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Water use, discrimination, and temporal change of life forms among C4 plants of Inner Mongolia grassland

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Contents

Abstract

Aims: The subject of present thesis was the variation of the carbon isotope discrimination (*Δ*) of C4 community in the Inner Mongolia grassland. Of particular interest were (i) to determine the seasonal water sources of dominant species intended to explore the contribution of winter precipitation to plant growth and water relationship among dominant species, (ii) the *Δ* variation of *Cleistogenes squarrosa*, a dominant C4 species, at tiller, local, catchment and regional scales intended to illustrate the driver and mechanism of the *Δ* of C4 species, and (iii) to obtain the *Δ* of C4 annuals and C4 perennials and their relative contribution to C4 community intended to reveal the *Δ* of C4 community in the Inner Mongolia grassland.

Material & Methods: Non-photosynthetic issues of plant at the interface between shoot and root systems were collected in three ungrazed plots and soil water content was measured in two years. Young mature leaves of *C. squarrosa* were collected in different scales, a grazing experiment, Xilin River catchment, and the grassland of the Mongolia plateau. Vegetation survey was conducted in the grassland of the Mongolia plateau covering an area of approximately 200000 km^2. Relative biomass of each C4 species was recorded at 280 sites and relative biomass of C4 annuals (R_{A4}) and C4 perennials (R_{P4}) was calculated. The samples were analyzed for their δD, $\delta^{13}C$ and leaf N content (N_{leaf}) respectively.

Results & Discussion: The fraction of water from winter half-year precipitation was an important water source. At species level, *Caragana microphylla* exhibited a complete access to deep soil water while *C. squarrosa* completely depended on summer rains. *Leymus chinensis, Agropyron cristatum,* and *Stipa grandis* showed a resource-dependent water use strategy, utilizing deep soil water when it was well available and shifting to rain water when subsoil water had been exploited. Differentiation of water sources among plants improves use of available soil water and lessens the interspecific competition for water in the semi-arid ecosystem.

The *Δ* of *C. squarrosa* diminished identically with increasing N_{leaf}, which increased with grazing intensity but decreased with leaf position and increasing precipitation in growing season. *Δ* could be influenced by biotic and abiotic factors that affect N_{leaf}. The leaf-level photosynthetic response scales up linearly across all scales from tiller to regional although the drivers controlling N_{leaf} may differ at different scales.

C4 community was either dominated by C4 perennials or by C4 annuals and sites where both life forms coexist were rather scarce. On average, R_{A4} was 11% with SD of 19% and R_{P4} was 13% with SD of 19%. C4 annuals significantly decreased and C4 perennials increased over years. C4 annuals preferred normal precipitation in growing season, while C4 perennials were dominant in dry growing season. Grazing had no effect on C4 annuals and C4 perennials. Both behaviors of C4 annuals and C4 perennials followed the prediction of Grime's plant strategy theory that C4 annuals profit from disturbances and are replaced by C4 perennials. The mean of Δ of C4 annuals and C4 perennials were 5.63‰ and 7.19‰. The Δ of C4 increased by almost 1‰ from 6.02‰ to 6.97‰ between 2001 and 2009 and the mean was 6.50‰ with SD of 0.69‰ during this period.

Conclusions: C4 plants mainly used topsoil water and had less competition for water with C3 plants mainly using deep soil water. The Δ of *C. squarrosa* was diminished with increasing N_{leaf}. C4 annuals and C4 perennials had different temporal dynamics with the increase in C4 plants during disturbance, but still the Δ of C4 community can be regarded as a constant value, 6.45‰, when it is an end member in calculating the C3/C4 ratio in the Inner Mongolia grassland.

Zusammenfassung

Ziele: Die Biomasseanteile von Arten mit C4-Photosynthese haben in der Steppe der Inneren Mongolei in den vergangenen Jahrzehnten zugenommen. Diese Zunahme ist überraschend, da auf Grund des Anstiegs der atmosphärischen CO_2-Konzentrationen eine Bevorzugung von Pflanzen mit C3-Photosynthese erwartet wird. Die vorliegende Arbeit beschäftigt sich daher mit der Ökophysiologie von Pflanzen mit C4-Photosynthese in der Steppe der Inneren Mongolei, insbesondere mit dem Gras *Cleistogenes squarrosa*, das die C4-Pflanzengemeinschaft dort dominiert. Besonderes Interesse galt dabei:

1) der saisonalen Veränderung der Wasserversorgung, vor allem hinsichtlich der Ausnutzung der Winterfeuchte, bei den dominanten C3- und C4-Arten,
2) der Umweltabhängigkeit der ^{13}C-Diskriminierung, ein Indikator der Wassernutzungseffizienz von *C. squarrosa*, auf unterschiedlichen Skalen.
3) der zeitlichen Variation über das letzte Jahrzehnt im Anteil von annuellen und perennierenden C4-Arten, die sich in der Ausbreitungsgeschwindigkeit unter den neuen, C4-fördernden Bedingungen unterscheiden sollten.

Material & Methoden:

zu 1) Photosynthetisch nicht aktives Gewebe am Übergang Wurzel/Spross wurde auf drei langjährig (>20 a) unbeweideten Flächen der drei wichtigsten Pflanzengesellschaften, der *Stipa-grandis*-Steppe, der *Leymus-chinensis*-Steppe und der *Caragana-microphylla*-Steppe beprobt. Es wurde auf die Deuteriumgehalte untersucht und mit den Deuteriumgehalten und Wasservorräten im Boden verglichen, um den Anteil von tiefem Bodenwasser (Winterfeuchte) an der Wasseraufnahme in Abhängigkeit von der Wasserverfügbarkeit zu bestimmen.

zu 2) Ausgewachsene, nicht-seneszente Blätter von *C. squarrosa* wurden auf den Skalen Trieb, Standort, Einzugsgebiet und Region in einem Beweidungsexperiment, im Einzugsgebiet des Xilin-Flusses und in der Steppenregion der Inneren Mongolei gesammelt, auf den ^{13}C-Gehalt und den N-Gehalt untersucht und mit den verschiedenen potentiellen Variationsursachen (Jahreszeit, Beweidungsintensität, Niederschlag, Boden etc.) korreliert.

zu 3) Vegetationsaufnahmen wurden in der Steppe der Inneren Mongolei in einem Gebiet von ca. 200000 km^2 zwischen 2001 und 2009 durchgeführt. Der relative Anteil der annuellen und der perennierenden C4-Arten an der C4-Biomasse wurde geschätzt und die Arten wurden auf ihre ^{13}C-Gehalte untersucht.

Ergebnisse & Diskussion:

zu 1) Bodenwasser, das aus dem Winterniederschlag stammte, trug wesentlich zur Wasserversorgung des Pflanzenbestandes bei. Auf Artebene verwendete *C. microphylla* überwiegend dieses Wasser, das während der Hauptvegetationsperiode noch unterhalb von 20 cm vorhanden war. *C. squarrosa* war dagegen ausschließlich von Sommerniederschlägen abhängig, die nur bis zu einer Tiefe von 20 cm in den Boden vordrangen. *L. chinensis*, *Agropyron cristatum* und *S. grandis* wiesen eine ressourcenabhängige Wassernutzungsstrategie auf. Sie griffen vor allem auf Sommerniederschlagswasser zurück, wenn die aus dem Winterniederschlag stammenden Vorräte erschöpft waren, während sie sonst beide Quellen nutzten.

zu 2) Die ^{13}C-Diskriminierung von *C. squarrosa* nahm mit zunehmendem N-Gehalt der Blätter ab, wobei der N-Gehalt der Blätter je nach Skalenniveau von unterschiedlichen Ursachen beeinflusst wurde. Auf Sprossebene nahm er im Lauf der Vegetationsperiode zu, auf der lokalen Ebene mit zunehmender Beweidungsintensität und auf der regionalen Ebene mit abnehmendem Niederschlag. Trotz dieser unterschiedlichen Ursachen der Variation im N-Gehalt war die Reaktion auf Blattebene hinsichtlich der Diskriminierung immer gleich. Damit verbesserte sich die Wassernutzungseffizienz auf Sprossebene im Laufe der Vegetationsperiode, auf lokaler Ebene mit zunehmender Beweidungsintensität, auf Einzugsgebietsebene mit abnehmendem Schluffgehalt der Böden und auf Einzugsgebietsebene mit abnehmendem Niederschlag.

zu 3) Die C4-Artengemeinschaft eines Standortes zu einem Zeitpunkt war entweder durch annuelle oder durch perennierende Arten dominiert. Im Schnitt trugen annuelle C4 11 % (Std. abw. 19 %) und perennierende C4 13 % (Std. abw. 19 %) zur gesamten Biomasse bei. C4-Annuelle dominierten in normalen und in feuchten Jahren, während die perennierenden C4 in Trockenjahren dominierten. Beweidung hatte dagegen keinen Einfluss auf die Präsenz der beiden Wuchsformen. Innerhalb des neunjährigen Untersuchungszeitraumes nahmen die C4-Annuellen signifikant ab, während die perennierenden C4 entsprechend zunahmen. Dieses Verhalten entspricht den Erwartungen hinsichtlich der Grime'schen C-S-R-Strategietypen, dass Annuelle von neuen Besiedlungsmöglichkeiten, wie sie z.B. durch Störungen geschaffen werden, zuerst profitieren und dann allmählich durch perennierende Arten ersetzt werden. In diesem Fall ergeben sich die neuen Besiedlungsmöglichkeiten durch die starke Zunahme der C4-Arten relativ zu den C3-Arten. Da sich die mittlere ^{13}C-Diskriminierung von annuellen (5,63 ‰) und perennierenden C4 (7,19 ‰) vor allem wegen der hohen Diskriminierung von *C. squarrosa* deutlich unterschieden, hat durch die Veränderung der funktionellen Gruppen auch die Diskriminierung der C4-Gemeinschaft zwischen 2001 und 2009 deutlich von 6,02 ‰ auf 6,97 ‰ zugenommen.

Schlussfolgerungen: C4-Pflanzen der innermongolischen Steppe nutzen überwiegend Sommerniederschlag, während C3-Pflanzen auch auf den unterhalb von 20 cm Tiefe gespeicherten Winterniederschlag zugreifen. Die Differenzierung zwischen den Arten hinsichtlich ihrer Wassernutzungsstrategie verbessert die Wasserausnutzung und vermindert die Konkurrenz zwischen den Arten in diesem semiariden Raum. Die Zunahme der C4-Anteile in der innermongolischen Steppe führt daher zu einer stärkeren Abhängigkeit von den räumlich und zeitlich stark variierenden Sommerniederschlägen und vermindert so die Resilienz gegenüber Niederschlagsschwankungen. Die Wassernutzungseffizienz der C4-Arten steigt im Laufe der Vegetationsperiode, mit zunehmender Beweidungsintensität und mit abnehmendem Wasserangebot (Bodenwasserspeicher + Niederschlag) an. Dies konnte speziell für *C. squarrosa* nachgewiesen werden, das die C4-Gemeinschaft dominiert, und auf Grund der hohen CO_2-Leckage der Bündelscheidenzellen mit seiner ^{13}C Signatur ein sensitiver Indikator der Wassernutzungseffizienz ist.

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CHAPTER I – General introduction

The present work is part of the subproject: Influence of grazing pressure on the carbon isotope composition of the grasslands of China: Spatio-temporal variations at multiple scales, which is in the second phase of the DFG founded project *Forschergruppe 536 MAGIM* (Matter fluxes in grasslands of Inner Mongolia as influenced by stocking rate, www.magim.net). MAGIM was intended to understand how extensive grazing regimes and environmental changes – as prevailing in Inner Mongolian grassland – provoke the degradation of the grassland and promote the associated alterations in e.g. soil erosion and desertification rate as well as in vegetation community structure. Therefore eleven subprojects were established to shed light on how different effects of grazing regimes and environmental changes affect the Inner Mongolian steppe. The goal was to offer a possible implementation of a sustainable grazing system to prevent the grasslands of further degradation, just as to allow for recovery of the grasslands. This work focused on the response of C4 plants to environmental change and tried to explore the pattern and mechanism of this response.

The C3/C4 ratio in the mixed grassland and global change

Grasslands are one of the most wide spread vegetation types worldwide, covering about one-fifth of the world's land surface, nearly 24×10^6 km^2 (Parton *et al.*, 1995). The biogeochemical cycles in grasslands have important influence on that of the whole world. Based on different photosynthesis types, grass has mainly two groups: C3 plants and C4 plants. Mixed C3/C4 grasslands contribute approximately 15% of the grassland area (Parton *et al.*, 1995). Compared with coexisting C3 plants, C4 plants generally exhibit higher photosynthesis rates at low CO_2 concentration and elevated temperature and have higher light, water, and nitrogen use efficiency in warm environment (Sage, 1999). Thus, the C3/C4 ratio will be varied with global change and further has large effect on seasonal distribution of biomass (Niu *et al.*, 2006) and nutrient content of products (Garden *et al.*, 2005), and linked carbon storage (Burke *et al.*, 1998; Derner *et al.*, 2006), water and nutrient cycles (Epstein *et al.*, 1998; Semmartin *et al.*, 2004; Nippert & Knapp, 2007).

There are two main factors to cause the variation of C3/C4: global climate change and human behaviours. Firstly, temperature and CO_2 concentration have significantly increased over last century. The increase in temperature favours C4 plants due to the higher effective quantum yield of CO_2 fixation and higher maximum photosynthetic rate at high temperature (Ehleringer & Björkman, 1977; Sage & Kubien, 2003). In contrast with the former the increase in CO_2 concentration prefers C3 plants (Collatz *et al.*, 1998) due to the increase of intercellular CO_2 concentration suppressing photorespiration. Hence, different trends of the C3/C4 ratio could be produced depending on the overall influence of CO_2 concentration and temperature effects. In addition, the variation of precipitation may modify the C3/C4 response to the rise of temperature and CO_2 concentration as this also influences the water use efficiency of plants (Sage, 1999). Secondly, human behaviours such as grazing and land use change may increase C4 plants in local scale (Wang, 1992), which again may modify the trend by the rise of CO_2 concentration and temperature. Thus, a complex C3/C4 response pattern may result due to these interactions. C3 and C4 plants differ in their ^{13}C signature and this significant contrast as the two end-member in the mixing model is recently more and more popular used to calculate the C3/C4 ratio (Murphy & Bowman, 2007; Auerswald *et al.*, 2009; Wittmer *et al.*, 2010). The equations are:

$$\Delta_{Cb} = \mathrm{P}_{\mathrm{C3}} \times \Delta_{C3} + \mathrm{P}_{\mathrm{C4}} \times \Delta_{C4} \qquad \text{(I.1)}$$

$$\Delta = \frac{\delta^{13}\mathrm{C}_{\mathrm{air}} - \delta^{13}\mathrm{C}_{\mathrm{sample}}}{1 + \delta^{13}\mathrm{C}_{\mathrm{sample}}} \qquad \text{(I.2)}$$

where $\delta^{13}C_{air}$ and $\delta^{13}C_{sample}$ are the $\delta^{13}C$ of air and sample, Δ_{Cb}, Δ_{C3}, and Δ_{C4} are the discrimination of bulk sample, C3 and C4 plants, P_{C3} and P_{C4} are the percent of C3 and C4 plants respectively.

Rearranging the equation (I.1) we obtained:

$$\mathrm{P}_{\mathrm{C4}} = 1 - \mathrm{P}_{\mathrm{C3}} = \frac{\Delta_{Cb} - \Delta_{C3}}{\Delta_{C4} - \Delta_{C3}} \qquad \text{(I.3)}$$

The available bulk material may be wool, feces, soil organic matter, root and others (Ehleringer *et al.*, 2000; von Fischer *et al.*, 2008; Auerswald *et al.*, 2009; Wittmer *et al.*, 2010). However, the uncertainty during calculation will be caused by the variation of the discrimination of the two end-members with environmental conditions and possible shifts of Δ during the formation of bulk material. It is well known that the Δ_{C3} varies with precipitation, altitude, latitude, and equations have been developed to standardize the shift of carbon isotope signature (Körner *et al.*, 1991; Mole *et al.*, 1994; Männel *et al.*, 2007; Wittmer *et al.*, 2008) , but for C4 plants a constant value is usually used which is generally justified with less variation than that of C3 plants (Li *et al.*, 2006a; von Fischer *et al.*, 2008). Comparing the density distributions for the Δ_{C3} and Δ_{C4} in a meta-analysis of published values from grassland species however showed that the overall variation among C4 is of about the same magnitude as that of C3 plants (Schnyder & Auerswald, 2008). This variation is caused by variation within a species due to environmental conditions (Chen *et al.*, 2002; Liu *et al.*, 2005) but also by differences between species in Δ (Schulze *et al.*, 1996; Murphy & Bowman, 2009). Hence, it is necessary to obtain more information about the variation of the Δ_{C4} not only for the application of C4 end-member but also for contributing to understand the physiology and ecology of C4 plants.

The Inner Mongolia grassland

The cool-season semi-arid grassland of Inner Mongolia is a part of the Eurasian steppe, one of the most contiguous biomes of the world. Of the total land area of 1.2 million km² in Inner Mongolia, 0.8 million km² are covered with grassland which includes meadow steppe, typical steppe and desert steppe (Xiao *et al.*, 1995). The Inner Mongolia grassland extends on the north on the Mongolia plateau to Mongolia, where also Gobi desert and forest steppe can be found. Precipitation is highly variable in the Inner Mongolia grassland. For instance, at a given site of IMGERS (Inner Mongolia Grassland Ecosystem Research Station), which is located in the typical steppe with MAP (mean annual precipitation) of 337 mm yr^{-1}, precipitation was 507 mm in 1999 similar to forest, but in 2005 only was 166 mm similar to desert steppe (data from IMGERS). More than 60% precipitation falls in the growth season, May - August (Bai *et al.*, 2007). This grassland is dominated by C3 plants (e.g. *Stipa grandis, S. baicalensis*, *Leymus chinensis*, *Caragana microphylla*) (Bai *et al.*, 2008). C4 plants are often co-dominant members of the grassland community (Chen, 2003) and contribute proximately 20% of total productivity (Auerswald *et al.*, 2009). Most C4 plants are Poaceae and Chenopodiceae (Pyankov *et al.*, 2000; Tang & Liu, 2001). C4 annuals and C4 perennials are reported to exist on the Mongolia plateau (Tang & Liu, 2001). Among the perennials *Cleistogenes squarrosa* dominates while annuals are dominated by *Salsola collina*.

