Houshmandfar Alireza (Orcid ID: 0000-0003-0592-4926)

The relationship between transpiration and nutrient uptake in wheat changes under elevated atmospheric CO_2

Alireza Houshmandfar[1,2,*], Glenn J. Fitzgerald[3,2], Garry O'Leary[3], Sabine Tausz-Posch[2,4], Andrew Fletcher[1], Michael Tausz[5,6]

1) CSIRO Agriculture and Food, Private Bag 5, P.O., Wembley, WA 6913, Australia
2) Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Creswick, Victoria 3363, Australia
3) Agriculture Victoria, Victoria State Department of Economic Development, Jobs, Transport and Resources, Horsham 3401, Australia
4) School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom
5) Birmingham Institute of Forest Research, The University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom
6) Department of Ecosystem and Forest Sciences, The University of Melbourne, Creswick, Victoria 3363, Australia

Corresponding author: Alireza Houshmandfar, CSIRO Agriculture and Food, Private Bag 5, P.O., Wembley, WA 6913, Australia. Email: alireza.houshmandfar@csiro.au
Faculty of Veterinary and Agricultural Sciences, University of Melbourne, 4 Water Street, Creswick, Victoria 3363, Australia. Email: alireza.houshmandfar@unimelb.edu.au

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ppl.12676

Abstract

The impact of elevated [CO_2] (e[CO_2]) on crops often includes a decrease in their nutrient concentrations where reduced transpiration-driven mass flow of nutrients has been suggested to play a role. We used two independent approaches, a Free-Air CO_2 Enrichment (FACE) experiment in the South Eastern wheat belt of Australia and a simulation study employing the Agricultural Production Systems Simulator (APSIM), to show that transpiration (mm) and nutrient uptake (g m^{-2}) of nitrogen (N), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), and manganese (Mn) in wheat are correlated under e[CO_2], but that nutrient uptake per unit water transpired is higher under e[CO_2] than under ambient [CO_2] (a[CO_2]). This result suggests that transpiration-driven mass flow of nutrients contributes to decreases in nutrient concentrations under e[CO_2], but cannot solely explain the overall decline.

Keywords: APSIM, FACE, Jarvis model, nitrogen uptake, *Triticum aestivum* L.

Abbreviations: a[CO_2], ambient [CO_2]; CNC, critical nitrogen concentration; EC, electrical conductivity; e[CO_2], elevated [CO_2]; FACE, Free-Air CO_2 Enrichment; LAI, leaf area index; ns, not significant; PAR, photosynthetically active radiation; RUE, radiation use efficiency; g_s, stomatal conductance; TE, transpiration efficiency; VPD, vapour pressure deficit

Introduction

Anthropogenic activities such as fossil fuel consumption and deforestation have caused atmospheric [CO_2] to increase rapidly (Canadell et al., 2007) from 315 µmol mol^{-1} in 1960 to approximately 400 µmol mol^{-1} in 2015. Future [CO_2] is likely to reach 550 µmol mol^{-1} or above by 2050 (IPCC, 2013). Plants respond to elevated [CO_2] (e[CO_2]) with increased photosynthesis (Ainsworth and Long, 2005; Nowak et al., 2004) and reduced stomatal conductance (g_s) (Bunce, 2004; Leakey et al., 2006), and these responses are fundamental for all other CO_2-driven effects on plants and their accompanying ecosystems (Ainsworth and Rogers, 2007; Long et al., 2004).

In C_3 crops, these changes improve water use efficiency and grain yield (Ainsworth et al., 2008; Leakey et al., 2012) but are likely to reduce nutrient concentrations in vegetative tissues, which, in turn, can result in lower nutrient concentrations in the grain (Conroy and Hocking, 1993; Hogy et al., 2009; Pleijel and Hogy, 2015). Concentrations of nutrients such as nitrogen (N), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), and manganese (Mn) have all been reported to decrease by up to 22% under e[CO_2] (Loladze, 2002; Loladze, 2014; McGrath and Lobell, 2013).

Limited uptake of nutrients per unit root mass is one of the hypotheses suggested to explain the decline in nutrient concentration under e[CO_2] (Taub and Wang, 2008). This reduced root uptake could result from changes in root systems, such as less efficient root system architecture or decreased uptake capacity per unit root length (BassiriRad et al., 2001; Pritchard and Rogers, 2000), or from reduced transpiration-driven mass flow of nutrients due to decreased g_s under e[CO_2] (BassiriRad et al., 2001; Del Pozo et al., 2007; McGrath and Lobell, 2013).

The significance of transpirational mass flow for nutrient uptake is supported by a wealth of circumstantial data (Masle et al., 1992; Polley et al., 1999; Russell and Barber, 1960; Sellin et al., 2013). Transpiration rate may affect nutrient uptake both directly, through effects on the rate of radial transport of nutrients through the apoplasm (Marschner and Marschner, 2012), and indirectly, by influencing the supply of nutrients to the plasma membrane of root cells (Cramer and Hawkins, 2009; Tinker and Nye, 2000). Decreased transpiration rates may reduce rhizosphere nutrient depletion resulting from plant uptake, especially for solute nutrients (Kupper et al., 2012; Scholz et al., 2007).

Few attempts have been made to address the effect of e[CO_2] on the relationship between transpirational mass flow and nutrient uptake. McDonald et al. (2002) used a short duration (7-day period) pot experiment with e[CO_2] (approximately 1000 μmol mol^{-1}) and high humidity under glasshouse conditions to report that transpiration rate and nitrate (NO_3^-) uptake were positively related. A similar correlation was found by McGrath and Lobell (2013) in a meta-analysis that included experiments mostly conducted in open-top chambers, growth cabinets or glasshouse conditions. The authors pointed out that none of the experiments had measured transpirational mass flow and nutrient uptake simultaneously under e[CO_2]. Glasshouse or chamber conditions may also distort responses and lead to conclusions that are not immediately transferable to field conditions (Long et al., 2006; Long et al., 2004; Mcconnaughay et al., 1993; McLeod and Long, 1999). In addition, pot experiments can change the water dynamics necessary to evaluate the relationship between transpirational mass flow and nutrient uptake. Because of the small pot volume, nutrients are easily available to the roots, regardless of whether transpiration is reduced or not (McGrath and

Lobell, 2013). Free-Air CO_2 enrichment (FACE) studies with plants rooted in the ground would likely give better estimates because roots have unrestricted access to soil volume, and water dynamics represent typical field conditions.

In this study, we used two independent approaches applied to the same wheat cultivars grown at the same site to investigate the potential relationship between transpiration and nutrient uptake. For the first approach, measurements in the Australian Grains Free-Air CO_2 enrichment (AGFACE) facility were used to simultaneously evaluate the nutrient uptake (N, K, S, Ca, Mg and Mn) and transpiration rates in wheat (*Triticum aestivum* L.) grown under ambient [CO_2] (a[CO_2], approximately 390 µmol mol^{-1}) and e[CO_2] (approximately 550 µmol mol^{-1}). During one growing season (2013) nutrient uptake was measured in detail and transpiration was estimated for the same period using the Jarvis-type (Jarvis, 1976) empirical g_s model. This model was parameterised from approximately 1500 leaf level g_s measurements on the same wheat cultivars at the AGFACE site (Houshmandfar et al., 2015a). For the second approach, nutrient uptake (for N only) and transpiration was simulated by the Agricultural Production Systems Simulator (APSIM) modeling framework over ten consecutive growing seasons from 2007 to 2016. APSIM is a well-tested agricultural model internationally recognised as a highly advanced simulator of cropping systems (Holzworth et al., 2014). The model simulates plant water uptake (approximately equal to transpiration (Taub, 2010)) and N uptake in wheat (APSIM-Wheat) for an adjustable duration of time, along with a wide range of other capabilities. APSIM-Wheat has been broadly evaluated in a range of experimental (Holzworth and Huth, 2011; Zhang et al., 2012) and farm conditions (Hochman et al., 2009), also under e[CO_2] scenarios (Asseng et al., 2004; Reyenga et al., 1999), and in particular at the AGFACE site (O'Leary et al., 2015).

If the relationship between transpiration and nutrient uptake is identical under a[CO_2] and e[CO_2] (and explains a significant proportion of the data variance), then changes in transpiration rate will be sufficient to explain changes in crop nutrition under e[CO_2].

Understanding the relative role of transpiration in lower nutrient concentration in crops grown under e[CO_2] would improve our currently limited ability to predict responses for different crops and regions, and help guide breeding and agronomic strategies to adapt crops to higher [CO_2].

