Transpiration Response of C3 and C4 Plants of Northern Chinese Steppe Ecosystem to Water Vapor Pressure Deficits (VPD)

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Introduction

Temperature and air humidity are almost always confounded in experiments because saturated vapor pressure increases exponentially with increasing temperature to impact on plant growth (Sinclair *et al.*, 2007). The difference between atmospheric saturation vapor pressure and the actual water vapor pressure at a given temperature, as an independent factor on growth and physiology of plants. Temperature and vapor pressure deficit (VPD) effects on plants growth are almost always confounded in experiments because VPD also commonly increases greatly in experiments with elevated temperature treatment. Therefore, VPD provides a very direct indication of the atmospheric moisture conditions independent of temperature. Atmospheric evaporative demand, and consequently plant transpiration increase with increasing atmospheric vapor pressure deficit (VPD) (Sinclair and Bennett, 1998).

Previous studies have shown that plant water loss, transpiration, is positively correlated with radiation level, air VPD and soil water potential. Transpiration is directly proportional to VPD. And threshold VPD levels have been identified above which water transport in the plant can no longer match the transpiration demand. At that point, stomatal conductance decreases to stabilize transpiration at a constant, maximum rate and decreased CO_2 diffusion limits photosynthesis. A number of plant species, stomata conductance has been observed to decrease between VPDs of 1.0 and 2.5 kPa. A limited maximum transpiration rate has been observed when atmospheric VPD exceeds ~2 kPa. Franks (1999) investigated four growth groups' plants responses to a change in VPD from temperate environments. Wherley (2009) discovered that C_3 and C_4 turf grass species differed substantially in their response and sensitivity to VPD.

The experiments were conducted in big, climate-controlled chamber, which allowed plants to be exposed to a LED light (800W) levels while being subjected to various VPD. Transpiration responses to vapor pressure deficit in well watered. The aim of this study was to examine specifically the influence of VPD on transpiration response of five grassland species to increasing VPD at a stable temperature. And how is plants response to low range VPD (VPD < 1, high air humidity).

Material and Methods

Plant Materials

This study included five dominant species from 3 different plant functional groups (PFG) originating from Northern Chinese Songnen meadow steppe ecosystem: two perennial C_3 rhizome grasses, *Leymus chinensis* (Trin.) Tzvel. and *Phragmites australis* (Cav.) Trin. *ex* Steud., two annual C_4 grasses, *Chloris virgata* Swartz, *Setaria viridis* (L.) Beauv. and a perennial forb *Kalimeris integrifolia* Turcz. ex DC (Table 1).

Growth Conditions

The plants were raised from seeds directly sown in nursery pots filled with standard compost and commercially available fertilized garden soil. After establishment seedlings were transplanted after 2-4 weeks into 2-L plastic pots filled with 700g soil which is composted the same as before. Pots were watered every day and maintain the soil water content at 80 (v/v %). A complete liquid fertilizer (Wuxal Liquid, AgNova Technologies Pty Ltd) was applied once a week to ensure full nutrient supply.

Transpiration Chamber

All plants were measured in a closed chamber (80×80×100cm). 4 individual balances put in four corners of the chamber and were connected to a datalogger for continuous monitoring of pot weight so that the weight change of the plants could be recorded at the same time by the computer (Grasslog sofeware was used to measured weight at the same time when the chamber working). The chamber was equipped with two computer box fans, one was used to mix the air inside the chamber and the other was blowing air

into the chamber continuously. LED light installed on the top of the chamber (bloom power white360, Spled Gmbh, Germany). Photosynthetic photon flux (PPF) of 600-1200 μmol m^{-2} s^{-1}, Continuous flow of air through the VPD chamber ensured that no carbon dioxide deficit developed in the chamber. A humidity/temperature sensor (TV-4505, tiny tag, Gemini Data Loggers (UK) Ltd.) was mounted through the side wall of the chamber and chamber temperature and relative humidity was logged every minute. Temperature and relative humidity were recorded every 60sec to calculate the actual atmospheric VPD in the chamber during measurements. Once the atmosphere in the chamber was equilibrated, the entire unit of plants and pot were weighed on a balance with a resolution of 0.01 g (Model KERN KB 2400-2N d=0.01g).

Data analysis
After measuring the transpiration, the plants were clipped at the soil surface and these were separated into stem and leaf fractions. The total leaf area was measured using a LI-3100 area meter. Then the leaves and the stem were all dried in an oven to a constant weight. These values were then used to calculate the ratio of root/shoot for each species. Transpiration rate for each plant was expressed per unit of leaf area.

$$Tr = \frac{(W0 - W1)/18}{Time \times Leaf\ area}$$

W0= the for the former weight
W1= after 180seconds weight
Time= 180 seconds

Air temperature and relative humidity data were used to calculate the mean atmospheric VPD for each measurement. The data were analyzed by plotting transpiration against VPD. There was a large amount of scatter among data points; therefore, the data for each species were grouped into cohorts of five consecutive values of VPD. The mean of both VPD and transpiration for these cohorts were used in further analysis.

