Acclimatory responses of stomatal conductance and photosynthesis to elevated CO_2 and temperature in wheat crops grown at varying levels of N supply, in a Mediterranean environment

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Abstract

The short and long-term responses of flag leaf stomatal conductance (g_s) and rate of photosynthesis (An) to elevated CO_2 (757 µmol mol^{-1}), 4 ºC warmer temperatures and N supply were investigated in spring wheat (*Triticum aestivum* L. cv. Alcalá) crops grown in two seasons in field conditions under temperature gradient tunnels, in a Mediterranean environment. Plants grown at elevated CO_2 had lower g_s and An measured at 700 µmol mol^{-1} CO_2 than ambient CO_2-grown plants, indicating acclimatory responses to elevated CO_2. N supply, which was lower in 2003 than 2002, reduced this acclimation in both years. Warmer temperatures at high nitrogen supply also decreased An acclimation. Acclimatory responses of An and g_s were highly correlated. The analysis of the g_s – An relationship indicated that both parameters are probably linked and respond in parallel to elevated CO_2 at ambient temperatures, but non-parallel responses were observed at above-ambient temperatures.

Abbreviations: An, net photosynthesis rate; Ca, atmospheric CO_2 concentration; Ci, CO_2 concentration in the intercellular air space of leaves; g_s, leaf stomatal conductance; Hr, air relative humidity; PFD, photosynthetic flux density; Rubisco, Ribulose, 1-5, bisphosphate carboxylase oxygenase.

Key-words: acclimation, elevated CO_2, elevated temperature, gas exchange, nitrogen, photosynthetic capacity, stomatal conductance.

Introduction

Although carbon assimilation is stimulated by CO_2 levels above that in the current atmosphere, plants growing in elevated CO_2 often show an acclimatory down-regulation of the photosynthetic capacity of leaves [1-5], as well as a reduction in stomatal conductance [1, 6-9] and hence transpiration rate [10,11].

In spring wheat growing in field conditions, increase of CO_2 in the air from 370 to 550 μmol mol^{-1} using FACE (free air CO_2 enrichment), enhanced the rate of leaf photosynthesis (An) by 28% and reduced the stomatal conductance (g_s) by 36% [12, 13]. In another study conducted in winter wheat, an increase of the ambient CO_2 from 350 to 700 μmol mol^{-1} using open topped chambers reduced g_s and An measured at 700 μmol mol^{-1}, indicating acclimation [14]. Whether stomata acclimate independently to elevated CO_2 or as a consequence of the mesophyll photosynthetic acclimation is still unclear [15]. The constancy of the ratio of CO_2 concentration in the intercellular air space to that around the leaf (Ci/Ca) when growth CO_2 varies [1], is indicative of a close coupling of An and g_s. However, studies on stomatal sensitivity to CO_2 changes [7], and on the relationship between An and g_s in water-stressed plants [8], suggest that stomatal acclimation is unrelated to the degree of photosynthetic acclimation.

The responses of An and g_s to elevated CO_2 appear to be very sensitive to nitrogen supply and environmental factors such as temperature. Downward photosynthetic acclimation to elevated CO_2 seems to be more pronounced in nitrogen deficient plants [2, 16, 17], although other studies indicated no acclimation [5, 18] or even the opposite, i.e., greater acclimation at high compared to low N supply [19]. Higher leaf temperatures can stimulate the rate of photosynthesis [20] and the short-term response of photosynthesis to elevated CO_2 in cereals [21]. Also, g_s was increased as leaf temperatures increased from 15 to 35 °C in eight herbaceous species [20], but increased less at elevated than at ambient growth CO_2 [22]. Since concomitant increases in temperature and CO_2 level in the atmosphere will likely occur, as a consequence of global climatic change, it is of great interest to evaluate the short (minutes) and long-term (months) responses of g_s and An to elevated CO_2 and warming (ambient + 4°C), in a wheat crop growing in field conditions.

In this paper, long-term (acclimatory) and short-term (direct) responses of g_s and rate of photosynthesis to elevated CO_2 were examined for two yeas in spring wheat crops growing in temperature gradient tunnels set at ambient and 4 °C warmer, and at two different levels of nitrogen supply each year. The following questions were addressed: is the response of leaf g_s and An to elevated CO_2 modified by temperature and nitrogen? And do g_s and leaf photosynthesis acclimate to elevated CO_2 in parallel or independently?

Materials and Methods

Site and experimental setup

The experimental site, a clay sand soil, was located at the IRNASA Muñovela Farm at Salamanca (41° N, 800 m.a.s.l.), Spain. Climate in Salamanca corresponds to a Mediterranean type; long term average of the minimum temperatures of the coldest month (January) is 0.0 °C and of the maximum temperatures of the warmest month (July) is 27.2 °C. Mean annual rainfall is 506 mm.

Spring wheat (*Triticum aestivum* L. cv. Alcalá) was sown at a rate of 180 kg ha^{-1} and 0.13 m between rows, on 30 January 2002 and 11 February 2003. In 2002, a mixture of N (52 kg ha^{-1} as ammonium nitrate), P (60 kg ha^{-1}) and K (60 kg ha^{-1}) was applied to the soil before sowing. In 2003, the crop was sown after turnip and no fertilizer was applied before sowing. The crop was watered weekly through a drip irrigation system and provided 198 mm between February and June, which is the average rainfall in the area during the period of the experiment. Weeds were controlled chemically.