Material and methods

Experimental setup and plant material

A field experiment using two cultivars of wheat (*Triticum aestivum* L. cv. Scout and cv. Yitpi) was conducted at the AGFACE site located in Horsham, Victoria, Australia (36°45′07.2″S, 142°06′52.2″E; 127 m above mean sea level) in the 2013 growing season. The soil type is a Vertosol clay with non-dispersive and pedal surface (Isbell, 2002), approximately 35% clay at the top increasing to 60% at 1.4 m depth. The AGFACE is located in a semi-arid cropping area, which has a Mediterranean type climate but with cooler and drier winters (Hutchinson et al., 2005). Long-term average annual rainfall of the area is 435 mm, with 274 mm typically falling during the wheat growing season (June to November). Long-term mean growing season temperature is 16.5 °C (Australian Bureau of Meteorology). The soil had a pH of 8.2, EC (electrical conductivity) of 0.14 µS cm^{-1}, Mehlich 3 extractable (Mehlich, 1984) Ca of 6.4 (±0.5) g kg^{-1}, K of 501.8 (±42.5) mg kg^{-1}, Mg of 1.1 (±0.0) g kg^{-1}, Mn of 0.1 (±0.0) mg kg^{-1}, Fe of 120 (±9.2) mg kg^{-1}, zinc (Zn) of 2.3 (±0.5) mg kg^{-1}, copper (Cu) of 1.6 (±0.1) mg kg^{-1}, Bray extractable (Bray and Kurtz, 1945) phosphate (PO_4^{3-}) of 19.2 (±2.2) mg kg^{-1}, as well as AQ2 measured (Automated Discrete Analyzer, Seal Analytical Ltd, UK) nitrate (NO_3^-) and ammonium (NH_4^+) of 6.1 (±0.2) and 3.4 (±0.1) mg kg^{-1}, respectively. These values are averages of 32 soil samples from 0 to 25 cm depth.

In 2013, the AGFACE was fully randomized in a complete block design with four replications, with eight octagonal plots (16 m diameter) of which four were a[CO_2] (approximately 390 µmol mol^{-1}) and four e[CO]$_2$ (approximately 550 µmol mol^{-1}). Each cultivar was sown into two randomly allocated replicate subplots (1.5 m × 4 m), one each in opposing halves of the ring. Measurements from the two subplots were averaged for each replicate plot. Each e[CO_2] plot was encircled by horizontal CO_2-release-tubes in an octagonal shape. The tubes were progressively raised as the crop grew so that the CO_2 was injected about 0.1 - 0.15 m above the canopy. A plot center [CO_2] value of 550 µmol mol^{-1} was targeted for the e[CO_2] treatment from sunrise to sunset starting from germination. Average plot central [CO_2] were recorded every minute with an infrared gas analyzers (IRGA, SBA-4, PP Systems, MA, USA) located at the central part of each plot. Detailed engineering specifications, performance of the FACE system, and treatment descriptions are found in Mollah et al. (2011) and Fitzgerald et al. (2016).

Plant sampling and nutrient analysis

Leaf area index (LAI, one-sided green leaf area per m^{-2} ground area) and nutrient uptake into aboveground biomass (g m^{-2} ground area) were simultaneously measured at four different growing stages from stem elongation until the end of anthesis: DC31, DC34, DC65 and DC69 (DC decimal

code, according to Zadoks et al. (1974) cereal growth scale). Labelled areas of 1.35 × 0.5 m^2 were harvested at DC31 and DC65, and 30 to 50 random tillers sampled at DC34 and DC69 experimental unit. Plant densities were 414.4 (±15.3) and 462.5 (±13.2) tillers m^{-2} under ambient and elevated [CO_2], respectively. For each sampling time, entire aboveground biomass including leaves, stems, and spikes (DC 65 and DC 69) were harvested, then, after measuring leaf area with a calibrated leaf area meter (Li-3100, Li-Cor, NE, USA), dried for 72 h in a 70 °C of oven temperature. N concentration was measured using LECO Nitrogen Macro Determinator (TruMac, LECO Corporation, MI, USA). K, S, Ca, Mg, and Mn concentrations were measured using inductively coupled plasma atomic emission spectrometry (ICP-AES) (Applied Research Laboratories, 3580B, Switzerland) after digestion in concentrated nitric acid (HNO_3) following the procedure described by Zarcinas et al. (1987). We studied N, K, S, Ca, Mg and Mn because these nutrients are considered to be transported to a significant extent by transpirational flow (solute transport) (Marschner and Marschner, 2012; Oliveira et al., 2010). Nutrient uptake into aboveground was calculated as nutrient concentration multiplied with dry weights expressed per m^2 ground area.

Transpiration estimates

Transpiration was estimated on a leaf area basis using the principles of Fick's law, and g_s computed with the Jarvis-type (Jarvis, 1976) empirical model previously parameterised for wheat using many measurements of the same cultivars at the AGFACE site (Houshmandfar et al., 2015a). The parameterised Jarvis-type model predicts g_s by multiplying maximum g_s (a[CO_2]: 0.823 (±0.250), e[CO_2]: 0.529 (±0.127) mol m^{-2} s^{-1}) measured under optimum conditions with functions of the main microclimate and phenological variables including temperature, vapour pressure deficit (VPD), soil moisture content, time of day, leaf aging, and photosynthetically active radiation (PAR), parameterised separately for ambient and elevated [CO_2] growing conditions (Houshmandfar et al., 2015a). Microclimatic variables were taken from a continuously logging (15-min intervals) meteorological station located in the AGFACE site, except for the 143rd day after germination when average daily weather observations were taken from the nearby (<20 km) meteorological station # 079028 (Australian Bureau of Meteorology). The soil water content was omitted because soil water was regarded as non-limiting during the measurement period of this experiment (an exceptionally high rainfall growing season for the site, Table 1). VPD of air was calculated using temperature and relative humidity as described by Monteith and Unsworth (2013) and ranged from 0.005 to 3.993 kPa. Stomatal conductance was calculated on a 15-min average basis. The equation (transpiration = g_s ×

VPD) described by Hunt et al. (1985) was used to calculate the estimates for transpiration rates (Wright et al., 2012). Leaf-level transpiration was estimated separately for each replicate subplot from germination until the time of nutrient data collection, i.e. from germination until DC31, DC34, DC65 or DC69. Transpiration was then (approximately) scaled up to stand-level by multiplying leaf-level transpiration with LAI (Wright et al., 2012). The air-temperature-based VPD was used as an approximation rather than leaf-temperature-based VPD (Wright et al., 2012). Transpiration was expressed as mm.

Table 1 Average values for temperature and solar radiation, as well as rainfall amount from 2007 to 2016.

	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Annual average minimum air temperature (˚C)	8.0	6.7	7.6	7.3	7.4	6.8	7.5	7.3	7.3	8.0
Annual average maximum air temperature (˚C)	22.4	21.4	22.2	20.8	21.1	21.5	21.7	22.5	22.3	21.6
Annual rainfall (mm)	428.9	335.4	399.8	559.4	507.0	287.2	378.0	221.8	269.8	550.4
Rainfall during the growing season (mm)	164.0	166.2	266.6	268.6	215.4	174.8	320.8	115.0	125.2	326.4
Annual average solar radiation (MJ m^{-2})	16.3	15.6	16.3	18.2	15.3	16.0	15.8	16.2	16.1	15.8

APSIM model

APSIM-Wheat (v 7.8) (Holzworth et al., 2014) was used to simulate monthly transpiration and N uptake values in cv. Yitpi and cv. Scout from germination until approximately DC69 under a[CO_2] (as per the ambient [CO_2] in the growing season) and e[CO_2] (550 µmol mol^{-1}) in ten consecutive wheat seasons (2007-2016). Ambient [CO_2] was set to 360 µmol mol^{-1} for 2007 and 2008, 370 µmol mol^{-1} for 2009 and 2010, 380 µmol mol^{-1} for 2011 and 2012, 390 µmol mol^{-1} for 2013 and 2014, and 400 µmol mol^{-1} for 2015 and 2016, the approximate values corresponding to changes in the ambient [CO_2] in the growing seasons. The model was run for ten years to broaden the range of the environmental conditions to test if the relationships remain unchanged by variations in the growing seasons. In APSIM-Wheat, e[CO_2] impacts upon simulated growth and resource use via changes to radiation use efficiency (RUE), transpiration efficiency (TE) and the critical N concentration (CNC) (Reyenga et al., 1999). To capture CO_2 effects on RUE, and interactions with temperature, the model scales RUE using the ratio of the light-limited photosynthetic response at the e[CO_2] to that at 350 µmol mol^{-1}. The responses of TE and leaf CNC to e[CO_2] are assumed to be linear with changes of +37 and -7%, respectively, for a doubling of [CO_2] to 700 µmol mol^{-1} (O'Leary et al., 2015). Actual transpiration is indirectly reduced under e[CO_2] through the gain in TE.

