 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, respectively. Although the soil respiration of meadow was stronger than that of wetland, the net gain in meadow was higher than wetland's gain. The relatively higher CAP (carbon fixation rate in per unit leaf area) and LAI (leaf area index) both accounted for the increased net gain in meadow. We inferred that the stronger net gain in meadow might induce more pasture yield.

Conclusion

Here we made comparisons of canopy photosynthetic characteristics between two typical alpine communities in two important phenological phases, and analyzed the environmental factors that affected the canopy photosynthetic rate. Meanwhile, the daily net carbon gain of two communities was got. Though contrastive measurements of having and not having aboveground plants, we divided CO_2 flux into aboveground parts (CAP) and soil respiration, and HO_2 flux into plant transpiration (CAT) and soil evaporation. Thus, this can help to advance our understanding of community photosynthetic characteristics.

Acknowledgments

The first author acknowledges the financial grant from the Strategic Priority Research Program of Chinese Academy of Sciences (XDA20050102), the National Natural Science Foundation of China (41701106) and the People's Livelihood Science and Technology Project of Gansu, China (17CX1FP076).

References

- Badgley, G., C.B. Field and J.A. Berry, 2017. Canopy near-infrared reflectance and terrestrial photosynthesis. *Sci. Adv.*, 3: e1602244
- Bai, J., Q. Lu, Q. Zhao, J. Wang and H. Ouyang, 2013 Effects of alpine wetland landscapes on regional climate on the Zoige Plateau of China. *Adv. Meteorol.*, 5: 1–7
- Baldocchi, D.D. and J.S. Amthor, 2001 Canopy photosynthesis: History, measurements, and models. *Terr. Glob. Prod.*, Chapter 2, pp: 10–32
- Bao, X.Y., X.X. Zhu, X.F. Chang, S.P. Wang, B. Xu and C.Y. Luo, 2016. Effects of soil temperature and moisture on soil respiration on the Tibetan Plateau. *Plos One*, 11: 1–14
- Boelman, N.T., M. Stieglitz, H.M. Rueth, M. Sommerkorn, K.L. Griffin, G.R. Shaver and J.A. Gamon, 2003. Response of NDVI, biomass, and ecosystem gas exchange to long-term warming and fertilization in wet sedge tundra. *Oecologia*, 135: 414–421
- Bowman, W.D., T.A. Theodose and M.C. Fisk, 1995. Physiological and production responses of plant growth forms to increases in limiting resources in alpine tundra: implications for differential community response to environmental change. *Oecologia*, 101: 217–227
- Burkart, S., R. Manderscheid and H.J. Weigel, 2007. Design and performance of a portable gas exchange chamber system for CO_2 - and H_2O - flux measurements in crop canopies. *Environ. Exp. Bot.*, 61: 25–34
- Carbone, M.S., G.C. Winston and S.E. Trumbore, 2015. Soil respiration in perennial grass and shrub ecosystems: Linking environmental controls with plant and microbial sources on seasonal and diel time scales. *J. Geop. Res. Biogeosci.*, 113: 426–426
- Carey, J.C., J. Tang, P.H. Templer, K.D. Kroeger, T.W. Crowther and A.J. Burton, 2016. Temperature response of soil respiration largely unaltered with experimental warming. *Proc. Nat. Acad. Sci. USA*, 113: 13797
- Diemer, M., 1994 Mid-season gas exchange of an alpine grassland under elevated CO_2 . *Oecologia*, 98: 429–435
- Flexas, J., F. Loreto and H. Medrano, 2012. *Terrestrial Photosynthesis in a Changing Environment: A Molecular, Physiological, and Ecological Approach*. Terrestrial photosynthesis in a changing environment, CUP, Cambridgeshire, UK
- Flexas, J. and H. Medrano, 2002. Drought-inhibition of photosynthesis in C_3 plants: stomatal and non-stomatal limitations revisited. *Ann. Bot.*, 89: 183–189