Two temperature gradient tunnels of 9.6 m long, 2.2 m wide and 1.7 m high at the ridge were placed after the emergence of the crop on 2 and 29 April in 2002 and 2003, respectively, on different plots every year. The tunnels were adapted from [23] and have been described in detail elsewhere [24]. One tunnel was kept at ambient (370 µmol mol^{-1}) and the other at elevated (700 µmol mol^{-1}) CO_2 concentration during light hours. Since there appears to be no, or only small positive direct effects of growth CO_2 on leaf dark respiration [25, 26], lack of CO_2 enrichment during the night is probably irrelevant. Temperature in the two extreme modules of the tunnels was set at ambient (T) and 4 °C warmer (T+4). Additionally, two levels of nitrogen supply were established by adding 70 kg ha^{-1} to one longitudinal half of the tunnels on 2 and 30 April of 2002 and 2003, respectively, giving a total of 52 and 122 kg ha^{-1} N in 2002, and none and 70 kg ha^{-1} N in 2003. Due to delayed sowing and fertilizer application, and to ensure a response to N was observed, lower fertilizer rates were applied in 2003. Figure 1 shows that CO_2 was effectively raised from 376± 34 to 757± 29 µmol mol^{-1} and the temperature difference between the extreme tunnel modules was kept close to 4 ºC (3.7± 0.9 and 4.0 ± 0.4 in ambient and elevated CO_2, respectively, in 2002, and 3.8 ± 0.3 in both tunnels in 2003). Air humidity in the central hours of the day was similar between CO_2 levels and temperature regimes, while at the end of the day and night it was lower with warmer temperatures. Temperature was similar and humidity higher in the lower temperature module of the tunnels than in outside air. Light intensity inside the tunnels was 89 ± 3 % that outside.

Gas exchange measurements

Stomatal conductance (g_s) and rate of photosynthesis (An) were determined in attached flag leaves of four replicate plants of each treatment using a portable open system infra-red gas analyser (CIRAS-2, PP Systems, Hitchin, Herts., UK), after the onset of ear emergence (20-22 May in 2002; 21-23 May in 2003) and 8-15 days after the beginning of anthesis (03-06 June in 2002; 04-06 June in 2003). Three to 8 hours after the start of the photoperiod, measurements were performed on 1.7 cm^2 leaf areas with 300 ml min^{-1} air flow rate, 370 or 700 µmol mol $^{-1}$ CO_2, a photon flux density (PFD) of 1500 µmol m^{-2} s^{-1} and leaf temperatures of 25 ºC. Vapour pressure deficit was maintained at 1.6 ˛0.23 kPa. Short-term responses to changes in CO_2 were recorded after enough time had passed for the response to be complete [27].

Analysis of results

Stomatal conductance, rate of photosynthesis and the ratio of internal to external CO_2 concentration (Ci/Ca) measured at 700 µmol mol^{-1} were used to compare long term (acclimatory) responses to growth CO_2. The acclimatory or long-term response and the direct or short-term responses of g_s to elevated CO_2 were quantified as ratios of g_s according to [14], and the net effect as the product of the acclimatory and the direct effects (Net effect = Acclimatory effect x Direct effect):

$$g_s\,(e,E)/\,g_s\,(a,A) = [g_s\,(e,E)/\,g_s\,(a,E)] \times [g_s\,(a,E)/\,g_s\,(a,A)] \qquad (1)$$

where a and e refer to ambient (376 µmol mol^{-1}) and elevated (757 µmol mol^{-1}) growth conditions, respectively, and A and E refer to ambient (360 µmol mol^{-1}) and elevated (700 µmol mol^{-1}) measurement conditions. Similar ratios were calculated for rate of photosynthesis.

The Ball et al. model [28] to describe stomatal conductance is based on the observed correlation between stomatal conductance and assimilation, and was used to assess whether An and g_s acclimate to elevated CO_2 in parallel or independently:

$$g_s = g_0 + g_1\ An\ Hr\ /\ Ca \qquad (2)$$

where An is the rate of photosynthesis (µmol m^{-2} s^{-1}), Hr is the relative humidity at the leaf surface, Ca is the environmental CO_2 (µmol mol^{-1}), and g_0 and g_1 are the regression parameters. Values for g_s and An were those recorded at 360 and 700 µmol mol^{-1} for ambient and elevated growth conditions, respectively. The Ci/Ca ratio was also used to analyse the coupling between An and g_s responses to elevated CO_2 [1].

Chlorophyll content was determined in flag leaves collected on the same dates as gas exchange measurements. Four samples of four flag leaves each were harvested from each plot (treatment) and immediately transferred to liquid nitrogen. The projected area of a subsample was measured by image analysis, and then weighed and ground in liquid nitrogen. Chlorophyll was extracted with acetone (80%) and determined according to [30].

Differences between treatments were determined through analysis of variance using a nested design according to [31], with temperature and nitrogen as a stratum included in CO_2, and replicates as a stratum included in that for temperature and nitrogen. On each year there were two measurement dates, therefore the date effect was included in the analysis as a further stratum. Additional details on this analysis are described elsewhere [24].

Fittings of the [28] model were performed by linear regression with groups with the GenStat 6.2 statistical package. The regressions for the various treatments were compared by analysis of parallelism [32], which successively fits three models with different degrees of parallelism among the curves for these treatments. Thus, the model is first fitted with the same parameters for all treatments, then different g_0 parameters (see above) are fitted for each of the treatments, and finally separate g_0 and g_1 parameters are fitted to each treatment. With these three models, an accumulated analysis of variance is carried out to assess whether common or separate parameters should be fitted to the regressions for the compared treatments.

