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   *Strikes a line through text and opens up a text box where replacement text can be entered.*
   
   **How to use it**
   - Highlight a word or sentence.
   - Click on the Replace (Ins) icon in the Annotations section.
   - Type the replacement text into the blue box that appears.

2. **Strikethrough (Del)** Tool – for deleting text.
   
   *Strikes a red line through text that is to be deleted.*
   
   **How to use it**
   - Highlight a word or sentence.
   - Click on the Strikethrough (Del) icon in the Annotations section.

3. **Add note to text** Tool – for highlighting a section to be changed to bold or italic.
   
   *Highlights text in yellow and opens up a text box where comments can be entered.*
   
   **How to use it**
   - Highlight the relevant section of text.
   - Click on the Add note to text icon in the Annotations section.
   - Type instruction on what should be changed regarding the text into the yellow box that appears.

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   *Marks a point in the proof where a comment needs to be highlighted.*
   
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   - Click at the point in the proof where the comment should be inserted.
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   Inserts an icon linking to the attached file in the appropriate place in the text.

   **How to use it**
   - Click on the **Attach File** icon in the Annotations section.
   - Click on the proof to where you’d like the attached file to be linked.
   - Select the file to be attached from your computer or network.
   - Select the colour and type of icon that will appear in the proof. Click OK.

6. **Drawing Markups Tools** – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

   Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks.

   **How to use it**
   - Click on one of the shapes in the Drawing Markups section.
   - Click on the proof at the relevant point and draw the selected shape with the cursor.
   - To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
   - Double click on the shape and type any text in the red box that appears.
Carbon and nitrogen allocation and partitioning in traditional and modern wheat genotypes under pre-industrial and future CO₂ conditions

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INTRODUCTION

The results of a simultaneous 13C and 15N labelling experiment with two different durum wheat cultivars, Blanqueta (a traditional wheat) and Sula (modern), are presented. Plants were grown from the seedling stage in three fully controllable plant growth chambers for one growing season and at three different CO₂ levels (i.e., 260, 400 and 700 ppm). Short-term isotopic labelling (ca. 3 days) was performed at the anthesis stage using 13CO₂ supplied with the chamber air and 15NH₄⁺15NO₃ applied with the nutrient solution, thereby making it possible to track the allocation and partitioning of 13C and 15N in the different plant organs. We found that photosynthesis was up-regulated at pre-industrial CO₂ levels, whereas down-regulation occurred under future CO₂ conditions. 13C labelling revealed that at pre-industrial CO₂ carbon investment by plants was higher in shoots, whereas at future CO₂ levels more C was invested in roots. Furthermore, the modern genotype invested more C in spikes than did the traditional genotype, which in turn invested more in non-reproductive shoot tissue. 15N labelling revealed that the modern genotype was better adapted to assimilating N at higher CO₂ levels, whereas the traditional genotype was able to assimilate N more efficiently at lower CO₂ levels.
rate in response to future [CO$_2$]. However, in experiments with prolonged exposure to future [CO$_2$] (over a long period or the entire plant life cycle), in contrast, down-regulation of photosynthesis has been observed (Leakey et al. 2004; Long et al. 2004). One of the parameters that can affect photosynthetic down-regulation is modification of the source–sink ratio (Urban 2003). By definition, if increases in carbohydride production (source) associated with elevated [CO$_2$] exceed the capacity of the plant to produce new sinks, net photosynthesis rates may decline in order to balance the source activity with the sink capacity (Thomas & Strain 1991).

In terms of grain filling in wheat, two types of C source contribute to this process: (i) current photoassimilates are transferred directly to the grain from green tissues (mainly flag leaves and spikes), and (ii) photoassimilates are redistributed from reserve pools to vegetative tissues (leaves, stems and roots; Aranjuelo et al. 2011a). Moreover, the use of photoassimilates is influenced by the genotype and growth conditions, such as stress and/or increases in [CO$_2$] (Tambussi et al. 2007; Aranjuelo et al. 2009a,b).

Additionally, nitrogen frequently controls or limits plant growth in many terrestrial ecosystems (Vitousek 1994) and is tightly coupled with the leaf C cycle (Fisher et al. 2010). The relationship between C and N inputs and metabolism are further complicated by the dynamic exchanges between plant organs and the effects of the environment. Therefore, the appropriate balance of C and N between sink and source strengths will be an essential objective for maximising the response of cereals to growth under different C and N availability conditions (Aranjuelo et al. 2013). With this aim in mind, through the use of stable isotopes, the allocation and partitioning of C and N throughout the plant and between organs can be traced and studied. For instance, it was observed that plants at pre-industrial [CO$_2$] have stimulated the allocation of C towards leaves and shoots because of increasing C demand (Gerhart & Ward 2010). However, at future [CO$_2$] plants can invest more C in roots due to the increased C source availability (Ghashghaie & Tcherkez 2013). Another example is the higher requirement for N during grain filling in wheat (Fuentes-Mendizabal et al. 2012), N is remobilised from different parts of the plant (stored before grain filling) or new N is taken up in the same period (Dupont & Altenbach 2003). N content in plants is modulated by [CO$_2$] and it is lower at future than at current or pre-industrial [CO$_2$] (Mitsutoshi et al. 2005). Explanations for this decline in N content under elevated [CO$_2$] include different processes: (i) dilution as a result of higher plant growth, (ii) down-regulation and lower protein content in the form of Rubisco induced by increases in carbohydrate, (iii) limitations in the N available to plants due to C enrichment of the rhizosphere, and (iv) inhibition of nitrate assimilation from the soil due to elevated [CO$_2$] (Bloom et al. 2014).

Understanding the mechanisms controlling whole wheat plant N and C isotope composition will further advance our knowledge of the acquisition and allocation of N and C in plants under different climate scenarios (Farrar & Jones 2000; Fisher et al. 2010).

The $^{13}$CO$_2$ isotope labelling technique was used in this paper to study recently fixed C in wheat organs and the respiratory metabolism. This allowed calculation of the contribution of stored C versus current photoassimilates to the production of CO$_2$ through respiration (Schnyder et al. 2003; Noguès et al. 2004, 2014). Labelling with $^{13}$CO$_2$ permitted us to calculate the proportion of ‘new’ (i.e. recently fixed) C in total organic matter (TOM) and respired CO$_2$ (Noguès et al. 2004).

Similarly, the $^{15}$NH$_4$$^{15}$NO$_3$ isotope labelling technique is used to understand the N cycle in plants under ambient conditions (Robinson 2001). $^{15}$N can act as a powerful tool to assess whether processes in the N cycle are influenced by the increasing concentration of atmospheric CO$_2$. The IPCC (2013) predict that with climate change, there will be a reduction in N availability (N limitation) with increasing CO$_2$. A large part of the uncertainty in models predicting climate change feedbacks lies in the role of the N cycle in modulating the exchange of CO$_2$ between plants, the ecosystem and the atmosphere (Hungate et al. 2003). Moreover, the pattern of changes in grain protein content in response to future [CO$_2$] could guide future studies in identification of the exact processes in the N cycle that respond to future climate change (BassiriRad et al. 2003).