The experimental site was defined by its soil type (Table 2), and daily weather conditions (solar radiation, maximum and minimum temperature and rainfall, Table 1) which were mostly taken from

the continuously logging meteorological station located in the AGFACE site. When on-site measurements were unavailable, daily weather observations were taken from the nearby meteorological station # 079028, extracted from the SILO climate data archive (Jeffrey et al., 2001). Additional experimental site related characteristics are found in O'Leary et al. (2015). Cultivar-specific parameters used to define wheat growth in the APSIM simulation are listed in Table 3. No change to the species parameters and the module source code was made.

Table 2 Soil profile data describing the bulk density (BD), air dry, crop lower limit (LL), drained upper limit (DUL), saturation (SAT), organic carbon (OC), and the water availability coefficients of KL and XF.

Depth (cm)	BD ($g\ cc^{-1}$)	Air dry ($mm\ mm^{-1}$)	LL ($mm\ mm^{-1}$)	DUL ($mm\ mm^{-1}$)	SAT ($mm\ mm^{-1}$)	OC (%)	APSIM KL (day^{-1})	APSIM XF (0-1)
0-10	1.14	0.15	0.20	0.39	0.46	1.248	0.06	1.00
10-20	1.30	0.18	0.23	0.40	0.47	0.708	0.06	1.00
20-40	1.37	0.25	0.27	0.42	0.48	0.354	0.04	1.00
40-60	1.40	0.27	0.30	0.43	0.47	0.177	0.02	0.80
60-80	1.40	0.28	0.33	0.45	0.47	0.089	0.02	0.80
80-100	1.40	0.30	0.35	0.45	0.47	0.044	0.02	0.60
100-120	1.40	0.32	0.36	0.45	0.47	0.022	0.02	0.60
120-140	1.40	0.33	0.37	0.45	0.47	0.011	0.02	0.20
140-160	1.40	0.34	0.37	0.45	0.47	0.011	0.02	0.20
160-180	1.40	0.34	0.37	0.45	0.47	0.011	0.02	0.20

Table 3 Key parameters used to define cultivar-specific settings in APSIM-Wheat. [a] The thermal time from emergence until end of juvenile is influenced by the number of cumulative vernalising days during the period. [b] The phase from end of juvenile until floral initiation is influenced by the photoperiod sensitivity.

Cultivar parameters	Yitpi	Scout
Sensitivity to vernalisation[a]	1.5	1.8
Sensitivity to photoperiod[b]	3.0	3.5
Kernel number per stem weight at the beginning of grain filling (g)	25	25
Potential daily grain filling rate ($g\ grain^{-1}\ day^{-1}$)	0.002	0.002
Grain growth rate from flowering to grain filling ($g\ grain^{-1}\ day^{-1}$)	0.001	0.001
Maximum grain size (g)	0.041	0.041
Thermal time from start grain filling to maturity (˚C days)	545	550
Thermal time from floral initiation to flowing (˚C days)	555	555
Thermal time needed in anthesis phase (˚C days)	120	120

Thermal time needed from sowing to end of juvenile (˚C days)	400	400
Maximum root depth (cm)	180	180

Statistical analyses

Analysis of variance for the effects of CO_2, cultivar, and their interaction on total transpiration and nutrient uptake into aboveground biomass were performed using split plot module (CO_2 × cultivar treatment structure) separately for each growth stage with "agricolae" package (de Mendiburu and de Mendiburu, 2016) in R software (v 3.0.3) (R Core Team, 2000). No cultivar or cultivar by CO_2 interaction effect was found to be statistically significant, therefore, we only report the CO_2-driven effects.

Simple regression analyses with R software were used to assess the relationship between transpiration and nutrient uptake under ambient and elevated [CO_2]. Significance of differences between the fitted coefficients (slopes and intercepts) of the regression lines under ambient and elevated [CO_2] were evaluated using 95% confidence intervals calculated from the standard error (Diem and Seldrup, 1982). Similar to the analysis of variance, we did not find a significant difference between the two cultivars and therefore (for the regression analysis only) data for both cultivars were pooled, increasing the degrees of freedom (from 14 to 30). Graphs were produced using "ggplot2" package (Wickham, 2009) of R software.

Results

FACE experiment - estimated g_s and transpiration

Lower mean g_s was estimated for plants grown under e[CO_2] than under a[CO_2] conditions (Fig. 1), and the difference was smaller earlier than later in the growing season. Elevated [CO_2] decreased g_s by a mean of 27% across all estimated values over the study period. This translated into a 31% (15 mm) lower total transpiration under e[CO_2] at DC34, 21% (45 mm) at DC65, and 24% (56 mm) at DC69 (Fig. 1). Total transpiration estimates, from germination until DC69, were 233.1 (±11.1) and 177.4 (±9.3) mm under ambient and elevated [CO_2], respectively.

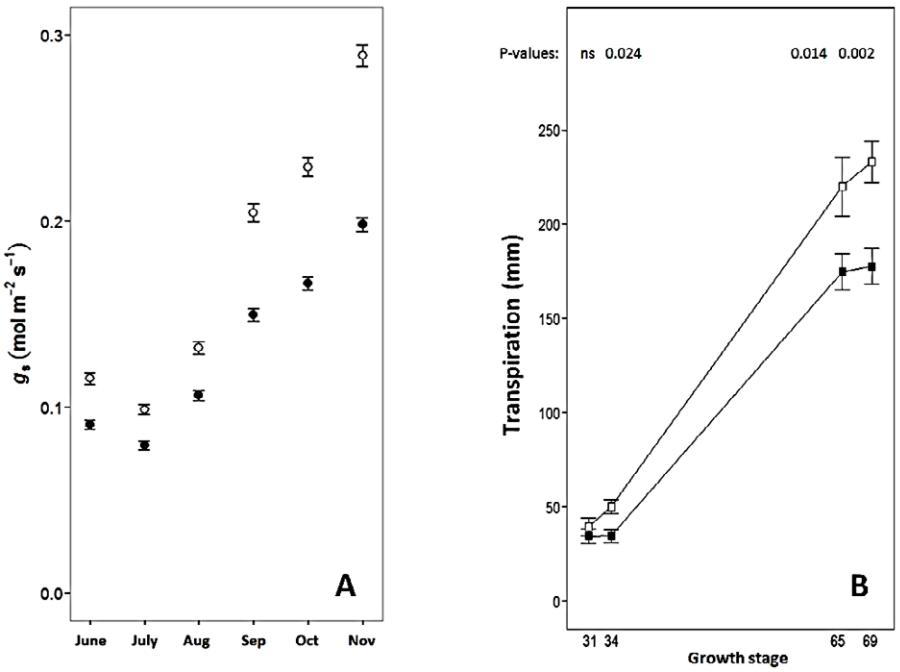

Fig. 1 Monthly-averaged stomatal conductance (g_s) (mol m^{-2} s^{-1}) under a[CO_2] (open circles) and e[CO_2] (filled circles) estimated using a Jarvis-type model developed at this site (Houshmandfar et al., 2015a) (A). Cumulative transpiration estimates (±standard error) from germination until DC31, DC34, DC65 and DC69 in wheat grown under FACE conditions (a[CO_2]: open squares; e[CO_2]: filled squares) (B). Each data point represents a mean of 4 replicates × 2 cultivars (±standard error). Transpiration was estimated as g_s multiplied with VPD and LAI.

FACE experiment - nutrient uptake

Total nutrient uptake into aboveground biomass was higher in plants grown under e[CO_2] than under a[CO_2] when measured at later stages of development, i.e. DC65 and DC69 (albeit not always significant) (Fig. 2). At DC31 and DC34 (early growth stages), this difference was not apparent (Fig. 2).