The grassland of Inner Mongolia has faced large change in climate and grazing pressure. The temperature increased approximately 2 °C during growing period from 1990 to 2000 (NOAA NCDC Climate Data Online, 2007). CO_2 concentration increased more than 50 ppm over the last century. Grazing pressure increased. For instance, the number of sheep increased by about 1250% from 1950 to 2007 (Inner Mongolia Statistics Office, 2008) and additionally the grazing changed from nomadic to sedentary in the 1960's. Hence one might expect a pronounced impact of above disturbances on the physiology, ecology, and competiveness of C4 plants and subsequently on the C3/C4 ratio of Inner Mongolia grassland.

In this semi-arid grassland available soil water is a main limited factor to plant growth (Bai *et al.*, 2004). It depends on the seasonal distribution of precipitation. As evapotranspiration exceeds rainfall during summer, summer rains mainly recharge the topsoil except for macropore flow, while deeper soil layers can only be recharged during the winter-half year (Schneider *et al.*, 2007). C4 plants have shallower root system compared to C3 plants (Chen *et al.*, 2001) which can better use the topsoil moisture (Nippert & Knapp, 2007). Phenologically, C4 plants start grow later than C3 plants mainly due to their preference for the combination of high temperature and precipitation in the summer. In years with low precipitation in the early summer, they will even start growth in July. Hence, C3 and C4 plants may have different water use patterns influenced by the seasonal soil moisture. In the leaf scale, water availability can influence CO_2 and water exchange controlled by stomata conductance, photosynthesis, and carbon isotope signature (Farquhar *et al.*, 1989). Thus, we needed to understand how C4 plants use precipitation together with coexisting C3 plants, which substantially may influence the relative proportion of C4 plants and their carbon isotope signature.

There are only few researches focused on the Δ variation of C4 plants (Δ_4) with environmental conditions and disturbance in a cool-season semi-arid grassland (Chen *et al.*, 2002). The grassland of the Mongolia plateau has large gradients in grazing pressure, climatic and edaphic conditions like flat and slope, which allow studying and making it necessary to study the influence of these gradients on the Δ_4. *Cleistogenes squarrosa,* is a dominant endemic C_4 (NAD-ME) perennial bunchgrass in the typical semi-arid steppe of the Mongolia plateau in Eurasia grassland. Understanding how the Δ of *C. squarrosa* varies with environmental conditions and disturbance is crucial for understanding the mechanism of Δ variation of C4 plants. Meanwhile, the increasing disturbances have been caused by the increase of grazing pressure, the increase use of motor vehicles, and the change of climate. Based on Grime's C-S-R theory (Grime, 1977), the increase of disturbance should increase the proportion of ruderals which should be annuals, especially C4 annuals in this grassland due to the high photosynthesis efficiency. Due to the dominance of *C. squarrosa* and *S. collina* in C4 perennials and C4 annuals respectively, this would affect the ratio of C4 perennials and C4 annuals. Hence, we expect that studying the temporal variation of C4 perennials and C4 annuals will contribute to understand the dynamics of them during the increase of C4 plants.

Aims

The subject of present study is to better understand the variation of C4 community in the Inner Mongolia grassland. The following three questions are focused: a) Do the water sources differ between C4 plants and C3 plants given that they differ strongly in their seasonal development? b) How is the response of C4 plants' discrimination and hence their water use efficiency to environmental conditions and grazing pressure? c) How did the relative biomass of annual and perennial components of the C4 community and their regional distribution change over time during/after the spread of C4? Hence, the first part of this thesis evaluates the water sources among dominant species and the contribution of winter precipitation to plant growth. The experiment was conducted in a dry year and a wet year on three long-term fenced and ungrazed plots around IMGERS. Two end-member mixing model was used to calculate the fraction of winter half-year precipitation making use of the pronounced difference in isotopic composition of winter and summer precipitation. Specially, the following questions were addressed: First, do the dominant plant species differ in water use originating from either the meteorological winter half-year (i.e. October through April) or summer (May through September) precipitation? Second, how does the water source of plants vary with seasonal and yearly changes in precipitation? Third, at community level, what is the relative contribution of winter precipitation to plant total water uptake? (Chapter II)

The second part focuses on the variation in the *Δ* and water use efficiency of *C. squarrosa* as the dominant C4 species on different scales from the tiller scale to the local, the catchment and the regional scale. The questions addressed in this part are: 1) How do the *Δ* of *C. squarrosa* and its controlling factors vary across the different scales (i.e., at tiller, local, catchment, and regional scale)? 2) What are the possible mechanisms underpinning the variation in *Δ* of *C. squarrosa* at different scales? 3) How do the water use efficiency of *C. squarrosa* vary on different scales? (Chapter III) The last part evaluates the variation of relative biomass of C4 perennials and C4 annuals from 2001 to 2009 and further contributes to understand the processes of the increase of C4 plants. Except for the rise of temperature causing the increase of C4 plants, highly variable precipitation is an important factor to modify the process of the dynamics of C4 perennials and C4 annuals as it is a limited factor of plant growth. Hence, the objectives of this part are: 1) What are the temporal dynamics of C4 annuals and C4 perennials during disturbance causing the increase of C4 plants in the grassland in the Mongolia plateau? 2) How does growing season precipitation and grazing influence the trends of both? (Chapter IV). Their roles during the increase of C4 plants will be discussed. Furthermore, the discrimination of C4 annuals and C4 perennials were obtained and the Δ_{C4} was calculated (Chapter V).

CHAPTER II – Complementarity in water sources among dominant species in typical steppe ecosystems of Inner Mongolia, China[1]

Abstract

Water is the most important factor controlling plant growth, primary production, and ecosystem stability in arid and semi-arid grasslands. Here we conducted a two-year field study to explore the contribution of winter half-year (i.e. October through April) and summer precipitation (May through September) to the growth of coexisting plant species in typical steppe ecosystems of Inner Mongolia, China. Hydrogen stable isotope ratios of soil water and stem water of dominant plant species, soil moisture, and plant water potential were measured at three steppe communities dominated by *Stipa grandis*, *Caragana microphylla*, and *Leymus chinensis*, respectively. The fraction of water from winter half-year precipitation was an important water source, contributing 45% to plant total water uptake in a dry summer after a wet winter period (2005) and 15% in a summer where subsoil moisture had been exploited in the previous year (2006). At species level, *C. microphylla* exhibited a complete access to deep soil water, which is recharged by winter precipitation, while *Cleistogenes squarrosa* completely depended on summer rains. *L. chinensis*, *Agropyron cristatum*, and *S. grandis* showed a resource-dependent water use strategy, utilizing deep soil water when it was well available and shifting to rain water when subsoil water had been exploited. Our findings indicate that differentiation of water sources among plants improves use of available soil water and lessens the interspecific competition for water in these semi-arid ecosystems. The niche complementarity in water sources among coexisting species is likely to be the potential mechanism for high diversity communities with both high productivity and high resilience to droughts.

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[1] Hao Yang, Auerswald K, Bai YF, Han XG (2010) *Plant and Soil*, 10.1007/s11104-010-0307-4.

Introduction

Water is the most limiting factor influencing species richness, productivity, and stability of grassland ecosystems (Sala *et al.*, 1988; Knapp *et al.*, 2001; Bai *et al.*, 2004; Bai *et al.*, 2008). In arid and semi-arid ecosystems, coexisting plant species may utilize water from different sources (Ehleringer & Dawson, 1992). For instance, the early spring species mainly use water resulting from snowmelt, while most warm-season species take advantage of the rain when the rainy season comes (Ehleringer *et al.*, 1991). This may also cause a systematic variation among photosynthetic types, as C4 plants dominate more in summer vegetation due to their preference for higher temperatures, while C3 plants are more efficient at cooler temperatures as found in spring (Ehleringer & Björkman, 1977).

For different species, the ability of extracting water from different soil layers depends mainly on their active rooting depth (Flanagan *et al.*, 1992; Leffler & Caldwell, 2005; Schwinning *et al.*, 2005), which can vary with the vertical distribution of water contents (Dodd *et al.*, 1998; Leffler & Caldwell, 2005). The water source can shift from shallow soil water to greater depth when the shallow soil water is depleted (Nippert & Knapp, 2007). Plants that have the ability to shift among different water sources might have competitive advantages in arid and semi-arid environments, because they can exploit water resources to maintain their growth under fluctuating environments (Ehleringer & Dawson, 1992). However, Bazzaz (1996) argued that most of the grassland plants could only use limited water resources due to the intrinsic nature of rooting depth and life history strategies. Empirical test of these hypotheses and predictions in semi-arid grassland ecosystems is still lacking, as most studies have been conducted in deserts, savannahs, and mesic grassland ecosystems (Dawson *et al.*, 2002; Nippert & Knapp, 2007). Thus, a comprehensive understanding of water use strategies of coexisting species in semi-arid grassland ecosystems is needed, particularly in the Eurasian steppe.

The grassland ecosystems in the Inner Mongolia steppe region of northern China are representatives of the Eurasian steppe (Chinese Academy of Sciences Integrative Expedition Team to Inner Mongolia and Ningxia, 1985; Bai *et al.*, 2007). In this semi-arid grassland with evapotranspiration exceeding rainfall during summer, summer rains mainly recharge the topsoil except for macropore flow, while deeper soil layers can only be recharged during the winter-half year, especially by snowmelt, which also drives catchment runoff (Schneider *et al.*, 2007). Consequently, water from winter half-year precipitation (mostly snow water) can be better used by deep rooted plants while shallow rooted plants are restricted to summer rains. When topsoil water is limiting, high fraction of winter precipitation in total water uptake of some species may mitigate the interspecific competition for water. Based on the theory of niche complementarity (Loreau *et al.*, 2001), the partitioning of water sources among coexisting species will improve the use of available soil water and thus increase ecosystem resilience to droughts.

To test the above hypothesis, we conducted a two-year field study at three typical steppe communities dominated by *Stipa grandis, Caragana microphylla,* and *Leymus chinensis*, respectively. Specifically, we want to address the following three research questions: First, do the dominant plant species differ in water use originating from either the meteorological winter half-year (i.e. October through April) or summer (May through September) precipitation? Second, how does the water source of plants vary with seasonal and yearly changes in precipitation? Third, at community level, what is the relative contribution of winter precipitation to plant total water uptake?

Material and Methods

Study site

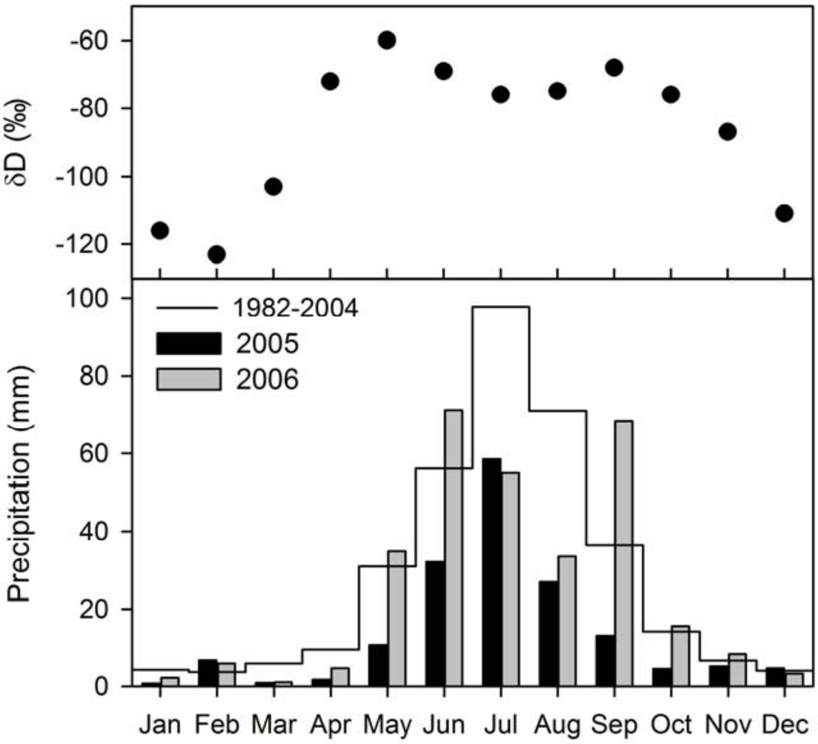

Fig. II.1 The monthly distribution of precipitation for 2005 and 2006 compared to the long-term mean (1982-2004) (bottom panel) and predicted hydrogen stable isotope ratio (δD) for the study area according to www.waterisotopes.org. (top panel).

The study was carried out at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS), which is located in the Xilin River Basin (116°42′E, 43°38′N), Inner Mongolia Autonomous Region of China (Bai *et al.*, 2004). The continental temperate semi-arid climate is characterized by a cold and dry winter but a warm and moist summer (Chen, 1988). The mean annual temperature in the study area is 0.4 ºC with mean monthly temperatures ranging from -21 ºC in January to 19 ºC in July (Bai *et al.*, 2008). The growing season begins in the end of April and ends in early October, lasting about 150 days. The mean annual precipitation is 337 mm with 60-80% falling during the growing season. Actual evapotranspiration (ET) nearly equals the precipitation within one single year (Wen, 2006). Winter precipitation (snow) begins to melt and penetrates to deep soil layers when temperature increases rapidly in April. The river discharge hence peaks in April exceeding the discharge during the precipitation peak in June and July (Schneider *et al.*, 2007).

Three typical steppe communities were selected as our experiment plots, which belong to the permanent field sites of IMGERS. The first plot was dominated by *Stipa grandis* and was fenced from animal gazing since 1979. The second plot was dominated by *Caragana microphylla* and was fenced since 1983. The third plot was dominated by *L. chinensis* and was fenced since 2001. More details about communities and soils are given in Table II.1

Table II.1 Characteristics (mean ± SE) of the plant communities and topsoils properties (0 - 20 cm).

		Stipa grandis plot		*Caragana microphylla* plot		*Leymus chinensis* plot	
Item		2005	2006	2005	2006	2005	2006
Species richness (n = 10)		10.5 ± 0.5	11.1 ± 0.7	11.2 ± 0.7	11.8 ± 0.7	15.1 ± 0.6	16.6 ± 1.1
Total aboveground biomass (g m^{-2}) (n = 10)		98.1 ± 5.1	135.2 ± 3.9	101.1 ± 5.6	211.6 ± 12.8	153.5 ± 6.5	135.1 ± 5.0
Litter weight (g m^{-2}) (n = 10)		110.4 ± 8.4	147.2 ± 12.6	104.9 ± 7.4	120.5 ± 11.8	172.2 ± 17.0	234.1 ± 31.6
Bulk density (g cm^{-3}) (n = 5)		1.30 ± 0.03		1.41 ± 0.00		1.29 ± 0.04	
Calcic layer		Yes (30 - 40 cm)		Yes (>50 cm)		No	
Soil texture (%)	Clay	7.44		7.65		11.61	
	Silt	23.46		11.29		18.85	
	Sand	69.10		81.06		69.54	

Note: The particle size is 0.05-2.0 mm for sand, 0.002-0.05 mm for silt, and < 0.002mm for clay.

Field sampling and stable isotope analysis

To explore the water sources of dominant species, we selected six species, i.e., *S. grandis*, *L. chinensis*, *Cleistogenes squarrosa*, *Agropyron cristatum*, *Carex korshinskyi* (only in *L. chinensis* plot) and *C. microphylla* (only in *C. microphylla* and *L. chinensis* plots). All of them are perennials and together account for more than 80% of the total aboveground biomass. Both *S. grandis* and *A. cristatum* are C3 bunchgrasses, *L. chinensis* is a C3 rhizome grass, and *C. microphylla* is a C3 legume shrub. *C. korshinskyi* is a C3 sedge. *C. squarrosa* is a C4 short bunchgrass characterized by a shallow rooting system (Chen *et al.*, 2001; Jigjidsuren & Johnson, 2003). All species start the growing season in April except for C4 species like *C. squarrosa*, which begins the growing season after May.

A 100-m transect was established within each plot. Samples were collected in early May (May 7-8) and mid August (August 17-20) respectively in both 2005 and 2006.

Plant samples from the non-photosynthetic tissues at the interface between shoot and root systems, often at or just below the soil surface, were collected for analyzing the hydrogen stable isotope ratios (Thorburn & Walker, 1993). For each species, the non-photosynthetic tissues from at least 20 individuals were collected as one replicate, enclosed in a screw-capped glass vial, and sealed immediately using Parafilm. Soil samples at different depths (i.e., 0-5, 5-10, 10-20, 20-40, 40-60, 60-80, and 80-100 cm) were also collected by a 5-cm diameter soil auger to analyze hydrogen stable isotope ratios of soil water. There were three replicates for each plant species and soil depth at each plot. Soil moisture at different depths (0-20, 20-40, and 40-110 cm, n = 3) for each plot were measured gravimetrically every ten days from early May to mid of October in 2005 and 2006, and then converted to volumetric basis. For each soil depth, plant available water was calculated as the difference between volumetric soil water content and unavailable soil water. For each site, the unavailable soil water was the average of the lowest water contents from three replicates at the top (0-20), middle (20-40 cm), and bottom (> 40 cm) layer that were measured in both years. These values, which were used to approximate the wilting points, should best reflect the plant-water relations under the local pedoclimatological situation (Li & Chen, 1999). At the *Stipa* site, for instance, these values (6% vol.) were close to those values (5.5 to 7.5 % vol.) determined by laboratory methods (Li & Chen, 1999).

Summer rain in Inner Mongolia usually occurs in short, high-intensity events. Rains occurring within four to five days before the plant sampling were collected at IMGERS with a dry bowl, which was checked for dryness every morning and sampled immediately after a rain as it was continuously under control at the research station and could be reached within < 2 min. Rainwater samples were immediately enclosed in air-tight glass vials and stored in a refrigerator at 4 °C. The plant tissue and soil samples were kept frozen in the laboratory until water was extracted using a cryogenic vacuum distillation apparatus (Ehleringer & Osmond, 1989). The water samples were pyrolyzed into CO and H_2 in an elemental analyzer (TCEA; Thermo Finnigan) interfaced (ConFlo III; Thermo Finnigan MAT) to a Thermo Finnigan Delta^{plus}XP isotope ratio mass spectrometer (Thermo, Waltham, USA) in the Institute of Botany, Chinese Academy of Sciences (Beijing, China). All samples were measured against laboratory working standard gases, which were previously calibrated against IAEA standards (IAEA SMOW for D, accuracy of calibration ± 0.10‰ SD). Two kinds of laboratory working standard water, which were also previously calibrated against IAEA standards (IAEA SMOW), were measured additionally after every twenty samples against these references. The precision for sample repeats was better than 1‰ for δD. The stable isotope ratio of hydrogen in water is expressed using standard delta notation (δ) in parts per thousand (‰) as:

$$\delta D = (R_{sample} / R_{standard} - 1) \times 1000 \qquad \text{(II.1)}$$

where R_{sample} and $R_{standard}$ are the molar ratios of D/H of the sample and standard water (V-SMOW), respectively.