The main objective of this study was to characterise the C and N allocation and its implications in terms of biomass, photosynthesis and reserves in traditional and modern wheat genotypes grown in pre-industrial, current and future CO$_2$ environments. To date, the mechanism conditioning C and N allocation responses to pre-industrial [CO$_2$] in wheat has not been sufficiently documented, and studying this may help us to understand the behaviour of plants in future climate change scenarios. In order to better understand C and N partitioning among the organs of these plants exposed to pre-industrial and future [CO$_2$], double labelling with $^{13}$CO$_2$ and $^{15}$NH$_4$$^{15}$NO$_3$ was conducted.

**MATERIAL AND METHODS**

**Plant material**

Wheat seeds were germinated in Petri dishes. After 4 days, seedlings were transferred to 4-l pots (one plant per pot) filled with quartz sand of 1-mm grain size. Plants were grown in three fully controllable plant growth chambers (Conviron E15; Controlled Environments Ltd, Winnipeg, MB, Canada) at a temperature of 22/18°C (day/night) and 60% relative humidity. Plants were supplied with a photosynthetic photon flux density (PPFD) of ca. 400 μmol·m$^{-2}$·s$^{-1}$ for a 16-h light period (day) and the remaining 8 h in darkness (night). Plants were watered with Hoagland complete nutrient solution. Each plant growth chamber was maintained at a different CO$_2$ level (i.e. 700, 400 and 260 ppm).

Two durum wheat genotypes (*Triticum turgidum* var. Sula and var. Blanqueta) were used in the experiment, both of which are cultivated in Spain. Blanqueta is a land race that was widely grown in Sicily and the west of Spain in the first half of the last century, but which is now grown in small areas mainly to satisfy local consumers who appreciate the properties of this variety. It is characterised by its tall stature, high tillering capacity, medium to late heading and maturity, moderate productivity and good adaptability to environments characterised by scarce water and nutrient resources. Sula (released in 1994) is a modern and commercially grown genotype. It is characterised by its short stature, early heading and maturity and high yield potential. It is grown in Andalucía, Catalonia and Extremadura in Spain.
Experimental design

The humidity, temperature and [CO₂] in the chamber air were continuously monitored at 5-min intervals with a combined sensor (CMPS3243; Controlled Environments Ltd.) and compared every 2 weeks with separate sensors (HMP75; humidity and temperature, and GMP222; for 0–2000 ppm CO₂; Vaisala M170; Vaisala, Helsinki, Finland) in order to maintain a good characterisation of environmental parameters.

Plants were grown during the whole life cycle (from September to January) under three different levels of CO₂ (700, 400 and 260 ppm) at the Experimental Fields Service at the University of Barcelona, Barcelona, Spain. Forty-eight plants were placed in the first plant growth chamber and were maintained at a high (future) [CO₂] (ca. 700 ± 18 ppm) during the whole life cycle. In order to raise the CO₂ level in the chamber, commercial CO₂ (99.5% pure CO₂, without H₂O, O₂, N₂, CO or hydrocarbons) was used (Carburos Metalicos S.A. Barcelona, Spain). An infrared gas analyser (IRGA) connected to the chamber continuously monitored CO₂ levels. When the level of CO₂ dropped below 700 ppm, commercial CO₂ was injected into the chamber, thus maintaining CO₂ concentration at 700 ppm. The air in the three plant growth chambers (future, current and pre-industrial CO₂) was collected using 10-ml vacuum containers and analysed using gas chromatography combustion isotope ratio mass spectrometry (GC/IRMS). Mixing of the commercial CO₂ (δ¹³C ca. −38.2‰) with the ambient air (δ¹³C ca. −12.5‰) resulted in a δ¹³C of CO₂ of ca. −22.6 ± 0.9‰ inside the plant growth chamber. Another 48 plants were placed in the second plant growth chamber and were maintained at current [CO₂] (ca. 400 ± 20 ppm) during the whole life cycle, with a δ¹³C of CO₂ of ca. −11.2 ± 0.6‰.

Finally, the same number of plants was placed in the third plant growth chamber and maintained at pre-industrial [CO₂] (ca. 260 ± 28 ppm) during the whole life cycle, with a δ¹³C of CO₂ of ca. −10.8 ± 0.5‰. In this chamber, CO₂ was removed using a pump that sent the air inside the chamber through a 1-l column filled with soda lime (soda lime with indicator QP; Panreac Quimica SA, Barcelona, Spain). The soda lime was changed every 2 weeks. The CO₂ levels of these two chambers (400 and 260 ppm) were also continuously monitored with an IRGA. Plants were rotated in the plant growth chamber each week to avoid chamber influences in the treatments.

Isotope labelling procedures with ¹³C and ¹⁵N

Simultaneous C and N labelling was conducted in the plants and at different CO₂ levels. Double labelling with ¹³C and ¹⁵N was carried out over 3 days during the anthesis period in order to ensure that both genotypes were labelled with the same amount of ¹³C and ¹⁵N in the different plant growth chambers. All plants assimilated the same amount of labelled CO₂ (ca. 3000 mmol C m⁻²) during ca. 3 days, but the assimilation time varied between the different CO₂ treatments and was calculated according to their net assimilation rates (Table 1; Nogués et al. 2014).

The δ¹³C composition of air inside the three plant growth chambers was modified during the labelling period. In each of the chambers, mixing of commercial CO₂ (¹³C ca. 99.9%; Euriso-top, Saint-Aubin, France) with the ambient air (δ¹³C ca. −22.6‰, −11.2‰ and −10.8‰, in future, current and pre-industrial plant growth chambers, respectively) resulted in a δ¹³C of CO₂ of ca. 165‰. Air samples from the chambers and air respired by plants in darkness were taken before and after labelling in order to analyse the ¹³CO₂ isotopic composition using GC/IRMS according to Nogués et al. (2004).

Labelling with ¹⁵N was also applied during the same period by replacing the ¹⁴N labelled ammonium nitrate (¹⁵NH₄NO₃) in the Hoagland solution with double ¹⁵N labelled ammonium nitrate (¹⁵NH₄NO₃) that had a ¹⁵N excess atom fraction of 5%. After labelling, ¹⁵N was removed by washing the quartz sand with distilled water. Plants were then irrigated with normal Hoagland solution.