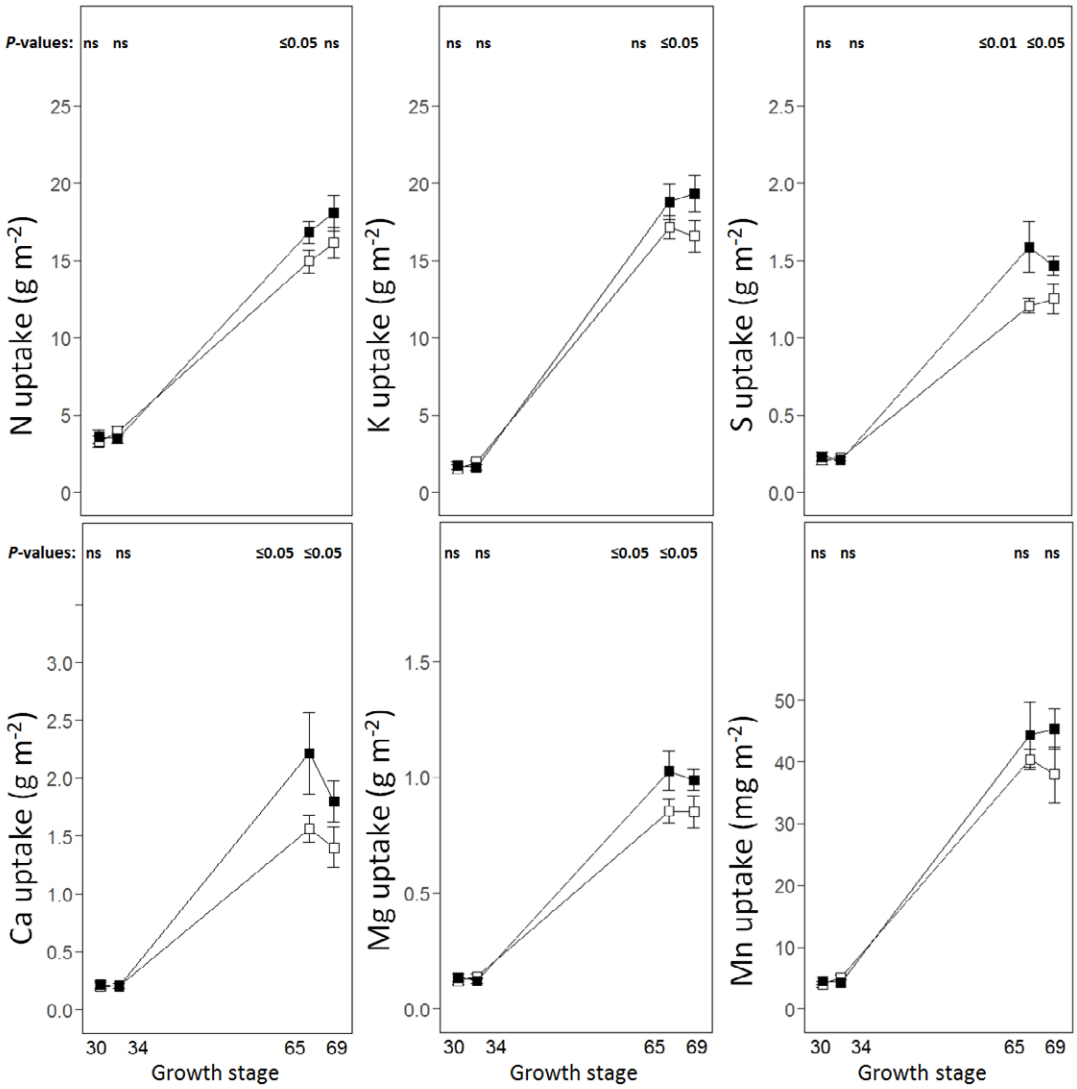

Fig. 2 Nutrient uptake into aboveground biomass from germination until DC31, DC34, DC65 and DC69 in wheat grown under FACE conditions (a[CO_2]: open squares; e[CO_2]: filled squares). Each data point represents a mean of 4 replicates plot × 2 cultivars (±standard error).

FACE experiment - relationship between transpiration and nutrient uptake

Transpiration (mm) was positively correlated with uptake m^{-2} of N, K, S, Ca, Mg, and Mn under both ambient and elevated CO_2 conditions (Fig. 3). The coefficient of determinations (R^2) were 0.96 and 0.93 for N, 0.90 and 0.94 for K, 0.96 and 0.78 for S, 0.89 and 0.64 for Ca, 0.95 and 0.83 for Mg, and 0.89 and 0.84 for Mn, under ambient and elevated CO_2, respectively (Fig. 3). The slopes of the relationships were consistently steeper under e[CO_2] than under a[CO_2] and this difference was

significant for all nutrients (*P*d0.05). Nutrient uptake per unit water transpired was on average 50% higher under e[CO_2] than a[CO_2].

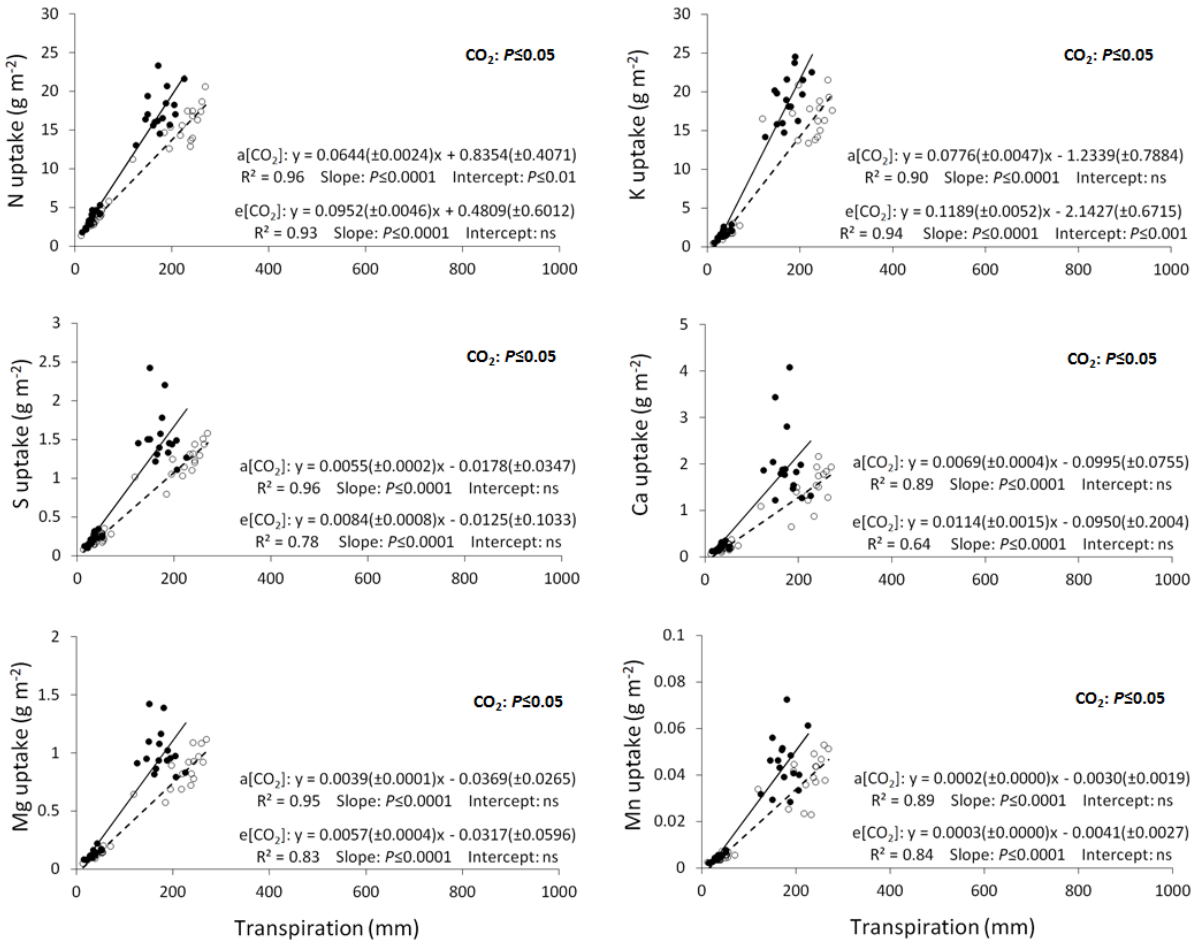

Fig. 3 Relationships between transpiration (mm) and uptake for nitrogen (N), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), and manganese (Mn) under a[CO_2] (dashed lines and open circles) and e[CO_2] (bold lines and filled circles). ±: standard error for the estimated parameters (slope and intercept of the linear relationship). ns: not significant. Each data point represents a replicate subplot (n=32).