Water potential of plant leaves

To explore the relationship between plant water potential and the δD value of plant water, leaf samples of four dominant species, *L. chinensis, S. grandis, A. cristatum* and *C. squarrosa*, were collected at the *S. grandis* plot during August 20-24, 2005. For each species, the whole aboveground plant tissues were taken, sealed in plastic bags, and stored immediately in an icebox. Predawn (05:00) water potential (ψ_{pd}) and midday (12:00) water potential (ψ_{md}) from three fully matured leaves of each species were measured shortly afterwards (within 2 h) by an HR33-T Dewpoint Potential Meter (Wescor, Logan, USA).

Aboveground biomass

Aboveground biomass was sampled within a period of August 17 to September 2, when the standing crop of the communities reached their annual peak in both 2005 and 2006. For each plot, ten 1×1 m^2 sampling quadrats were arranged randomly. All living biomass within each quadrat was collected by clipping to the soil surface, separated to species, oven dried at 65 °C to constant mass, and weighed. The height, number of individuals, and coverage of each species were also recorded within each quadrat. Aboveground biomass and species richness for each community were estimated by averaging the ten quadrats. We approximated ANPP with the annual peak community biomass as is commonly done for semi-arid grassland communities (Sala *et al.*, 1988; Bai *et al.*, 2004; Bai *et al.*, 2008) as the plots were fenced.

Data analyses

We used simple mass balance (Ehleringer *et al.*, 1991; Williams & Ehleringer, 2000) between lower and upper limit δD of possible water sources to calculate the fraction of total plant water uptake constituted by winter precipitation (*fw*) based on the following reasoning. In the semi-arid Inner Mongolian grassland, the deep soil water is only recharged by precipitation after the growing season and by snowmelt in the early spring because of potential evapotranspiration exceeding rainfall in summer. Therefore, the δD value of deep soil water is a good indicator of winter half-year water although without measurement we do not know how much fractionation occurred during the snowmelt and soil water evaporation (Winograd *et al.*, 1998; Li *et al.*, 2006b). The δD value of soil water at 100 cm depth then approximates the lower limit of δD value of plants because the maximum rooting depth for most perennial grasses and forbs is less than 100 cm (Cheng *et al.*, 2006). Even for the deep rooting species, *C. microphylla*, the δD value of 100 cm soil water can also be used for calculating f_w because the δD value below this depth is less variable (Brunel *et al.*, 1995; Cook & O'Grady, 2006). To further validate the above assumption, we measured δD values of soil samples in the early May and mid August in both 2005 and 2006 across the three plots. The δD of 100 cm depth was relative stable (δD_{mean} = -94.2 ‰, SE = 1.4 ‰, n = 6) (see results), which was very close to another measurement in 2003 in *L. chinensis* plot (δD = -94 ‰, depth = 90 cm; δD = -96 ‰, depth = 120 cm) (Qing, unpublished data). Thus, the average value of δD at 100 cm soil depth was used for estimating the f_w of plants.

We used the δD value of last rain before each sampling date as the upper limit of plant xylem water (Williams & Ehleringer, 2000). At the *C. microphylla* plot, no effective rainfall occurred within two weeks before plant sampling in August, 2006. Thus, we used the average δD at 2-10 cm soil depth (δD_{mean} = -37.18‰, SE = 1.25 ‰, n = 3) for calculating the f_w because the δD value of soil water at 0-2 cm depth was highly enriched due to strong evaporation.

The fraction of plant total water uptake constituted by winter precipitation (f_w) can then be estimated:

$$f_w = \frac{\delta D_P - \delta D_R}{\delta D_{DSW} - \delta D_R} \qquad \text{(II.2)}$$

with the indices *P* for the water from non-photosynthetic tissues at the interface between shoot and root, *R* for last rain water, and *DSW* for deep soil water (100 cm). The f_w was set to 0 when the δD value of plant was above the δD value of rain probably attributed to using enriched dew or surface soil water, particularly in the arid and semi-arid ecosystems (Cheng *et al.*, 2006; Li *et al.*, 2006b; Zhao *et al.*, 2009). When the δD value of plants was below the δD value of 100 cm soil water, the f_w was set to 1 (Cheng *et al.*, 2006).

Statistical analyses for isotope data and volumetric soil water were performed by using general linear model (Univariate analysis) in SPSS Version 16.0 (SPSS Inc., Chicago, USA, 2007). Experimental factors, including plot, species, year, season, and all interactions were treated as fixed effects. One-Way ANOVA, followed by a least-significant difference (LSD) multiple-range test, was applied to compare the predawn water potential, midday water potential, and the diurnal range of water potential for the four species. Independent-samples *t*-test for the f_w was used to examine the statistic significance at $P < 0.05$ and $P < 0.01$ levels between May (early growing season) and August (peak growing season) in both 2005 and 2006.

Results

Precipitation, soil moisture, and soil water δD

In comparison with the long-term average (1982-2006), annual precipitation was 51% and 11% lower in 2005 and 2006, respectively (Fig. II.1). The main growing season precipitation from April to August was 53% higher in 2006 (199 mm) than that in 2005 (130 mm). In 2005 the main share of rain fell in July, while rain distribution was bimodal in 2006 with two peaks appearing in June and September, respectively. Hence precipitation in 2006 was about normal in May and June but also below average in July and August.

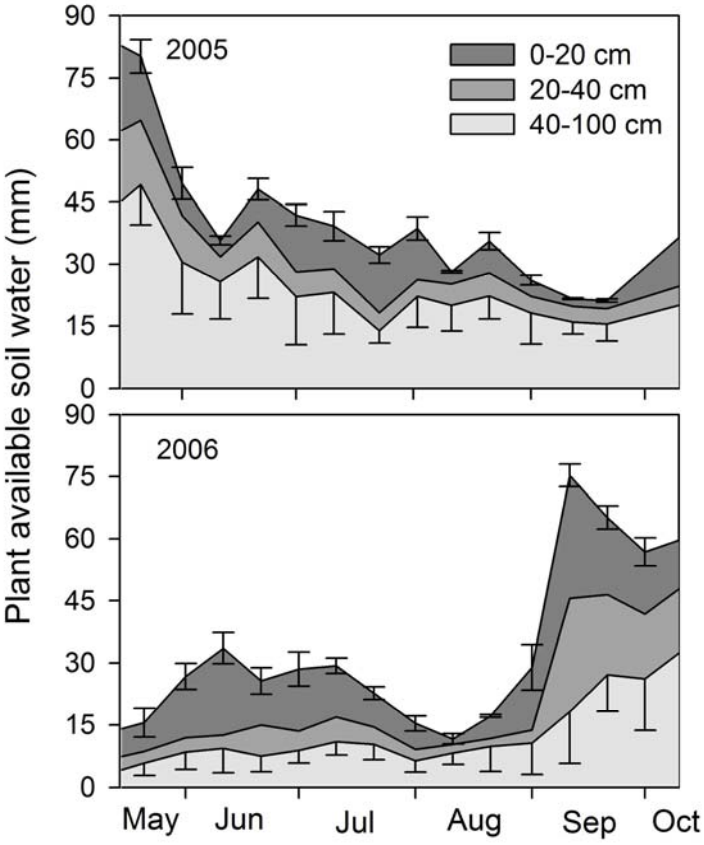

Fig. II.2 Plant available soils moisture (volumetric water content minus unavailable water) accumulated over three depths during the growing seasons in 2005 and 2006 and averaged over the *Stipa grandis* plot, the *Caragana microphylla* plot, and the *Leymus chinensis* plot. The total annual precipitation was 166 mm in 2005 and 304 mm in 2006. Vertical bars denote the standard deviation (n = 9). For readability, error bars are not given for the 20-40 cm layer (on average half of the standard deviation in the < 20 cm layer) and only the lower half error bar is shown for the > 40 cm layer.

For all three plots, soil moisture within 0-20 cm fluctuated more than at other depths (Fig. II.2). It was continuously depleted by evapotranspiration and recharged by rain in the top layer while the soil moisture at greater depth – especially > 40 cm – only became depleted because rain was not sufficient to cause percolation to that depth. At the end of the 2005 growing season, almost all plant available soil water had been removed from all depths. Precipitation during the winter half-year 2005/2006 was only 25 mm and thus not sufficient to recharge soil moisture at > 20 cm depths. Consequently, the depleted subsoil was inherited in the winter 2005/2006. Thus 2005 had a dry summer but soil moisture derived from previous winter precipitation was available below 20 cm depth, while 2006 was a normal year regarding early summer precipitation but it was dry in terms of deep soil moisture originated from the previous winter precipitation. Rains at the end of the growing season 2006 exceeded the already low evapotranspiration at that time and started to recharge the plant available water > 40 cm depth.

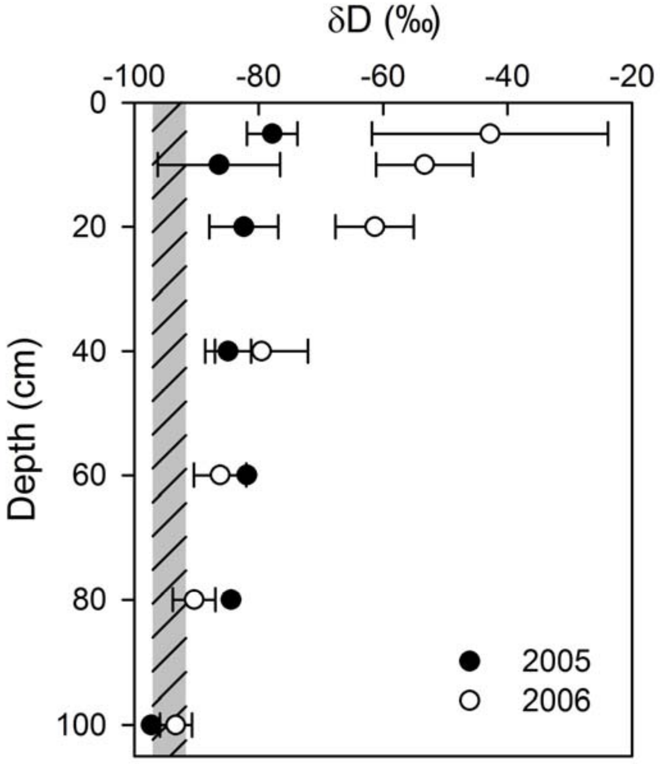

Fig. II.3 Hydrogen stable isotope ratio (δD) of soil water. Gray area indicates the range of the δD values at 100 cm soil depth. Horizontal bars denote the standard deviation of means (n = 6) for each data point.

Soil water δD (Fig. II.3) decreased with increasing depth at all the plots and across sampling times. Greater variation was found at the topsoil (20 cm) while the δD values were less variable at the deep soil layers. For all the three plots, the δD value of the 100 cm soil was most negative, similar for all profiles and similar to what can be expected for the precipitation during the winter-half year (Fig. II.1). In the dry summer 2005 with large contribution of cool-season precipitation, δD was lower than that in 2006 when soil water mainly resulted from summer rains.

δD in plant water at the stem base

There were significant differences in δD values among species (S), plots (P), years (Y), and months (M) and the interactions of S × P, S × Y, S × M, P × Y, Y × M, and S × P × Y were also significant (Table II.2). The main effects (S, P, Y, M) had by far the largest influence except for M (see mean square in Table II.2) while the interactions of two factors explained one order of magnitude less except for Y × M indicating that the influence of M in contrast to the other three main factors differed strongly between the years. The interaction of three factors explained another order of magnitude less. Among species, the most distinct differences were found between *C. microphylla* and *C. squarrosa* in their utilization of winter precipitation (see below).

Table II.2 Univariate analyses of stem base δD values using Species, Plot, Year, Month, and all interactions as fixed-effects.

Source	Type III Sum of Squares	df	Mean Square	F	*P*
Species (S)	9655.34	5	1931.07	82.60	0.000
Plot (P)	3335.66	2	1667.83	71.34	0.000
Year (Y)	3648.33	1	3648.33	156.05	0.000
Month (M)	139.08	1	139.08	5.95	0.018
S × P	808.33	7	115.48	4.94	0.000
S × Y	3182.77	4	795.69	34.03	0.000
S × M	646.29	3	215.43	9.21	0.000
P × Y	1518.30	1	1518.30	64.94	0.000
Y × M	765.25	1	765.25	32.73	0.000
S × P × Y	243.67	3	81.22	3.47	0.021
S × Y × M	42.10	2	21.05	0.90	0.412
Error	1449.53	62	23.38		
Total	456318.08	93			
Corrected Total	27905.82	92			

Fraction of winter precipitation to plant water uptake (f_w)

The δD of rain (Fig. II.4) was within the expected range (Fig. II.1) and differed significantly from δD at 100 cm soil depth in both the early growing season and peak growing season and across the two years ($P < 0.05$). In 2005 the plant stem water was intermediate indicating that the plants took up rain water from the topsoil but also subsoil water (Fig. II.4) with two exceptions. *C. microphylla* only used deep soil water while *C. squarrosa* completely depended on shallow water from summer rains.

In 2006, when deep soil water was low due to the previous dry year δD in plant stem water was close to or even above precipitation water for most species; this suggested that the plants were mainly using rain water or even the water in the very top soil, which was enriched by evaporation. Again, there were two exceptions. *L. chinensis*, which had already used slightly more subsoil water in 2005, still used some subsoil water in May 2006. *C. microphylla* did not switch to rain water; its stem water even was slightly below δD in 100 cm depth, implying that *C. microphylla* accessed even deeper soil water.

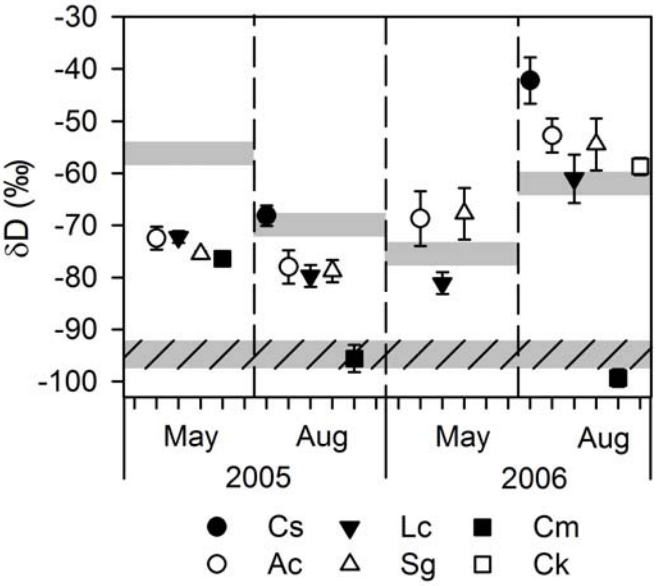

Fig. II.4 Hydrogen stable isotope ratios (δD) of the non-photosynthetic plant tissues (the interface between the shoot and root systems). Upper gray area indicates the range of rain δD values. Lower gray diagonal area indicates the range of the δD values at 100 cm soil depth. Vertical bars denote the standard deviation (n = 3-9). Cs, *C. squarrosa*; Lc, *L. chinensis*; Cm, *C. microphylla*; Ac, *A. cristatum*; Sg, *S. grandis*; and Ck, *C. korshinskyi*.

The large contrast between δD in rain and subsoil water allowed calculating the f_w. In the dry summer year with sufficient subsoil moisture (2005), f_w was three times larger than that in 2006 when averaged across all species and seasons (2005: mean = 0.45, SE = 0.05, n = 42; 2006: mean = 0.15, SE = 0.04, n = 51). Less water was delivered by rain while winter-derived water was available in 2005. However, in 2006 more rain but only little winter-derived water was available. Thus, f_w was lowest in May 2006. But it increased slightly again in August 2006 when there was enough moisture from the rain though available moisture was low in the subsoil (Fig. II.5). The dry July and

August in 2006 then forced the plants to extract some subsoil moisture even though the subsoil was largely depleted by the previous year (Fig. II.2).

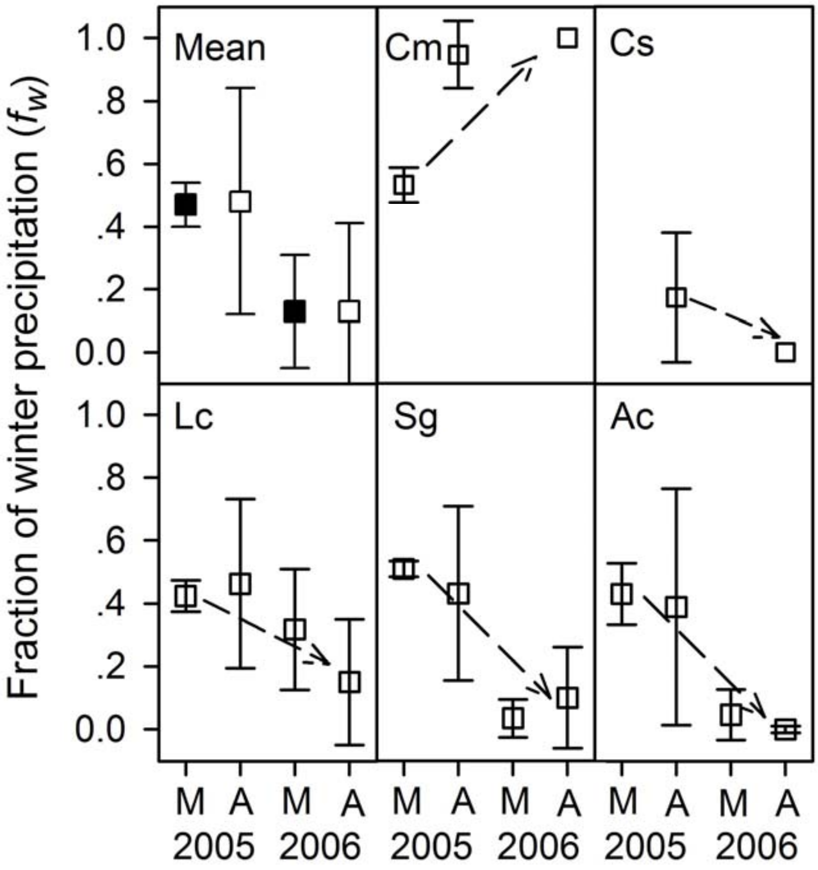

Fig. II.5 Fraction of total plant water uptake constituted by winter precipitation (f_w) calculated from the δD values of plant water in non-photosynthetic tissues. Vertical bars denote the standard deviation for each data point. Mean is the average over all species. Species are abbreviated as in Fig II.4. Note: *C. squarrosa* could only be sampled in August due to its late onset of growth. M = May and A = August.