Stomatal Conductance Calculation
Stomatal conductance is a more sensitive measure of stomatal regulation than transpiration because it corrects for the effects of changes in temperature and relative humidity during the measurement period. Transpiration rate, chamber air temperature and relative humidity measurements were used to calculate average whole-plant stomatal conductance using Equation 1,
gs = Tr/ VPD   Equation 1
Where gs is stomatal conductance in mmol H_2O m^{-2} s^{-1}, Tr is the transpiration rate in mmol H_2O m^{-2} s^{-1}, and VPD (vapor pressure deficit) the dimensionless (mol/mol) driving deficit for water vapor diffusion out of the stomata's.

Statistical analysis
Data were analyzed by regressing transpiration rate against VPD using observations different species, for each species, the data were analyzed using both linear and quadratic terms (PROC REG, SAS). Slopes and intercepts for individual experiments were compared to one another and no differences were found (α=0.05), indicating that the relationship between VPD and transpiration did not differ over experiments within a species. Data were then pooled for a species for further analysis using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, 2007) (Wherley and Sinclair, 2009)
Pool over all data was analyzed by plotting all individual transpiration rate (Tr) data against VPD for each species. Two regression equations were applied to the pool over data using least-squares regression in GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, 2007) for each species.
Initially a double linear regression was applied to the data:
If VPD < X0, Tr = Slop1 (VPD) + Intercept1 (Line 1)
If VPD ≥ X0, Tr = Slop2 (VPD) + Intercept2 (Line 2)
Where X0 is the break point between the two line segments, Slop1 and Slop2 are the slopes of the first and second line segments, respectively, and Intercept1 and Intercept2 are the constants of the first and second line segments, respectively. In the regression, the second line segment is constrained to intersect with the first line segment at X0. The slopes of the two linear regressions (Slop1 and Slop2) were statistically compared to determine if they differed significantly (p < 0.05). If the slopes differed, the double-linear regression was retained. When the slopes were not significantly different, a simple linear regression was applied to all the data.

Normalizing the response to change in VPD
All groups of plants measured here, being wide ranging growth form and natural habitat, operate over widely differing ranges of stomatal conductance. This presents some difficulty when attempting to compare the responses of these plants to perturbations. Our solution to this was to normalize the VPD response for a standard increment in VPD from the highest value to the other value. The normalized response is expressed as the final steady-state transpiration rate, for a given increase in VPD, relative to what it would have been if no change in stomatal conductance had occurred. Referring to Fig.1, this ratio Φ is given by Φ=b/(a+b), where b is the actual transpiration rate at the second value and (a+b) the rate of transpiration that would have occurred had where been no change in steady-state stomatal conductance following the VPD change. We will refer to Φ as an index of stomatal sensitivity to VPD, although, as Φ is negatively related to the magnitude of the VPD response, it could be referred to as an index of insensitivity. In theory the lower limit of Φ is zero, occurring if stomata were to close completely following the VPD change, reducing transpiration rate to zero. This would also require a cuticualr conductance of zero. The upper limit of Φ is 1, and occurs if there is no change in stomatal conductance following the increase in VPD. In practice Φ will lie somewhere between 0 and 1, and in almost all case between 0.4 and 1. In most cases the increment in VPD was applied in one single step to minimize the confounding effects of diurnal rhythms and photosynthetic time elapsed (Franks et al 1997).

Results and Discussion

   The two-segment linear regression provided the best fit for *L. chinensis* (Fig. 1). As VPD increased within the low VPD range, transpiration of the *L. chinensis* increased sharply at rates ranging from 1.4 mmol H_2O m^{-2} s^{-1} for each 1 kPa increase in atmospheric VPD. While, data for the other four species were found not fit to the two-segment model, and hence, a single linear regression generally fit these data well with the R^2 ranging from 0.69 to 0.87 (Table 2). The slope of these species with single-linear transpiration response, ranged from 0.4 to11.6 mmol H_2O m^{-2} s^{-1}, with *K. integrifolia* has a highest slop 3.98 mmol H_2O m^{-2} s^{-1} per 1 VPD.