APSIM study – simulated transpiration and N uptake

Differences between simulated values for transpiration under ambient and elevated [CO_2] were small in any of the studied years from 2007 to 2016 (Table 4). Total N uptake into aboveground biomass from germination until approximately DC69 was 20% higher in 2007, 24% in 2008, 21% in 2009,

17% in 2010, 18% in 2011, 21% in 2012, 20% in 2013, 16% in 2014, 13% in 2015, and 17% in 2016 in plants grown under e[CO_2] than under a[CO_2], respectively (Table 4).

Table 4 APSIM-simulated values of transpiration (mm) and N uptake (g m^{-2}) from germination until approximately DC 69. ±: standard error for the difference between simulated values for the two studied cultivars (cv. Yitpi and cv. Scout).

Simulated year	Transpiration (mm)		Nitrogen uptake (g m^{-2})	
	a[CO_2]	e[CO_2]	a[CO_2]	e[CO_2]
2007	119.1±0.9	121.5±0.7	11.2±0.0	13.5±0.1
2008	113.0±2.1	117.0±1.7	10.3±0.4	12.8±0.4
2009	144.6±4.8	148.4±3.0	14.4±0.7	17.5±0.6
2010	115.2±2.0	118.2±0.9	10.7±0.6	12.6±1.2
2011	126.5±1.9	128.7±1.4	13.2±0.1	15.6±0.2
2012	117.9±2.7	122.2±2.3	11.2±0.6	13.6±0.7
2013	139.6±9.0	145.0±6.9	13.0±0.9	15.7±0.8
2014	91.3±0.4	92.5±0.3	9.1±0.1	10.6±0.2
2015	101.0±0.7	102.1±0.6	11.1±0.1	12.6±0.2
2016	139.8±7.4	142.1±5.5	13.5±1.1	15.8±1.0

APSIM study - relationship between transpiration and N uptake

The simulated transpiration and N uptake were positively correlated under ambient and elevated CO_2 conditions (Fig. 4). The slope of the relationship under e[CO_2] was steeper than under a[CO_2]: N uptake per unit water transpired was approximately 15% higher under e[CO_2] than a[CO_2] (*P*d0.05) (Fig. 4).

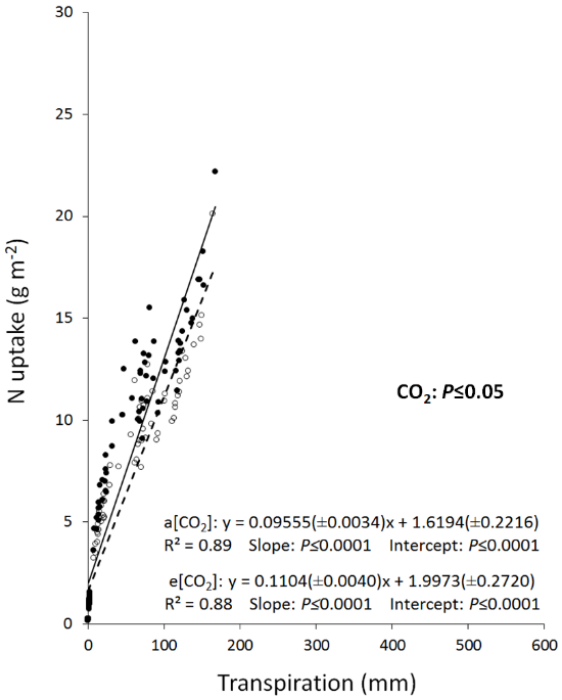

Fig. 4 Relationship between simulated transpiration (mm) and nitrogen (N) uptake (expressed as g m^{-2} ground area) under a[CO_2] (dashed line and open circles) and e[CO_2] (bold line and filled circles), simulated monthly average values using APSIM modeling frame work (2007- 2016). ±: standard error for the estimated parameters (slope and intercept of the linear relationship). Difference between for the slopes of the relationships under ambient and elevated CO_2 was significant at *P*d0.05. The difference was not significant for intercepts (n=100).

Discussion

The Jarvis-type empirical model has been widely used for estimating g_s in transpiration studies (Jarvis, 1976; Stewart, 1988; Wright et al., 2012). We employed the Jarvis model to allow estimation from repeated measurement days to the whole growing season. The Jarvis model was parameterised previously for wheat under ambient and elevated CO_2 growing conditions at the AGFACE site, employing the same set of cultivars (cv. Yitpi and cv. Scout) (Houshmandfar et al., 2015a). Stomatal conductance was on average 27% lower under elevated than under ambient CO_2. This is in agreement with CO_2-driven decreases of between 18 to 30% reported by earlier FACE trials measuring *in situ* g_s in wheat, depending on intraspecific variations and environmental conditions under which plants were

grown or g_s measurements were collected (Garcia et al., 1998; Houshmandfar et al., 2016; McGrath and Lobell, 2013; Wall et al., 2000).

The difference between estimated g_s under ambient and elevated CO_2 was smaller earlier than later in the growing season. It has been shown in previous papers that wheat grown under e[CO_2] can have increased stomatal sensitivity to environmental factors (Bunce, 2004; Houshmandfar et al., 2015a), in particular response functions to temperature and VPD in a Jarvis model changed (Houshmandfar et al. 2015a). Temperature and VPD are likely to have higher values during the late than early growing season, which can explain the increasing difference in g_s.

The simple approach used to upscale from leaf-level g_s to an estimate of canopy transpiration (multiplying g_s with VPD and LAI; Wright et al 2012) ignores within-canopy differences in stomatal responses and micro-environment as well as potential boundary layer effects, and is therefore likely to overestimate transpiration. We have no independent transpiration to further evaluate our estimates directly, but a study on soybean in FACE showed that stand-level evapotranspiration scaled well with g_s measured on upper canopy leaves, implying strong coupling between canopy and atmosphere and consequently high boundary layer conductance (Bernacchi et al., 2007). Since soybean canopies are denser than dryland wheat, and the AGFACE site sees average wind speeds, it is a reasonable assumption that the wheat canopy in our study was at least as well coupled to the atmosphere and strong bias by boundary layer effects (as e.g. in dense forest canopies; Kauwe et al. (2013)), is unlikely. Calculating TE for biomass production with our transpiration estimates resulted in about 4 g dry aboveground biomass L^{-1} transpiration (using anthesis biomass data for ambient CO_2 reported in Houshmandfar et al. (2016)). Given that 2013 was a very high rainfall season at the site, this compares well to corresponding values between 4-6 g L^{-1} determined at anthesis for a number of Australian wheat cultivars under non-limiting water conditions (Fig. 4 in Fletcher and Chenu (2015)).

Uncertainties in upscaling aside, our focus was on the comparison between ambient and elevated CO_2 grown wheat, and the estimated 23% decrease for stand-level transpiration under e[CO_2] compared to a[CO_2] is in good agreement with reports in the literature that suggest up to 22% lower stand-level transpiration in wheat grown under FACE, depending on growing conditions (Hunsaker et al., 1996; Hunsaker et al., 2000; Leakey et al., 2009; Tausz-Posch et al., 2012). The soybean FACE study cited above also showed that differences in transpiration between ambient and elevated CO_2 were governed by differences in g_s, but not by changes in LAI or canopy structure (Bernacchi et al., 2007). Increasing [CO_2] has been shown to cause partial stomatal closure, which reduces transpiration per unit of leaf (e.g. Wall et al. (2000)), and in the above mentioned reports, per unit ground area. Conversely, CO_2

stimulation of growth can result in larger plants with higher LAI, which would tend to increase stand-level transpiration (Kimball et al., 1995; Rosenberg et al., 1990), so that the actual change depends mainly on the relative magnitude of effects on leaf level transpiration and LAI. Some studies found that the leaf area effect may dominate, especially in dry soils where e[CO_2] grown wheat could produce greater LAI (Samarakoon et al., 1995).

