

RESEARCH ARTICLE

Stable Water Use Efficiency of Tibetan Alpine Meadows in Past Half Century: Evidence from Wool $\delta^{13}\text{C}$ Values

Hao Yang*, Nianpeng He, Yongtao He, Shenggong Li, Peili Shi, Xianzhou Zhang

Key Laboratory of Ecosystem Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China

* yangh@igsnr.ac.cn



CrossMark
click for updates

OPEN ACCESS

Citation: Yang H, He N, He Y, Li S, Shi P, Zhang X (2015) Stable Water Use Efficiency of Tibetan Alpine Meadows in Past Half Century: Evidence from Wool $\delta^{13}\text{C}$ Values. PLoS ONE 10(12): e0144752. doi:10.1371/journal.pone.0144752

Editor: Alexandra Weigelt, University of Leipzig, GERMANY

Received: May 24, 2015

Accepted: November 23, 2015

Published: December 11, 2015

Copyright: © 2015 Yang et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by Natural Sciences Foundation of China (<http://www.nsf.gov.cn/>) grants 31470506 (NH) and 31100336 (HY), and Knowledge Innovation Project of the Chinese Academy of Sciences (<http://english.igsnr.cas.cn/>) grant 201003011 (HY). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Understanding the influences of climatic changes on water use efficiency (WUE) of Tibetan alpine meadows is important for predicting their long-term net primary productivity (NPP) because they are considered very sensitive to climate change. Here, we collected wool materials produced from 1962 to 2010 and investigated the long-term WUE of an alpine meadow in Tibet on basis of the carbon isotope values of vegetation ($\delta^{13}\text{C}_{\text{veg}}$). The values of $\delta^{13}\text{C}_{\text{veg}}$ decreased by 1.34‰ during 1962–2010, similar to changes in $\delta^{13}\text{C}$ values of atmospheric CO_2 . Carbon isotope discrimination was highly variable and no trend was apparent in the past half century. Intrinsic water use efficiency (W_i) increased by $18 \mu\text{mol}\cdot\text{mol}^{-1}$ (approximately 23.5%) during 1962–2010 because the increase in the intercellular CO_2 concentration ($46 \mu\text{mol}\cdot\text{mol}^{-1}$) was less than that in the atmospheric CO_2 concentration (C_a , $73 \mu\text{mol}\cdot\text{mol}^{-1}$). In addition, W_i increased significantly with increasing growing season temperature and C_a . However, effective water use efficiency (W_e) remained relatively stable, because of increasing vapor pressure deficit. C_a , precipitation, and growing season temperature collectively explained 45% of the variation of W_e . Our findings indicate that the W_e of alpine meadows in the Tibetan Plateau remained relatively stable by physiological adjustment to elevated C_a and growing season temperature. These findings improve our understanding and the capacity to predict NPP of these ecosystems under global change scenarios.

Introduction

The Tibetan Plateau, referred to as the Earth's "third pole," is highly sensitive to climate change, and climate warming has been widely observed here during the past several decades [1–2]. The mean annual temperature (MAT) increased from 1961 to 2010, accompanied by a strong increase in atmospheric CO_2 concentration (C_a) and a slight decrease in photosynthetically active radiation, although the mean annual precipitation (MAP) did not vary apparently during this period [2–3]. The way in which these changes in climate and C_a influence the net primary productivity (NPP) of alpine meadows is of great concern [2]. The NPP of grasslands

generally increases with increasing promotion of plant growth by precipitation or increased water use efficiency (WUE) related to the leaf area index [4]. The observed increases in growing season temperature (GST) and C_a in the Tibetan Plateau may increase Rubisco enzyme activity, stimulate leaf photosynthesis, enhance NPP, and result in higher WUE [5–6]. Here, we assumed that the WUE of alpine meadows would increase under scenarios of warming and increasing C_a and result in higher NPP in the Tibetan Plateau as shown by Piao and others [2].

Various methods have been used to evaluate WUE of vegetation, including: 1) estimation from measurements of photosynthetic parameters [7]; 2) indirect estimation from the carbon isotope composition ($\delta^{13}\text{C}$) of leaves [8]; 3) calculation from measurements of vegetation biomass and the biomass/precipitation index [9]; and 4) calculation from eddy covariance measurements of CO_2 and H_2O fluxes [4]. Photosynthesis- and $\delta^{13}\text{C}$ -based methods are generally used at the species or individual plant levels; biomass- and eddy covariance-based methods are performed for ecosystem-level assessments. Because of limitations related to plant lifespan, sampling, and measurement techniques, reports on long-term ecosystem-level WUE of grassland are limited. In the Tibet Plateau, most previous studies on WUE or $\delta^{13}\text{C}$ values focused on the species or individual plant levels. For example, the WUE of perennials were found to be higher than that of annuals in terms in individual plants as shown by plant carbon isotope measurements [10]. At the ecosystem level the WUE of alpine meadow is higher at the middle of the growing season and low at the beginning and end of the growing season during a year, and it is higher in a wet year than in a normal year as revealed by eddy covariance [4, 11]. However, it is not clear how the WUE of grasslands changes over longer time scales at the ecosystem level.

New approaches using $\delta^{13}\text{C}$ values of animal tissues such as horns have been successfully used and the stable long-term and ecosystem-level WUE in the Alps alpine grassland was previously reported [12]; the isotopic composition of these tissues can reflect spatiotemporal information about vegetation in grazed areas. An apparent ^{13}C enrichment of wool related to the animal's diet, called the carbon isotopic "diet-wool shift" (ϵ) [13–15], results from ^{13}C fractionation during digestion or metabolism [16]. The ^{13}C discrimination of vegetation ($^{13}\Delta_{veg}$) can be calculated, and long-term and ecosystem-level WUE can be deduced based on ϵ , the $\delta^{13}\text{C}$ values of animal tissues, and the $\delta^{13}\text{C}$ value of atmospheric CO_2 ($\delta^{13}\text{C}_{air}$).