The species differed in their access to water. *C. microphylla* always had access to deep soil water and even increased this access during dry conditions. In contrast, *C. squarrosa* completely depended on shallow water from summer rains even in 2005 when winter moisture was available (Fig. II.5). This is not only due to the fact that *C. squarrosa* appears after May and could only be sampled in August. Even in 2006, when there was more water in the topsoil due to more rain, *C. squarrosa* even restricted water uptake to the very top 10 cm, where the soil water was enriched by evaporation compared to rain (compare Figs. II.3 and II.4). All other species showed a more resource-dependent water use strategy. They used subsoil water in the early growing season 2005 when there was still enough water in the subsoil and then gradually shifted to rain water as the subsoil became depleted. In 2006, when there was not much winter moisture stored in the subsoil, they almost entirely used rain water. Among the species with resource dependent strategy, *L. chinensis* tended to use more winter-derived water than *S. grandis* and *A. cristatum*. This was especially evident in 2006 and caused a slower shift between water sources than with the two other species (see slope of the arrows in Fig. II.5).

Plant leaf water potential

Both the predawn leaf water potential (ψ_{pd}) and midday leaf water potential (ψ_{md}) differed significantly among the four species examined (Table II.3). For a given species, the mean ψ_{md} was more negative than that of the ψ_{pd} ($P < 0.01$). As compared to the other three species, *C. squarrosa* showed the highest diurnal variation of ψ_{md} and ψ_{pd}, which was mainly due to a pronounced recovery overnight (Table II.3). Among the four species, *L. chinensis* exhibited the least negative ψ_{pd}, which was followed by *C. squarrosa, A. cristatum*, and *S. grandis* (Table II.3). In addition, the δD value increased significantly with less negative ψ_{pd} across all species except for *L. chinensis* ($R^2 = 0.73$, $P < 0.003$, n = 9), indicating that the use of deep water increased with increasing plant water stress.

Table II.3 Predawn (ψ_{pd}) and midday (ψ_{md}) leaf water potentials of the four dominant species at the *S. grandis* plot in August, 2005.

Species	ψ_{pd}	ψ_{md}	$\Delta_{\psi pd-\psi md}$
C. squarrosa	-3.44 ± 0.10a	-7.02 ± 0.40a	3.58 ± 0.49a
S. grandis	-4.71 ± 0.10b	-7.04 ± 0.21a	2.33 ± 0.15b
A. cristatum	-3.96 ± 0.24c	-5.58 ± 0.09b	1.62 ± 0.64b
L. chinensis	-3.33 ± 0.04a	-4.93 ± 0.13b	1.60 ± 0.17b

Note: Data in columns are shown with mean ± SE (n = 3). The different letters indicate a significant difference ($P < 0.05$) in a Least-Significant Difference (LSD) test for species within each column. The statistical difference between ψ_{pd} and ψ_{md} within a row was examined using t-test; it was $P < 0.01$ in all cases.

Discussion

Hydrology

Both years differed considerably in precipitation. Summer precipitation alone was, however, not sufficient to characterize the water supply to the vegetation, because a considerable amount of the property of the antecedent year was carried over. After a wet antecedent year the subsoil supplied moisture whereas after a dry year the vegetation had to rely solely on summer precipitation. Our assessment based on soil moisture measurements is corroborated by eddy covariance measurements, on the *L. chinensis* plot, which showed that evapotranspiration was about 50 mm larger than summer rainfall in 2005 whereas it equaled precipitation in the growing season of 2006 until soil recharge began at the end of August 2006 and precipitation exceeded evapotranspiration (Wang *et al.*, 2010). The huge inter-annual variability in summer precipitation, typical for this area (Wittmer *et al.*, 2008), is thus attenuated if the soil is recharged before a growing season. Several dry years in succession, even if only moderately dry, should be more severe than a dry year after a wet year. Strategies to switch water use between topsoil and subsoil should hence be advantageous for the plants as none of both sources is reliable.

On average, the δD values of precipitation in the early growing season and peak growing season closely followed the predictions by www.waterisotopes.org with a mean deviation of 2‰, but the individual months of both years varied considerably with a mean absolute deviation from the prediction of 9‰. Summer rains could not recharge the deeper soil depths and the δD values of 100 cm soil water varied in a narrow range (δD_{mean} = -94.24‰, SE = 1.40‰, n = 6) while the variation of rain was larger (mean = -70.03‰, SE = 3.30‰, n = 11). Thus, the fraction of winter precipitation contributing to plant water uptake could be calculated. The δD of dew was -40‰ on average (unpublished data), which was above the δD of plant water. The extremely negative ψ_{pd}, however, indicated that in our case the contribution of dew to total plant water uptake was too small even to cause a significant relaxation of leaf water potential during the night. Previous studies have proposed that some halophytes and woody xerophytes discriminate against deuterium during water uptake, which seems to be caused by the specific root morphology of such plants (Ellsworth & Williams, 2007). Based on the measurement of δD and $\delta^{18}O$ in stem water for one sampling date in 2005, we found that there was a significant correlation between δD and $\delta^{18}O$ in stem water ($R^2 = 0.77$, $n = 18$), which followed the mixing line between soil water and groundwater with no species exhibiting more negative δD. This indicates that no discrimination against deuterium occurred during plant water uptake.

Relationship between δD and leaf water potential

The ψ_{pd} provides a good indicator for leaf water stress and recovery during night when leaf transpiration is small. The ψ_{pd} was extremely negative and considerably lower than what was found in a South American grassland (Colabelli *et al.*, 2004) but within the range reported in Inner Mongolia (Liu *et al.*, 2004), indicating a pronounced cell wall rigidity (Kramer & Boyer, 1995).

The close correlation between predawn water potential and δD (respective f_w) indicates that with increasing water stress the plants increasingly exploit winter moisture stored at greater depth. Among the four species, the ψ_{pd} value of *S. grandis* was most negative and that of *L. chinensis* was the least negative, while those of *A. cristatum* and *C. squarrosa* were intermediate. Similar to the South American grassland (Colabelli *et al.*, 2004), there was a large variation between species but this did not relate to the photosynthetic pathway.

Root architecture seems to be responsible for the large variation of leaf water potential among species. Chen *et al.* (2001) have reported that the roots of *C. squarrosa* only extend to 14 cm depth and that of *A. cristatum* to 25 cm depth, while *S. grandis* and *L. chinensis* have deeper roots than the former two species. Thus, both the lowest and the highest leaf water potential occur with deep rooted species, while the shallow rooted species are intermediate. The discrepancy in between the deep rooted species presumably relates to the fact that *L. chinensis* seems to be able to better buffer diurnal fluctuations in water deficit due to its rhizomes and thus exhibits the lowest range in leaf water potential. Also, *L. chinensis*, which extracted winter moisture from below 70 cm depth as indicated by higher f_w, seems to be competitively superior to other perennial grasses in exploiting the subsoil moisture.

Possible relationship between the water-use strategy and species coexistence

The survival of perennial species through extended drought periods depends on both the ability of roots to acquire limited soil water and the ability of shoots to tolerate water stress (MacMahon & Schimpf, 1981), and the differences in rooting niche separation among species contribute to minimize competition for water during prolonged drought periods when upper soil layers become dry (Mooney *et al.*, 1980). In this study, we found that plants made use of soil moisture at greater depth when it was available (early growing season of the dry 2005) and otherwise shifted to the upper layer. This suggests that plant competition for water is intensified when water is limited.

Among the species, which were able to shift water extraction between topsoil and subsoil, *L. chinensis* made more use of subsoil water than the others. The large share of *L. chinensis* in total aboveground biomass (Chen *et al.*, 2005a) supports the notion that deep rooted species, which use the deep soil water, had competitive advantage in these steppe ecosystems. First, the rhizomes may take up water from soil and serve as a water storage organ (Wang *et al.*, 2003) and buffer diurnal variation in water potential leading to the narrow range of diurnal leaf water potential. Second, the leaf water potential of *L. chinensis* was less negative than those of the other species. Third, even without a large water stress *L. chinensis* extracted much more winter moisture than the other species. *C. squarrosa* does not seem to have the plasticity to shift among water sources as it only used topsoil moisture, which is reasonable as it is the last species to start growth (June). During dry summer years it may even delay the start of growing season (until early July) and thus compensate for the lacking plasticity in water extraction depth.

Our findings support the general predictions of niche complementarity theory that communities with high plant diversity tend to be more productive because of fuller resource utilization (Loreau *et al.*, 2001). Furthermore, our study gives more insights into the relationship between plant water sources, species coexistence and productivity. The niche complementarity in water sources among the coexisting species may lead to better use of available soil water. This is likely to be the potential mechanism for high diversity communities with both high productivity and high resilience to droughts (Bai *et al.*, 2004). Similarly, the reduced community productivity and the intensified severity of droughts in the overgrazed community may be attributable to both the losses of biodiversity and diminishing complementarity in plant water use (unpublished data). Our general conclusion was also corroborated by recent studies (Bai *et al.*, 2004; Cui *et al.*, 2005; Bai *et al.*, 2007; Querejeta *et al.*, 2007; Bai *et al.*, 2008).

Long-term observations showed that frequent drought and warming together with overgrazing in this region, which decrease subsoil water recharge and promote shallow rooting species, are likely to be key drivers for the shifts in species composition, i.e., steppe communities originally dominated by *L. chinensis* and *S. grandis* were replaced by *C. squarrosa*, *Artemisia frigida* but also by *C. microphylla* (Li, 1989; Xiong *et al.*, 2003; Wittmer *et al.*, 2010). Thus the dominant species that utilize subsoil moisture in the undisturbed communities become less abundant in the degraded communities. This may decrease the water use efficiency and further intensify the impacts of drought, overgrazing, and regional warming.

CHAPTER III –Variation in carbon isotope discrimination in *Cleistogenes squarrosa*: Patterns and drivers at tiller, local, catchment, and regional scales

Abstract

Understanding patterns and drivers of carbon isotope discrimination in C4 species is critical for predicting the effects of change in C3/C4 ratio of plant community on ecosystem processes and functionning. *Cleistogenes squarrosa* is a dominant C4 perennial bunchgrass of arid and semi-arid ecosystems across the Mongolia plateau of the Eurasian Steppe. Its carbon isotope discrimination (Δ) during photosynthesis is relatively large and variable among C4 species, which implies variable water use efficiency. We examined the Δ of *C. squarrosa* and its potential drivers at a nested set of scales: within a tiller, within a local site (5 km^2), within a catchment (5 x 10^3 km^2), and within the region (5 x 10^5 km^2) in the grassland of the Mongolia plateau. Within cohorts of tillers, Δ of leaves increased from 5.1‰ to 8.1‰ from old to young leaves. At the local scale, Δ of mature leaves varied from 5.8‰ to 8.4‰, increasing with decreasing grazing intensity. At the catchment scale, Δ of mature leaves varied from 6.2‰ to 8.5‰ and increased with topsoil silt content. At the regional scale, Δ of mature leaves varied from 5.5‰ to 8.9‰, increasing with growing season precipitation. Within pooled samples, Δ decreased with increasing leaf nitrogen content (N_{leaf}), which was positively correlated with grazing intensity and leaf position along tillers, but negatively correlated with precipitation. Our results suggest that both bundle sheath leakiness (Φ) and the ratio of internal to ambient CO_2 concentration (C_i/C_a) had an effect on Δ in *C. squarrosa* when interpreted in terms of Farquhar's model of Δ in C4 plants. Our findings indicate that Δ is influenced by biotic and abiotic factors that affect N_{leaf} and further C_i/C_a and Φ. The leaf-level effect of N_{leaf} on Δ appeared at all scales although the drivers affecting N_{leaf} differed with scale.

Introduction

Grassland has been experiencing rapid shifts in botanical composition, structure and functioning driven primarily by global climate change and human disturbances (Alward *et al.*, 1999; Grime *et al.*, 2000; Sage & Kubien, 2003). Such changes may also shift the dominance of the two distinct photosynthetic pathways, C3 and C4, which differ in the mode of initial CO_2 fixation (Farquhar *et al.*, 1989; Sage & Kubien, 2003). Most of the world's C4 species are composed of grasses (Teeri & Stowe, 1976; Ehleringer *et al.*, 1997). The ratio of C3 to C4 photosynthetic types is an important component for understanding ecosystem processes, because it can affect biomass production, carbon sequestration, and water cycling (Tieszen *et al.*, 1997; Pyankov *et al.*, 2000; Sage & Kubien, 2003). It can also influence the terrestrial carbon isotope signal, which is used for the land-ocean partitioning of CO_2 fluxes (Still *et al.*, 2003; Suits *et al.*, 2005).

The actual C3/C4 ratio is often obtained from the stable carbon isotope compositions of C3 and C4 plants, and bulk samples in a two-member mixing model (Murphy & Bowman, 2007) where bulk samples may comprise soil organic matter (Wittmer *et al.*, 2010), n-alkanes (Zhang *et al.*, 2003), wool (Auerswald *et al.*, 2009) or other materials. This approach is based on the difference in discrimination against the heavy carbon stable isotope (Δ) during photosynthesis between C3 and C4 plants (Farquhar *et al.*, 1989). A single mean C4 end member value is usually assumed in the two-member mixing model (Murphy & Bowman, 2007; Wittmer *et al.*, 2010), whereas variable C3 end member values are estimated, based on the well known correlations between the Δ of C3 plants and environmental conditions (Mole *et al.*, 1994; Männel *et al.*, 2007; Wittmer *et al.*, 2008). The reason for this is that Δ of C4 plants is reported to be less responsive to environmental variables than that of C3 plants (Van de Water *et al.*, 2002; Murphy & Bowman, 2009). However, this view has been challenged by a number of studies that have examined variations in Δ of C4 plants, and which suggest that the Δ of C4 species can vary with environmental variables (Henderson *et al.*, 1992; Buchmann *et al.*, 1996; Wang *et al.*, 2005). For instance, the Δ of *Amaranthus cruentus* was about 6‰ higher in low light leaves than in high light leaves (Tazoe *et al.*, 2006). The Δ of *Bothriochloa ischaemum* increased 2‰ with the increase in annual precipitation from 350 mm yr^{-1} to 700 mm yr^{-1} (Liu *et al.*, 2005). In

Saccharum spp., Δ declined by 2‰ with increasing leaf nitrogen content in a nitrogen-stress experiment (Meinzer & Zhu, 1998).

The Δ of a plant can be obtained from the carbon isotope composition of a plant ($\delta^{13}C_{plant}$) and that of the air ($\delta^{13}C_{air}$) in which it grows:

$$\Delta = \frac{\delta^{13}C_{air} - \delta^{13}C_{plant}}{1 + \delta^{13}C_{plant}} \qquad \text{(III.1)}$$

The Δ mainly results from the biochemical fractionation in mesophyll and bundle sheath cells. According to Farquhar *et al.* (1983; 1989), the Δ of C4 plants depends on bundle sheath leakiness (Φ) and the ratio of internal and ambient CO_2 concentrations (C_i/C_a):

$$\Delta = a + (b_4 + b_3 \cdot \phi - a) \cdot C_i / C_a \qquad \text{(III.2)}$$

where a is the discrimination of ^{13}C during diffusion of CO_2 through stomata (4.4‰), b_3 is the fixation by Rubisco (27‰ for C_4 plants; von Caemmerer *et al.* (2008)), and b_4 is the hydration of CO_2 to HCO^{3-} and fixation by PEP carboxylase (PEPc).

From equation (III.2), it is clear that Δ variation of C4 plants depends on b_4, Φ, and on C_i/C_a. These variables in turn depend on a host of environmental conditions. Temperature is the biggest influence on b_4, inducing variation from -4.8 at 33.8 °C to -6.2 at 21.0 °C (Henderson *et al.*, 1992). Φ is mostly affected by the CO_2 concentration gradient between bundle sheath and mesophyll cells, and thus by factors influencing the activity ratio of Rubisco to PEPc (Farquhar, 1983) such as leaf nitrogen content (N_{leaf}) (Sage *et al.*, 1987), temperature (Pittermann & Sage, 2001), and light (Tazoe *et al.*, 2008). C_i/C_a depends on stomatal conductance, which is influenced by vapor pressure deficit and leaf water potential (Turner *et al.*, 1984). C_i/C_a also varies with the photosynthetic capacity of the plant, which is affected by the concentration and light-dependent activity of Rubisco and PEPc (Farquhar & Sharkey, 1982). Because there is an interaction between the influences of Φ and C_i/C_a on Δ, Δ can increase or decrease with C_i/C_a depending on the term ($b_4+b_3\Phi$-a) (Farquhar, 1983). For $\Phi > 0.37$, this term is greater than zero with $\Delta > 4.4‰$ and increasing with C_i/C_a. For $\Phi < 0.37$, this term is smaller than zero with $\Delta < 4.4‰$ and decreasing with C_i/C_a. For Δ differing from 4.4‰ Δ thus also indicates C_i/C_a and hence water use efficiency.

Studies of the Δ of C4 plants and its response to environmental variables have been conducted primarily in grasslands in North America, Europe, and Australia (Schulze *et al.*, 1996; Murphy & Bowman, 2009) or in experiments under controlled conditions (Ranjith *et al.*, 1995; Tazoe *et al.*, 2006). Few studies of this type have been reported from the mixed C3/C4 grassland of the Mongolia plateau where the C4 community of the semi-arid steppe is dominated by *Cleistogenes squarrosa,* a C4 (NAD-ME) perennial bunchgrass that has a wide ecological range from the meadow steppe to the typical steppe dominated by *Stipa grandis* and *Leymus chinensis* as well as desert steppe and sand dunes. The Δ in *C. squarrosa* is well above 4.4‰ (Pyankov *et al.*, 2000; Chen *et al.*, 2002; Gong *et al.*, 2008) and it is known to vary with soil moisture (Chen *et al.*, 2002), which agrees with the prediction from equation (III.2) that Δ indicates water use efficiency.

In this study we investigated the Δ variability in *C. squarrosa* and its controlling factors across a range of scales on the Mongolia plateau. We hypothesized that the Δ variability in *C. squarrosa* is driven by factors controlling the N_{leaf}, which influence C_i/C_a and Φ. Factors influencing N_{leaf} may include leaf age, grazing pressure, soil properties or weather conditions, all of which vary at different scales. Hence we studied the Δ of *C. squarrosa* with a nested set of scales (i.e., tiller, local, catchment, and regional scale) to identify and quantify controls of N_{leaf} and, in turn, Δ.

Material and methods

Study area

The study area was located between 106.15 °E and 117.13 °E longitude and 40.62 °N and 45.57 °N latitude in the Inner Mongolia Autonomous Region, China (Fig. III.1). This area of approximately 200000 km^2 constitutes the regional scale. We sampled at altitudes ranging from 1010 to 1635 m elevation. The temperate, semi-arid climate is characterized by warm summers and cold, dry winters (Chen, 1988) with mean annual temperatures (MAT) between 0.3 and 5.6 °C and mean July temperatures (MJulT) between 18.7 and 22.7 °C. Mean annual precipitation (MAP) increases from 160 mm yr^{-1} in the western part to 350 mm yr^{-1} in the eastern part, and 60-80% of the annual total occurs in the May-August growing season (Bai *et al.*, 2007). Regional sampling took place in five different years (2005-2009) to take account of the large inter-annual variation in precipitation in the region.