APSIM simulated whole-season water use and wheat yield of three previous seasons at AGFACE well, but results for intermediate growth stages, as in this study, were less accurate (O'Leary et al., 2015). Discrepancies of transpiration assessments by different methods warrant further investigation, but for the purpose of this study the differences between ambient and elevated CO_2 were more important. In contrast to the transpiration estimates extrapolated from leaf level measurements, our APSIM simulations suggested no considerable differences between the transpiration rates in plants grown under ambient and elevated [CO_2]. This result is in line with O'Leary et al. (2015) who tested various modeling frameworks including APSIM-Wheat with AGFACE data under various environmental conditions (e.g. differential time of sowing and watering regimes) in 2007, 2008, and 2009 growing seasons. Their results suggested that APSIM overestimated stimulation of LAI by e[CO_2], especially during early growing stages, with the result that although measured water use during the growing season was reduced by e[CO_2], the simulation by APSIM-Wheat showed no such reduction (O'Leary et al., 2015). For the purpose of this paper the important question was whether the simulation by APSIM indicates a change for the transpiration-nutrient uptake relationship. An overestimation of transpiration under e[CO_2] would only give false negative, but not false positive results, that is, the disparity between the relationships under ambient and elevated [CO_2] may be underestimated.

Total nutrient uptake into aboveground biomass was higher in plants grown under e[CO_2] than under a[CO_2] (albeit not always significant) in the AGFACE experiment. This result was consistent with APSIM output where higher N uptake into aboveground biomass was simulated under elevated than under ambient [CO_2] in all the simulated years with an acceptable absolute error for 2013. Tissue concentration of nutrients was mostly, albeit not always significantly, lower in plants grown under e[CO_2] (e.g. meta-analyses by Loladze (2002), Loladze (2014), and McGrath and Lobell (2013)) but plants take up more nutrients on an area basis because of increased biomass production (Adam et al., 2000; Brooks et al., 2000; O'Leary et al., 2015; Wechsung et al., 1995). In this particular growing season (2013) nutrient concentrations, for the most part, were not significantly lower (data not shown), but over multiple years the same tendency towards lower concentrations was observed at this

site (e.g. Walker et al. (2016), Buchner et al. (2015), Fernando et al. (2014), and Panozzo et al. (2014)).

The nutrient uptake per unit water transpired was higher under e[CO_2] than under a[CO_2]. Although we have not measured xylem nutrient concentrations in this study directly, our results indicate that on average nutrient concentrations in the transpiration stream would be greater under e[CO_2], assuming nutrients are transported in the transpiration stream. This is in apparent contrast with our earlier work reporting e[CO_2] decreases both transpiration flow and concentrations of nutrients in the xylem sap of wheat (Houshmandfar et al., 2015b). In that study, Ca and Mg concentrations in the xylem were evaluated at anthesis stage (DC 60 and DC 69). That result was also supported by Li et al. (2016), whose measurements taken ten days after DC 65 also demonstrated that xylem sap concentrations of Ca, Mg, and K were lower in plants grown under e[CO_2] than under a[CO_2]. It is important to note that those results may be specific to the phenological stage when the measurements were done - around or just after anthesis, which corresponds to end of the phenological period investigated in this present paper. Our results here imply that such lower nutrient concentrations in the xylem stream at the later stage have been offset during earlier growth, possibly up to anthesis, to arrive at an overall greater uptake per unit transpiration.

There are a number of possible mechanisms to modify the effect of e[CO_2] on nutrient uptake during phenological development. It is of vital importance for plant adaptation to respond flexibly to changes in environmental conditions (Forde and Lorenzo, 2001; Robinson, 1994). Although reports about root growth under e[CO_2] are highly variable in details (e.g. Burkart et al. (2004) and Pacholski et al. (2015)), root biomass is generally stimulated by e[CO_2] in line with shoot biomass. Earlier in the season, this may allow good access to soil nutrients and high uptake rates. Nutrient concentrations in soil and plant tissues act as signals continuously modifying lateral and seminal root formation (Lopez-Bucio et al., 2003; Morgan and Connolly, 2013). There are some suggestions that e[CO_2] stimulates lateral root growth preferentially over the elongation of primary roots, leading to highly branched, shallower root system architecture (Burkart et al. (2004) and Pacholski et al. (2015)). Such changes can make the root systems less efficient in soil exploration and thus nutrient uptake (Berntson, 1994; Pritchard and Rogers, 2000; Taub and Wang, 2008), and this may only become manifest towards the end of the season when exploration of deeper soil layers becomes more important.

Transpiration and nutrient uptake were strongly correlated. This supports the literature, e.g. as reviewed in Taub and Wang (2008), suggesting that limitations to the transpiration-driven mass flow rate of nutrients due to decreased g_s is a contributing factor to the a decrease in nutrient concentration

under e[CO_2]. If this reduction resulting from decreased transpirational flow was the only mechanism, the nutrient uptake per unit water transpired should not have been different under ambient and elevated CO_2 conditions, and in the graphs (Fig. 3 and 4) measurements from e[CO_2] and a[CO_2] grown plants should be part of the same relationship. Because in our data the nutrient uptake per unit water transpired was consistently higher under e[CO_2] than a[CO_2], we conclude that mechanisms other than mass flow of nutrients are involved. On average, these mechanisms increase nutrient uptake into the transpiration stream, and may therefore to some extent mitigate the decrease in transpiration. Such mechanisms could be associated with changes in root system architecture or function (Berntson, 1994; Pritchard and Rogers, 2000; Taub and Wang, 2008).

Conclusions

We used a FACE experiment and the APSIM modelling framework with wheat to investigate potential changes of the relationship between nutrient uptake and transpiration rates under e[CO_2] to test whether limited transpiration-driven mass flow of nutrients can explain nutrient decline under e[CO_2]. Our results suggest that transpiration and nutrient uptake of N, K, S, Ca, Mg and Mn are correlated under e[CO_2], but that on average across the active growing season nutrient uptake per unit water transpired is higher in plants grown under e[CO_2] than a[CO_2]. We therefore concluded that limited transpiration-driven mass flow of nutrients contributes to decreases in nutrient concentrations under e[CO_2], but cannot solely account for the overall, more complex relationship between plant nutrition and e[CO_2].

Acknowledgements

The AGFACE facility is jointly run by the University of Melbourne and the Victorian Department of Economic Development, Jobs, Transport and Resources (Agriculture Victoria) and received funding support from the Australian Commonwealth Department of Agriculture, the Grain Research and Development Corporation (GRDC), and the Australian Research Council (ARC). We gratefully acknowledge the contribution of the AGFACE field team lead by Russel Argall for agronomic trial management, Mahabubur Mollah for operating the CO_2 enrichment technology, Najib Ahmadi for laboratory management, and Chao Chen for her help on the APSIM simulations. AH received a Melbourne International Research Scholarship.

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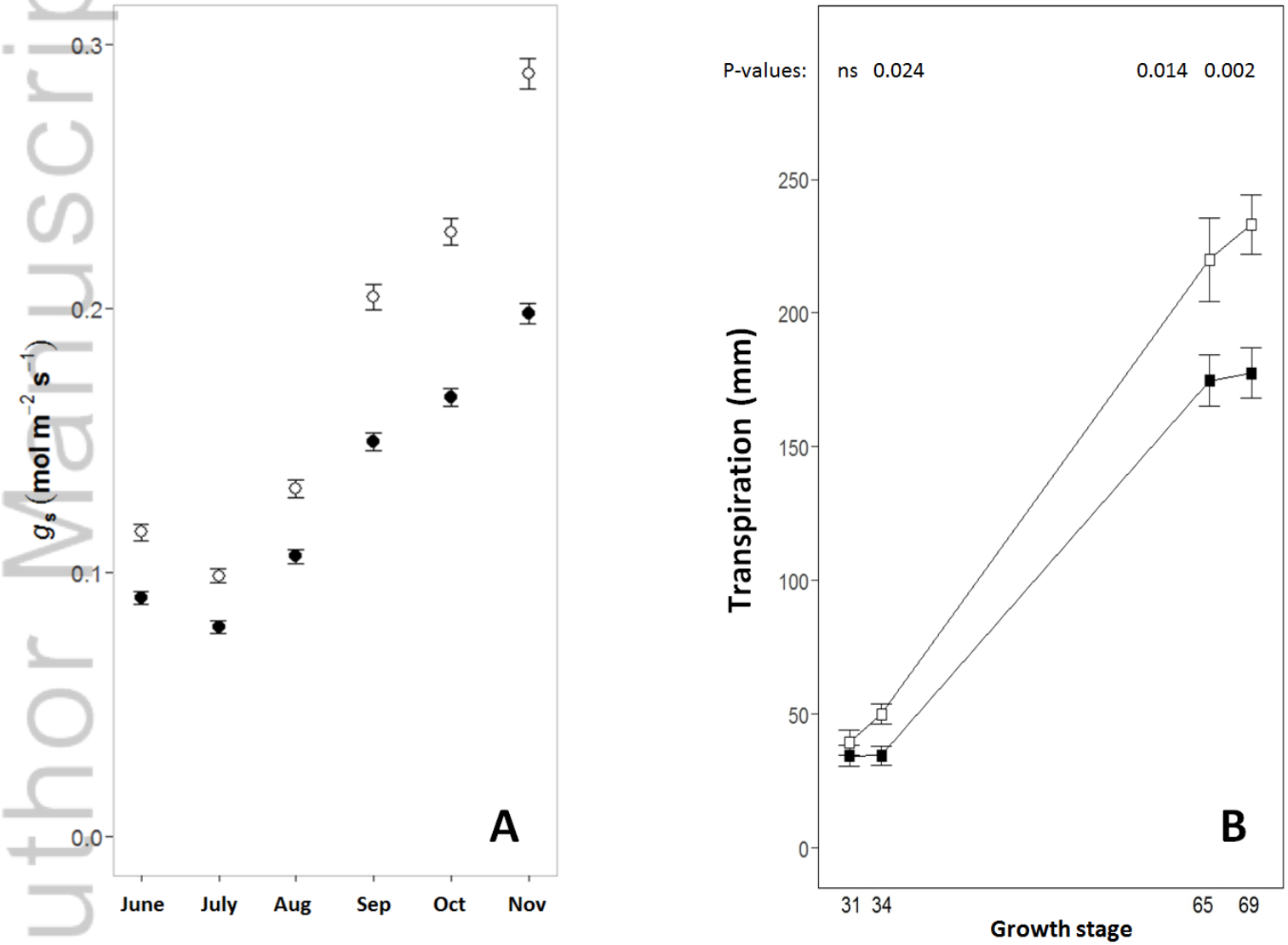