On the Tibetan Plateau, wool-based products such as fur coats and rugs have been preserved by farmers and monks, and these materials provide an opportunity to assess samples produced in different years. In this study, we collected samples of wool products produced from 1962 to 2010 in a natural alpine meadow region of the Tibetan Plateau and analyzed the $\delta^{13}\text{C}$ values of the samples. Our objectives were to 1) assess long-term changes in WUE of alpine meadows at the ecosystem level, 2) explore the underlying drivers of WUE, and 3) examine the hypothesis that WUE of alpine meadows increases and may result in higher NPP on the Tibetan Plateau under a warming climate with increasing C_a . Our findings can improve the capacity to predict NPP of alpine meadows under global change scenarios.

Materials and Methods

Study area

The study area, a natural alpine meadow on the southern Tibetan Plateau, is located in Damxung County ($90^{\circ}45' - 91^{\circ}31'\text{E}$, $29^{\circ}31' - 30^{\circ}25'\text{N}$, 10036 km^{-2} including 30% grassland area) in the Xizang Autonomous Region, People's Republic of China (Fig 1). Damxung County has a mean elevation of 4200 m and a plateau monsoon climate. MAT is approximately 1.3°C , and MAP is 477 mm, the majority of which (90%) falls during the growing season (June–September). The dominant plant species in the meadow are *Stipa capillacea*, *Carex montis-everestii*,

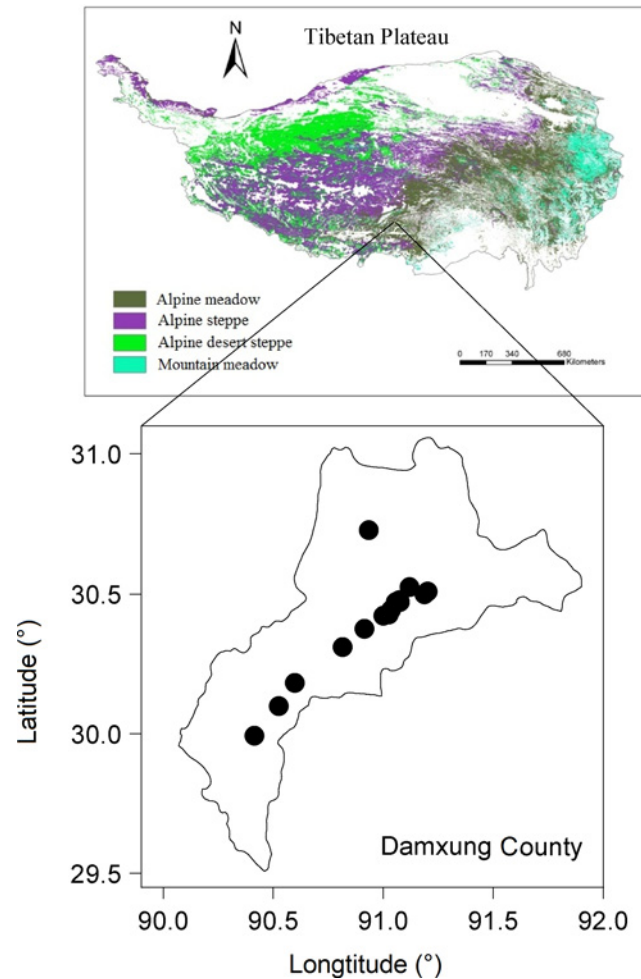


Fig 1. Sampling sites in Damxung County, Tibetan Plateau. This figure is a modification from the Vegetation Map of The People's Republic of China (1:1000000) [38].

doi:10.1371/journal.pone.0144752.g001

and *Kobresia pygmaea*, together accounting for 69% of total aboveground biomass (measured during 2009–2012 by the Damxung Grassland Research Station [91°05'E, 30°25'N], Chinese Academy of Sciences). Twenty-one plant species were observed during 2009–2012, and all are C_3 species. The study area is freely grazed by sheep and yak, along with a low number of goats; the mean number of livestock in 1985–2010 was 0.55 ± 0.02 million on an area of approximately 0.3 million ha, which suggests that the grazing pressure was low and varied little. Winter forage consists mainly of dry grasses that are harvested locally in August.

Sampling

In September 2011, 15 villages in Damxung County (4230–4300 m a.s.l.) were selected randomly as sampling sites (Fig 1). Wool materials from local sheep, including fur coats, rugs, and recently sheared wool, were obtained from farmers and monks. Wool samples were dated by asking farmers and monks about the age of the samples. The sample size for each year varied from 1 to 12 depending on how many samples we were able to collect. No specific permission was required for these sampling sites and activities because the temples were open and sampling was permitted by monks and farmers. The wool was only produced by sheep, not

endangered or protected species. In total, 106 wool samples dating from 1962 to 2010 were collected. Three assumptions are made for deriving temporal information about vegetation from these samples [12]: that (i) sheep grazed on the same meadows every year, (ii) the dietary preferences of sheep did not change over time, and (iii) the value of ϵ was stable among individual sheep.