Carbon and N isotope composition of TOM

Samples from different parts of the plant (i.e. leaves, stems, roots and spikes) were collected before, 1 and 10 days after labelling, dried in an oven at 60 °C for 48 h and ground to a fine powder. Then, 1 mg was weighed in tin capsules and C and N isotope composition determined using an elemental analyser (Flash EA 112; Carlo Erba, Milan, Italy) coupled to an

---

**Table 1.** CO₂ effects (700, 400 and 260 ppm) on physiological parameters in the flag leaf of durum wheat Sula and Blanqueta genotypes.

<table>
<thead>
<tr>
<th>CO₂ treatment</th>
<th>Genotype</th>
<th>Vc,max</th>
<th>Jmax</th>
<th>Rₛ</th>
<th>gs</th>
<th>Aₘₜ</th>
<th>A₁₅₀</th>
<th>Amax</th>
<th>C/C₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>700</td>
<td>Blanqueta</td>
<td>77.0 ± 12.2</td>
<td>126.1 ± 9.1</td>
<td>−0.6 ± 0.5</td>
<td>14.0 ± 1.3</td>
<td>14.4 ± 1.5</td>
<td>16.7 ± 1.5</td>
<td>23.0 ± 1.3</td>
<td>0.59 ± 0.01</td>
</tr>
<tr>
<td>700</td>
<td>Sula</td>
<td>68.9 ± 5.1</td>
<td>166.4 ± 4.5</td>
<td>−1 ± 0.5</td>
<td>22.7 ± 3.5</td>
<td>15.2 ± 0.8</td>
<td>19.6 ± 0.2</td>
<td>28.4 ± 0.8</td>
<td>0.66 ± 0.009</td>
</tr>
<tr>
<td>400</td>
<td>Blanqueta</td>
<td>80.6 ± 14.2</td>
<td>171.9 ± 36.6</td>
<td>−0.3 ± 0.8</td>
<td>19.2 ± 1.9</td>
<td>17.4 ± 3.0</td>
<td>21.4 ± 3.3</td>
<td>28.9 ± 5.2</td>
<td>0.48 ± 0.006</td>
</tr>
<tr>
<td>400</td>
<td>Sula</td>
<td>84.2 ± 15.4</td>
<td>144.3 ± 30.9</td>
<td>−1.7 ± 0.9</td>
<td>15.9 ± 1.7</td>
<td>18.8 ± 2.4</td>
<td>22.5 ± 3.1</td>
<td>26.5 ± 3.7</td>
<td>0.74 ± 0.008</td>
</tr>
<tr>
<td>260</td>
<td>Blanqueta</td>
<td>88.5 ± 17.9</td>
<td>181.5 ± 41.8</td>
<td>−0.7 ± 0.3</td>
<td>19.1 ± 0.5</td>
<td>18.4 ± 4.9</td>
<td>22.7 ± 5.9</td>
<td>28.7 ± 4.3</td>
<td>0.62 ± 0.006</td>
</tr>
<tr>
<td>260</td>
<td>Sula</td>
<td>74.3 ± 3.6</td>
<td>148.2 ± 20.3</td>
<td>−1.8 ± 2.3</td>
<td>15.0 ± 1.0</td>
<td>18.5 ± 0.0</td>
<td>22.0 ± 0.5</td>
<td>26.8 ± 0.7</td>
<td>0.74 ± 0.006</td>
</tr>
</tbody>
</table>

Vc,max = maximum carboxylation velocity of Rubisco; Jmax = rate of photosynthetic electron transport; Rₛ = rate of daytime respiration; gs = stomatal conductance; Aₘₜ = assimilation rate at saturation of light; A₁₅₀ = assimilation rate at 360 ppm CO₂; Amax = maximum assimilation rate; C/C₄ = internal CO₂ concentration/ambient CO₂ ratio.

Data are means ± SE, n = 4.

ANOVA Tukey-b (n.s., non-significant; *P < 0.05; **P < 0.01; ***P < 0.001).
isoTrac isotope ratio mass spectrometer (Delta C with Conflo III; Thermo Finnigan, Bremen, Germany).

Results of C isotope ratio analyses are reported as δ\(^{13}\)C in per mil (%\(\text{oo}\)) and referenced against the international standard V-PDB (Vienna Pee Dee Belemnite) according to the following equation:

\[
\delta^{13}\text{C} (\%\text{oo}) = \left( \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1000 \tag{1}
\]

where \(R\) is the \(^{13}\)C/\(^{12}\)C ratio.

Carbon isotope discrimination (\(\Delta^{13}\)C) was calculated for unlabelled plants from \(\delta_s\) and \(\delta_p\) (Farquhar et al. 1989) as in the following equation:

\[
\Delta^{13}\text{C} = \frac{\delta_s - \delta_p}{\delta_p + 1} \tag{2}
\]

where \(a\) and \(p\) refer to \(\delta^{13}\)C of air CO\(_2\) and plant material, respectively.

Nitrogen results were also expressed in \(\delta^{15}\)N notation (%\(\text{oo}\)), using the international secondary standards with known \(^{15}\)N/\(^{14}\)N ratio (IAEA N\(_1\) and IAEA N\(_2\) ammonium sulphate and IAEA NO\(_3\) potassium nitrate) with reference to the international primary standard air N\(_2\), which has a \(\delta^{15}\)N value of 0%\(\text{oo}\) (Werner & Brand 2001):

\[
\delta^{15}\text{N} (\%\text{oo}) = \left( \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1000 \tag{3}
\]

where \(R\) is the \(^{15}\)N/\(^{14}\)N ratio.

Labelled samples were expressed in atom fraction (%) \(^{15}\)N as recommended in the international guidelines for stable isotope ratio (Coplen 2011):

\[
\text{Atom fraction} (\%) = \frac{\delta + 1000}{\delta + 1000 + \frac{1000}{R_{\text{standard}}}} \tag{4}
\]

where \(\delta\) is the isotopic signature of \(^{15}\)N samples. \(R_{\text{standard}}\) is the international standard reference.

The \(N\) isotope discrimination (\(\Delta^{15}\)N) of TOM for unlabelled plants was calculated from \(\delta_s\) and \(\delta_p\) (Farquhar et al. 1989) as:

\[
\Delta^{15}\text{N} = \frac{\delta_s - \delta_p}{\delta_p + 1} \tag{5}
\]

where \(s\) and \(p\) refer to \(\delta^{15}\)N of solution and plant, respectively.