The Xilin River Basin covers an area of 10786 km^2 in the northeast of the region. Within this catchment, MAP increases from 275 to 346 mm yr^{-1} along a northwest-southeast precipitation gradient, with elevation ranging from 1019 to 1482 m. MAT varies from 0.3 in the southeast to 2.5 °C in the northwest of the basin, with MJulT ranging from 18.7 to 20.9 °C. The soils in the Xilin River Basin include phaeozems, shallow and stony soils at rocky outcrops, and sandy soils of low water holding capacity in sand dune areas (Barthold *et al.*, 2010).

A grazing experiment (Schönbach *et al.* (2009), 116.67 °E, 43.56 °N) near the Inner Mongolia Grassland Ecosystem Research Station (IMGERS) of the Chinese Academy of Sciences at the middle reach of the Xilin River represents the local scale. MAT, MJulT, and MAP at IMGERS are 0.4 ºC, 19.0 ºC, and 337 mm yr^{-1}, respectively (Zheng *et al.*, 2010).

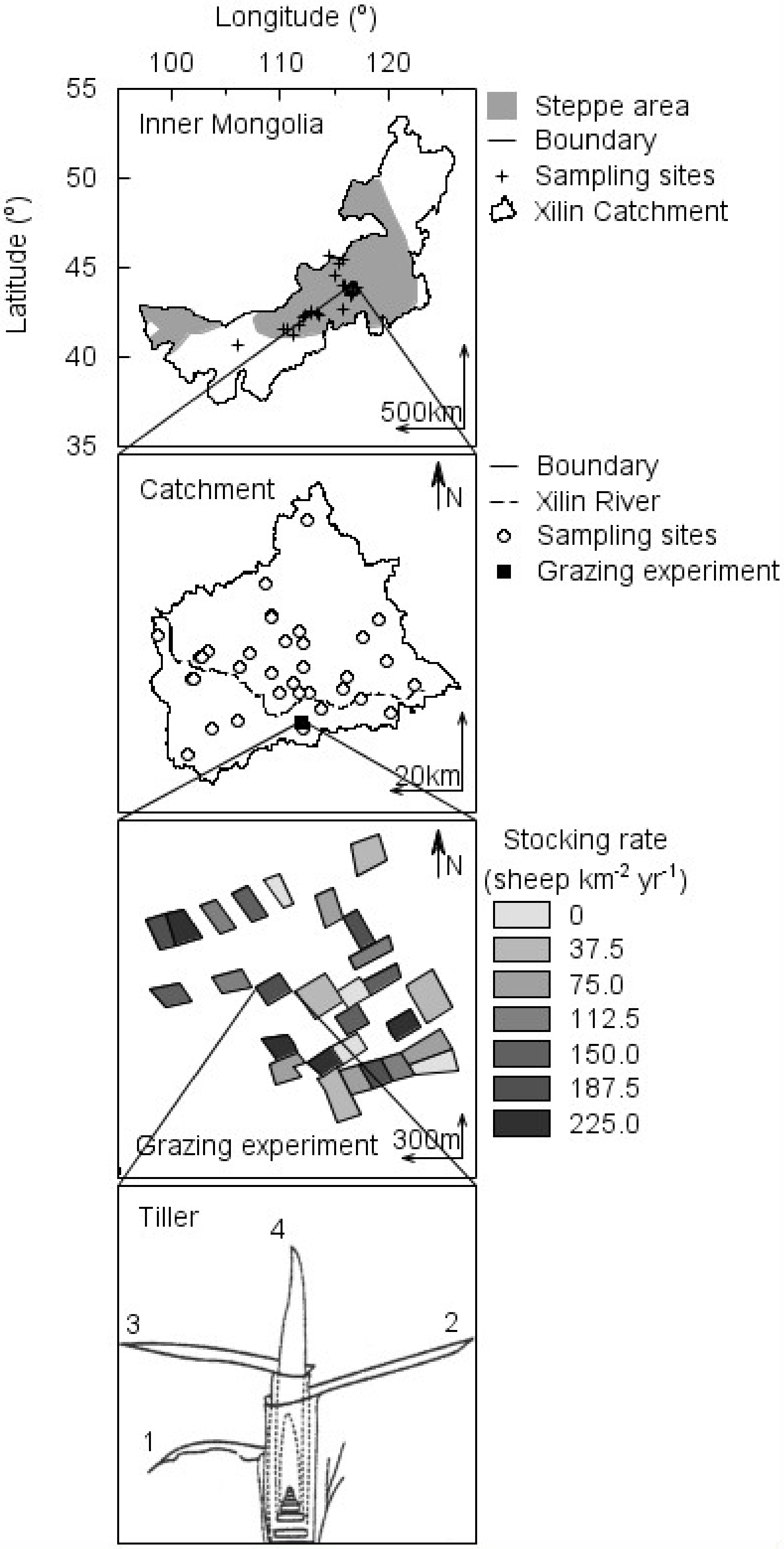

Fig. III.1 Nested hierarchy of sampling sites in the Inner Mongolia grassland. Leaf position along a tiller constitutes the tiller scale, plots in a grazing experiment constitute the local scale, the Xilin River Basin constitutes the catchment scale, and the steppe area of Inner Mongolia constitutes the regional scale. Numbers on the tiller scale are phytomer units from base.

Meteorological data and $\delta^{13}C$ of atmospheric CO_2

Growing season precipitation was geostatistically determined according to Wittmer *et al.* (2008) for each sampling location and time of sampling. This was done by interpolating between meteorological stations and taking into account 1.5 x 1.5 km^2 resolution maps of MAP, mean monthly precipitation, MJulT, and MAT (Climate Source Inc., Corvallis, Oregon). We calculated the mean daily precipitation (mm day^{-1}) between 1 May, which is the date that usually corresponds with the onset of growth of *C. squarrosa* (Liang *et al.*, 2002), and the day of sampling. This measure of precipitation takes account of the fact that samples were collected on different days and in different years.

$\delta^{13}C_{air}$ was obtained from a long-term monitoring station in Ulan Uul (NOAA NCDC Clamiate Data Online, 2009), Mongolia, about 460 km northwest of IMGERS.

Soil analyses

On the catchment scale sampling locations were selected to cover the full range of soils as known from soil mapping campaigns. Soil pits were dug to 1 m at locations representing the mapping units and the soils were sampled horizon-wise. A comprehensive set of soil properties was measured (for details see Wiesmeier *et al.* (2009), including bulk density, texture, pH, and nitrogen content. We used either topsoil properties (e.g., silt content) because water uptake by *C. squarrosa* is restricted to the topsoil (Yang *et al.*, 2010), or whole-soil properties (e.g. N stocks).

Grazing experiment

The grazing experiment had 28 annually grazed plots (Fig. III.1, grazing experiment) within two experimental blocks differing in topography (flat and gently sloping). The plots were grazed between June and September each year since 2005 at seven stocking rates: 0, 37.5, 75.0, 112.5, 150.0, 187.5, and 225.0 sheep km^{-2} with three times higher stocking densities during the four months of grazing. Further information about the grazing experiment can be found elsewhere (Schönbach *et al.*, 2009).

C. squarrosa sampling

The tiller scale was sampled in an ungrazed plot, where no leaves were lost by grazing. Leaves from several individuals were sampled and pooled according to their phytomer positions (Fig. III.1). Leaves were collected in mid July, end of July, and mid August 2008, respectively. Resampling over time allowed us to distinguish between both new leaves and old leaves grown between sampling dates. Old leaves at low phytomer positions were then compared between consecutive sampling dates to identify changes over time.

At the local, catchment, and regional scales we sampled mature, fully expanded and sun-exposed leaves from several non-flowering individuals. At the local scale, we collected leaves from the plots of the grazing experiment in June, July and August of 2007, and in May, June and July of 2008. These two years differed in precipitation: 2007 was dry with 240 mm yr^{-1}, and 2008 was an average year with 362 mm yr^{-1}. At the catchment and regional scales, samples were collected in each of the five years, 2005-2009. The precise locations and elevations of all sampling positions were recorded with a mobile GPS. In total, we collected 51, 144, 64, and 47 samples at the tiller, local, catchment, and regional scales, respectively.

Isotope and N$_{\text{leaf}}$ analysis

All samples were dried at 60 °C for 48 hours in a forced-draught oven and ground to homogeneity with a ball mill. The carbon isotope composition and nitrogen content were then measured using an elemental analyzer (NA 1110; Carlo Erba, Milan) interfaced (ConFlo III; Finnigan MAT, Bremen) to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT) following the protocol by Wittmer *et al.* (2008). Carbon isotope data were specified as δ^{13}C relative to the Vienna Pee Dee Belemnite standard:

$$\delta^{13}\mathrm{C} = \frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1 \qquad \text{(III.3)}$$

where R_{sample} and R_{standard} are the ratios of ^{13}C/^{12}C in the sample and standard. The precision for sample repeats was better than 0.15‰ for δ^{13}C and 0.04% for nitrogen content in dry matter.

Statistical analysis

Linear regressions were used to evaluate relationships between Δ, N_{leaf}, and the environmental variables of interest at a certain scale: leaf position, grazing intensity, soil properties, and growing season precipitation. The coefficient of determination was tested with a two-sided test for significance of the regression. Confidence intervals (95%) were calculated for the relationship between Δ and N_{leaf} and for the population. All procedures were carried out in SPSS Version 16.0 (SPSS Inc., Chicago, USA, 2007).

Results

Relationship between Δ and leaf position at tiller scale

The Δ of *C. squarrosa* decreased and N_{leaf} increased from the lowermost leaf to the uppermost leaf (Fig. III.2), indicating that young leaves have low Δ but high N_{leaf}. The Δ mainly changed from leaf #5 to younger leaves, while N_{leaf} mainly changed among leaves older than leaf #7. Hence in a bulk sample of undefoliated plants, the variation in Δ can mainly be attributed to younger leaves whereas the variation in N_{leaf} can mainly be attributed to older leaves.

The N_{leaf} of leaves between leaf #1 and leaf #6 was the same in mid-July (average = 1.6%, SD = 0.2%) and in August (average = 1.6%, SD = 0.3%), which suggested no nitrogen redistribution over time. N_{leaf} of the three uppermost (newly grown) leaves (average = 2.4%, SD = 0.1%) was significantly higher ($P < 0.01$) in August than those grown in July (average = 1.7%, SD = 0.2%).

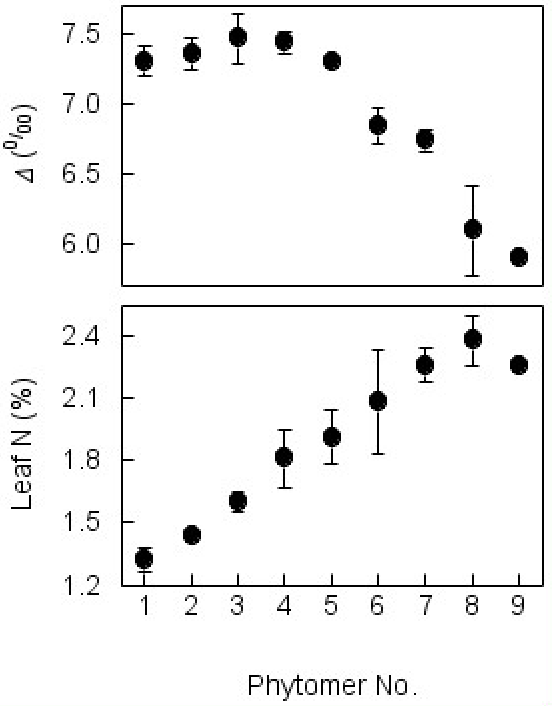

Fig. III.2 Influence of leaf position (phytomer #1 is the first leaf from base) on the carbon isotope discrimination and leaf nitrogen content of *C. squarrosa.* Means of three sampling dates in an ungrazed plot; error bars denote the standard error. Note that more than 7 phytomers were found in August and only one sample was available for phytomer #9.

Δ, stocking rate, and topography at local scale

At the local scale, *Δ* varied between 5.8‰ and 8.4‰ with a SD of 0.5‰. N_{leaf} varied between 1.8% and 3.8% with a SD of 0.4%. *Δ* decreased ($R^2 = 0.04$, $n = 144$, $P < 0.05$) while the N_{leaf} increased ($R^2 = 0.11$, $n = 144$, $P << 0.001$) with stocking rate (Fig. III.3). *Δ* and N_{leaf} also varied with year ($P < 0.001$), month ($P < 0.001$), and stocking rate ($P < 0.001$), but not with topographic position ($P > 0.05$).

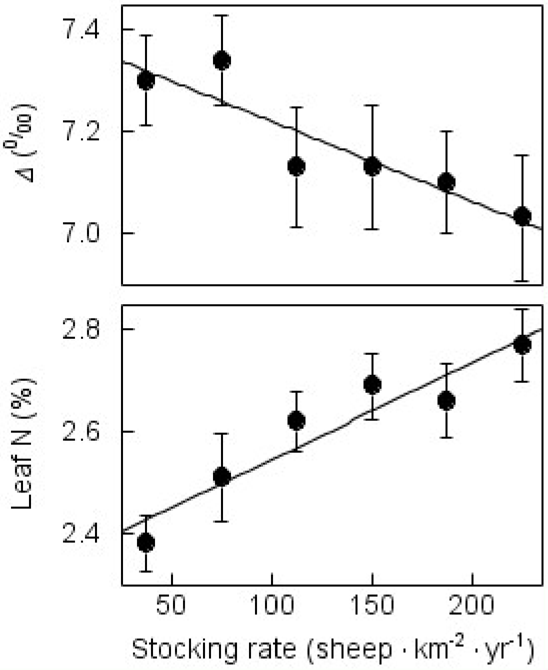

Fig. III.3 Influence of stocking rate on the carbon isotope discrimination and leaf nitrogen content of *C. squarrosa*. Each data point shows the mean and standard error of 24 samples taken from four plots with different topographic positions and management systems.

Δ and soil variables at catchment scale

Across the Xilin River catchment, *Δ* of *C. squarrosa* varied between 6.2‰ and 8.5‰ (SD = 0.5‰), N_{leaf} varied between 1.5% and 3.7% (SD = 0.5%), and topsoil silt content varied from 2% to 40% (SD = 10%). *Δ* increased with topsoil silt content ($R^2 = 0.42$, $n = 24$, $P < 0.001$) despite there being differences in slope between the two years ($P < 0.05$), but N_{leaf} was not correlated with silt content ($P > 0.05$) (Fig. III.4). Similar patterns were found with soil nitrogen stocks. The *Δ* increased significantly with soil nitrogen stocks ($R^2 = 0.40$, $n = 25$, $P < 0.01$), but there was no relationship between soil nitrogen stocks and N_{leaf} ($P > 0.05$). The influence of soil nitrogen stocks and silt content on *Δ* could not be separated due to their correlation with each other; this also applied to other soil properties.

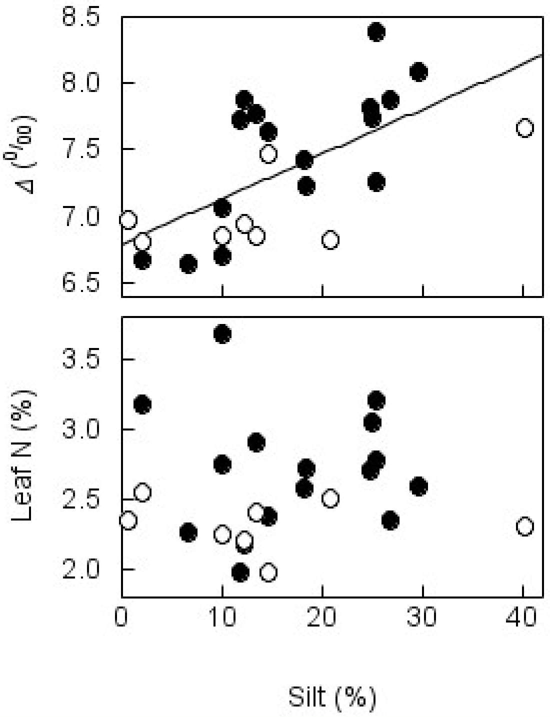

Fig. III.4 Influence of topsoil silt content on the carbon isotope discrimination and leaf nitrogen content of *C. squarrosa* at the catchment scale in 2007 (filled symbols), a dry year, and in 2008 (open symbols), a normal year.

Δ and precipitation at regional scale

As expected, the range of *Δ* of *C. squarrosa* (3.4‰) was considerably larger at the regional scale than at the local (2.6‰) and catchment scales (2.3‰). Precipitation during the growing season had a significant, positive effect on *Δ* ($R^2 = 0.31$, $n = 47$, $P < 0.001$) and a negative effect on N_{leaf} ($R^2 = 0.56$, $n = 47$, $P << 0.001$) (Fig. III.5). The *Δ* of *C. squarrosa* increased ($P < 0.05$) whereas N_{leaf} decreased ($P < 0.01$) from the desert steppe to the typical steppe, and to the meadow steppe.

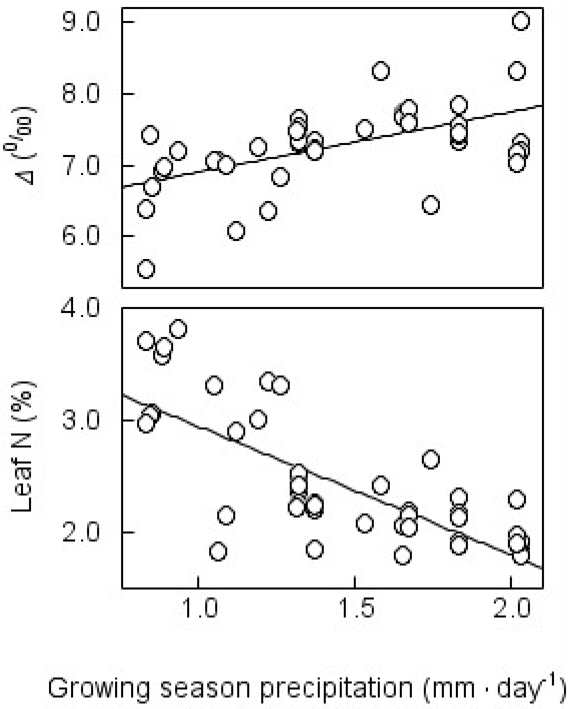

Fig. III.5 Influence of growing season precipitation on the carbon isotope discrimination and leaf nitrogen content of *C. squarrosa* at the regional scale. Growing season precipitation was calculated based on year and time of sampling (see Methods section).