Sample preparation and carbon isotope analysis

The wool samples were cleaned following the procedure of Schwertl and others [17], and samples (0.2–0.4 mg) were packed into tin cups for isotope analysis. The $\delta^{13}\text{C}$ values of wool ($\delta^{13}\text{C}_{\text{wool}}$) were measured using an elemental analyzer (NA 1110; Carlo Erba, Milan) interfaced (ConFlo III; Finnigan MAT, Bremen) with an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT). The $\delta^{13}\text{C}_{\text{wool}}$ values were specified as $\delta^{13}\text{C}$ relative to the Vienna Pee Dee Belemnite (VPDB) standard:

$$\delta^{13}\text{C}_{\text{wool}} = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \quad (1)$$

where R_{sample} and R_{standard} are the ratios of $^{13}\text{C}/^{12}\text{C}$ in the samples and standard, respectively. Each sample was measured against a laboratory working CO_2 standard, which was previously calibrated against an International Atomic Energy Agency secondary standard (IAEA-CH6, calibration accuracy of 0.06‰ SD). After every tenth sample, a solid internal lab standard (SILS) with a C/N ratio similar to that of the sample material was run as a blind control. The SILSs were previously calibrated against IAEA-CH6. The precision of the repeated sample was 0.11‰.

Estimating vegetation $\delta^{13}\text{C}$

The values of ϵ , the vegetation to wool fractionation, are approximately 2–4‰ in C_3 or C_3/C_4 mixed vegetation [15, 17, 18, 19] and are independent of altitude [13]. Based on a previous study by Maennel and others [13] from a region with similar background (C_3 plants grazed by sheep, high altitude), 3.2‰ was used as ϵ . Hence, $\delta^{13}\text{C}_{\text{veg}}$ was estimated as

$$\delta^{13}\text{C}_{\text{veg}} = \delta^{13}\text{C}_{\text{wool}} - 3.2\text{‰} \quad (2)$$

Estimating WUE

The $^{13}\Delta_{\text{veg}}$ is derived from $\delta^{13}\text{C}_{\text{veg}}$ and $\delta^{13}\text{C}_{\text{air}}$:

$$^{13}\Delta_{\text{veg}} = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{veg}}}{1 + \delta^{13}\text{C}_{\text{veg}}/1000} \quad (3)$$

The $^{13}\Delta_{\text{veg}}$ value captures the main drivers of photosynthetic carbon isotope fractionation [20]. Farquhar and others [8] reported that $^{13}\Delta_{\text{veg}}$ depends on the relationship between the photosynthetic carbon assimilation rate (A) and stomatal conductance (g_s), which determines the ratio of intercellular to atmospheric CO_2 concentrations (C_i/C_a) in C_3 plants:

$$\frac{C_i}{C_a} = \frac{13\Delta_{\text{veg}} - a}{b - a} \quad (4)$$

where a is the discrimination of ^{13}C during the diffusion of CO_2 through stomata (4.4‰) and b is the net fractionation by carboxylation (27‰).

Hence, intrinsic water use efficiency (W_i) can be calculated as follows:

$$W_i = \frac{A}{g_s} = \frac{C_a - C_i}{1.6} = \frac{C_a(1 - \frac{C_i}{C_a})}{1.6} \quad (5)$$

where g_s is leaf stomatal conductance of water vapor and 1.6 is the ratio of the diffusivity of water vapor and C_a . W_i is regarded as the potential WUE assuming a constant evaporative demand and is used to assess long-term trends in the balance between carbon gain and intrinsic water loss of plants. Under variable environment conditions, effective WUE (W_e) is used as the actual WUE because it considers the effect of the water vapor pressure concentration gradient between intercellular spaces and the atmosphere (v) [8]:

$$W_e = \frac{A}{E} = \frac{C_a - C_i}{1.6v} = \frac{C_a(1 - \frac{C_i}{C_a})}{1.6v} \quad (6)$$

where E is the leaf transpiration rate.

Climate data, C_a , and $\delta^{13}C_{air}$

Climate data for Damxung station were obtained from the China Meteorological Administration (CMA). C_a and $\delta^{13}C_{air}$ were estimated following Wittmer and others [21] and Barbosa and others [12] and were required for the calculation of $^{13}\Delta_{veg}$ (Eqs 3 and 4) and WUE (Eqs 5 and 6). The $\delta^{13}C_{air}$ values for 1991–2010 were obtained from the US National Oceanic and Atmospheric Administration using data from the Waliguan station (100°54'E, 36°17'N, 3810 m a.s.l.), the closest meteorological station on the Tibetan Plateau.

$$C_a = 16081 \times t^2 - 62345 \times t + 60735 \quad (7)$$

where t is the sampling year/1000. The root mean squared error for the overall C_a model was $1.4 \mu\text{mol}\cdot\text{mol}^{-1}$. A cubic function was fitted to $\delta^{13}C_{air}$ to estimate mean annual values. The model was:

$$\delta^{13}C_{air} = 13675.5085 \times t^3 - 81341.3526 \times t^2 + 161233.8290 \times t - 106514.4913 \quad (8)$$

where t is the sampling year/1000. The root mean squared error for the overall $\delta^{13}C_{air}$ was 0.08‰. To calculate mean growing-season $\delta^{13}C_{air}$, a seasonal correction factor of 0.14‰ was added because the growing-season mean is 0.14‰ greater than the annual mean [21].

Estimation of vapor pressure concentration gradient (v)

The value of v was estimated by vapor pressure deficit (VPD) and was used to calculate W_e (Eq 6), with the assumption that leaf and air temperature were the same. The saturation vapor pressure (e) was related to air temperature (T) and was obtained as follows [22]:

$$e(T) = 0.6108e^{\frac{17.27T}{T+237.3}} \quad (9)$$

Daily mean saturation vapor pressure (e_s) was calculated as

$$e_s = \frac{e(T_{max}) - e(T_{min})}{2} \quad (10)$$

Actual vapor pressure (e_a) was calculated as

$$e_a = e_s \times RH \quad (11)$$

where RH is relative humidity. Then, VPD was given by:

$$VPD = e_s - e_a \quad (12)$$

Eq 12 was the relative humidity fraction and did not include the decreasing effect of high altitude on total atmospheric pressure (P). Thus, we calculated v as

$$v = \frac{VPD}{P} \quad (13)$$

where P is total atmospheric pressure (60.6 kPa at 4200 m a.s.l.).