Fig 1.tif

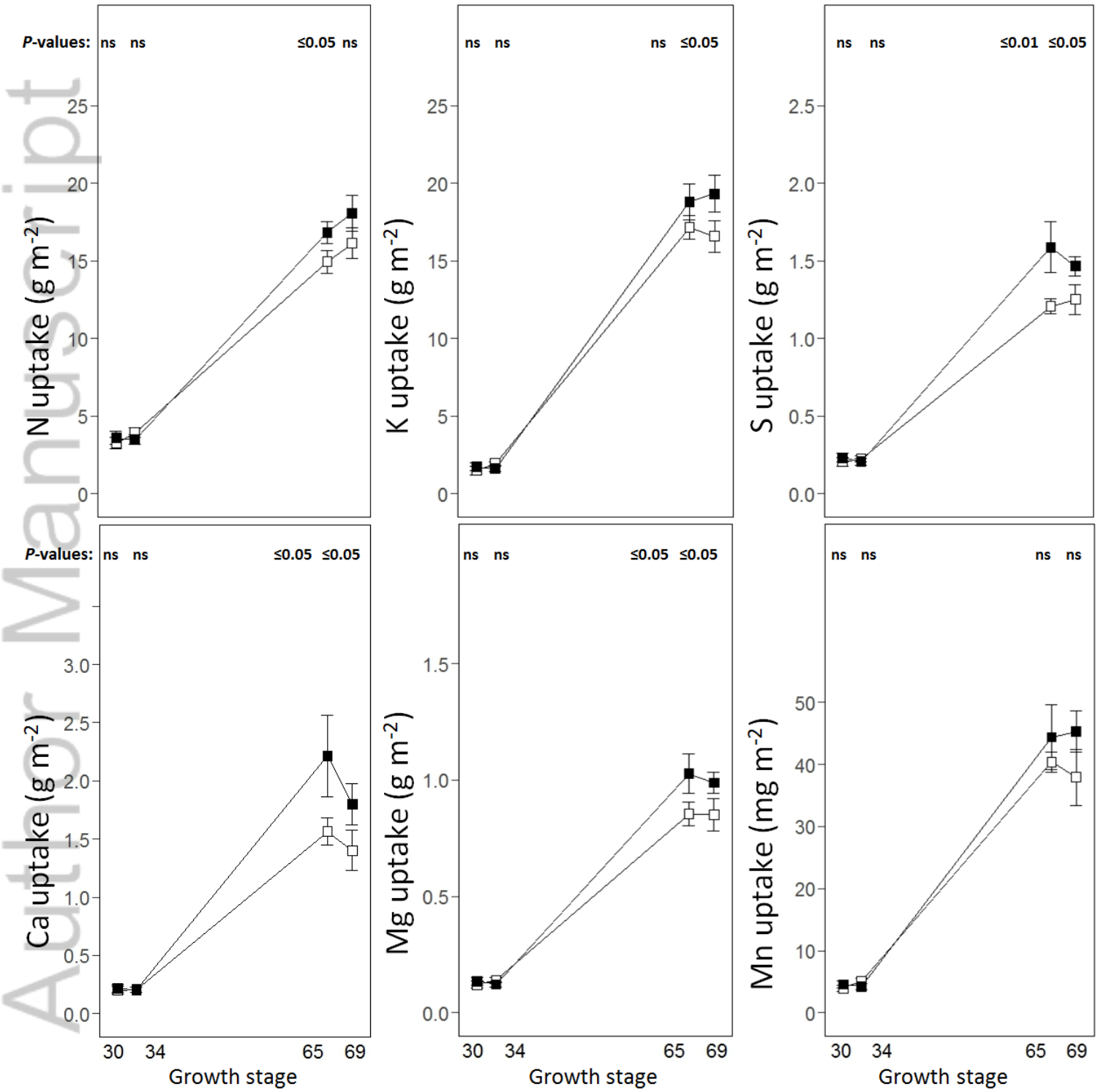

Fig 2.tif

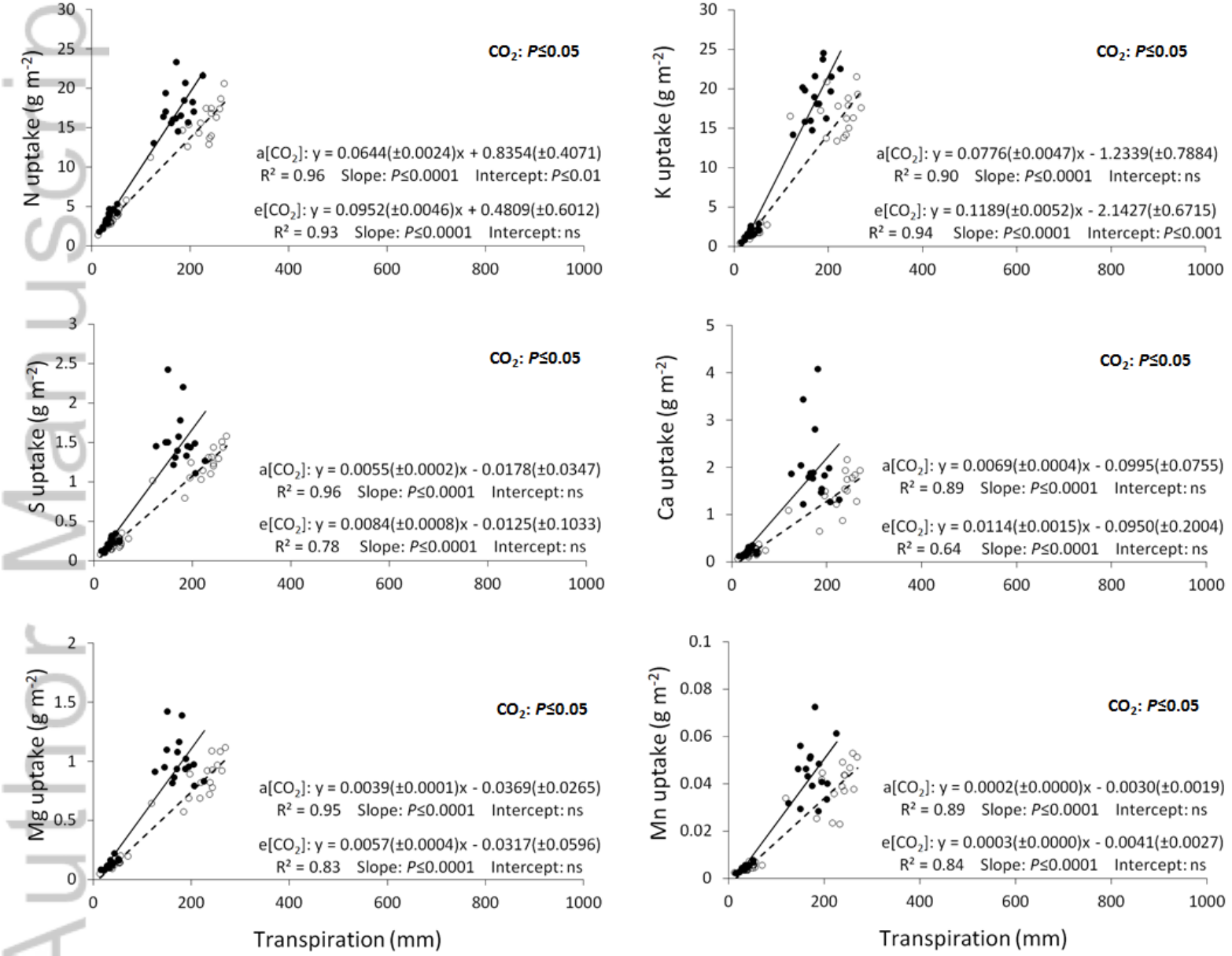

Fig. 3.tif

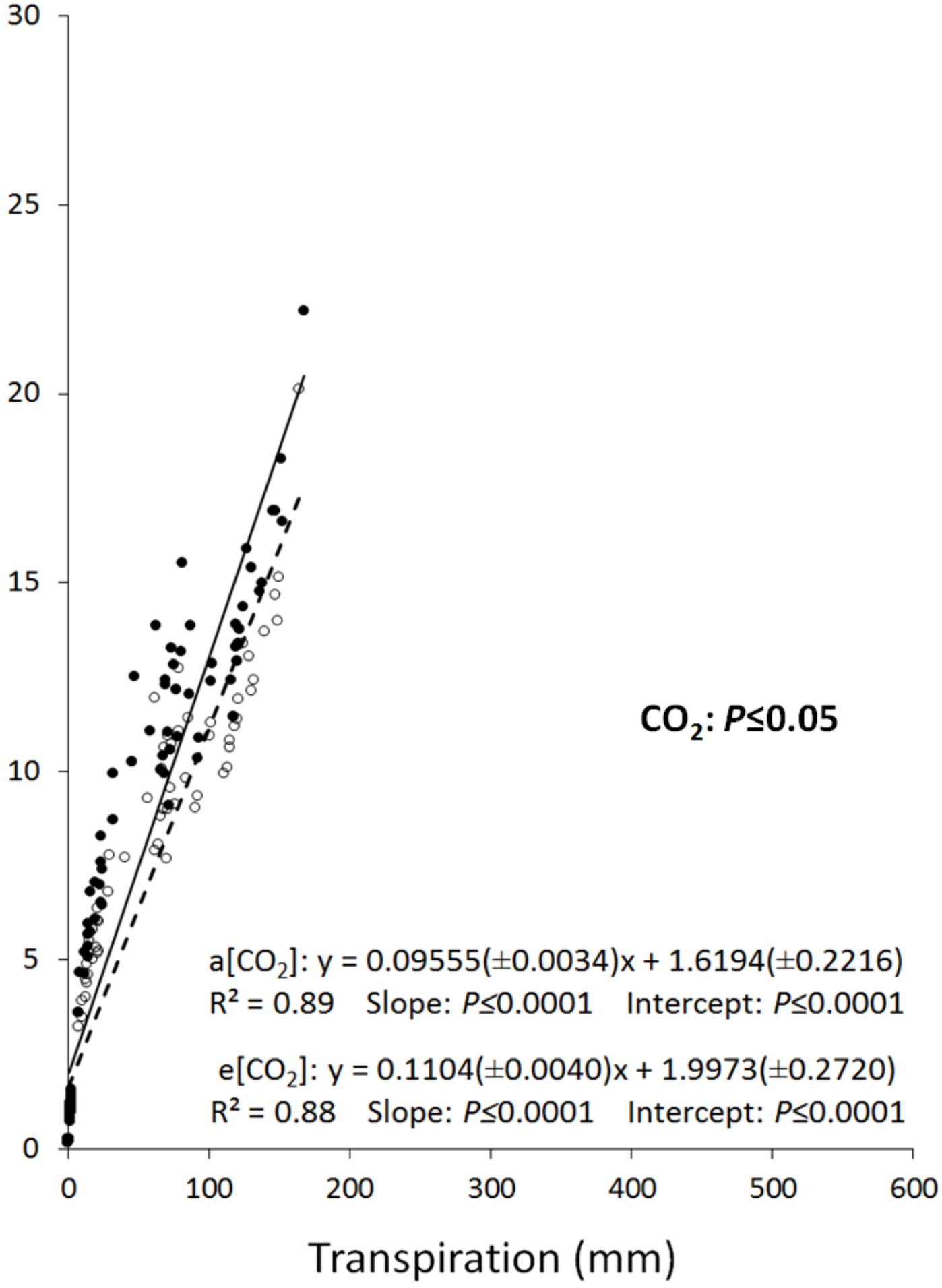

Fig. 4.tif

	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Annual average minimum air temperature (˚C)	8.0	6.7	7.6	7.3	7.4	6.8	7.5	7.3	7.3	8.0
Annual average maximum air temperature (˚C)	22.4	21.4	22.2	20.8	21.1	21.5	21.7	22.5	22.3	21.6
Annual rainfall (mm)	428.9	335.4	399.8	559.4	507.0	287.2	378.0	221.8	269.8	550.4
Rainfall during the growing season (mm)	164.0	166.2	266.6	268.6	215.4	174.8	320.8	115.0	125.2	326.4
Annual average solar radiation (MJ m^{-2})	16.3	15.6	16.3	18.2	15.3	16.0	15.8	16.2	16.1	15.8

Depth (cm)	BD (g cc^{-1})	Air dry (mm mm^{-1})	LL (mm mm^{-1})	DUL (mm mm^{-1})	SAT (mm mm^{-1})	OC (%)	APSIM KL (day^{-1})	APSIM XF (0-1)
0-10	1.14	0.15	0.20	0.39	0.46	1.248	0.06	1.00
10-20	1.30	0.18	0.23	0.40	0.47	0.708	0.06	1.00
20-40	1.37	0.25	0.27	0.42	0.48	0.354	0.04	1.00
40-60	1.40	0.27	0.30	0.43	0.47	0.177	0.02	0.80
60-80	1.40	0.28	0.33	0.45	0.47	0.089	0.02	0.80
80-100	1.40	0.30	0.35	0.45	0.47	0.044	0.02	0.60
100-120	1.40	0.32	0.36	0.45	0.47	0.022	0.02	0.60
120-140	1.40	0.33	0.37	0.45	0.47	0.011	0.02	0.20
140-160	1.40	0.34	0.37	0.45	0.47	0.011	0.02	0.20