Different response to transpiration in C$_3$ and C$_4$ species
Our experiment is a new method to measure four plants in the same time in different VPD condition. Figure 1 shows the responses of canopy transpiration to changes in VPD. For *L. chinensis* there is a breakpoint when VPD equal to 1.14. When VPD>1.14 the slop is smaller than VPD<1.14 (Fig. 1). Such a limitation is likely a result of stomatal closure in response to elevated VPD (Bunce 2006); due to the fact that water transport in the plant could not meet transpirational demand (Sinclair *et al.*, 2007).This decrease in transpiration with increasing VPD could be loosely referred to as the feed back regulation response. In contrast, the transpiration of other four species has a positive liner relationship with VPD increase. But the slop of this four species are different, *K. integrifolia* has a larger slop because it has a big root system (Table 2). In addition, these five species belong to different pathway of photosynthesis (Table 1). *C. virgata* and *S. viridis* are C4 species and have a small root system; the ratio of the LA/R is about 550 cm^{-2} g m^{-1}. Unless transpiration is restricted by stomata conductance, plant transpiration is anticipated to increase linearly with increases in the VPD.

Stomata conductance response to VPD increase
Stomatal are the primary site of water loss in plants, therefore stomatal control is important in avoiding water stress under conditions of atmospheric and soil water change. Mott and Parkhurst (1991) demonstrated that stomatal conductance did not respond directly to VPD, but to VPD corrected for the diffusion coefficient of water vapour in the medium around the leaf. And Bunce (2006) point out that stomatal responses to VPD are controlled by peristomatal transpiration rate and there was no transient increase in transpiration as the increase in VPD was imposed. The most of species show a reduction in Gs with increasing VPD (Schulz and Hall, 1982). And for many C3 species have breakpoint when VPD≈1. The few studies that have measured Gs over a range of VPD have found the majority of species show no effect of growing well-watered plants under different magnitudes of VPD (Kawamitsu et al., 1993; Mooney and Chu, 1983; Roy and Mooney, 1982). Stomatal conductance is larger in C$_3$ plants than C$_4$ was observed in this study. *L. chinensis* and *C. virgata* have a peak Gs when VPD equal to 1.07 and 0.96 respectively, the value belong to the other paper's results. But for C$_4$ plant *C. virgata* also have a breakpoint under VPD<1. This result is different from other study results, maybe because the VPD often limit between 1 and 4 in other experiments. We calculate Gs from the canopy basis of the plant which includes the old leaves, while in our experiments Gs often contributed by the fresh leaves. And for C$_4$ species they has a narrow stomata conductance range with the VPD change, Hogg and Hurdle also found that Gs has a liner response with the VPD from 0 to 1 (Hogg and Hurdle, 1997). Moreover, it is more likely that stomatal conductance is influenced by the difference in water potential between guard cells and other epidermal cells (Bunce, 2006). For *L. chinensis* and *C. virgata* although there is evidence that lower stomata conductance reduced transpiration at higher VPD, there is no evidence of any feed forward behavior. Farquhar and Sharkey (1982) have argued that stomata usually impose only a slight limitation on net assimilation, and further, that stomata do not limit assimilation in C$_4$ species more than in C$_3$ species.

Normalizing the response to change in VPD
VPD increase enhanced the peristomatal and cuticular transpiration more than stomatal transpiration, resulting in water potential elevation of the guard cell and its swelling, so stomata opened the pores and Gs increased. It implied that positive feedback control worked in this process (Raschke, 1970). The sensitivity of stomatal conductance to VPD was linearly proportional to the magnitude of stomatal conductance (Morison and Gifford, 1983). Photosynthesis affects by stimulating chlorophyll formation and protein synthesis and at the same time promote stomatal opening so that the stomata would not limit the increased capacity for photosynthesis. Farquhar and Sharkey (1982) argued that stomata usually impose only a slight limitation on net assimilation, and further, that stomata do not limit assimilation in C$_4$ species more than in C$_3$ species. Although *L. chinensis*, *P. australis* and *K. integrifolia* all belong to C3 photosynthetic pathway. Growing plants under a low VPD increased stomatal sensitivity to increasing VPD in most species (Cunningham, 2004). These high stomatal conductances may contribute to very high rates of transpiration under natural conditions. It could be argued that while water availability is non-limiting, these plants achieve their relatively high rates of productivity with correspondingly high rates of water loss.

Conclusions and Outlook

   These results have important implications in predicting plant response to climate change. *L. chinensis*, and other four species, could experience a substantial benefit with temperature increases expected in temperate grassland if VPD were to remain unchanged. During the past 50 years, VPD has remained virtually constant (Szilagyi *et al.*, 2001). In Songnen grassland the VPD range is from 1.0 to 2.5 during growing season (Dong *et al.*, 2011). The sensitivity to VPD indicates that if VPD remains stable in future climates as it has in the past, growth of *L. chinensis* could well be stimulated rather than decreased by global warming in temperate grassland.

References
Bunce J.A. (1981) Comparative responses of leaf conductance to humidity in single attached leaves. *J Exp Botany* 32, 629–634
Bunce J. A. (2006) How do leaf hydraulics limit stomatal conductance at high water vapour pressure deficits? Plant Cell Environ 29:1644–1650

Comstock, J., Ehrleringer, J. (1993) Stomatal response to humidity in common bean (Phaseolus vulgaris): implications for maximum transpiration rate, water-use efficiency and productivity. *J. Plant Physiol*. 20, 669–691.