Because plant photosynthesis and transpiration occurred in daytime when sun-light was present, the daytime VPD was more reasonable to use than the VPD here; thus the calculation was performed using the daily VPD as follows.

First, the daily VPD during 1962–2010 were calculated from the climate data (daily) of the CMA using Eqs 9–12. The daily VPD in the growing seasons from July 19, 2003 to August 16, 2010 were validated by the daily VPD calculated using the climate data (hourly) measured by the eddy covariance tower at Damxung Grassland Research Station. The linear regression was:

$$y = 0.9924x - 0.012, R^2 = 0.88, n = 883 \quad (14)$$

where y was the daily VPD from the eddy covariance tower and x was the daily VPD from CMA. Second, the climate data (hourly) measured by the eddy covariance tower and the sunshine time calculated according to the longitude and latitude were used to calculate the relationship between daytime VPD and daily VPD. The linear regression was:

$$y = 1.2582x + 0.037, R^2 = 0.97, n = 883 \quad (15)$$

where y was the daytime VPD and x was the daily VPD. Third, we assumed that Eq 15 was correct during our study period, and the daytime VPD during 1962–2010 was then calculated using Eq 15 and the daily VPD from the CMA.

Statistical analyses

Because wool is generally shorn in July (the beginning of the growing season), $\delta^{13}C_{\text{wool}}$ mainly reflects the isotopic signature of vegetation during the previous growing season. Hence, C_a and $\delta^{13}C_{\text{air}}$ values for the previous growing season were used to calculate C_i , W_i , and W_e and to explore the relationships among them.

All isotope data were tested for normality using the Kolmogorov–Smirnov test and for equality of error variance using Levene’s test. Linear regression was used to explore the changing trends of W_e with time, and the absolute values of the partial correlation coefficient obtained from a partial correlation analysis were used to identify the relative importance of explanatory variables. All statistical analyses were performed using SPSS Version 17.0 (SPSS, Inc., Chicago, IL).

Results

Long-term changes in $\delta^{13}C_{\text{wool}}$, $\delta^{13}C_{\text{veg}}$, and $^{13}\Delta_{\text{veg}}$

The average value of $\delta^{13}C_{\text{wool}}$ was -22.22‰ , ranging from -23.57‰ to -20.04‰ . The $\delta^{13}C_{\text{wool}}$ and $\delta^{13}C_{\text{veg}}$ values decreased over time ($R^2 = 0.35$, $n = 106$, $P < 0.001$) (Fig 2), similar to the trend observed for $\delta^{13}C_{\text{air}}$. Unexpectedly, $^{13}\Delta_{\text{veg}}$ was relatively stable over the past half century (Fig 3A) with an average value of 18.10‰ (range, 16.52‰ to 19.43‰).

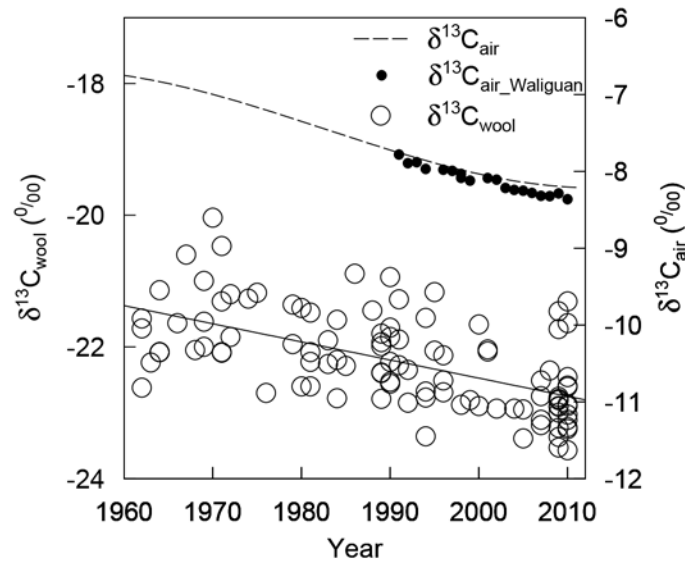


Fig 2. Carbon isotopic composition of wool ($\delta^{13}\text{C}_{\text{wool}}$) from Tibetan alpine meadows and of atmospheric CO_2 ($\delta^{13}\text{C}_{\text{air}}$, $\delta^{13}\text{C}_{\text{air_Waliguan}}$). The wool data were fitted with a linear model ($y = -0.028x + 32.561$, $n = 106$, $P < 0.001$).

doi:10.1371/journal.pone.0144752.g002

Long-term changes in C_i , C_i/C_a , and WUE

As described by Eq (4) and $^{13}\Delta_{\text{veg}}$ values, the C_i/C_a ratio changed little during the four decades examined. C_i values increased by $46 \mu\text{mol}\cdot\text{mol}^{-1}$ ($R^2 = 0.69$, $P < 0.001$) but did not balance the increase in C_a ($73 \mu\text{mol}\cdot\text{mol}^{-1}$, Fig 3B). Hence, C_a-C_i increased linearly over time ($R^2 = 0.58$, $P < 0.001$; Fig 3C). W_i and mean VPD in the growing season increased linearly (W_i : $R^2 = 0.56$, $P < 0.001$, Fig 4A; VPD: $R^2 = 0.14$, $P = 0.009$, Fig 4B). W_i increased by 23.5% (approximately $18 \mu\text{mol}\cdot\text{mol}^{-1}$) from 1962 to 2010, which was an increase of 3.20% per $10 \mu\text{mol}\cdot\text{mol}^{-1}$ increase in C_a . Mean VPD in the growing season increased by 0.11 kPa; as a result, the observed W_e did not increase significantly from 1962 to 2010 (Fig 4C).