Results

The negative effect of elevated growth CO_2 on g_s measured at 700 µmol mol^{-1} CO_2 was less pronounced in 2002 than in 2003 (Fig. 2). In the first year, the decrease in g_s with growth in high CO_2 tended to be smaller at high N supply - g_s actually increased at elevated CO_2 with high N after anthesis -, although the CO_2 x N interaction did not reach significance ($P > 0.05$, Table 1). The N x date and the temperature x N x date interactions were significant ($P < 0.05$, Table 1), indicating that warm temperatures decreased g_s at high nitrogen after anthesis, but increased it in other cases. In 2003, elevated growth CO_2 severely reduced g_s at both temperatures and N supplies – which were lower than in 2002 - (Fig. 2 and Table 1). Also, the temperature x N interaction was significant ($P < 0.05$) in 2003, as a result of the increase in g_s with temperature at high but not at low N (Fig. 2).

The long-term or acclimatory response of g_s (measured at 700 µmol mol^{-1}) to elevated CO_2 was greater in 2003 compared to 2002 (Table 2). At high N supply there was no acclimation of g_s in 2002, and there were no differences in the acclimatory response between the two growing temperatures in the two years (Table 2). In plants growing at ambient CO_2, an increase of the measurement CO_2 from 360 to 700 µmol mol^{-1} CO_2 (the short-term or direct response) reduced g_s by 0-13% (Table 2). Thus, the net response of g_s to elevated CO_2 was a reduction at low N supply in 2002 and at both N supplies in 2003, at both temperatures (Table 2).

In both years, An measured at 700 µmol mol^{-1} CO_2 was significantly ($P < 0.05$) lower at elevated than at ambient growth CO_2. The date effect was also significant ($P < 0.05$, Table 1), due to the reduction of An after anthesis (Fig. 2). In 2003, the effect of growth in elevated CO_2 on An varied with nitrogen and temperature ($P < 0.05$ for CO_2 x temperature x N interaction); the decrease of An was lower at ambient + 4 ºC and high N. Thus, acclimation of photosynthesis occurred in both years but it was greater in 2003 compared to 2002 (Table 2). Due to the large stimulation of the photosynthetic rate when the measurement CO_2 increased from 360 to 700 μmol mol^{-1} CO_2 (the direct effect), the net effect of elevated growth CO_2 on photosynthesis was an increase in An, except in plants growing at low N supply and at warmer temperatures in 2003, which experienced the greatest acclimation (Table 2).

The intercellular to around-leaf CO_2 ratio (Ci/Ca) in flag leaves measured at 700 μmol mol^{-1} was similar for plants grown at ambient and elevated CO_2 in 2002 (average 0.48 ± 0.03), but in 2003 the mean Ci/Ca ratio was lower in plants grown at elevated CO_2 (0.58 ± 0.02) compared to those grown at ambient CO_2 (0.68 ± 0.02).

Curvilinear relationships were found between Ci/Ca and g_s, all measured at 700 μmol mol^{-1} CO_2 (Fig. 3). The relationships were similar for plants grown at ambient and elevated CO_2. No correlation was found between Ci/Ca and An (r value was 0.2 in 2003, and 0.35 in 2003). The linear regression with groups analysis showed no significant differences between years and measurement dates within each combination of CO_2, temperature and nitrogen, and so a single [28] model fitting was used for each treatment (Fig. 4). Moreover, when data of g_s and An, both measured at their respective growth CO_2, were fitted to the [28] model, the linear regression with groups yielded a common relationship for plants grown at both CO_2 levels under ambient temperature, indicating that stomata acclimated to CO_2 in parallel with photosynthesis (Fig. 4). In contrast, there was a significant difference between the relationships for ambient and elevated CO_2 among plants grown at ambient + 4 ºC temperatures, and the relationship for plants at ambient temperature significantly departed from that for ambient + 4 ºC. Nitrogen supply did not significantly modify the model.

Chlorophyll concentration per unit leaf area was not affected by growth CO_2 ($P > 0.05$), but it was significantly reduced ($P < 0.05$) by low N supply (Table 1). In 2002 the rate of photosynthesis and g_s were not correlated with chlorophyll concentration (Fig. 5a, c), but in 2003 the decrease in photosynthesis and g_s in plants growing in elevated compared with ambient CO_2 was correlated with lower chlorophyll concentration in leaves (Fig. 5b, d).