Cunningham S. C. (2004) Stomatal sensitivity to vapour pressure deficit of temperate and tropical evergreen rainforest trees of Australia, *Trees*, 18, 399-407.

Dong G, Guo J, Chen J, Sun G, Gao S, Hu L, Wang Y (2011) Effects of Spring Drought on Carbon Sequestration, Evapotranspiration and Water Use Efficiency in the Songnen Meadow Steppe in Northeast China. *Ecohydrology*, 4: 211-224.

Farquhar G.D. & Sharkey T.D. (1982) Stomatal conductance and photosynthesis. *Annu Rev Plant Physiol*, 33, 317-345.

Franks P.J., Farquhar G.D. (1999) A relationship between humidity response, growth form and photosynthetic operating point in C_3 plants, *Plant, Cell and Environment* 22, 1337-1349.

Franks P.J., Cowan I.R., Farquhar G.D. (1997) The apparent feed-forward response of stomata to air vapour pressure deficit: Information revealed by different experimental techniques with two rainforest trees, *Plant, Cell and Environment* 20, 142-145.

Hirasawa, T., Hsiao, T.C. (1999) Some characteristics of reduced leaf photosynthesis at midday in maize growing in the field. *Field Crop Res*. 62, 53–62.

Hogg E. H., Hurdle P. A. (1997) Sap flow in trembling aspen: implications for stomatal responses to vapor pressure deficit, *Tree Physiology*, 17, 501—509.

Isoda, A., Wang, P. (2002) Leaf temperature and transpiration of field grown cotton and soybean under arid and humid conditions. *Plant Prod. Sci*. 5, 224–228.

Raschke K. (1970) Leaf hydraulic system: rapid epidermal and stomatal responses to changes in water supply. Science, 167, 189-191.

Kawamitsu Y., Yoda S. and Agata W. (1993) Humidity Pretreatment Affects the Responses of Stomata and CO2 Assimilation to Vapor Pressure Difference in C_3 and C_4 Plants, *Plant Cell Physiol*.. 34, 113-119.

Mooney HA, Chu C (1983) Stomatal responses to humidity of coastal and interior populations of a Californian shrub. Oecologia 57:148–150.

Morison James I.L., Gifford Roger M. (1983) Stomatal Sensitivity to Carbon Dioxide and Humidity a comparison of two C_3 and two C_4 grass species. *Plant Physiol*.. 71, 789-796.

Mott K. A., Parkhurse D. F. (1991) Stomatal response to humidity in air and helox. *Plant, Cell and Environment*, 14, 509-515.

Roy J., Mooney H.A. (1982) Physiological adaptation and plasticity to water stress of coastal and desert populations of Heliotropium curassavicum L. *Oecologia* 52, 370–375.

Salih, A.A., Ali, I.A., Lux, A., Luxova, M., Cohen, Y., Sugimoto, Y., Inanaga, S. (1999) Rooting, water uptake, and xylem structure adaptation to drought of two sorghum cultivars. Crop Sci. 39, 168–173.

Schulze E.D. & Hall A.E., (1982) Stomatal responses, water loss, and COT assimilation rates of plants in contrasting environments. In Physiological Plant Ecology II. Encyclopedia of Plant Physiology. New Series. Vol. I2B (eds O, L, Lange, P, S, Nobel, C, B, Osmond & H, Zelgler), pp, 181-230, Springer-Verlag, Berlin,

Sinclair T., Fiscus E., Wherley B., Durham M., Rufty T. (2007) Atmospheric vapor pressure deficit is critical in predicting growth response of "cool-season" grass Festuca arundinacea to temperature change. *Planta* 227, 273–276

Sinclair T.R., Bennett J.M. (1998) Water. In: Sinclair, T.R., Gardner, F.P. (Eds.), Principles of Ecology in Plant Production. CAB International, pp. 103–120.

Szilagyi J., Katul G.G., Parlange M.B. (2001) Evapotranspiration intensi- Wes over the conterminous United States. *J Water Res Plann Manage* 127, 354–362

Turner N.C., Schulze E-D, Gollan T. (1984) The responses of stomata and leaf gas exchange to vapour pressure deficits and soil water content I. Species comparisons at high soil water contents. *Oecologia* 63,338–342

Wherley B.G., Sinclair T.R. (2009) Differential sensitivity of C_3 and C_4 turf grass species to increasing atmospheric vapor pressure deficit. *Environmental and Experimental Botany*, 67, 372–376.

Fig. 1 Transpiration rate response of five species to increasing vapor pressure deficit (VPD) as two patterns of response that was found for the tested five species. The detail of regression information was presented in Table 3.