Relationship between W_i and W_e and environmental parameters

GST increased linearly over time ($R^2 = 0.51$, $n = 49$, $P < 0.001$), and growing season precipitation (GSP) was relatively stable ($R^2 = 0.01$, $n = 49$, $P = 0.495$). W_i increased significantly with increasing GST ($R^2 = 0.38$, $n = 106$, $P < 0.01$) and C_a ($R^2 = 0.55$, $n = 106$, $P < 0.01$). GSP had no apparent influence on W_i ($R^2 = 0.04$, $n = 106$, $P = 0.041$).

W_e was positively related to GSP ($P < 0.05$) but negatively related to GST and C_a ($P < 0.05$). Linear regression showed that GSP , GST , and C_a jointly explained 45% of the variability in W_e ($W_e = 17.671 + 0.046C_a + 0.021GSP - 2.332GST$; $R^2 = 0.452$, $n = 106$, $P < 0.001$). Partial correlation analysis showed that the relative importance of the three variables was GSP (partial correlation coefficient, 0.506) > GST (partial correlation coefficient, -0.350) > C_a (partial correlation coefficient, 0.237).

Discussion

Intrinsic water use efficiency increased in alpine meadows

The average $^{13}\Delta_{\text{veg}}$ value (18.10‰) and its intra-annual variation (~2.5‰) were reasonable because they were close to the values of other C_3 plants on the Tibetan Plateau (average,

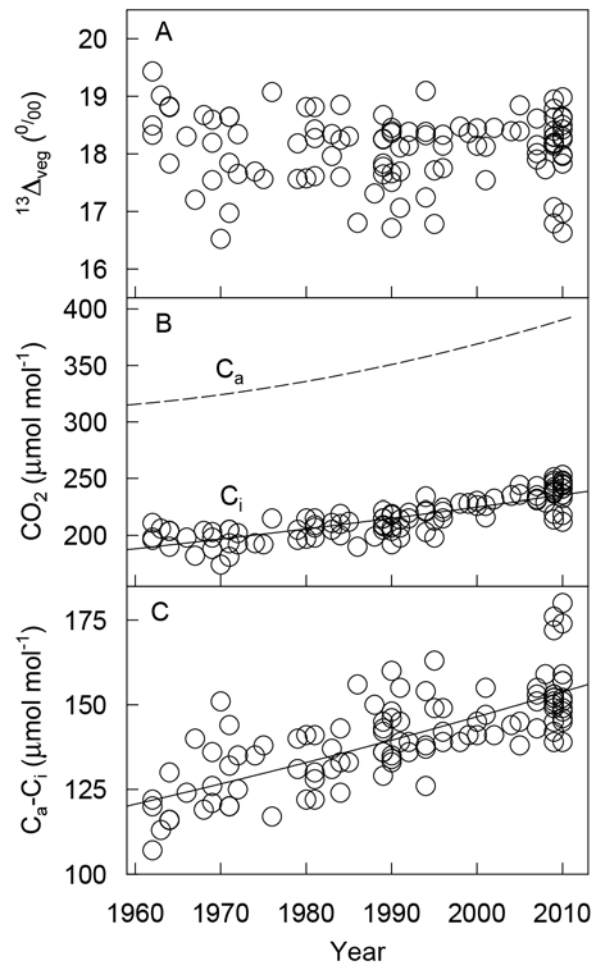


Fig 3. Ecophysiological parameters of Tibetan alpine meadows reconstructed from the $\delta^{13}\text{C}$ time series of wool samples. (A) Carbon isotope discrimination ($^{13}\Delta_{veg}$); (B) CO_2 concentration in the atmosphere (C_a , dashed line) and in intercellular space (C_i , solid line); (C) the difference between C_a and C_i ($C_a - C_i$). The parameter trend lines were calculated using the values derived from $\delta^{13}\text{C}$ of wool samples ($n = 106$). The models fitting C_i and $C_a - C_i$ data were $y = 0.029e^{0.004x}$ and $y = 0.009e^{0.005x}$ respectively.

doi:10.1371/journal.pone.0144752.g003

~18‰; variation, 4.5‰) [10, 23] and the Mongolian Plateau (average, ~17‰; variation, 1.9‰) [21]. Intra-annual variation of $^{13}\Delta_{veg}$ might have been a result of the spatial differences in GST and GSP. The observed C_i/C_a ratio varied little over the past half century, which indicated a proportional adjustment of C_i in vegetation to the increase in C_a . Three theoretical responses of C_i to increasing C_a include (i) the difference between C_a and C_i remains constant when the increase in C_i and C_a is equal, (ii) the C_i/C_a ratio remains consistent because C_i and C_a increase proportionally, and (iii) C_i is constant [12]. The first response suggests that W_i remains constant; the second and third responses indicate that W_i increases with increasing C_a . Our results demonstrated that, with the increase in C_a and GST, C_i increased in the alpine meadows of the Tibetan Plateau to keep the C_i/C_a ratio relatively stable, which supports the second response.