Discussion

The short-term response to doubling CO_2 was a small reduction of g_s (0-13%) but an increase of An by 65-104% (Table 2), showing that the long known [33] decrease in conductance in response to increased CO_2 is more than compensated by the larger substrate for carboxylation. In contrast, the long-term response to elevated CO_2 was a significant reduction of both g_s and An (measured at 700 µmol mol^{-1}) indicative of a downward acclimation to CO_2 enrichment. A survey of the literature covering 28 species revealed that the ratio g_s (e,E)/ g_s (a,A), i.e. the net effect, was on average 0.80 [1]. Bunce [14] found values of 0.66 and 0.89, respectively, for wheat and barley grown in field conditions in Maryland, USA. In the present study in a Mediterranean environment, the net effect of elevated CO_2 on g_s was 0.71 and 1.11 at low and high N in 2002 and 0.36 and 0.45 at low and high N supply, respectively, in 2003. The greater reduction in g_s observed in 2003 compared with 2002 could be attributed to the lower N fertilization in 2003, which caused N deficiency symptoms in the low N treatment. Clearly, the acclimatory responses of g_s and An to elevated CO_2 were more pronounced in nitrogen deficient plants (Table 2). In other crop species the acclimation of photosynthesis was also enhanced under reduced N supply [2, 16, 17, 34], although N supply had no significant effect on photosynthetic acclimation in 13 grassland species [5], and with N addition in direct proportion to plant growth rate, no acclimation to elevated CO_2 was found in low-N plants [18]. Our finding of increased acclimation of g_s to CO_2 under N deficiency contrasts with results of a meta-analysis of woody species grown for more that 1 year under CO_2 enrichment [8]; we do not know whether the length of the exposure to elevated CO_2, or differences between species and functional groups [8] can account for the contrasting results. So far, it is not clear whether the An-leaf nitrogen relationship is affected by growth CO_2 [35], since there are no direct comparisons over a wide range of N supplies. However, common relationships of An and g_s to chlorophyll concentration - which is closely related with N concentration - were found for ambient and elevated CO_2-grown plants (Fig. 5), suggesting that the relative acclimation of g_s and An to elevated CO_2 was due to decreased leaf N content. Because stomata appear to respond to the intercellular CO_2 concentration [36], it is likely that increased mesophyll demand for CO_2 at high N diminished g_s acclimation. Other, direct effects of N on stomatal opening cannot be excluded.

In wheat and barley, the direct response of An to, and the net effect on g_s of elevated CO_2 increased with temperature [21, 22], but more information is needed on how temperature affects the long-term or acclimatory response of An to elevated CO_2. In our experiments, the acclimatory response of g_s to elevated CO_2 was not affected by warmer temperatures, while that of An only increased with temperature at low N in 2003, but decreased at high N in both years. This is in agreement with our earlier finding that the downward regulation of photosynthesis by elevated CO_2 was decreased by a 4 ºC increase in temperature at high N supply [37]. The differential response of g_s and An suggests that altered acclimation of An to elevated CO_2 at ambient + 4 ºC temperatures was not mediated by changes in stomatal opening, but rather in mesophyll reactions. In vitro assays of total Rubisco activity (EC 4.1.1.39, data not shown) showed lower down regulation of this enzyme by elevated CO_2 at ambient + 4 ºC temperatures in both years.

Previous studies have reported similar Ci/Ca ratios, close to 0.7, in both ambient and elevated growth CO_2 [1, 7, 38, 39], indicative of coupling between stomata and mesophyll ([15]). The Ci/Ca ratio measured at 700 μmol mol^{-1} in our experiments was similar for plants grown in ambient and elevated CO_2 in 2002 (average 0.48), but was lower at elevated (0.58) than at ambient CO_2 (0.68) in 2003. The Ci/Ca ratio seems to be highly dependent on g_s, as indicated by the close relationship between these two parameters (Fig. 3); therefore, the lower values of Ci/Ca observed in 2002 and at elevated CO_2 in 2003 can be explained by the lower values of g_s, which decreased the diffusion of CO_2 into the leaf. It is noteworthy that, the decrease in Ci/Ca at elevated CO_2 in 2003 would argue against parallel acclimation of stomata and mesophyll. However, as indicated by [6], this ratio is not a very sensitive indicator for parallel or independent acclimations.

The acclimatory response of An (Table 2) was highly correlated with the acclimatory response of g_s ($r = 0.88$, $N = 8$). To better assess whether g_s and An acclimate to elevated CO_2 in parallel or independently, the [28] model has been used [8]. We have found a single relationship between An and g_s in the [28] model (Fig. 4) – for two years and two developmental stages - for plants grown at ambient and elevated CO_2 in ambient temperature, showing that stomata and photosynthesis of wheat respond in parallel to elevated CO_2. Similar responses have been found in several tree species [7, 8]. However, the significant separation of regressions for the two CO_2 levels at ambient + 4 ºC in the [28] model indicates that, with warmer temperatures, uncoupled stomata and mesophyll acclimation to CO_2 occurs. In addition, differences in model fit between growth temperatures show this environmental parameter causes differential acclimation of stomatal conductance and carbon assimilation. Some evidence of independent g_s and An acclimation to CO_2 has also been obtained under water stress [8], and antisense plants with decreased Rubisco activity show that stomatal conductance is not directly determined by the photosynthetic capacity of guard or mesophyll cells [40].

In conclusion, wheat plants grown at elevated CO_2 had significantly reduced g_s and An measured at 700 μmol mol^{-1} when compared to plants grown at ambient CO_2, indicating acclimatory responses to elevated CO_2. Increased nitrogen supply reduced the acclimatory responses of g_s and An to elevated CO_2, and warm temperatures at high N supply also reduced An acclimation. The g_s – An relationship and the high correlation between the acclimatory response of g_s and An indicated that both parameters are probably linked and respond in parallel to elevated CO_2 at ambient temperatures, but at above-ambient temperatures, non-parallel responses suggest unconnected An and g_s acclimations. Warm temperatures also modified the coupling of mesophyll and stomata.

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Table 1. Analysis of variance (F-values) for flag leaf stomatal conductance and rate of photosynthesis measured at 700 µmol mol^{-1} CO_2, and chlorophyll concentration of wheat grown in the field in temperature gradient tunnels either at elevated (757 µmol mol^{-1}) or ambient (376 µmol mol^{-1}) CO_2, ambient or ambient +4 °C temperature, and low or high nitrogen supply, in 2002 and 2003. Date corresponds to measurements taken at two developmental stages (ear emergence and after anthesis). Numbers in bold type represent significant effects ($P < 0.05$).