Δ and N_{leaf}

At tiller, local, and regional scales, Δ was negatively correlated with N_{leaf} (Fig. III.6) although the range of values and factors that affected the variation in N_{leaf} differed among scales (Figs. III.2, III.3, and III.5). In spite of no significant relationship at the catchment scale ($P > 0.05$), all except four of the total of 47 data points were included in the 95% confidence intervals for all pooled samples. N_{leaf} explained 23% of the variation in Δ when all samples were pooled. When regressed separately, the slope of the regression was significantly steeper at the tiller scale than other scales ($P < 0.01$), but remained within the 95% confidence interval of the common regression slope for the other scales (Fig. III.6).

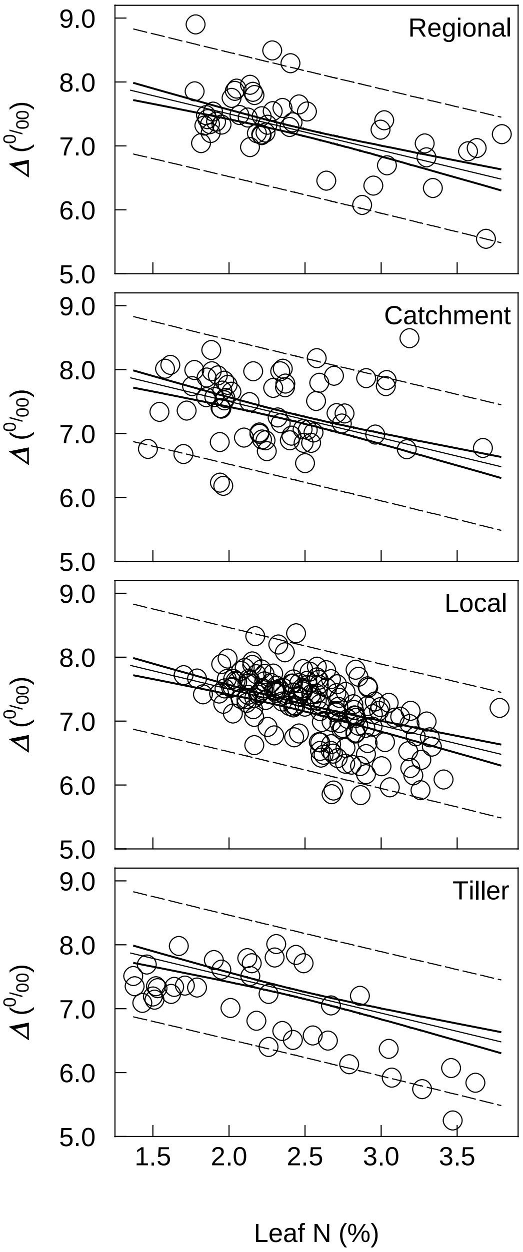

Fig. III.6 Correlations between the carbon isotope discrimination and leaf nitrogen content of mature leaves of *C. squarrosa* at the regional (steppe area of Inner Mongolia), catchment (Xilin River catchment), local (grazing experiment), and tiller scales (an ungrazed plot and a grazed plot). The central line in each panel denotes the regression for the pooled samples, the inner envelopes (solid lines) are the 95% confidence intervals for the regression and the outer envelopes (dashed lines) are the 95% confidence intervals for individual samples.

Discussion

This study represents the first comprehensive examination of the *Δ* variability of a C_4 (NAD-ME) perennial bunchgrass, *Cleistogenes squarrosa,* and its controlling factors across a range of scales (i.e., tiller, local, catchment, and regional scale) on the

Mongolia plateau. Our results have shown that Δ of *C. squarrosa* was large (on average: 7.2‰) and, at the region scale, it varied over a 4‰ range (5 to 9‰) across the Inner Mongolia steppe. This range is larger than that for other C_4 species in the study area. For instance, *Salsola collina*, sampled together with *C. squarrosa* in the grazing experiment, varied by 3‰ under identical conditions (data not shown). A previous study has reported that *Bothriochloa ischaemum*, a dominant C4 perennial bunchgrass on the Chinese Loess Plateau, varied only by 2‰ across its range (Liu *et al.*, 2005). This suggested that *C. squarrosa* can be a model species for studying the effect of environmental conditions on the Δ of C_4 plants, and that Δ of the C_4 community is likely to vary, largely due to the change in relative abundance of *C. squarrosa*.

Although many studies have demonstrated that foliar $\delta^{13}C$ of plants are negatively correlated with water availability (e.g., soil moisture and annual precipitation) across an environmental gradient (Farquhar *et al.*, 1989; Stewart *et al.*, 1995; Liu *et al.*, 2005). The mechanisms underpinning these patterns, however, have yet to be explored. Our results indicate that N_{leaf} has a strong and consistent effect on Δ of *C. squarrosa* at all scales and explained 23% of the variation in Δ, even though the factors influencing N_{leaf} varied at different scales. Our general findings are also corroborated by results of a nitrogen addition experiment (Chen *et al.*, 2005b). Therefore we can have great confidence in this relation, and we may expect that other environmental variables which affect N_{leaf} would also affect Δ in a similar way.

Factors influencing N_{leaf}

Factors that affect N_{leaf} will include factors that control N availability, such as fertilization (Bai *et al.*, 2010), factors that influence N uptake, such as root density and root activity (Farooq *et al.*, 2009), and factors that relate to ontogenesis and organogenesis, such as the availability of water and nutrients, which determine the velocity of leaf expansion and leaf dimensions where grazing could contribute (Anten *et al.*, 1998).

The influence of leaf position on N_{leaf} is most likely to have been caused by increasing soil temperature, which was about 12.6 °C in late May and increased to 21.1 °C in mid August (average of 10 days at 10 cm depth, data from IMGERS) as N uptake of C4 is especially sensitive to low temperature (Farooq *et al.*, 2009). Remarkably, neither N_{leaf} nor Δ at early leaf positions changed at later sampling dates, indicating that the influence of leaf position was not caused by redistribution of N from old to young leaves. The increase of leaf nitrogen with grazing intensity is well known and relates to the faster cycling of N and decrease in mean leaf age (Mattson, 1980). Decreasing N_{leaf} with increasing precipitation is likely to be related to the dilution effect caused by additional plant growth. Temperature-related N uptake may also add to the effect of precipitation, as regional temperatures and precipitation are closely negatively correlated ($R^2 = 0.93$, Bai *et al.* (2007)) and hence cannot be separated in their effects. Finally, the Birch effect of an enhanced mineralization after a pronounced dry spell (Birch, 1964; Austin *et al.*, 2004) could also contribute to an improved N availability in drier areas where rainfall occurs infrequently.

At the catchment scale, the influences on N_{leaf} are less clear. Δ increased with silt content whereas N_{leaf} did not, although the relation between Δ and N_{leaf} was similar at this scale as for the other scales. At this scale the variation of soil is expected to have a strong influence but, in contrast to leaf position, grazing intensity or precipitation acting on the other scales, the soil cannot be arranged along only one single variable like silt content. Soil is an aggregation of several soil properties, which may exert influence on N_{leaf} and in turn on Δ.

The effect of N_{leaf} on Δ

In principle, the variability in Δ can originate from discrimination associated with photosynthesis and from post-photosynthetic fractionation caused CO_2 release in dark respiration, and/or by catabolic processes converting new photosynthate into plant biomass. Although the latter processes have not yet been studied in C4 plants, evidence for the contribution of post-photosynthetic fractionation comes from the fact that Δ obtained from online measurement of photosynthetic $^{13}CO_2/^{12}CO_2$ exchange is often less than Δ estimated from the carbon isotope composition of leaf dry mass (Evans *et al.*, 1986; Kubásek *et al.*, 2007). Respiration and carbon allocation are responsive to environmental conditions including temperature and water stress (Ryan, 1991; Flexas *et al.*, 2005) and thus can contribute to the observed variability. However, post-photosynthetic fractionation is rather unlikely as a consistent explanation of the correlation of Δ with N_{leaf}. Proteins differ isotopically from cellulose only by 4‰ (Benner *et al.*, 1987). A difference in N_{leaf} of 2%, corresponding to a difference in protein content of 12%, is insufficient to explain a difference in Δ of 3‰. Also, secondary compounds, which increase with leaf age and may thus relate to N_{leaf}, cannot explain the variability of Δ at the tiller scale, because the young leaves present in mid July did not change in isotopic composition until mid August. Hence variation in discrimination during photosynthesis most likely caused the variation in leaf Δ.

In the Farquhar model, Δ mainly depends on Φ and C_i/C_a as b4 changes little within the 4 °C range in air temperature in the study area. C_i/C_a should increase with stomata openness (Turner *et al.*, 1984), but decrease with the activity of carboxylating enzymes (Sage & Pearcy, 1987; Sage *et al.*, 1987), which should increase with N_{leaf} (Sage *et al.*, 1987; Ranjith *et al.*, 1995). Φ depends on the activity ratio of Rubisco and PEPc, which is influenced by the allotment of nitrogen to them (Sage *et al.*, 1987). At the local scale, C_i/C_a was measured by Zheng *et al.* (2010) simultaneously to our sampling. This allows calculating Φ and comparing C_i/C_a and Φ with Δ, although this can only be a rough comparison because the temporal scales differ considerably between instantaneous gas exchange measurements and the isotopic composition of leaves that may be several weeks old. Zheng *et al.* (2010) measured C_i/C_a and found that it varied between 0.41 and 0.66, similar to the range of 0.34 to 0.59 found by Chen *et al.* (2005a) for *C. squarrosa* (Table III.1). The comparison to N_{leaf} from our study indicates that both C_i/C_a and Φ were most likely to have been influenced by N_{leaf} (Table III.2) but the range in Φ was small (0.56 to 0.58) compared to the range in C_i/C_a. Owing to the large Φ of *C. squarrosa*, which exceeds the range of most other NAD-ME plants, Δ is a sensitive indicator of C_i/C_a and water use efficiency. Our results, therefore, indicate that water use efficiency increased for leaves that developed later in the growing season, it also increased with increasing grazing intensity, with decreasing silt content, and hence water holding capacity of the soil, and it increased with decreasing precipitation during the growing period.

Table III.1 Leakiness (Φ) and ratio of internal to ambient CO_2 concentrations (C_i/C_a) of NAD-ME plants from published sources.

Species	Φ	C_i/C_a	References
Amaranthus caudatus	0.37	0.43 - 0.46	Kubásek *et al.* (2007)
A. edulis	0.32	0.43	Henderson *et al.* (1992)
A. retroflexus	0.40	0.40 - 0.90	Sage and Pearcy (1987)
Atriplex lentiformis	0.54 - 0.62	-	Zhu *et al.* (1999)
A. rosea	0.45	0.47	Henderson *et al.* (1992)
Cleistogenes squarrosa	-	0.34 - 0.59	Chen *et al.* (2005a)
Cynodon dactylon	-	0.12 - 0.28	Mantlana *et al.* (2008)
Eleusine coracana	0.40	0.38	Henderson *et al.* (1992)
E. indica	0.37, 0.27	0.48, 0.58	Farquhar (1983)
Eragrostis lehmanniana	0.45 - 0.57	0.08 - 0.70	Fravolini *et al.* (2002), Mantlana *et al.* (2008)
Panicum schinzii	0.32	0.38	Henderson *et al.* (1992)
Total range	0.27 - 0.62	0.08 - 0.90	
Mean	0.42	0.44	

Table III.2 Variation in the ratio of internal to ambient CO_2 concentrations (C_i/C_a) and leakiness (Φ) for the local scale and correlations to leaf nitrogen content (N_{leaf}). Gas exchange measurements were taken from (Zheng *et al.*, 2010).

Variable	Minimum - maximum	Correlation to N_{leaf}
C_i/C_a	0.41 – 0.66	- 0.866
Φ	0.56 – 0.58	+ 0.832

CHAPTER IV – Temporal variation of C4 annuals and C4 perennials in the grassland of Mongolian plateau

Abstract

C4 plants have increased during the past several decades in the grassland of the Mongolia plateau mainly due to pronounced regional warming. However, how the composition of the C4 plants changed during/after this increase is not known. Based on Grime's C-S-R plant strategy theory, C4 annuals as a ruderal group and C4 perennials as a stress-tolerant group should have different responses to disturbances. Here, we report the temporal change of relative biomass of C4 annuals (R_{A4}) and C4 perennials (R_{P4}) in the grassland of the Mongolian plateau between 2001 and 2009. Our results showed that C4 plants were either dominated by C4 perennials or by C4 annuals; sites where both life forms coexist were scarce. On average, R_{A4} was 11% with a SD of 19% and R_{P4} was 13% with a SD of 19%. C4 annuals significantly decreased and C4 perennials increased over time. Both behaviors of C4 annuals and C4 perennials followed the prediction of Grime's C-S-R plant strategy theory that C4 annuals profit from disturbances and are replaced by C4 perennials. Our results also indicated that C4 annuals preferred normal growing season precipitation, while C4 perennials were dominant in the dry growing season. Grazing as a main human disturbance changed neither the percent of C4 plants nor the R_{A4} and R_{P4}. Precipitation, not grazing, modified the trends of R_{A4} and R_{P4}. This study provides the first evidence on the differentiated evolution of components of C4 plants under global change.

Introduction

Atmospheric CO_2 concentration and temperature have risen substantially over the last century, which potentially changes the distribution of C3 and C4 plants. The cool-temperature mixed C3/C4 grassland of the Mongolian plateau, has seen a CO_2 concentration increase of more than 50 ppm over the last century (Yu *et al.*, 2003). During this period, growing season temperature has increased 2 °C and stocking rates have doubled (NOAA NCDC Clamiate Data Online, 2008; Wittmer *et al.*, 2010). Furthermore, land use change from nomadic to sedentary grazing in the 1960s (Wittmer *et al.*, 2010). The C3/C4 ratio and its variation in this area were recently determined by measuring the carbon isotope signature of wool and soil organic matter (Auerswald *et al.*, 2009; Wittmer *et al.*, 2010). The abundance of C4 plants has increased by 10% due to the increase in growing season temperature (Wittmer *et al.*, 2010). This is because C4 plants have higher maximum photosynthesis rates (Sage & Kubien, 2003) and higher effective quantum yield of CO_2 fixation (Ehleringer & Björkman, 1977) under high temperature. Wittmer *et al.* (2010) also documented a spread of C4 plants towards northern latitudes and higher altitudes in the grassland of the Mongolian plateau. C4 annuals and C4 perennials possibly responded differently to above disturbances during the increase in C4 plants. However, how they change during the rise of C4 plants is not yet clarified.

Grime's C-S-R plant strategy concept is widely used to explain the mechanism of species coexistence and vegetation succession (Grime, 1977; Craine, 2005). C, S, and R indicate competitive, stress-tolerant, and ruderal strategy respectively. Plants with a competitive strategy have a higher competitive ability than neighbor plants to utilize the same resources in undisturbed environments. Perennials herbs, shrubs, and trees are usually included in this group. Plants with a stress-tolerant strategy can survive under the unfavorable environmental conditions such as drought or shade, which normally includes lichens, perennial herbs, shrubs and trees. Ruderal species, mainly annuals, have higher competitive ability in disturbed environments. The plants comprising each group vary in different ecosystems. In the cool-temperature grassland of the Mongolia plateau, annuals employ a ruderal strategy, opportunistically and rapidly colonizing new open habitats. Perennials are long-lived and devote only a small proportion of annual production to seeds, which can be stress tolerant strategy. Facing disturbance annuals and perennials will have different responses based on the predictions of Grime's concept (Grime, 1977). Annuals will rapidly produce large quantity of seeds to ensure rapid rehabilitation after disturbance in despite of the high risk of failure with small seeds. Perennials will conservatively use the resources and decline the production of seeds to survive. After disturbance, perennials usually replace annuals because of their higher competition ability in an environment where disturbance is infrequent. Hence, increases in C4 annuals and C4 perennials may not equally attributed to the substantial increase in C4 plants in the grassland of the Mongolia plateau in the past several decades. It may be expected that C4 annuals profit faster from a change in environmental conditions favoring C4 plants than C4 perennials and after disturbance C4 perennials will gradually replace C4 annuals as shown in Fig IV.1. However, this is not validated to date.

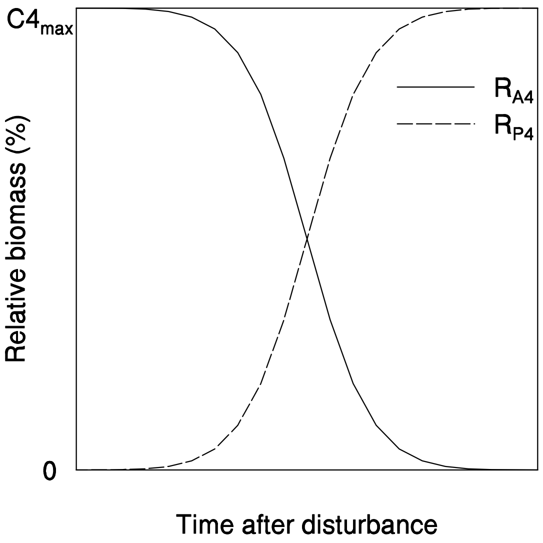

Fig. IV.1 Concept of R_{A4} (the relative biomass of C4 annuals) and R_{P4} (the relative biomass of C4 perennials) change after climate became better suitable for C4 plants.

In the semi-arid grassland of the Mongolia plateau, water is a limiting resource to plant growth (Bai *et al.*, 2004; Bai *et al.*, 2008). C4 plants have a shallow root system and mainly depend on rainfall during the growing season (Nippert & Knapp, 2007; Yang *et al.*, 2010). Their growth is restricted to summer months when high precipitation and temperature occurred together. C4 annuals exhibit an opportunistic growth and germination can be triggered by a critical minimal amount of rainfall in warm months (Ehleringer, 1985). Thus, the proportion of C4 annuals and C4 perennials may partly depend on the distribution of rainfall within the short C4 growing period (May-August). This amount of growing season precipitation is also highly variable over years (Xiao *et al.*, 1995; Wittmer *et al.*, 2008). Hence, the effect of growing season precipitation on C4 annuals and C4 perennials need to be considered when exploring the changes of both with disturbance.

The grassland of the Mongolia plateau has faced large increase in grazing pressure, especially in the Inner Mongolia grassland which is located in northern China. The number of sheep increased by about 1250% from 1950 to 2007 (Inner Mongolia Statistics Office, 2008). Most of the Inner Mongolia grassland has been degraded mainly by overgrazing (Jiang *et al.*, 2006) which has reduced species richness and aboveground biomass (Bai *et al.*, 2007) and resulted in less plant cover. C4 annuals have advantage colonizing the new bare ground since they have large seedbanks (Coffin & Lauenroth, 1989) and exhibit opportunistic growth, especially after grazing (Kinucan, 1992). Hence, we will ask if grazing can influence the proportion of C4 annuals and C4 perennials and further modify the trend of both during disturbance.