In terms of gas-exchange (Eq 5) the increase in W_i must result from increased assimilation or decreased stomatal conductance. Previous studies, such as the FACE and OTC experiments [24], are consistent with our findings that elevated CO_2 stimulates photosynthesis and decreases or has no influence on g_s . The significant positive correlations between W_i and GST

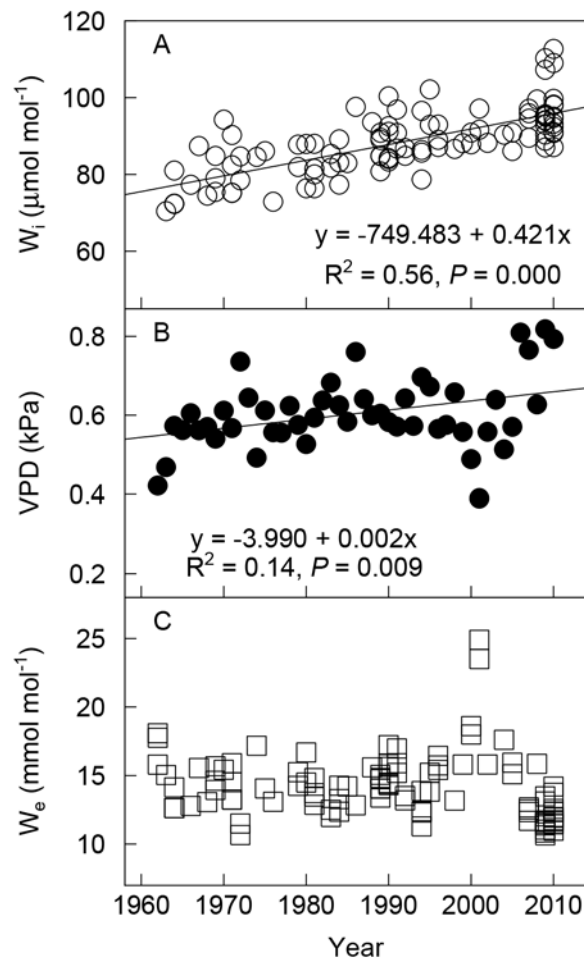


Fig 4. Ecophysiological parameters of Tibetan alpine meadows. (A) Intrinsic water use efficiency (W_i), $n = 106$; (B) atmospheric vapor pressure deficit (VPD), $n = 49$; (C) effective water use efficiency (W_e), $n = 106$. The parameter trend lines were calculated using values derived from $\delta^{13}C$ of wool samples.

doi:10.1371/journal.pone.0144752.g004

and between W_i and C_a suggested that the rapid increases in GST and C_a had positive effects on photosynthesis. For GST , the main reason was that the increase of GST can increase leaf temperature, improve Rubisco enzyme activity in chloroplasts, and enhance A although the increasing GST would also enhance leaf respiration. Additionally, g_s could be decreased by the increase of GST to decrease water loss. For C_a , the major reason was that the increase of C_a could increase the supply of CO_2 to leaf mesophyll cells and then improve photosynthesis. In addition, increased grazing pressure and decreased GSP could also theoretically explain the increase in W_i , but only to a lesser extent considering that these were not major factors during the study period because they were almost unchanged. Hence, under the background of warming and the increase of C_a , the plants tried to adapt to these changes in environment conditions via the physiological adjustment of the WUE.

Previous studies have reconstructed the responses of WUE in trees on the Tibetan Plateau over the past two centuries using Eq (5) [25–26] and found that W_i increased in response to elevated C_a and drought. Our findings for W_i were consistent with previous studies that examined this variable in trees in various ecosystems worldwide and in Tibetan alpine meadows (Table 1). These findings suggest that the long-term responses of W_i to increasing C_a are

Table 1. Long-term changes in intrinsic water use efficiency (W_i) of trees and alpine meadow and in atmospheric CO_2 concentration (C_a).

Systems	Locality	Period	C_a increase ($\mu\text{mol}\cdot\text{mol}^{-1}$)	Increase in W_i (%)		References
				Total	Per 10 $\mu\text{mol}\cdot\text{mol}^{-1}$ increase of C_a	
Forests						
<i>Pinus</i> sp., <i>Picea sitchensis</i> , <i>Quercus lobata</i> , <i>Fitzroya cupressoides</i> , <i>Juniperus phoenicea</i>	Western North America and Chile (<i>Fitzroya</i>)	1800–1990	72	5–45	0.70–6.25	[36]
<i>Larix</i> sp., <i>Pinus</i> sp., <i>Picea</i> sp.	Northern Eurasia	1861–1990	67	19.2 ±0.9	2.87±0.13	[36]
<i>Fagus sylvatica</i> (Coppice with standards)	Northeastern France	1850–1990	69	23	3.33	[37]
<i>Fagus sylvatica</i> (High forest)	Northeastern France	1850–1990	69	44	6.38	[37]
<i>Sabina przewalskii</i>	Tibet, China	1850–2000	83	23.6	2.84	[25]
<i>Picea crassifolia</i>	Tibet, China	1850–2000	83	35.5	4.28	[25]
<i>Picea crassifolia</i>	Tibet, China	1890–2002	79	34	4.30	[26]
Grasslands						
Alpine meadow	Switzerland	1938–2006	81	17.8	2.20	[12]
Alpine meadow	Tibet, China	1962–2010	73	23.5	3.20	This study

doi:10.1371/journal.pone.0144752.t001

similar in alpine forests and alpine meadows. However, the long-term response of W_i observed here was 1.5-fold higher than that of alpine grasslands in the Swiss Alps from 1938 to 2006 [12] (Table 1). Possible reasons for this difference include lower air pressure due to higher altitude in our study (4200 m) relative to the Swiss grasslands (2200 m) and difference in species composition.