	Stomatal conductance		Rate of photosynthesis		Chlorophyll	
	2002	2003	2002	2003	2002	2003
CO_2 (C)	2.47	**11.93**	**6.61**	**13.07**	0.20	3.23
Temperature (T)	1.59	**5.77**	2.68	1.16	**7.27**	0.11
Nitrogen (N)	0.45	**4.77**	0.00	**24.09**	**31.94**	**114.87**
CT	0.00	2.47	2.15	0.00	0.11	0.20
CN	3.64	0.03	2.27	2.25	1.29	0.08
TN	1.79	**7.50**	0.00	**5.13**	2.96	1.42
CTN	0.06	0.84	0.35	**5.30**	2.12	0.50
Date (D)	3.92	4.18	**10.33**	**46.96**	**96.40**	**26.50**
CD	6.69	0.01	0.00	0.85	5.95	0.20
TD	7.37	2.12	4.90	3.38	0.29	2.28
ND	**17.20**	1.33	3.73	0.62	4.21	0.76
CTD	3.06	0.06	4.88	0.03	1.64	0.31
CND	0.34	0.00	1.14	1.56	0.53	0.21
TND	**18.88**	0.05	5.65	0.13	1.47	0.88
CTND	1.04	0.33	1.70	0.88	1.62	0.39

Table 2. Acclimatory, direct and net responses of stomatal conductance and rate of photosynthesis to elevated CO_2 in wheat grown in the field in temperature gradient tunnels either at ambient or at ambient +4 °C temperature, and low or high nitrogen supply, in 2002 and 2003. Values are means of two developmental stages (ear emergence and after anthesis) and four replicate plants. See Materials and Methods for more explanation.

Year	Temperature	Nitrogen	Stomatal responses			Photosynthetic responses		
			Acclimation	Direct	Net effect	Acclimation	Direct	Net effect
			[$g_s(e,E)/ g_s(a,E)$]	[$g_s(a,E)/g_s(a,A)$]	[$g_s(e,E)/g_s(a,A)$]	[An(e,E)/An(a,E)]	[An(a,E)/An(a,A)]	[An(e,E)/An(a,A)]
2002	Ambient (T)	Low	0.71	1.01	0.71	0.80	1.92	1.53
		High	1.04	1.07	1.11	0.87	2.04	1.75
	T + 4	Low	0.78	0.89	0.69	0.83	1.77	1.53
		High	1.07	0.94	1.00	1.05	1.83	1.93
2003	Ambient (T)	Low	0.42	0.87	0.36	0.67	1.73	1.16
		High	0.50	0.89	0.45	0.65	1.79	1.17
	T + 4	Low	0.35	0.96	0.35	0.47	1.69	0.79
		High	0.50	0.92	0.46	0.80	1.65	1.32

Figure legends

Figure 1. Mean daily courses of CO_2 concentration (a), temperature (c, d) and humidity (e, f) in 2002 (c, e) and 2003 (d, f) in temperature gradient tunnels set at either ambient (376 µmol mol^{-1}; ○, □) or elevated (757 µmol mol^{-1}; ●, ■) CO_2 and ambient (○,●) or ambient + 4 ºC (□,■) temperatures. The irradiance outside and inside (○ and ●, respectively) the tunnels is shown in (b) and the temperature and humidity outside (▲) the tunnels are sown in (d) and (f).

Figure 2. Mean values (± S.E.) of stomatal conductance (g_s, a-d) and rate of photosynthesis (An, e-h) measured at 700 µmol mol^{-1} CO_2 for flag leaves of wheat grown in the field in temperature gradient tunnels at either ambient (white columns, 376 µmol mol^{-1}) or elevated (black columns, 757 µmol mol^{-1}) CO_2, ambient (T) or ambient + 4 ºC (T+4) growth temperatures and low (LN) or high (HN) nitrogen supply. Measurements were taken at ear emergence (a, c, e, g) and 8-15 days after anthesis (b, d, f, h) in 2002 (a, b, e, f) and 2003 (c, d, g, h). PFD was 1500 µmol m^{-2} s^{-1} and leaf temperature was 25 ºC.

Figure 3. Relationships of intercellular to ambient CO_2 ratio (Ci/Ca) to stomatal conductance (g_s) of wheat grown in the field in temperature gradient tunnels either at ambient (○) or elevated (●) CO_2, in 2002 (a) and in 2003 (b). Measurements were made at 700 µmol mol^{-1} CO_2 at ear emergence and soon after anthesis. Regression lines are: a) $Ci/Ca = -0.54 + 0.19 \ln(g_s)$, $r^2 = 0.63$; b) $Ci/Ca = 0.04 + 0.11 \ln(g_s)$, $r^2 = 0.62$. N = 64 in both cases.

Figure 4. Fits of the Ball et al. [28] model for stomatal conductance (g_s) at ear emergence and soon after anthesis of wheat grown in the field in temperature gradient

tunnels at two levels of N supply in 2002 and 2003. Values for gs and An were those recorded at 360 and 700 µmol mol^{-1} for ambient and elevated growth conditions, respectively. ○) ambient CO_2 with ambient temperature; ●) elevated CO_2 with ambient temperature; □) ambient CO_2 with ambient +4 ºC ; ■) elevated CO_2 with ambient +4 ºC. Regression lines: dotted line, ambient CO_2 with ambient+4 ºC; broken line, elevated CO_2 with ambient+4 ºC; filled line, ambient and elevated CO_2 with ambient temperature. Parameters for the regression $g_s = g_0 + g_1$ An Hr/CO_2 are indicated below. D is the percentage of variance explained by the fitted lines using linear regression with groups (GenStat 6.2, 2002).