- Gao, S., P.X. Su, Q.D. Yan and S.S. Ding, 2010. Canopy and leaf gas exchange of *Haloxylon ammodendron* under different soil moisture regimes. *Sci. Chin. Life Sci.*, 53: 718–728
- Good, N.E. and S. Izawa, 2017. Inhibition of photosynthesis. *Centr. Jpn. J. Ortho. Surg. Traum.*, 43: 805–806
- Haldimann, P. and U. Feller, 2004. Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens* L.) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1, 5-bisphosphate carboxylase/oxygen. *Plant Cell Environ.*, 27: 1169–1183
- Hawkes, C.V., B.G. Waring, J.D. Rocca and S.N. Kivlin, 2017. Historical climate controls soil respiration responses to current soil moisture. *Proc. Nat. Acad. Sci. U.S. Amer.*, 114: 6322
- Henneberger, R., S. Cheema, A.G. Franchini, A. Zumsteg, J. Zeyer, 2015. Methane and carbon dioxide fluxes from a European alpine fen over the snow-free period. *Wetlands*, 35: 1149–1163
- Körner, Ch., 1982 CO₂ exchange in the alpine sedge *Carex curvula* as influenced by canopy structure, light and temperature. *Oecologia*, 53: 98–104
- Körner, Ch. and M. Diemer, 1987. In situ photosynthetic responses to light, temperature and carbon dioxide in herbaceous plants from low and high altitude. *Funct. Ecol.*, 1: 179–194
- Liu, M.X., Y.Y. Liu, S.W. Chen and Q. An, 2015. Plant photosynthetic characteristics along slope gradients in an alpine meadow region on the Eastern edge of Qinghai-Tibetan Plateau. *Soil Crop*, 4: 104–112
- Lösch, R. and P. Mülders, 1994. Scientific results of the BRYOTROP expedition to Zaire and Rwanda 3. photosynthetic gas exchange of bryophytes from different forest types in eastern Central Africa. *Trop. Bryol.*, 9: 169–185
- Rada, F., 1998. Effects of temperature on photosynthesis of two morphologically contrasting plant species along an altitudinal gradient in the tropical high Andes. *Oecologia*, 114: 145–152
- Salvucci, M.E. and S.J. Crafts-Berner, 2004. Inhibition of photosynthesis by heat stress: the activation state of rubisco as a limiting factor in photosynthesis. *Physiol. Plant.*, 120: 179–186
- Shaver, G.R., E.B. Rastetter and M. Williams, 2007. Functional convergence in regulation of net CO₂ flux in heterogeneous tundra landscapes in Alaska and Sweden. *J. Ecol.*, 95: 802–817
- Singh, J.S. and S.R. Gupta, 1997. Plant decomposition and soil respiration in terrestrial ecosystems. *Bot. Rev.*, 43: 449–528
- Street, L.E., G.R. Shaver, M. Williams and W. Mtván, 2007. What is the relationship between changes in canopy leaf area and changes in photosynthetic CO₂ flux in arctic ecosystems? *J. Ecol.*, 95: 139–150
- Su, P.X., Z.J. Zhou, R. Shi and T.T. Xie, 2018. Variation in basic properties and carbon sequestration capacity of an alpine sod layer along moisture and elevation gradients. *Acta Ecol. Sin.*, 38: 1–13
- Tappeiner, U. and A. Cernusca, 2006. Microclimate and fluxes of water vapor, sensible heat and carbon dioxide in structurally differing subalpine plant communities in the central Caucasus. *Plant Cell Environ.*, 19: 403–417
- Thomey, M.L., S.L. Collins, R. Vargas, J.E. Johnson, R.F. Brown, D.O. Natvig and M.T. Friggens, 2015. Effect of precipitation variability on net primary production and soil respiration in a Chihuahuan Desert grassland. *Glob. Change Biol.*, 17: 1505–1515
- Tian, Y.Q., Q. Gao, Z.C. Zhang and K. Zhu, 2009. The advances in study on plant photosynthesis and soil respiration of alpine grasslands on the Tibetan Plateau. *Ecol. Environ. Sci.*, 18: 711–721
- Vieira, J., F. Campelo, S. Rossi, A. Carvalho, H. Freitas and C. Nabais, 2015. Adjustment capacity of maritime pine cambial activity in drought-prone environments. *Plos One*, 5: e0126223
- Wang, Z., M.Y. Bader, X. Liu, Z.M. Zhu and W.K. Bao, 2017. Comparisons of photosynthesis-related traits of 27 abundant or subordinate bryophyte species in a subalpine old-growth fir forest. *Ecol. Evol.*, 7: 7454–7461
- Wohlfahrt, G., M. Anderson-Dunn, M. Bahn, F. Berninger, and C. Campbell, 2008. Biotic, abiotic, and management controls on the net ecosystem CO₂ exchange of European mountain grassland ecosystems. *Ecosystems*, 11: 1338–1351
- Yao, H.S., Y.L. Zhang, X.P. Yi, X.J. Zhang and W.F. Zhang, 2016. Cotton responds to different plant population densities by adjusting specific leaf area to optimize canopy photosynthetic use efficiency of light and nitrogen. *Field Crops Res.*, 188: 10–16
- Zhang, L.M., G.R. Yu, X.M. Sun, X.F. Wen, C.Y. Ren, Y.L. Fu, Q.K. Li, Z.Q. Li, Y.F. Liu, D.X. Guan and J.H. Yan, 2006. Seasonal variations of ecosystem apparent quantum yield (α) and maximum photosynthesis rate (P_{max}) of different forest ecosystems in China. *Agric. For. Meteorol.*, 137: 176–187
- Zhao, N.N., S. Gou, B.B. Zhang, Y.L. Yu and S.J. Han, 2017. Changes in pan evaporation and their attribution to climate factors in the Zoige alpine wetland, the eastern edge of the Tibetan Plateau (1969–2014). *Water*, 9: 971
- Zhong, Z.P., K.B. Wang, J.X. Shan, L.J. Feng, Y.Y. Xin, L.B. Li and T.Y. Kuang, 2001. Studies on photosynthetic characteristics and adaption to environment of some alpine plants in Qinghai-Xizang Plateau. *Sci. Access*, 3: 1–7
- Zhou, Z.J., P.X. Su, R. Shi and T.T. Xie, 2017. Light use efficiency of plants and environmental impact factors in different alpine ecosystems. *Chin. J. Ecol.*, 36: 1570–1577

(Received 22 March 2018; Accepted 13 April 2018)