A plant community consisting solely of C_3 species is an important assumption of the use of $\delta^{13}C_{veg}$ to estimate C_i/C_a and W_i . In general, C_4 plants are more abundant in areas with mean monthly growing season temperature above 22°C and precipitation above 25 mm [27–29], conditions that do not occur above 3500 m on the Tibetan Plateau [28–30]. Our investigation of plant composition validated the assumption of C_3 species only in our study area; thus, W_i values estimated from wool were reliable for this region. Another potentially influencing factor of $\delta^{13}C_{veg}$ could be changes in species composition over time. Unfortunately, we are not aware of any long-term vegetation analyses in our study region or similar ones. However, a short term simulated warming experiment in alpine meadows in Tibet [31] revealed no significant changes in species composition and data from the Damxung Grassland Research Station also showed only minor variation in the relative aboveground biomass of Poaceae (mean, 34.2%; SD, 12%) and Cyperaceae (mean, 30.4%; SD, 9.1%) during 2005–2011, a period of significant warming and C_a increase (Yongtao He, unpublished data). We would therefore conclude that the potential effect of species compositional changes should be minor.

Effective water use efficiency remained stable

Alpine meadows on the Tibetan Plateau have made physiological adjustments to increase WUE under climate warming and elevated C_a , but, unexpectedly, W_e remained relatively stable over the past half century (Fig 4C). Warming and drying trends of the atmosphere as shown by

the increase of VPD and the results of Xie and others [32] hindered the improvement of plant WUE. Our assumption that the increase in NPP depended on the increase of WUE was not supported by the results. Plant W_e and available soil water [33] determine the NPP of alpine meadows. Based on the model results of Piao and others [2] and Chen and others [34], the NPP in this region increased during our study period. In this case, a major reason for the increase of NPP may be the increase of water available to the plants. Although GSP did not change during 1962–2010, the amount of annual precipitation increased in Damxung County ($R^2 = 0.16$, $P = 0.005$) and in the whole Tibetan Plateau ($R^2 = 0.24$, $P < 0.001$) [2]. These results suggest that winter precipitation may be an important water resource in our study area. Similar findings have been reported in the Mongolia grasslands, where the water from winter half-year precipitation contributed 15–45% of the total water uptake by plants [35].

Supporting Information

S1 Dataset. The values of stable carbon isotope, WUE and related climate data in the study. (XLSX)

Acknowledgments

We are grateful to Dr. Leiming Zhang and the staff of the Lhasa Plateau Ecosystem Research Station, Chinese Academy of Sciences, for their help in field sampling and data sharing.

Author Contributions

Conceived and designed the experiments: HY. Performed the experiments: HY. Analyzed the data: HY NH YH. Contributed reagents/materials/analysis tools: HY NH YH. Wrote the paper: HY NH SL. Revised the manuscript: PS XZ.

References

1. Yao S, Liu X, Wang L. Questions on the range of climate change in the Tibetan Plateau. *Chin Sci Bull.* 2000; 45: 98–106.
2. Piao S, Tan k, Nan H, Ciais P, Fang J, Wang T, et al. Impacts of climate and CO₂ changes on the vegetation growth and carbon balance of Qinghai-Tibetan grasslands over the past five decades. *Glob Planet Change.* 2012; 98–99: 73–80.
3. Tang W, Qin J, Yang K, Niu X, Zhang X, Yu Y, et al. Reconstruction of daily photosynthetically active radiation and its trends over China. *J Geophys Res Atmos.* 2013; 118: 13292–13302.
4. Hu Z, Yu G, Fu Y, Sun X, Li Y, Shi P, et al. Effects of vegetation control on ecosystem water use efficiency within and among four grassland ecosystems in China. *Glob Change Biol.* 2008; 14: 1609–1619.
5. Von caemmerer S, Farquhar GD. Some relationships between the biochemistry of photosynthesis and the gas-exchange of leaves. *Planta.* 1981; 153: 376–387. doi: [10.1007/BF00384257](https://doi.org/10.1007/BF00384257) PMID: [24276943](https://pubmed.ncbi.nlm.nih.gov/24276943/)
6. Farquhar GD, Sharkey TD. Stomatal conductance and photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol.* 1982; 33: 317–345.
7. Zheng S, Lan Z, Li W, Shao R, Shan Y, Wan H, et al. Differential responses of plant functional trait to grazing between two contrasting dominant C3 and C4 species in a typical steppe of Inner Mongolia, China. *Plant Soil.* 2011; 340: 141–155.
8. Farquhar GD, Ehleringer JR, Hubick KT. Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol.* 1989; 40: 503–537.
9. Bai Y, Wu J, Xing Q, Pan Q, Huang J, Yang D, et al. Primary production and rain use efficiency across a precipitation on the Mongolia Plateau. *Ecology.* 2008; 89: 2140–2153. PMID: [18724724](https://pubmed.ncbi.nlm.nih.gov/18724724/)
10. Li M, Liu H, Song D, Li L. Water use efficiency and nitrogen use efficiency of alpine plants grown in the east of Qinghai-Tibet Plateau. *Xibei Zhiwu Xuebao.* 2007; 27: 1216–1224.
11. Yan W, Zhang X, Shi P, Yang Z, He Y, Xu L. Carbon dioxide exchange and water use efficiency of alpine meadow ecosystems on the Tibetan Plateau. *J Nat Resour.* 2006; 21: 756–767.