Temperature	CO_2	g_0	g_1	r^2	D%
T + 4	Ambient	-117.6	20.17	0.80	79.2
	Elevated	12.4	11.279	0.78	
Ambient (T)	both levels	-19.5	11.279	0.61	

Figure 5. Relationships with chlorophyll concentration per unit of leaf area (g m^{-2}) of: rate of photosynthesis (An) (a, b), and stomatal conductance (g_s) (c, d), for the flag leaf of wheat grown in the field in temperature gradient tunnels either at ambient (376 µmol mol^{-1}; ○) or elevated (757 µmol mol^{-1}; ●) CO_2, in 2002 (a, c) and 2003 (b, d). Coefficients of determination were a) $r^2 = 0.04$, n.s. ; b) $r^2 = 0.51$, $P < 0.001$; c) $r^2 = 0.09$, n.s.; d) $r^2 = 0.40$, $P < 0.001$. N = 64 in all cases.

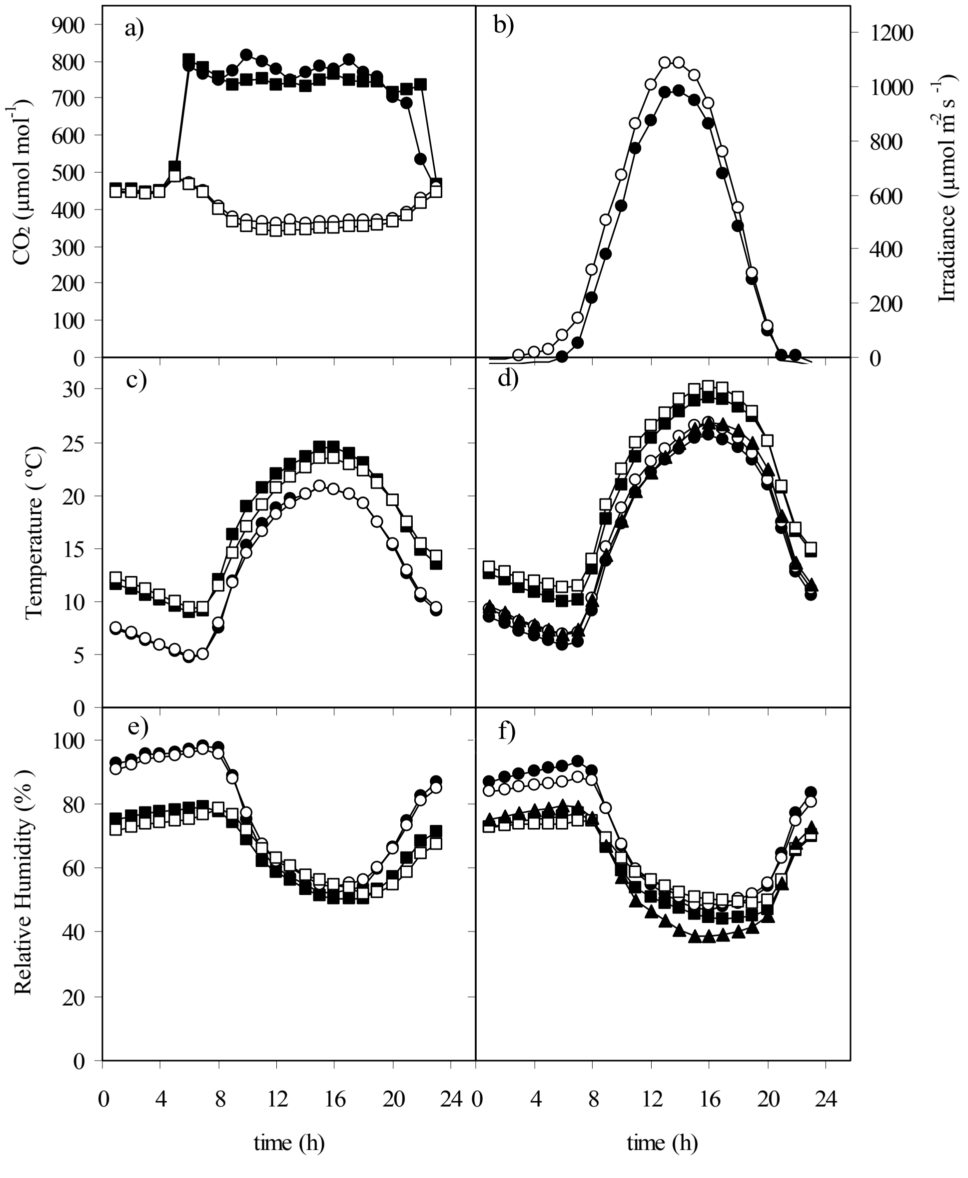
a)
b)
c)
d)
e)
f)
CO2 (µmol mol-1)
Irradiance (µmol m-2 s -1)
Temperature (°C)
Relative Humidity (%)
time (h)
time (h)
0
100
200
300
400
500
600
700
800
900
0
200
400
600
800
1000
1200
0
5
10
15
20
25
30
0
20
40
60
80
100
0
4
8
12
16
20
24