Open system for isotopic dark respiration determinations

The \(\delta^{13}\)C of the CO\(_2\) respired after 20 min in darkness of the different plant organs (i.e. flag leaf, remaining leaves, spikes, stems and root) was studied in a respiration chamber as previously described (Nogués et al. 2004). The chamber was connected in parallel to the sample air hose of a portable gas exchange analyser (Li-Cor 6400; Li-Cor Inc., Lincoln, NE, USA). The PPFD inside the chamber was maintained at 0 \(\mu\text{mol photon m}^{-2} \text{s}^{-1}\) by covering the chamber with a black piece of, which kept the chamber in darkness. The organ was first placed in the chamber with ambient air (\(\delta^{13}\)C ca. \(-10.3 \pm 0.5\%\text{oo}\)). The chamber was then flushed with CO\(_2\)-free air and the CO\(_2\) respired by the organ was allowed to accumulate over a period of 10 min. This was then collected using gas syringes (SGE International Pty Ltd, Australia) and stored in 10-ml vacutainers.

The air in the three growth chambers (e.g. pre-industrial, current and future CO\(_2\)) was also sampled using 10-ml vacutainers in order to obtain isotopic composition of the air (the source of C). The CO\(_2\) inside the vacutainers was analysed using GC/C/IRMS.

All the GC/C/IRMS and EA/IRMS analyses were performed at the Scientific Technical Services of the University of Barcelona.

Calculation of the proportion (\(p\)) of new C and N

We assumed that 100% of C and N supplied during short-term labelling could be assimilated by the different parts of the plant, and that this C and N is allocated throughout the plant. The proportion of ‘new’ carbon (derived from the labelling) in CO\(_2\) respired in darkness after illumination and the proportion of ‘new’ C and N in TOM was calculated as described in Nogués et al. (2004):

\[
x = 100 \times \frac{\delta_{\text{after}} - \delta_{\text{control}}}{\delta_{\text{fixed}} - \delta_{\text{control}}} \tag{6}
\]

where \(\delta_{\text{control}}, \delta_{\text{fixed}}\) and \(\delta_{\text{after}}\) are the isotopic compositions of the fraction of interest (CO\(_2\) and TOM) of the control (not labelled), of C and N atoms fixed during labelling and of the sample after labelling, respectively. The isotopic composition of fixed C and N was calculated as:

\[
\delta_{\text{fixed}} = \frac{\delta_{\text{source}} - \Delta}{1 + \Delta} \tag{7}
\]

where \(\Delta\) is the isotope discrimination (equations 2 and 5) and \(\delta_{\text{source}}\) is the isotopic composition of the source during the labelling.

Leaf C and N content

Leaves, spikes, stems and roots used for gas exchange were collected and dried at 65 °C until constant weight and ground to a powder. An aliquot of 1 mg dry powder was analysed for the C (mg C mg\(^{-1}\)) and N content (mg N mg\(^{-1}\)) with an elemental analyser at the Scientific Technical Services at the University of Barcelona, Spain.

Gas exchange analyses

For simultaneous measurements of gas exchange and chlorophyll fluorescence in an expanded flag leaf, the Li6400 was connected to a leaf chamber fluorometer (Li6400-40). A/C\(_i\) curves with chlorophyll fluorescence and dark respiration rates were determined. A/C\(_i\) curve determinations were conducted on totally expanded flag leaves for each CO\(_2\) treatment and in each genotype. The A/C\(_i\) curves were repeated in four different
plants for each treatment and genotype and were measured from 0 to 2000 μmol·mol\(^{-1}\) CO\(_2\). The curves were generated at 1200 μmol photon·m\(^{-2}\)·s\(^{-1}\) PPFD and 25 °C. Measurements were carried out before anthesis (T0). CO\(_2\) assimilation rates (A), CO\(_2\) assimilation rate at light saturation (A\(_{\text{sat}}\)), maximum CO\(_2\) assimilation rate at CO\(_2\) saturation (A\(_{\text{max}}\)) and stomatal conductance (g\(_s\)) were estimated using equations developed by von Caemmerer & Farquhar (1981). Estimations of the maximum carboxylation velocity of Rubisco (V\(_{\text{cmax}}\)), the rate of photosynthetic electron transport based on NADPH requirement (J) and the rate of respiration (R\(_d\)) were made by fitting a maximum likelihood regression below and above the inflexion of the A/I/C response using the method of Nogués & Baker (2000).

### Biomass parameters

Biomass parameters in durum wheat genotypes grown at three different CO\(_2\) levels (700, 400 and 260 ppm) were analysed. Leaves were scanned with a commercial scanner (HP ScanJet 3400C; Hewlett-Packard, Palo Alto, CA, USA) and images were analysed with leaf area meter software (Comprises WinDIAS; Delta-T Devices Ltd. Cambridge, UK) to obtain the total leaf area (TLA; cm\(^2\)). Harvest index (HI), reproductive biomass (RB; g), shoot dry weight (shoot DW; g), root dry weight (root DW; g) and shoot/root were also studied. HI was calculated as the ratio between grain DW and total DW.

### Data analysis

The effects of CO\(_2\) in both wheat genotypes were tested with two-factor (CO\(_2\) treatment and durum wheat genotype) ANOVA. The statistical analysis was conducted with spss 17.0 software (SPSS Inc., Chicago, IL, USA). The means ± SE were calculated for each parameter. When a particular test was significant, we compared the means using the Duncan multiple comparison test. The results were accepted as significant at P < 0.05.

### RESULTS

Before labelling (T0), TOM was more \(^{13}\)C-enriched at pre-industrial than at current [CO\(_2\)] (with increases of 5.4\%\(_{\text{respired}}\) and 7.3\%\(_{\text{respired}}\) in spikes, 7.7\%\(_{\text{respired}}\) and 6.3\%\(_{\text{respired}}\) in leaves, 7.1\%\(_{\text{respired}}\) and 8.6\%\(_{\text{respired}}\) in stems, 8.7\%\(_{\text{respired}}\) and 7.4\%\(_{\text{respired}}\) in roots for Blanqueta and Sula, respectively) and more \(^{13}\)C-depleted at future [CO\(_2\)] than at current [CO\(_2\)] (with decreases of 9.7\%\(_{\text{respired}}\) and 8.2\%\(_{\text{respired}}\) in spikes, 6.4\%\(_{\text{respired}}\) and 9.5\%\(_{\text{respired}}\) in leaves, 8.6\%\(_{\text{respired}}\) and 8.6\%\(_{\text{respired}}\) in stems, 8.3\%\(_{\text{respired}}\) and 9.7\%\(_{\text{respired}}\) in roots for Blanqueta and Sula, respectively; Fig. 1, Figure S1). In general, the Blanqueta genotype was between 2.7\%\(_{\text{respired}}\) and 5.9\%\(_{\text{respired}}\) more \(^{13}\)C-enriched than Sula in all organs and CO\(_2\) treatments, with some exceptions, e.g. root and stem at elevated CO\(_2\). It is worth noting that plants at future CO\(_2\) levels were grown with a \(\delta^{13}\)C of ca. −22.6 ± 0.9\%\(_{\text{respired}}\) inside the plant growth chamber, while the \(\delta^{13}\)C of the other two plant growth chambers were ca. −10.8 ± 0.5\%\(_{\text{respired}}\) and −11.2 ± 0.6\%\(_{\text{respired}}\) for 260 and 400 ppm, respectively (see Material and Methods).