In this study, we tried to explain the increase in C4 plants by Grime's C-S-R plant strategy theory. We examined the change in C4 annuals and C4 perennials over 9 years in the grassland of the Mongolia plateau. Survey work was conducted in July/August of 2001-2009. Our hypothesis is that the changes of C4 annuals and C4 perennials follow the prediction of Grime's plant strategy theory and precipitation and grazing will influence their trends. Specially, the following questions are addressed: 1) What are the temporal dynamics of C4 annuals and C4 perennials during disturbance causing the increase of C4 plants in the grassland in the Mongolia plateau? 2) How do growing season precipitation and grazing influence both trends?

Material & Methods

Study area

The study area was situated between 106° and 119° E (approximately 1200 km) and 40° and 48° N (approximately 800 km) in the grassland of the Mongolian plateau located in the Autonomous Region of Inner Mongolia in China and in the Republic of Mongolia (Fig IV.2). Sampled altitudes ranged between 800 and 1700 m above sea level (a.s.l.). Mean annual precipitation (MAP) increases from 100 mm yr^{-1} in the west part to 400 mm yr^{-1} in the eastern and northern part of the study area. Most of the precipitation (approximately 75%) falls during the growing period (May-August).

Mean annual temperature (MAT) and mean temperature of July (MJulT) vary between -2 and 8 °C and between 16 and 24 °C, respectively. The spatial variation of temperature and precipitation follows altitudinal and latitudinal trends. Almost the entire study area is grazed by sheep and goats with little agriculture. C4 perennials usually start growth earlier than C4 annuals. For instance, *Cleistogenes squarrosa*, a dominant perennial, starts growth at the beginning of May. *Salsola collina*, an annual, starts growth in July or August depending on the precipitation.

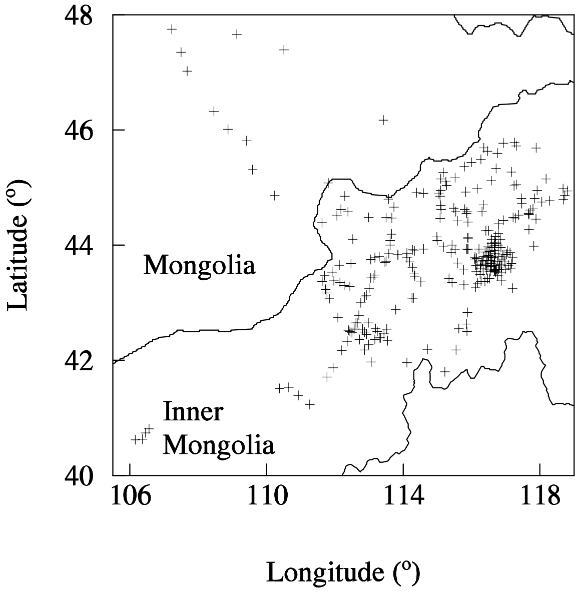

Fig. IV.2 Sampling sites (+) in the grassland of the Mongolian plateau. Lines denote the country border between Inner Mongolia in China and the Republic of Mongolia and the district border of Inner Mongolia, China. Sites are only where C4 plants occur.

Survey

We conducted surveys in July and August of 2001-2009. Not everywhere in study area were surveyed every year because of large survey area and limited road. Altogether, the number of locations was 390 in 9 years. All C4 species in a 100 m^2 plot were recorded at each location (Table IV.1). Of the 390 survey locations, 280 had C4 plants. Positions and altitudes of sampling locations were recorded with a mobile GPS. Relative biomass of individual C4 species and the whole C4 plants in total were visually estimated using $1 \times 1 m^2$ quadrat. C4 species were assigned to annuals and perennials according to literatures (Tang & Liu, 2001; Jigjidsuren & Johnson, 2003;

Wang, 2003). Land use types were classified as grazed and ungrazed based on the presence of fresh faeces or sheep.

Table IV.1 Frequency and life form of C4 species during survey.

Species	Frequency	Life form	
Cleistogenes squarrosa	0.6	Perennial	Grass
Salsola collina	0.5	Annual	Forb
Kochia prostrata	0.1	Perennial	Semi-shrub
Setaria viridis	0.1	Annual	Grass
Amaranthus retroflexus	< 0.1	Annual	Forb
Aristida heymannii	< 0.1	Annual	Grass
Artemisia sieversiana	< 0.1	Annual	Forb
Bassia dasyphylla	< 0.1	Annual	Forb
Chloris virgata	< 0.1	Annual	Grass
Digitaria ischaemum	< 0.1	Annual	Grass
Eragrostis minor	< 0.1	Annual	Grass
Euphorbia humifusa	< 0.1	Annual	Forb
Micropeplis arachnoidea	< 0.1	Annual	Forb
Tribulus terrestris	< 0.1	Annual	Forb
Anabasis brevifolia	< 0.1	Perennial	Semi-shrub
Artemisia adamsii	< 0.1	Perennial	Forb
Cleistogenes songorica	< 0.1	Perennial	Grass
Kochia krylovii	< 0.1	Perennial	Forb
Salsola passerina	< 0.1	Perennial	Semi-shrub

Meteorological data

The precipitation and temperature within the different years deviated considerably from MAP and MJulT and this deviation differed among sites. Hence, growing season precipitation and temperature were determined for each sampling location based on the year and time of sampling. This was done following the approach by Wittmer *et al.* (2008), which interpolates between meteorological stations based on 1.5 x 1.5 km^2 resolution maps of MAP, mean monthly precipitation, MJulT, and MAT (Climate Source Inc., Corvallis, Oregon). In brief, the difference between each climatic variable under consideration and its long-term average of the last normal period (1961-1990) was calculated for each meteorological station ($n = 46$). These differences were then geostatistically interpolated and added to the high-resolution maps of the means. This procedure incorporates the temporal details of the individual sampling years while retaining the spatial details of the long-term maps, which account for factors such as topography, rain shadows, lake effects, and temperature inversions.

Growing season (May - August) precipitation in sampling year (PG) was calculated for each site. Comparing PG with the long-term average of growing season precipitation in each site, we divided all sampling sites into three groups: dry, normal, and wet sites. Dry sites were sites where PG was 10% lower than long-term average, wet sites were sites where PG was 10% higher than long-term average, and normal sites were in the middle.

Statistical analysis

Relative biomass of C4 plants (P_{C4}), C4 annuals (R_{A4}), and C4 perennials (R_{P4}) in total were calculated. In statistical analyses, we excluded the locations where either C4 annuals or C4 perennials were not found. Relative biomass of C4 annuals in C4 plants was also calculated to show the proportion of C4 annuals and C4 perennials in C4 community.

Geostatistical analysis of meteorological data was carried out using the GNU R 2.7.2 (R Development Core Team, 2008) with the auxiliary packages geoR (Ribeiro & Diggle, 2001) and gstat (Pebesma, 2004). Kernel density (Silverman, 1986) was calculated by Gaussian kernels using the GNU R 2.7.2 to yield probability distribution of the relative biomass of C4 annuals in C4 plants. The relative biomass of C4 annuals in C4 plants, being bounded between 0 and 1, was achieved by using the reflection method according to Silverman (1986) at the boundaries. Bandwidth of density estimation was defined *via* Silverman's "rule of thumb". R_{A4} and R_{P4} were log transformed. Because values of zero cannot be log transformed, the data were slightly compressed with the formula:

$$\text{Adjusted } R_{A4}/R_{P4} = (R_{A4}/R_{P4} + 0.001) \times 0.998 \qquad \text{(IV.1)}$$

Statistical analyses on data with this adjustment did not differ importantly from those on other data compressions that bring the extreme values closer to zero (Clary, 2008).

Linear, linearized, and multiple regressions were used to evaluate the relations between R_{A4} and R_{P4} and environmental variables and the change of R_{A4} and R_{P4} over years. The coefficient of determination was tested with a two-sided test for significance of the regression. Analysis of variance (ANOVA) followed by the LSD multiple range tests was used to evaluate the effect of precipitation and grazing on the relative biomass of all C4 plants, C4 perennials, and C4 annuals. All regression procedures were carried out using SPSS Version 16.0 (SPSS Inc., Chicago, USA, 2007).

Results

C4 abundances and environmental conditions

Altogether 7 perennial C4 species and 12 annual C4 species were observed. *C. squarrosa* and *S. collina* were the dominant in C4 perennials and C4 annuals, respectively. Other C4 species were rare in sampling locations (Table IV.1). Grasses and forbs had higher frequency than semi-shrubs. Based on our observation, meadow steppe sites or other sites with high plant cover seldom supported C4 plants. The relative biomass of C4 annuals in C4 plants was almost a binomial variable. The C4 community was either dominated by C4 perennials or by C4 annuals while locations where both life forms coexist were rather scarce (Fig IV.3).

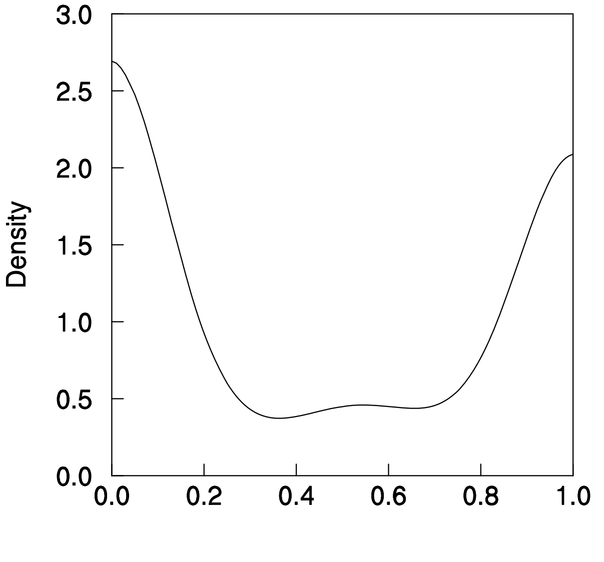

Fig. IV.3 Density distribution of the relative biomass of C4 annuals in C4 plants. The integral density is 1.

On average, R_{A4} was 11% with a SD of 19% and R_{P4} was 13% with a SD of 19%. R_{A4} was significantly correlated ($P < 0.001$) to MAP, PG, MJulT, JulT, and year, but R_{P4} was only significantly correlated ($P < 0.05$) with year (Table IV.2). However, most variables explain less than 10% variation of R_{A4} and R_{P4}, which was at least partly due to the zero values of R_{A4} and R_{P4} produced by the quasi binominal behavior in Fig IV.3 and can't be described by continuous variables like environmental properties in logistic regression. Year explained 22% of R_{A4}. R_{A4} decreased with year and precipitation (either MAP or PG) but increased with temperature (MJulT and JulT). MJulT had the strongest effect on R_{A4} than others since R_{A4} increased 13% (data not shown in Table IV.2) as the temperature increased from 16.2 °C to 23.4 °C. The combination of year and MAP in multiple regressions obviously improved the linear regression. R_{P4} increased with year, but no significant with precipitation (either MAP or PG) and temperature (MJulT and JulT). All correlations were not independent because there were also close relations between the environmental variables (all $P < 0.000$). As a result, the effects of environmental variables on R_{A4} and R_{P4} could not be separated and each response function of R_{A4} and R_{P4} to an environmental variable included direct and indirect effects.

Table IV.2 Linear regression parameters for regressions of the form $y = \beta_0 + x_1 \times \beta_1$ with y denoting R_{A4} or R_{P4} with the log transformation (n = 280). Climate means apply for the last normal period 1961-1990. MAP, mean annual precipitation; PG, precipitation in growing season of sampling year; MJulT, mean July temperature; JulT, July temperature in sampling year; Year, number of years after 2000.

		R_{A4}			R_{P4}		
Variable	Range, unit	Response	r^2	*P*	Response	r^2	*P*
MAP	116-386, mm yr^{-1}	-	0.08	0.000	n/a	0.00	0.926
PG	57-343, mm yr^{-1}	-	0.06	0.000	n/a	0.00	0.574
MJulT	16.2-23.4, °C	+	0.13	0.000	+	0.00	0.688
JulT	18.5-24.6, °C	+	0.04	0.000	+	0.00	0.318
Year	1-9, yr	-	0.22	0.000	+	0.08	0.000
Year x MAP		-	0.26	0.000	+	0.08	0.000

Temporal dynamic of C4 annuals and C4 perennials

P_{C4} didn't relate to year although higher in 2002 (upper panel, Fig IV.4). The mean P_{C4} is 24.0±23.5%. Among all years, PG in 2002 (157 mm) was 44 mm lower than that in other years (average of 201 mm). Thus, the lower PG was probably one of the reasons leading to higher P_{C4} in 2002. R_{A4} decreased significantly (R^2 = 0.92, P = 0.000, n = 8) during the study period (middle panel, Fig IV.4). R_{P4} increased over the study period, but not significantly (R^2 = 0.12, P = 0.392, n = 8) (lower panel, Fig IV.4). If 2002 is excluded from the analysis, the increasing R_{P4} trend becomes significant (R^2 = 0.91, P = 0.001, n = 7).

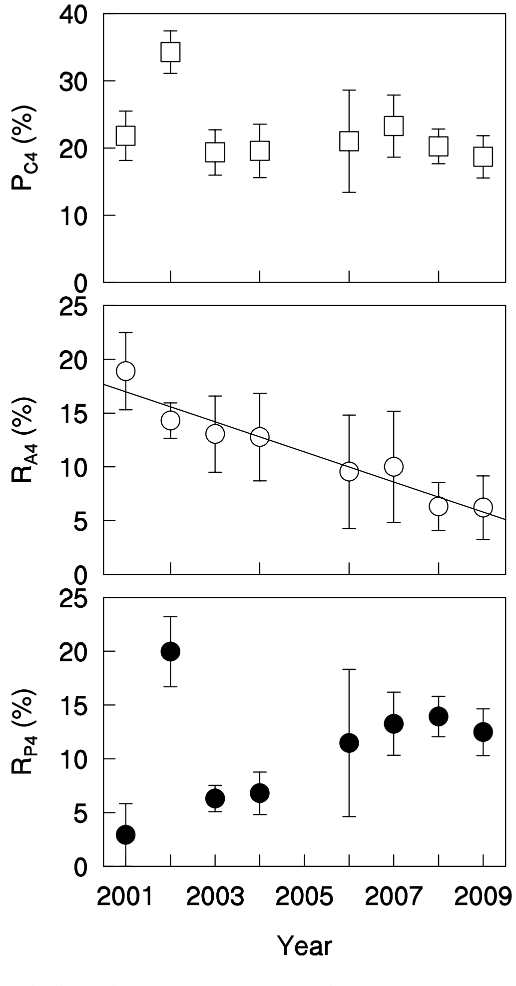

Fig. IV.4 Variation in relative biomass of C4 plants (P_{C4}), C4 annuals (R_{A4}), and C4 perennials (R_{P4}) in total over years. Each point is the average of relative biomass of survey sites within each year. The measurement numbers are 15 in 2001, 75 in 2002, 45 in 2003, 33 in 2004, 12 in 2006, 20 in 2007, 65 in 2008, and 15 in 2009. Error bars are standard error.

Responses of C4 plants, C4 annuals, and C4 perennials to growing season precipitation

The number of dry, normal, and wet sites was 59, 103, and 118 respectively. C4 plants did not significantly change to PG ($P > 0.05$) in spite of a decreasing trend from dry to wet (Fig IV.5). However, C4 annuals and C4 perennials responded differently to PG. C4 perennials were dominant in the dry growing season, while C4 annuals were dominant in the normal growing season. They were almost half biomass of C4 plants in the wet growing season.

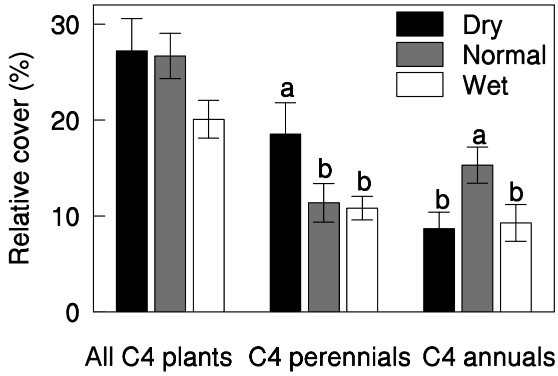

Fig. IV.5 Responses of the percentage of all C4 plants, C4 annuals, and C4 perennials to growing season precipitation in sampling year (PG). Dry, normal, and wet sites were divided by comparing PG with the long-term average of growing season precipitation in 1961-1990. Each value is the average of relative biomass of dry, normal, and wet sites. Error bars are standard error.

Responses of C4 plants, C4 annuals, and C4 perennials to grazing

We surveyed a total of 248 grazed sites and 32 ungrazed sites. Ungrazed locations had large SE than grazed locations (Fig IV.6). Grazing had no significant effect on $P_{C4,}$ R_{A4}, or R_{P4}.

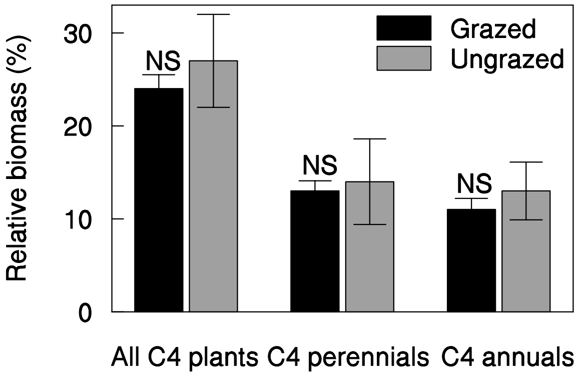

Fig. IV.6 Responses of the percentage of all C4 plants, C4 annuals, and C4 perennials to grazing. Each value is the average of relative biomass of grazed/ungrazed sites. Error bars are standard error. NS donates no significant difference.

Discussion

In the past several decades, the grasslands of the Mongolia plateau have experienced intensive increase in temperature. This causes the spread of C4 plants to areas with high latitudes and high altitudes (Wittmer *et al.*, 2010). Our findings further show a part of the spread process that the dominance of C4 annuals was gradually replaced by C4 perennials after intensive increase of C4 plants, which follows the prediction of Grime's C-S-R plant strategy theory. Both trends are influenced by growing season precipitation rather than by grazing. To our knowledge, this is the first survey evidence on how C4 plants spread.

Possible process of the increase of C4 plants

MJulT indicating growing season temperature increased 2 °C in the grassland of the Mongolia plateau in the past several decades and has been relatively stable since 2000 (NOAA NCDC Clamiate Data Online, 2008). We assumed that this serious increase in temperature was a kind of disturbance because it was much different with the variation of 0.5 °C in last normal period of 1960-1990. Hence, we can say that our study period is just after the serious increase of C4 plants caused by the increase in temperature and sampling year represents the succession time after disturbance. Our results shows that C4 annuals significantly decreased over sampling years and C4 perennials increased (Fig IV.4). Sampling year explained 22% the variation of C4 annuals which was highest in all relations (Table IV.2). Hence, our case is fit to the ruderal strategy of C4 annuals.