Fig 1

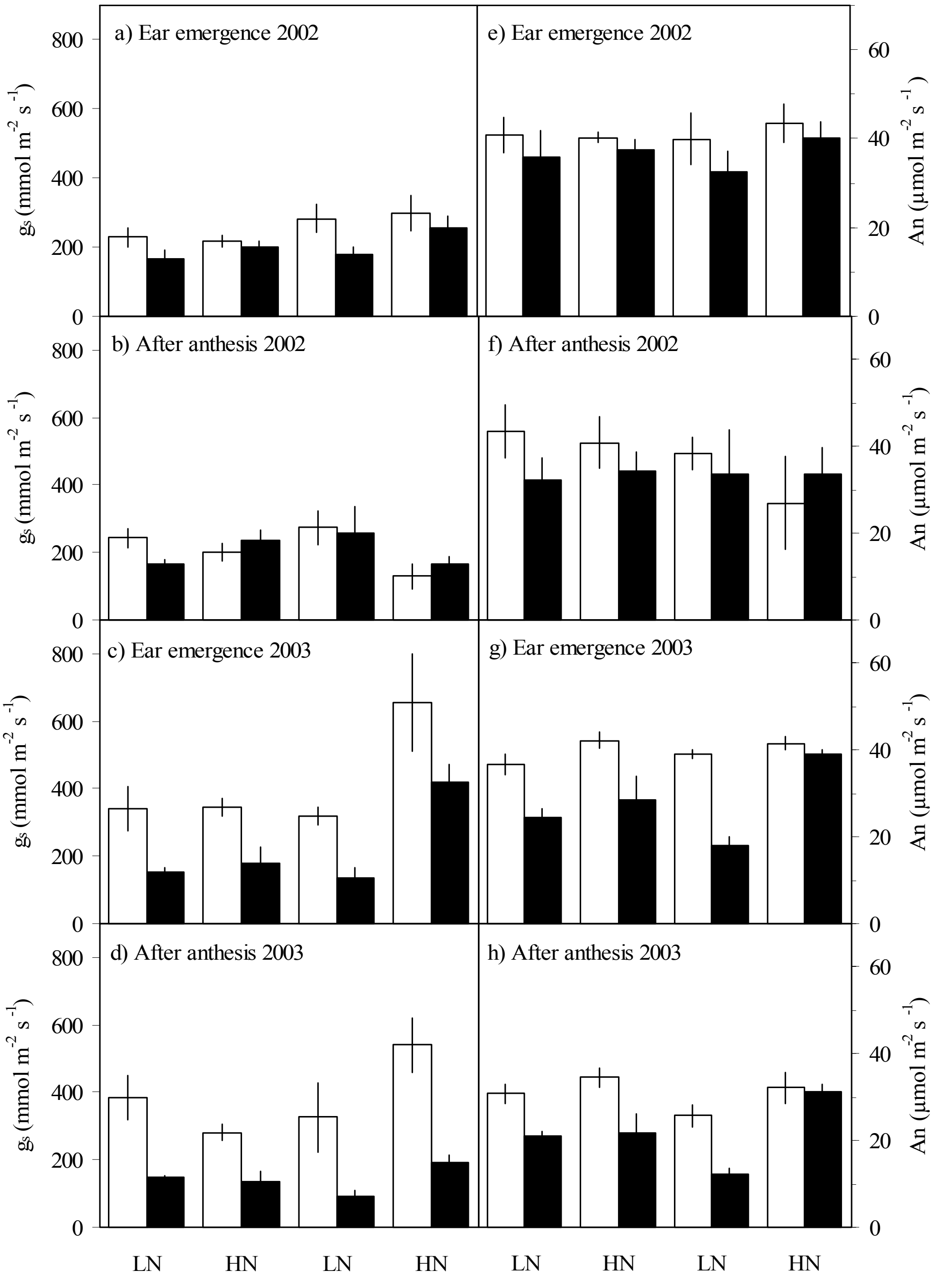
a) Ear emergence 2002
b) After anthesis 2002
c) Ear emergence 2003
d) After anthesis 2003
e) Ear emergence 2002
f) After anthesis 2002
g) Ear emergence 2003
h) After anthesis 2003
g_s (mmol m^{-2} s^{-1})
An (µmol m^{-2} s^{-1})
0
200
400
600
800
0
20
40
60
LN
HN
LN
HN
LN
HN
LN
HN
T
T+4
T
T+4