During labelling, the \(\delta^{13}\)C of the air in the three plant growth chambers was ca. 165\%\(_{\text{respired}}\). After labelling (T1), the \(\delta^{13}\)C of TOM in labelled plants was more \(^{13}\)C-enriched than the corresponding non-labelled plants in both wheat genotypes (Fig. 1), where spikes were the main C sink. In general, Blanqueta was more \(^{13}\)C-enriched than Sula in all organs and CO\(_2\) treatments. However, plants were more \(^{13}\)C-depleted at future than at current CO\(_2\) conditions and more \(^{13}\)C-enriched at pre-industrial than current CO\(_2\) (F = 23.05, P < 0.001). We also observed that on the last sampling day (T2), plants were less \(^{13}\)C-enriched at higher [CO\(_2\)].

The \(\delta^{13}\)C of respired CO\(_2\) (\(\delta^{13}\)CO\(_2\) Respired) in the dark in the different plant organs was analysed with a GC-C-IRMS. It was observed that, \(^{13}\)C losses through dark respiration (Fig. 2) were larger in Blanqueta than in Sula. Although respiration is an important C sink in all organs, respiration of recently fixed C was higher in spikes and stems compared to flag and other leaves. Before labelling (T0), \(\delta^{13}\)CO\(_2\) Respired was ca. −26\%\(_{\text{respired}}\) for leaves, −26\%\(_{\text{respired}}\) for roots, −30\%\(_{\text{respired}}\) (Sula) and −34\%\(_{\text{respired}}\) (Blanqueta) for spikes, and −34\%\(_{\text{respired}}\) for stems in the 700-ppm treatment. In the other CO\(_2\) treatments, respired CO\(_2\) was more \(^{13}\)C-enriched. After labelling (T1), the \(\delta^{13}\)CO\(_2\) Respired was positive in all organs, indicating that plants had assimilated labelled C, however, the largest quantities were found in spikes and stems. Moreover, the \(\delta^{13}\)CO\(_2\) Respired values increased with decreasing [CO\(_2\)] (Fig. 2). We also observed that on the last sampling day (T2), the \(\delta^{13}\)CO\(_2\) Respired was negative once again.

Figure 3 shows a simplified diagram of recently fixed C and respired CO\(_2\) for wheat at 700 (Fig. 3A), 400 (Fig. 3B) and 260 (Fig. 3C) ppm. We assumed that 100% of C supplied during short-term labelling could be assimilated by the different parts of the plant, and that this C was allocated through three main processes: (i) storage in the plant tissues, (ii) translocation to other organs of the plant and (iii) losses through plant respiration.

The percentage of new C in TOM and CO\(_2\) respired after the labelling at T1 revealed that the C stored was higher in Blanqueta than in Sula for the different wheat organs and that the spike, followed by leaves, was the organ with the highest percentage of new C; Blanqueta showed double or higher percentage of new C in pre-industrial [CO\(_2\)], up to seven times more in current [CO\(_2\)] and almost the same value at future [CO\(_2\)] than Sula. However, 10 days after labelling (T2), the percentages of new C in both varieties decreased. Plants at future [CO\(_2\)] had less new labelled C than treatments at current [CO\(_2\)], and plants at pre-industrial [CO\(_2\)] had a higher percentage of new C overall. After labelling, plants exposed to future and pre-industrial [CO\(_2\)] had higher percentages of new C in respired CO\(_2\) than at current CO\(_2\) treatments. Interestingly, Sula showed a higher percentage of new respired C in the different parts of the plant except in the flag (and only at future CO\(_2\)), whereas Blanqueta showed higher percentages than Sula in the other treatments. Figure 3 shows that, in both genotypes, losses of assimilated new C after the labelling through respiration were between 20–35% at 700 ppm and 10–30% at 260 ppm.

Before \(^{15}\)N labelling (Figure S1), significant differences in \(\delta^{15}\)N were found between CO\(_2\) treatments (F = 9.61, P < 0.05), but not between genotypes (F = 0.527, P = 0.471). At pre-industrial [CO\(_2\)], organs were more \(^{15}\)N-depleted than at current [CO\(_2\)] (0.7\%\(_{\text{respired}}\) and 0.5\%\(_{\text{respired}}\) in spikes, 0.4\%\(_{\text{respired}}\) and 1\%\(_{\text{respired}}\) in leaves, 0.6\%\(_{\text{respired}}\) and 1\%\(_{\text{respired}}\) in stems, 5\%\(_{\text{respired}}\) and 4\%\(_{\text{respired}}\) in roots) and more \(^{15}\)N-enriched at future CO\(_2\) levels than at current [CO\(_2\)] (18\%\(_{\text{respired}}\) and 2.7\%\(_{\text{respired}}\) in spike, 21\%\(_{\text{respired}}\) and 2.2\%\(_{\text{respired}}\) in leaves, 14\%\(_{\text{respired}}\) and 4\%\(_{\text{respired}}\) in stem, 3\%\(_{\text{respired}}\) and 2\%\(_{\text{respired}}\) in roots, for Blanqueta and Sula, respectively;
Figure S1). After labelling with $^{15}$NH$_4$$^{15}$NO$_3$, at future [CO$_2$], Sula had a higher $\delta^{15}$N than Blanqueta. In the other CO$_2$ treatments, Blanqueta was more $^{15}$N-enriched. Furthermore, at 260 ppm, between labelling and the end of grain filling, spikes and leaves were more $^{15}$N-enriched, while roots and stems showed decreases in $\delta^{15}$N. In this same period, plants at pre-industrial [CO$_2$] had more $^{15}$N than plants in current conditions, which in turn were more $^{15}$N-enriched than plants at future [CO$_2$] (Fig. 1).

The percentage of N absorbed during the labelling ($^{15}$NH$_4$$^{15}$NO$_3$) was calculated as a percentage of new N in T1 and T2. Overall, the percentage of new N found in the different plant organs and genotypes ranged from 0.6% (i.e. spike in Blanqueta at future CO$_2$) to 8.8% (i.e. roots in Blanqueta at pre-industrial CO$_2$; Table 2). Sula presented a higher percentage of new N in the spike at future CO$_2$ (3.6%) than Blanqueta (0.6%), whereas Blanqueta presented higher percentages of new N than Sula in the other treatments (2.8% and 4.7% higher at current [CO$_2$], 3.6% and 4.8% at pre-industrial CO$_2$ in Sula and Blanqueta, respectively). This percentage of new N was higher in plants growing at lower [CO$_2$] in both genotypes. We also found that at T2, percentages were increased in spikes and leaves, since spikes are an important N sink. Percentage of new N content decreased in roots as N was redirected to other organs. In stems, the percentage of new N decreased at future [CO$_2$] since N was allocated to the leaf or spike where it was used for storage or the production of new proteins and metabolites. This was not the case at pre-industrial [CO$_2$], where the percentage of new N increased in the stem.