160-180	1.40	0.34	0.37	0.45	0.47	0.011	0.02	0.20

Cultivar parameters	**Yitpi**	**Scout**
Sensitivity to vernalisation[a]	1.5	1.8
Sensitivity to photoperiod[b]	3.0	3.5
Kernel number per stem weight at the beginning of grain filling (g)	25	25
Potential daily grain filling rate (g grain^{-1} day^{-1})	0.002	0.002
Grain growth rate from flowering to grain filling (g grain^{-1} day^{-1})	0.001	0.001
Maximum grain size (g)	0.041	0.041
Thermal time from start grain filling to maturity (˚C days)	545	550
Thermal time from floral initiation to flowing (˚C days)	555	555
Thermal time needed in anthesis phase (˚C days)	120	120
Thermal time needed from sowing to end of juvenile (˚C days)	400	400
Maximum root depth (cm)	180	180

Simulated year	Transpiration (mm)		N uptake (g m^{-2})	
	a[CO_2]	e[CO_2]	a[CO_2]	e[CO_2]
2007	119.1±0.9	121.5±0.7	11.2±0.0	13.5±0.1
2008	113.0±2.1	117.0±1.7	10.3±0.4	12.8±0.4
2009	144.6±4.8	148.4±3.0	14.4±0.7	17.5±0.6
2010	115.2±2.0	118.2±0.9	10.7±0.6	12.6±1.2
2011	126.5±1.9	128.7±1.4	13.2±0.1	15.6±0.2
2012	117.9±2.7	122.2±2.3	11.2±0.6	13.6±0.7
2013	139.6±9.0	145.0±6.9	13.0±0.9	15.7±0.8
2014	91.3±0.4	92.5±0.3	9.1±0.1	10.6±0.2
2015	101.0±0.7	102.1±0.6	11.1±0.1	12.6±0.2
2016	139.8±7.4	142.1±5.5	13.5±1.1	15.8±1.0