12. Barbosa ICR, Köhler IH, Auerswald K, Lüps P, Schnyder H. Last-century changes of alpine grassland water-use efficiency: a reconstruction through carbon isotope analysis of a time-series of *Capra ibex* horns. *Glob Change Biol.* 2010; 16: 1171–1180.
13. Maennel T, Auerswald K, Schnyder H. Altitudinal gradients of grassland carbon and nitrogen isotope composition are recorded in the hair of grazers. *Glob Ecol Biogeogr.* 2007; 16: 583–592.
14. Schnyder H, Auerswald K. Isotopes as natural recorders of grassland ecosystem functioning and change. In: Multifunctional Grasslands in a changing world (ed. Conference OColl), p. 46. Guangdong People's Publishing House; 2008.
15. Auerswald K, Wittmer MHOM, Maennel TT, Bai Y, Schaeufele R, Schnyder H. Large regional-scale variation in C3/C4 distribution pattern of Inner Mongolia steppe is revealed by grazer wool carbon isotope composition. *Biogeosciences.* 2009; 6: 795–805.
16. De Niro MJ, Epstein S. Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta.* 1978; 42: 495–506.
17. Schwertl M, Auerswald K, Schaeufele R, Schnyder H. Carbon and nitrogen stable isotope composition of cattle hair: ecological fingerprints of production systems? *Agric Ecosyst Environ.* 2005; 109: 153–165.
18. Jones RJ, Blunt CG, Ludlow MM, Troughton JH. Changes in the natural carbon isotope ratios of the hair from steers fed diets of C4, C3 and C4 species in sequence. *Search.* 1981; 12: 85–87.
19. Sponheimer M, Robinson T, Ayliffe L, Passey B, Roeder B, Shipley L, et al. An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores. *Can J Zool.* 2003; 81: 871–876.
20. Cernusak LA, Ubiernad N, Winter K, Holtum JAM, Marshall JD, Farquhar GD. Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phyto.* 2013; 200: 950–965.
21. Wittmer MHOM, Auerswald K, Tungalag R, Bai Y, Schaeufele R, Schnyder H. Carbon isotope discrimination of C3 vegetation in Central Asian grassland as related to long-term and short-term precipitation patterns. *Biogeosciences.* 2008; 5: 913–924.
22. Allen RG, Pereira LS, Raes D, Smith M. Crop evapotranspiration-Guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. Food and Agriculture Organization of the United Nations; 1998.
23. Zhou Y, Fan J, Zhang W, Harris W, Zhong H, Hu Z, et al. Factors influencing altitudinal patterns of C3 plant foliar carbon isotope composition of grasslands on the Qinghai-Tibet Plateau, China. *Alp Bot.* 2011; 121: 79–90.
24. Long SP, Ainsworth EA, Rogers A, Ort DR. Rising atmospheric carbon dioxide: plants face the future. *Annu Rev Plant Biol.* 2004; 55: 591–628. PMID: [15377233](#)
25. Liu X, Shao X, Liang E, Zhao L, Chen T, Qin D, et al. Species-dependent responses of juniper and spruce to increasing CO₂ concentration and to climate in semi-arid and arid areas of northwestern China. *Plant Ecol.* 2007; 193: 195–209.
26. Liu X, Shao X, Wang L, Liang E, Qin D, Ren J. Response and dendroclimatic implications of ¹³C in tree rings to increasing drought on the northeastern Tibetan Plateau. *J Geophys Res.* 2008; 113: G03015.
27. Ehleringer JR, Cerling TE, Helliker BR. C4 photosynthesis, atmospheric CO₂, and climate. *Oecologia.* 1997; 112: 285–299.
28. Collatz GJ, Berry JA, Clark JS. Effects of climate and atmospheric CO₂ partial pressure on the global distribution of C4 grasses: present, past, and future. *Oecologia.* 1998; 114: 441–454.
29. Still CJ, Berry JA, Collatz GJ, Defries RS. Global distribution of C3 and C4 vegetation: Carbon cycle implications. *Global Biogeochem Cycles.* 2003; 17: 1006.
30. Lu H, Wu N, Gu Z, Guo Z, Wang L, Wu H, et al. Distribution of carbon isotope composition of modern soils on the Qinghai-Tibetan Plateau. *Biogeochemistry.* 2004; 70: 275–299.
31. Wang SP, Duan JC, Xu GP, Wang YF, Zhang ZH, Rui YC, et al. Effects of warming and grazing on soil N availability, species composition, and ANPP in an alpine meadow. *Ecology.* 2012; 93: 2365–2367. PMID: [23236908](#)
32. Xie H, Ye J, Liu X, E C. Warming and drying trends on the Tibetan Plateau. *Theor Appl Climatol.* 2010; 101: 241–253.
33. Yang Y, Fang J, Pan Y, Ji C. Aboveground biomass in Tibetan grasslands. *J Arid Environ.* 2009; 73: 91–95.
34. Chen B, Zhang X, Tao J, Wu J, Wang J, Shi P, et al. The impact of climate change and anthropogenic activities on alpine grassland over the Qinghai-Tibet Plateau. *Agric For Meteorol.* 2014; 189–190: 11–18.

35. Yang H, Auerwald K, Bai Y, Han X. Complementarity in water sources among dominant species in typical steppe ecosystems of Inner Mongolia, China. *Plant Soil*. 2011; 340: 303–313.
36. Saurer M, Siegwolf RTW, Schweingruber FH. Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. *Glob Change Biol*. 2004; 10: 2109–2120.
37. Duquesnay A, Bréda N, Stievenard M, Dupouey JL. Changes of tree-ring $\delta^{13}\text{C}$ and water-use efficiency of beech (*Fagus sylvatica* L.) in north-eastern France during the past century. *Plant Cell Environ*. 1998; 21: 565–572.
38. Editorial Committee of Vegetation Map of China, Chinese Academy of Sciences. *Vegetation Map of The People's Republic of China (1:1000000)*. Beijing: Geological Publishing House; 2000.