All genotypes showed higher leaf and spike N content (mg N g$^{-1}$) at lower [CO$_2$] at T0 and T1. However, at T2, leaf N content was higher at current [CO$_2$] than in any other CO$_2$ treatment (Table 3). On the other hand, data was more variable in stems with the lowest N content at current [CO$_2$], at T0 and...
T1 but not at T2. In roots, the trend was more constant where N content decreased with higher levels of CO\textsubscript{2} for both Sula and Blanqueta genotypes, except for Blanqueta at T2. The relationship between \textit{d}\textsubscript{15}N and \textit{d}\textsubscript{13}C in TOM in Sula and Blanqueta genotypes before and after labelling and at the different \textit{CO}_2 levels (700, 400 and 260 ppm) showed a positive correlation ($r^2 = 0.834$, $P < 0.05$; Fig. 4). Table 1 shows that plants underwent an acclimation process under the different \textit{CO}_2 treatments, which can also in part explain C allocation. At future [CO\textsubscript{2}], we observed that carboxylation activity was diminished by the reduction in $A_{\text{max}}$ and $A_{\text{sat}}$. Furthermore, plants had a lower rate of $V_{c_{\text{max}}}$ and $J_{\text{max}}$ contributing to RuBP regeneration. $V_{c_{\text{max}}}/J_{\text{max}}$, $A_{\text{sat}}$, $A_{360}$ and $A_{\text{max}}$ increased after prolonged exposure to pre-industrial [CO\textsubscript{2}], although differences between \textit{CO}_2 treatments and genotypes were not significant.

In terms of biomass parameters, significant differences were found between \textit{CO}_2 treatments and genotypes. It was observed that \textit{CO}_2 treatments had a larger effect on the biomass of Sula than Blanqueta (Table 4), although there were no significant differences in TLA between \textit{CO}_2 treatments ($F = 1.872$, $P > 0.05$) and shoot biomass between genotypes ($F = 2.715$, $P > 0.05$). Shoot DW and root DW were lower at lower \textit{CO}_2 levels. Sula showed higher TLA, spike biomass, shoot and root DW at future [CO\textsubscript{2}] ($F = 27.264$, $P < 0.001$). Higher values in the spike biomass, shoot DW and TLA, and lower values in root DW were found at pre-industrial [CO\textsubscript{2}] compared with current [CO\textsubscript{2}]. Finally, Blanqueta had more vegetative and less reproductive biomass than Sula.

**DISCUSSION**

In our study, the partitioning and allocation of C and N in two wheat genotypes Sula (modern) and Blanqueta (traditional) in three different \textit{CO}_2 concentrations were characterized by means of \textit{C} and \textit{N} allocation under different \textit{CO}_2 concentrations.

**Photosynthetic acclimation at different \textit{CO}_2 concentrations**

Plant photosynthesis showed acclimation to the different \textit{CO}_2 levels. Currently, photosynthetic acclimation to different...
CO₂ levels is one of the key issues in CO₂ research, and it has been demonstrated that acclimation to pre-industrial and future CO₂ during long-term exposure can compensate for the effects of CO₂ variation in plant processes (Sage & Coleman 2001). Plants used in this study, which were maintained at optimal water and nutritional conditions, showed an
Table 2. CO₂ effects (700, 400 and 260 ppm) on the percentage of new N in spikes, leaves, stems and roots in durum wheat Blanqueta and Sula genotypes 1 day (beginning grain filling, T1) and 10 days (end of grain filling, T2) after labelling. Labelling was carried out during the anthesis period.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Organ</th>
<th>700</th>
<th>400</th>
<th>260</th>
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<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Blanqueta</td>
<td>Spike</td>
<td>0.6 ± 0.2</td>
<td>1.4 ± 0.6</td>
<td>4.7 ± 0.8</td>
</tr>
<tr>
<td>Sula</td>
<td>Spike</td>
<td>3.6 ± 0.5</td>
<td>2.6 ± 0.1</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>Blanqueta</td>
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<td>0.7 ± 0.3</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>Sula</td>
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<td>1.1 ± 0.1</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>Blanqueta</td>
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<td>0.9 ± 0.3</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>Sula</td>
<td>Stem</td>
<td>5.7 ± 0.4</td>
<td>2.0 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Blanqueta</td>
<td>Root</td>
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<td>0.9 ± 0.2</td>
<td>6.6 ± 0.7</td>
</tr>
<tr>
<td>Sula</td>
<td>Root</td>
<td>5.7 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>3.1 ± 0.2</td>
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</tbody>
</table>

Data are means ± SE, n = 4.

Table 3. CO₂ effects (700, 400 and 260 ppm) on N content (mg g⁻¹) in leaf, spike, stem and root of durum wheat Blanqueta and Sula genotypes before labeling (before anthesis, T0), 1 day after labeling (beginning of grain filling, T1) and 10 days after labeling (end of grain filling, T2).

<table>
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<tr>
<th>CO₂ treatment</th>
<th>Timing</th>
<th>Genotype</th>
<th>Organ</th>
<th>N content</th>
<th>± SE</th>
<th>N content</th>
<th>± SE</th>
<th>N content</th>
<th>± SE</th>
<th>N content</th>
<th>± SE</th>
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<th>± SE</th>
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<tbody>
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<td></td>
<td></td>
<td>Blanqueta</td>
<td>Leaf</td>
<td>1.6</td>
<td>0.3</td>
<td>1.9</td>
<td>0.3</td>
<td>2.5</td>
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<td>2.4</td>
<td>0.2</td>
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<td>1.3</td>
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<td>3.5</td>
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<td>2.0</td>
<td>0.2</td>
<td>2.4</td>
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<tr>
<td></td>
<td></td>
<td>Blanqueta</td>
<td>Leaf</td>
<td>1.7</td>
<td>0.3</td>
<td>2.5</td>
<td>0.3</td>
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<td></td>
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<td>Sula</td>
<td>Leaf</td>
<td>1.6</td>
<td>0.4</td>
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</tr>
<tr>
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<td>Blanqueta</td>
<td>Spike</td>
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<td>2.5</td>
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</tr>
<tr>
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<td>Spike</td>
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<td>Blanqueta</td>
<td>Stem</td>
<td>1.5</td>
<td>0.4</td>
<td>0.8</td>
<td>0.1</td>
<td>1.4</td>
<td>0.2</td>
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<td></td>
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<tr>
<td></td>
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<td>Root</td>
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<td>2.1</td>
<td>0.3</td>
<td>1.6</td>
<td>0.3</td>
<td>2.1</td>
<td>0.3</td>
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</tbody>
</table>

Data are means ± SE, n = 4.