C4 plants favors open space created by the rise of temperature since the rise of temperature stimulates C4 plants more than C3 plants (Ehleringer & Björkman, 1977; Sage & Kubien, 2003). Life history traits determine which group quickly habitat the open space and how the dominance shift among groups (Chapin *et al.*, 2002). Compared to C4 perennials, C4 annuals have some advantages to colonize the open space as they have short life spans, high growth rates, and can produce large quantities of seeds using short-term optimal resources. Hence, the germination of seeds of annuals accelerates (Baskin & Baskin, 1998) and thus C4 annuals can rapidly colonize in open space.

Previous works reported that annuals-soil feedback changed soil structure (Norton *et al.*, 2004), biotic communities (Hawkes *et al.*, 2006), water relations (Leffler *et al.*, 2005), and ultimately enhance the mineralization-driven nutrient cycles (Sperry *et al.*, 2006). Hence, C4 annuals were likely improved microenvironment, which prefers the development of C4 perennials. Conservative use of resources of C4 perennials also pushes this process. The behaviors of C4 perennials and C4 annuals follow the predictions of ruderal group and stress-tolerant group responding to disturbances in Grime’s C-S-R plant strategy theory (Grime, 1977).

Uncertainty of C4 annuals and C4 perennials trends

Our findings show C4 annuals are favored by normal growing season precipitation and C4 perennials prefer dry growing season (Fig IV.5). Hence, C4 annuals will be higher in years with normal growing season precipitation and C4 perennials will be higher in years with dry growing season. As no available data of R_{A4} and R_{P4} can be used in 2005 (Fig IV.4), we speculate that C4 annuals are lower and C4 perennials are higher than the prediction from regression because of low growing season precipitation.

Grazing has no influence on C4 plants and its components although it decreases the aboveground net primary productivity (Bai *et al.*, 2007). This result, consistent with results from a long-term grazing experiment in our study area, indicates that increases in stocking rate have no influence on the P_{C4} (Wittmer, 2010).

Implications

Climate change is bringing the increase in temperature and the altered intensity and amount of precipitation in the coming years (Intergovernmental Panel on Climate Change, 2001). Our findings have important implications for understanding the response of grassland ecosystem to climate change and the parameterization of models using C4 annuals and C4 perennials.

First, annuals have the lowest relative biomass and highest variability when compared to other plant functional groups in the grassland of the Mongolia plateau (Bai *et al.*, 2004). Thus, the increase in C4 annuals contributes to the high variability and low stability of the steppe ecosystem. In addition, the increase in C4 annuals will contribute to the uncertainty of annual primary production, which will affect livestock production.

$\delta^{13}C$ of C4 annuals is 1.5‰ higher than that of C4 perennials in this area (see Chapter V). Large variation in the C4 annual: C4 perennial ratio over years is likely to cause the $\delta^{13}C$ variation of the C4 community and further atmospheric $\delta^{13}C$. Given the high percent of C4 annuals, the $\delta^{13}C$ of C4 community will be high and that of atmospheric $\delta^{13}C$ will be low. One should pay more attention to the setup of related parameters in models, such as the accurate partitioning of land and ocean carbon sinks through inversion of atmospheric $^{13}CO_2$ and CO_2 data.

CHAPTER V– General and summarizing discussion

In the grassland of the Mongolia plateau, a rise of C4 plants has been reported recently (Wittmer *et al.*, 2010). However, only few publications focused on the relation of C4 and C3 plants in this grassland and its implications. This study therefore concentrates on the water relation between dominant C4 plants and coexisting C3 plants (Chapter II), spatial pattern of discrimination of dominant C4 species and its underlying mechanism (Chapter III), and temporal dynamics of C4 annuals and C4 perennials over time (Chapter IV). Finally, the water relations between C3 and C4 plants and the Δ of C4 plants will be discussed in this chapter.

The water relations between C3 and C4 plants

In C4 communities *Cleistogenes squarrosa* on average contributes more than 70% and hence can serve as representative of the C4 community. *C. squarrosa* depended on the shallow soil water while dominant C3 species like *L. chinensis* and *S. grandis* to a considerable extent used the deep soil water except for shallow soil water (Chapter II). Given the relevance of these species for the C3 and C4 communities, we can say that C3 and C4 plants in the grassland of the Mongolia plateau differ in patterns of water use, which is consistent with other results from tallgrass prairie (Nippert & Knapp, 2007).

C4 plants usually start growth in May or June, later than C3 plants because they need the precipitation in warm months in the grassland of the Mongolian plateau (showed in Fig V.1). The competition for water between coexisting C3 and C4 plants in May or June will be increased because the available shallow soil water is decreased due to less precipitation and the increase in evapotranspiration. However, some dominant C3 plants like *L. chinesis* and *S. grandis* have plasticity to shift water sources from shallow to deep soil water during drought, which may alleviate the competition for shallow soil water between C3 and C4 plants and further contribute to the increase of C4 plants.

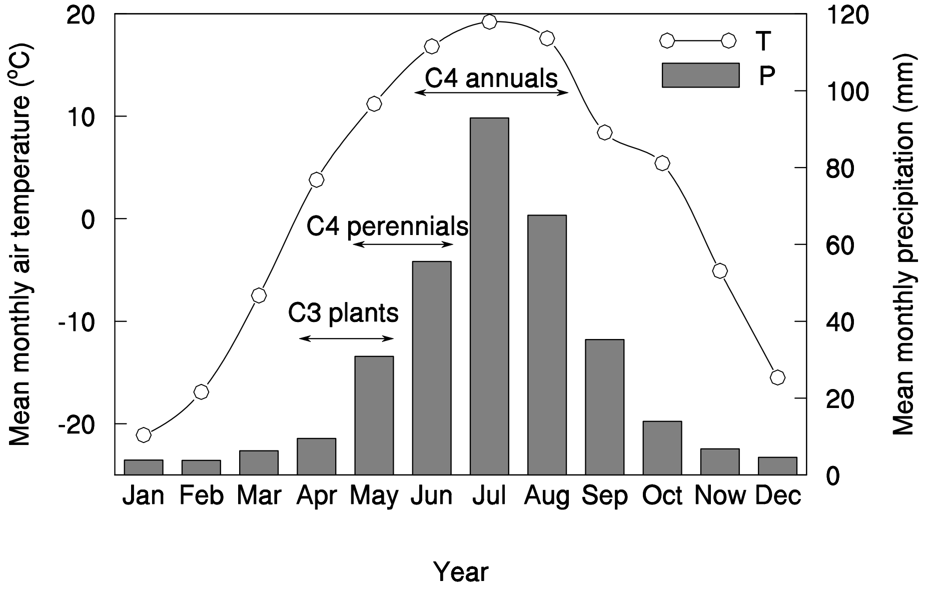

Fig V.1 Mean seasonal distribution of precipitation and temperature in 1982-2008 and the typical onset of growth of C3 plant, C4 perennials and C4 annuals (Bai & Xu, 1994; Liang *et al.*, 2002). Data of air temperature and precipitation were taken from IMGERS.

In the grassland of the Mongolia plateau, small rain with amount less than 5 mm occurred frequently (Cheng *et al.*, 2006) and can recharge only shallow soil water. The exclusive use of shallow soil water by C4 plants means that they can use these frequent small summer rain during the germination of seeds and plant growth. Hence, C4 annuals with large soil seedbank can have high recruitment rate and then rapidly colonize open space produced by disturbance. This becomes one of the reasons to the rapid increase of C4 annuals during disturbance.

The Δ_{C4} in the grassland of the Mongolia plateau

For the interpretation of $\delta^{13}C$ of bulked material like bulk vegetation, hair of grazers or soil organic matter, which makes use of the pronounced contrast on the discrimination between C3 and C4 plants, Δ_{C4} is needed as one of two end-members. Generally Δ_{C4} has been regarded to be constant (Liu *et al.*, 2005; Wittmer *et al.*, 2010). Auerswald *et al.* (2009) deduced for the scale of multiple years and several square kilometres that the likely variation of Δ_{C4} in this grassland only can cause a negligible change on the C3/C4 ratio due to large contrast between C3 and C4 plants and high relative biomass of C3 plants. However, any variation of Δ_{C4}, which is ignored, will contribute to the uncertainty in calculating the C3/C4 ratio of grassland. This thesis allows a better differentiated estimate of the Δ_{C4} in the grassland of the Mongolia plateau. This estimate will differ depending on the location, the year but also the scale of the analysis. This rather complex behaviour is essentially the result of *C. squarrosa*, a dominant contributor to the C4 community in the grassland of the Mongolia plateau. The Δ of *C. squarrosa* has a remarkably large range of 4‰ in all cases. It decreased with the increase of grazing intensity and leaf position on tiller and increased with the increase of soil silt content and precipitation. The Δ variation of *C. squarrosa* at different scales was mainly caused by the variation of N_{leaf} (Chapter III).

Δ_{C4} then depends on the discrimination of individual C4 species or groups and their relative contributions to the C4 community. The two main species of the C4 community are *C. squarrosa* and *S. collina* which in total contributed approximately 90% of the C4 community in most sampling locations (Chapter IV). *C. squarrosa* is a perennial grass and *S. collina* is an annual forb. In general, annuals and perennials do not differ in Δ among C4 species (Fig. V.1a). The comparison of 233 perennial species and 154 annual species taken from literatures and own data shows an almost perfect match with a mean of 5.37‰ and a SD of 1.12‰ (Fig. V.1a). This is, however, not the case for the grassland of the Mongolia plateau. Combining the data from Pyankov *et al.* (2000), Chen *et al.* (2002) and own data, resulted in an average Δ of C4 annuals of 5.63 ± 0.20‰ while that of C4 perennials was 7.19 ± 0.07‰, both differing by 1.5‰ in the grassland of the Mongolia plateau (Fig. V.1b). This difference between the two functional groups is caused by the dominance of *C. squarrosa* and *S. collina.* While *S. collina* occupies about the same range as other C4 species, this is not the case for *C. squarrosa* (Fig. V.1c). The Δ of *C. squarrosa* was significantly 1.9‰ higher than that of *S. collina* ($P = 0.000$) or other C4 species.

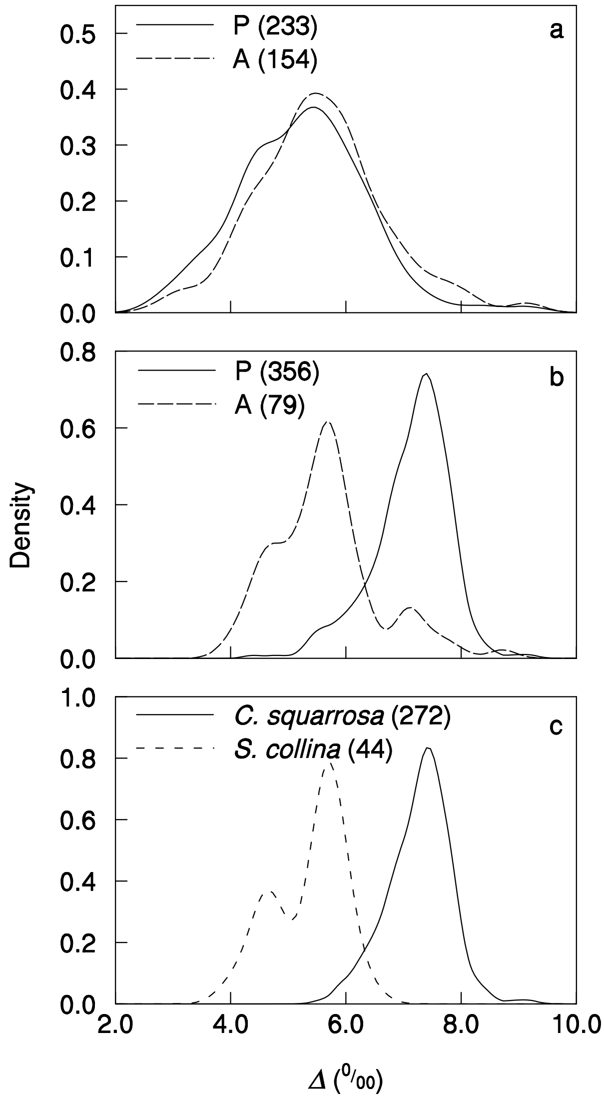

Fig. V.1 Density distributions of discrimination of different C4 populations; numbers in parenthesis give the number of samples. Total density under each curve is set to 1 ignoring the differences in size of the data sets. (a) averages for 154 annual C4 species (A) and 233 perennial C4 species (P) from Asia and Africa calculated from data taken from Ziegler *et al.*(1981), Batanouny *et al.*(1988), Redmann *et al.* (1995), Schulze *et al.* (1996) , Pyankov *et al.*(2000), Chen *et al.*(2002) and own data, (b) individuals of A and P from the grassland of the Mongolia plateau including data from Pyankov *et al.*(2000), Chen *et al.*(2002) and own data, (c) individuals of *Cleistogenes squarrosa* and *Salsola collina* including data from Pyankov *et al.* (2000), Chen *et al.* (2002) and own data

For a better prediction of Δ_4 in the grassland of the Mongolia plateau it is hence necessary to answer two questions: (i) What influences the ratio of the two functional groups, annuals and perennials, in C4 plants? (ii) What influences the Δ of *C. squarrosa*, the dominant species within C4 perennials? The first question was answered in Chapter IV where the relative biomass of every C4 species at surveyed sites was determined. The relative biomass of annuals in the C4 community decreased significantly from 2001 to 2009 (Rel. Biomass = – 0.076 x Year + 153.1, $R^2 = 0.82$, *P*

= 0.002, n = 8). This regression showed that on average relative biomass decreased from 75% to 14% between 2001 and 2009 while consequently the relative biomass of C4 perennials in C4 plants increased. This was mainly due to the ruderal strategy of C4 annuals which were able to profit faster from the change in conditions favoring C4 plants and causing their relative increase (Chapter IV). The second question was answered in Chapter III. By combining the information of both a better resolved estimate of the discrimination of the C_4 community can be obtained. It is given by the mean discrimination of C_4 annuals (Δ_{A4}) and perennials (Δ_{P4}) and the ratio of annuals within the C4 community (x) according to:

$$\Delta_{C4} = x \times \Delta_{A4} + (1 - x) \times \Delta_{P4} \tag{V.1}$$

From the decrease of annuals contributing to the total C4 biomass and the averages of Δ_{A4} and Δ_{P4} follows that Δ_{C4} has increased by almost 1‰ from 6.02‰ to 6.97‰ between 2001 and 2009 (Fig V.2). The mean Δ_{C4} during this period was 6.50‰ with SD of 0.69‰.

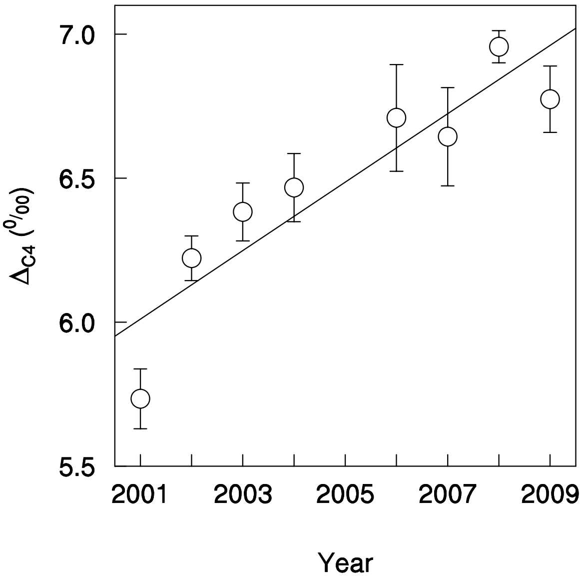

Fig. V.2 Variation of the discrimination of C4 community (Δ_{C4}) over time. Each point is calculated by equation V.1 using Δ_{A4}, Δ_{P4}, and the relative biomass of C4 annuals in C4 plants in each year. Δ_{A4}, and Δ_{P4} are 5.63‰ and 7.19‰ respectively for all years. The relative contribution of annuals to the C4 biomass is the average of all surveyed sites within a year with 15 sites in 2001, 75 in 2002, 45 in 2003, 33 in 2004, 12 in 2006, 20 in 2007, 65 in 2008, and 15 sites in 2009. Error bars are standard error for Δ_{C4}.

While the relative biomass of C4 annuals to C4 biomass is responsible for a pronounced change of Δ_{C4} over time, the Δ of *C. squarrosa* changed in space as shown in Chapter III. This causes Δ_{P4} and in turn Δ_{C4} to change in space. The combination of both then yields a complex spatio-temporal variation of Δ_{C4}. The lower boundary of this variation is 5.63‰. It will apply in places, where *C. squarrosa* is missing, which can especially be expected in 2001. The upper boundary is 7.19‰. This boundary will be found in places, where only *C. squarrosa* is present and where additionally, *C. squarrosa* has a low N_{leaf} (~1% N). Such a low content can especially be expected in relatively wet areas, early in the growing season in places where grazing intensity is low.

Outlook

The present work mainly focuses on C4 plants in the Inner Mongolia grassland, either in the ecophysiology of *C. squarrosa* including the water use and discrimination or in the yearly dynamics of the proportions of C4 annuals and C4 perennials and their relationships with environmental conditions. However, much work is still needed to understand the role of C4 plants in this cool-season semi-arid grassland ecosystem.

The high leakiness of *C. squarrosa* decreases its photosynthetic efficiency when light is limited. We may speculate that it would be replaced by other C4 species with lower leakiness in dense stands. However, it has been the dominant C4 species in a long-term ungrazed plot with cover more than 80% (including litter) and height more than 40 cm (data from IMGERS) indicating that even under these conditions light is not limiting but other factors are more limiting. Most likely water is the most limiting factor in this semi-arid grassland. The high leakiness of *C. squarrosa* thus opens opportunities for researches as it allows calculating stomatal conductance from discrimination and by doing so to study water use efficiency or nitrogen use efficiency of the major C4 plant in this area. This would not be possible for typical C4 plants with leakiness close to 0.4; this leakiness follows from the typical discrimination around 5.5‰ (Fig. V.1a).

During the study period, the R_{A4} decreased and R_{P4} increased significantly. However, the drivers are not clear. All of them, the rise of temperature and CO_2 concentration may be the reasons. As all changed in time, their influences can not be distinguished in a field study but studies under controlled environment are needed to find out which one is more important than others and what kind of physiological traits will be influenced and further change the competition between C4 annuals and C4 perennials.

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