Fig 2

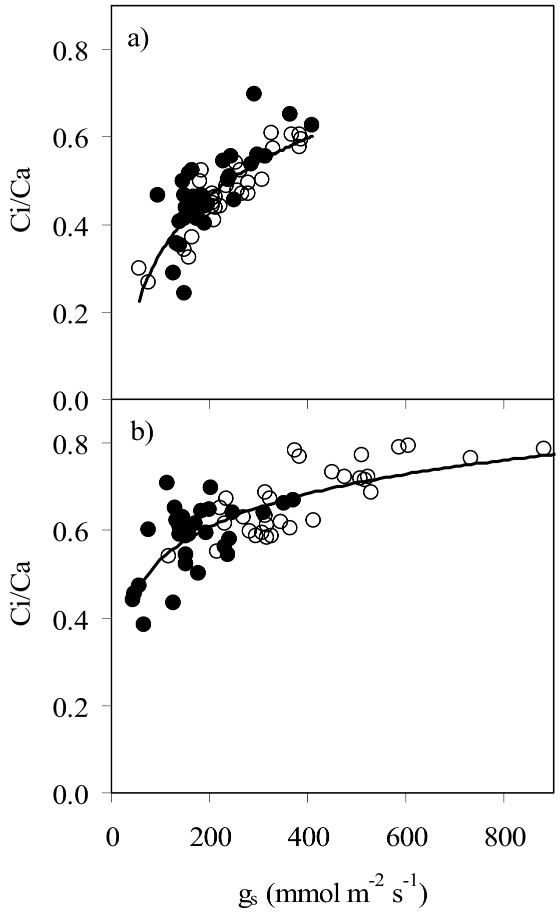

Fig. 3

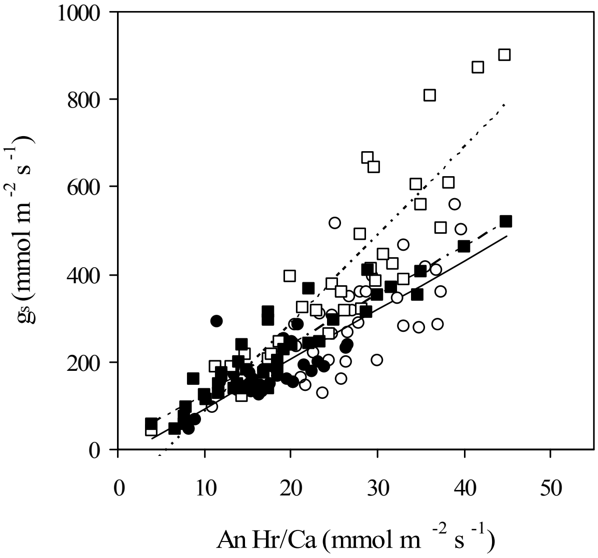
1000
800
600
400
200
0
0
10
20
30
40
50
gs (mmol m -2 s -1)
An Hr/Ca (mmol m -2 s -1)

Fig. 4

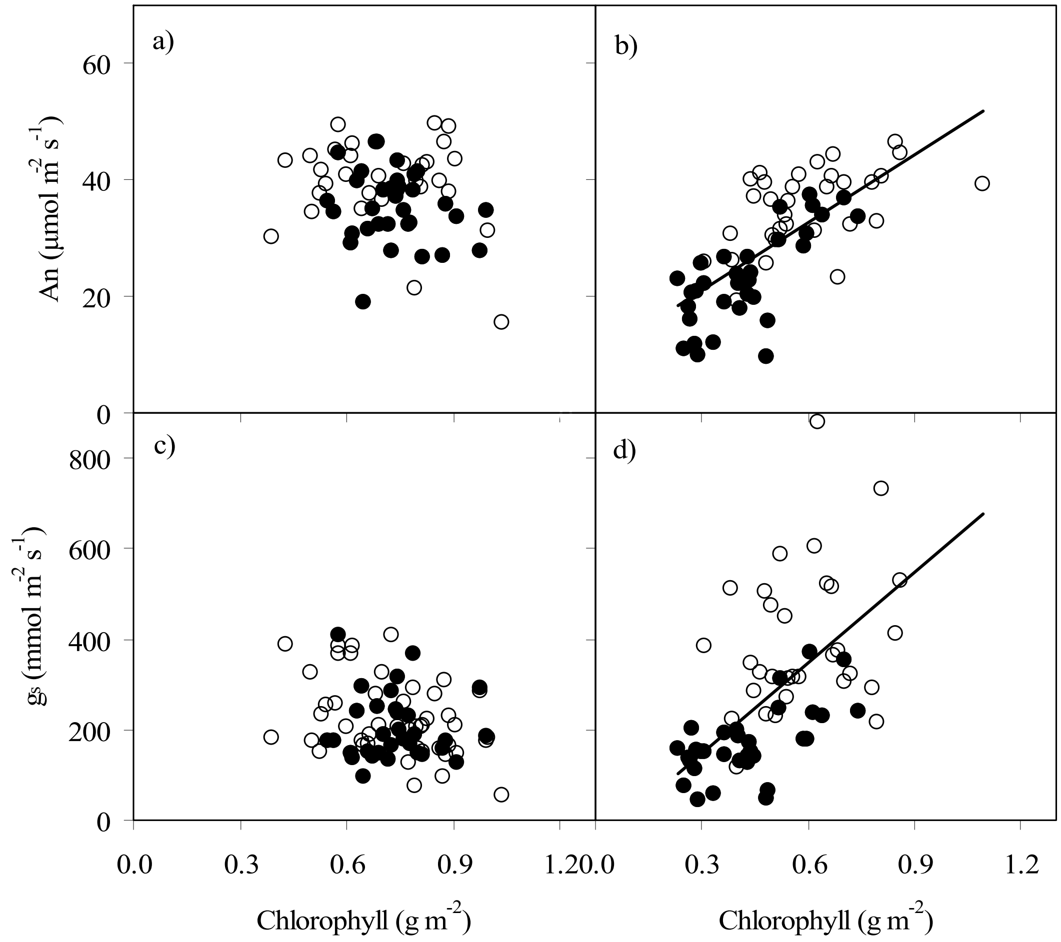
a)
b)
c)
d)
An (µmol m-2 s-1)
gs (mmol m-2 s-1)
0
20
40
60
0
200
400
600
800
0.0
0.3
0.6
0.9
1.2
0.0
0.3
0.6
0.9
1.2
Chlorophyll (g m-2)
Chlorophyll (g m-2)

Fig. 5