Fig. 4. δ¹³C and δ¹⁵N correlation in TOM of the leaf in wheat plants and three CO₂ treatments (700, 400 and 260 ppm), before labelling (before anthesis, T0), 1 day after labelling (beginning of grain filling, T1) and 10 days after labelling (end of grain filling, T2). Labelling was carried out during 3 days in anthesis. Linear regression is significant (r² = 0.834; P < 0.05).
up-regulation of photosynthesis with an increase and/or maintenance of physiological parameters such as \( V_{c,max} \) and \( A_{sat} \) in response to pre-industrial \([CO_2]\) (Sage & Reid 1992; Cowling & Sage 1998; Anderson et al. 2001). On the other hand, many studies have shown that photosynthesis also accelerates to future \([CO_2]\) over long-term experiments through down-regulation (Ainsworth et al. 2003; Urban 2003; Long et al. 2004; Aranjuelo et al. 2009b; Pardo et al. 2009) and, consequently, photosynthetic capacity decreases. Here, it was found that \( V_{c,max} \), \( I_{max} \), \( A_{sat} \) and \( A_{360} \) (Table 1) were lower at future \([CO_2]\) than at current levels, clearly indicating a down-regulation of photosynthesis. This implies that significant photosynthetic adjustments (together with increases in N, Rubisco and protein content at pre-industrial \([CO_2]\) and decreases at future \([CO_2]\)) may have taken place in the past (up-regulation) and will certainly be likely to occur in the future (down-regulation), thereby changing the allocation and balance of C and N in plants.

Different plant responses are conditioned by the genetics of the different genotypes (Reynolds et al. 1999) and the availability of C and N pools (Fuertes-Mendizabal et al. 2010). In our study, traditional genotypes produced more vegetative and less reproductive biomass than did modern genotypes. Plant growth and C and N distribution was also modulated by the different \([CO_2]\) treatments. Sula plants showed a large capacity to increase biomass of the spike and root at future \([CO_2]\), but less capacity at current or pre-industrial \([CO_2]\) (Aranjuelo et al. 2013). However, in the case of the traditional genotype, vegetative shoots (i.e. leaves and stems) were the main C and N sinks. On the other hand, this growth response was associated with increased C allocation from assimilation areas to the spike, especially when C was limited, thereby resulting in an increase in the shoot/root ratio. Lehmeier et al. (2005) found changes in shoot/root ratios at future and pre-industrial \([CO_2]\). Optimal C allocation favours those parts of the plant that incur the most severe growth limitations due to a lack of resources (McConnaughay & Coleman 1999). In our case, the shoot/root ratio increased in the Sula genotype with decreasing \([CO_2]\), from future to pre-industrial \([CO_2]\) (Table 4). Plants at pre-industrial \([CO_2]\) may have stimulated C allocation to shoots organs for grain filling (Anderson et al. 2010). Blanqueta showed a decrease in shoot/root ratio from future to current \([CO_2]\); however, this effect was not seen at pre-industrial \([CO_2]\). This could be explained by the fact that (i) the C requirement of the reproductive shoot area is lower in the traditional genotype than in the modern genotype, and/or (ii) traditional plants have a bigger vegetative shoot area than Sula (plants were also up-regulated). For this reason, it may be possible that Blanqueta can invest more C in the root at pre-industrial \([CO_2]\).

### Dynamics of C and N

New \(^{13}\text{C}\) and \(^{15}\text{N}\) was allocated to all the organs of the plant in both genotypes in the different \([CO_2]\) treatments. The largest C sinks were the spike and respiration, since a large percentage of \(^{13}\text{C}\) was quickly respired in the first 24 h after labelling (Tcherkez et al. 2003; Noguès et al. 2004). In our case, 10 days after labelling, plants had lost most \(^{13}\text{C}\) through respiration, and a large proportion of the remaining \(^{13}\text{C}\) had been allocated to the spike. Plants at future \([CO_2]\) were less enriched in \(^{13}\text{C}\) than at current or pre-industrial conditions because, on the one hand, they were photosynthetically down-regulated and the rate of assimilation was lower, and on the other hand, plants at pre-industrial \([CO_2]\) discriminated less against \(^{13}\text{C}\) because the availability of \([CO_2]\) was lower than in plants grown at higher levels of \([CO_2]\) (Farquhar et al. 1989). Thus, \(^{13}\text{C}\) in TOM of plant organs increased proportionally with lower \([CO_2]\) concentrations in the environment.

The percentage of new C in TOM after the labelling confirmed that <20% of C in the different organs, genotypes and treatments was derived from recently assimilated C (Fig. 3; Noguès et al. 2004, 2014; Aranjuelo et al. 2009a). The majority of photoassimilates formed in leaves might be destined for the following processes: (i) respiration, (ii) storage and (iii) export to other tissues (Noguès et al. 2004). However, the photoassimilates in the spike are mainly destined for respiration and storage in the grain. In our case, the traditional genotype fixed more new C than did the modern genotype, however traditional plants also respired more the new C and had less new C fixed in the different organs than did modern plants 10 days after labelling (T2). This suggests that in modern plants, the spike was the largest C sink, whereas this was respiration in the traditional genotype. \(^{13}\text{C}\) increased in leaves and stems at T2, but decreased in the spike due to respiration and in roots through translocation and respiration. This suggests that at pre-industrial \([CO_2]\), photoassimilates are stored in stems.
and/or leaves as plants cannot increase the capacity of the spike as a new sink.

Respired CO₂ by Blanqueta and Sula (exposed to the three levels of CO₂) was enriched in ¹³C at T1 (Fig. 2), implying that some of the C assimilated during labelling was immediately respirated (Noguès et al. 2014). However, the fact that respired CO₂ had low levels of ¹³C enrichment implies that a large part of the respired C originated in C stored in the organs prior to labelling (Fig. 1; Aranjuelo et al. 2009a). C losses through dark respiration were high in the spike and less so in the flag or leaves since these allocated photoassimilates to grain filling (Evans et al. 1975; Schnyder 1993). Ten days after labelling (T2), δ¹³C_CO₂ respired values were similar to those of δ¹³C_CO₂ respired before labelling, suggesting that leaves and other organs had used almost all of the labelled C substrate. The δ¹³C values of TOM at T1 suggest that plant organs used the labelled C, and the δ¹³C of TOM at T2 shows that some ¹³C still remained in the plants. After anthesis and during grain filling, the spike had a high demand for photoassimilates, which are supplied by spike photosynthesis and by C translocation from flag leaves, other leaves and stem internodes (Gebbing & Schnyder 1999; Tambussi et al. 2007; Aranjuelo et al. 2009a, 2011b). Our data showed that exposure to future [CO₂] modified the ¹³C enrichment of respired CO₂, as the CO₂ used for establishing the 700 ppm atmosphere would have an ca. 10% more negative δ¹³C value, which is also reflected in the respired CO₂ of the plants grown at 700 ppm, which was lower than at current and pre-industrial [CO₂] prior to labelling (Fig. 2). However, after labelling, plants exposed to pre-industrial [CO₂] had higher values of δ¹³C (in TOM and respired CO₂) and needed more time to lose the enriched ¹³C in TOM after labelling because in pre-industrial environments plants discriminate less and are more enriched in ¹³C (Fig. 1) than at current [CO₂].

Labelled ¹⁵N was applied with the ¹⁵NH₄⁺¹⁵NO₃ of the solution. Spikes and roots were the main N sinks. Plants were less enriched in ¹⁵N at future [CO₂] than at pre-industrial [CO₂] (Fig. 1) as photosynthesis was down-regulated and N demand was lower. This suggests that acclimation and CO₂ treatments also affected the allocation and distribution of N. We found significantly higher levels of leaf N in both genotypes at pre-industrial CO₂. Anderson et al. (2001) showed that the up-regulation of assimilation might be related to increases in leaf N content as well as to the re-allocation of N within leaves. Moreover, increases in leaf N and Rubisco would be needed for plants grown at 260 ppm CO₂ to achieve a similar level of photosynthetic activity as plants grown at 400 ppm (Sage & Reid 1992). Cernusak et al. (2011) showed that N and C content decreased in plants in response to future [CO₂], as was seen in our study in leaves, spikes and roots for both genotypes, which would provide additional evidence for down-regulation in plants (Figure S2).

After labelling and at future [CO₂], both genotypes had lower ¹⁵N than at current or pre-industrial [CO₂] (Fig. 1). However, Blanqueta was more ¹⁵N-depleted than Sula at future [CO₂], whereas in the other CO₂ treatments the opposite was true (with Sula being more δ¹⁵N-depleted). Also, we found different δ¹⁵N levels between organs. These data suggest that changes in organ δ¹⁵N (i.e. leaves, stems, roots or spikes) can be attributed to internal processes related to the assimilation and loss of ¹⁵N (as, e.g. translocation between organs or root exudates) in the plant and may depend on the genotype and environmental conditions (BassiriRad et al. 2003). Many studies have attempted to explain why there are differences in the δ¹⁵N signal between roots and shoots, and between different CO₂ levels: (i) changes in the fractionation processes within the plant–mycorrhizal system and/or changes in nitrate assimilation enzymes, which discriminate heavily against ¹⁵N (BassiriRad et al. 2003); (ii) the influence of C availability and soil moisture on microbial activity, thereby enriching plant-available N (Dijkstra & Cheng 2008); (iii) the correlation between the influence of plant transpiration in the N acquisition from the soil (Cernusak et al. 2009); and (iv) isotopic fractionations along metabolic reactions (Tcherkez 2010; Gauthier et al. 2013). Furthermore, there is evidence that plant assimilation of nitrate can vary at different CO₂ levels (Bloom et al. 2014). In our experiment, we assume a reduction of nitrate assimilation in the shoot under future [CO₂], in agreement with previous work (Robinsson 2001; Kruse et al. 2002). With regard to the percentage of new N, the same pattern was found in plants at future [CO₂] (i.e. at future [CO₂] Sula had a higher percentage of new N than Blanqueta). This suggests that the modern plant (with large C sinks) at future CO₂ conditions will be better adapted to assimilate more N than traditional plants. However, at current and pre-industrial [CO₂], traditional plants had higher percentages of new N, suggesting that Blanqueta is better adapted to the assimilation of N at these levels of CO₂.

Furthermore, the correlation between δ¹⁵N and δ¹³C (r² = 0.834, P < 0.05; Fig. 4) confirms that the distribution of labelled C and N was different between treatments and genotypes, suggesting that the partitioning and allocation of C and N was affected both by the genetics of the different genotypes of wheat and the CO₂ growth conditions. This allocation is reflected in Figure S2, where the N/C ratio decreases as the overall CO₂ increases in leaves, suggesting that at future [CO₂], leaves are more N- and C-limited. This is in accordance with Cernusak et al. (2011).

CONCLUSIONS

The effects of future and pre-industrial [CO₂], after exposure for a whole growing season, on two genotypes of durum wheat (traditional and modern) were studied. Our data showed, in accordance with photosynthetic parameters, a reduction in net photosynthesis rates and Vc,max at future [CO₂], indicating a clear down-regulation. Plants showed acclimation at future and pre-industrial [CO₂], with down- and up-regulation of photosynthesis, respectively. However, at future [CO₂], this photosynthetic acclimation was disrupted when a new C sink appeared during grain filling. The pre-industrial CO₂ treatment decreased growth and biomass production in both genotypes; however, these effects decreased over time, demonstrating a clear up-regulation of photosynthesis. Also, Blanqueta and Sula modulated the assimilation of ¹³C in accordance with CO₂ level, i.e. plants were less enriched in ¹³C at future [CO₂] and more enriched in ¹³C at pre-industrial [CO₂].

In our study, we observed the importance of the sink in terms of the response of plants to different CO₂ scenarios. Plants invested more C in shoots than roots at pre-industrial [CO₂], and specifically, in the case of the traditional genotype, vegetative parts were seen to be the main C sink. At current and future [CO₂], the source of C is higher and plants can redirect more from assimilation compartments (shoots) towards
Figure S1. Effects of three different CO₂ treatments (700 ppm, δ13CO₂-air −22.6‰, 400 ppm, δ13CO₂-air −11.2‰, and 260 ppm δ13CO₂-air −10.8‰) on the natural abundance of δ13C and δ15N in total organic matter (TOM) of flag leaf, other leaves, spikes, stems and roots in two different genotypes of durum wheat: Blanqueta (open bars) and Sula (close bars).

Figure S2. CO₂ effects (700, 400 and 260 ppm) on C and N content (mg·mg⁻¹) in spikes, leaves, stems and roots of durum wheat. Table S1. Statistical analysis of CO₂ effects (700, 400 and 260 ppm) on δ13C (‰) and atom% 15N values of total organic matter (TOM) in spikes, leaves, stems and roots of two different genotypes of durum wheat: Blanqueta (open bars) and Sula (close bars) before labelling (before anthesis, T0), 1 day after labelling (beginning of grain filling, T1) and 10 days after labeling (end of grain filling, T2).

References


Climate Change, Working group 1, Geneva, Switzerland.

IPCC (2013) Fifth assessment report on climate change 2013: the physical science basis, final draft underlying scientific-technical assessment. Intergovernmental Panel on Climate Change, Working group 1, Geneva, Switzerland.


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