Journal of Plant Sciences
2018; 6(3): 68-86
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20180603.11
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

Photosynthesis, Resource Acquisition and Growth Responses of Two Biomass Crops Subjected to Water Stress

Elena Sánchez, Gladys Lino, Claudia Arias, Xavier Serrat, Salvador Nogués*

Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Barcelona, Catalonia, Spain

Email address:
salvador.nogues@ub.edu (S. Nogués)

*Corresponding author

To cite this article:
Elena Sánchez, Gladys Lino, Claudia Arias, Xavier Serrat, Salvador Nogués. Photosynthesis, Resource Acquisition and Growth Responses of Two Biomass Crops Subjected to Water Stress. *Journal of Plant Sciences*. Vol. 6, No. 3, 2018, pp. 68-86.
doi: 10.11648/j.jps.20180603.11

Received: May 2, 2018; **Accepted**: May 19, 2018; **Published**: June 25, 2018

Abstract: This study compares photosynthesis, growth, ^{13}C and ^{15}N labelling patterns of two biomass crops (*Arundo donax* L. and *Panicum virgatum* L.) grown under water stress in greenhouse conditions. Plants were exposed to three water stress levels: control (*C*, 100% Pot Capacity), mild stress (*MS*, 50% PC) and severe stress (*SS*, 25% PC). Photosynthesis, fluorescence parameters and relative water content were measured at the beginning (Ti) and the end of the experiment (Tf). Biomass parameters were measured at Tf. Short-term double labelling with ^{13}C and ^{15}N stable isotopes was performed in both species. Isotopic analyses of total organic matter, total soluble sugars and the CO_2 respired were undertaken at T0 (pre-labelling), T1 (24h after labelling) and T2 (7 days after labelling). Immediately after the ^{13}C and ^{15}N labelling, stems and rhizomes seemed to be the main sinks for labelled carbon and nitrogen in both species. Moreover, not all of the labelled carbon and nitrogen substrate was used by plant metabolism after seven days. Decreases in photosynthesis parameters were observed as a consequence of the increase in water stress (*WS*) in both species, with a greater magnitude decline in giant reed than in switchgrass. A decrease in height, number of green leaves and total dry weight due to *WS* was observed in both species. Both species were more ^{13}C-enriched and more ^{15}N-depleted during the increases in *WS* due to lower stomatal conductance and transpiration. In general, *WS* accelerated plant phenology and, consequently, the accumulation of storage compounds in the rhizome occurred in response to stress. This effect was more clearly visible in switchgrass than in giant reed.

Keywords: *Arundo donax* L., *Panicum virgatum* L., ^{13}C and ^{15}N Isotope Labelling, Biomass, Water Stress

1. Introduction

The use of biomass for energy production as a substitute for fossil energy is often seen as an attractive option to reduce fossil-fuel dependency and help lower greenhouse gas emissions. Some species used as biomass sources are perennial rhizomatous grasses, belonging to the second generation of biofuels, which are produced by non-food crops such as *Arundo donax* L. (giant reed) and *Panicum virgatum* L. (switchgrass) [1, 2]. Both species have been used as energy crops in Europe in recent decades [1].

Giant reed, which is widely distributed in warm temperate regions all over the world [3], and switchgrass, which is a native perennial warm-season grass [1], both belong to the *Gramineae* family. The two species have high biomass productivity and are harvested annually [1, 4]. In relation to the photosynthetic pathway, giant reed is a C_3 species, whereas switchgrass utilizes the C_4 pathway [1, 5, 6]. Although giant reed has been described as a C_3 plant with a high photosynthetic potential [5], it is well known that the C_4 pathway is more efficient than the C_3 pathway, due to its Kranz anatomy that avoids photorespiration by raising the $[CO_2]$ around Rubisco (i.e. ribulose-1, 5-biphosphate carboxylase/oxygenase). In C_4 species the initial CO_2 fixation occurs in the mesophyll cytosol where CO_2 is converted to bicarbonate and then fixed by phosphoenolpyruvate

carboxylase (PEPc), the primary carboxylating enzyme in C_4 species. The C_4 photosynthetic pathway is classified into three biochemical subtypes based on the primary C_4 decarboxylase enzyme [7] and specialized leaf anatomy and physiology are associated with each of the C_4 subtypes [8]. In the case of switchgrass (the Alamo cultivar), it has been described as a lowland NAD-ME ecotype [9–12].

Water stress (*WS*) is one of the most serious environmental stresses affecting plant performance and agricultural yield. To maximize productivity, plants optimize the morphology, physiology and metabolism of their organs and cells, so plants have various mechanisms of adaptation to water-limiting environments [13]. During progressive *WS*, plants reduce water losses by closing their stomata [13, 14], which reduces the assimilation rate due to a depletion of intercellular [CO_2] (c_i) [15–17]. Among the consequences of *WS* are decreases in water content (i.e. measured as RWC) as well as a decrease in cell elongation. Consequently, plant growth (i.e. biomass production) could be affected. Many plant species can tolerate *WS* by maintaining low osmotic potential or accumulating solutes (i.e. osmolytes) through osmotic adjustment. *WS*-tolerant species have the advantage of maintaining cell turgor and growth under adverse conditions, whereas those displaying dehydration avoidance mechanisms (such as stomatal closure, leaf abscission or leaf rolling) display greatly reduced growth during stress [18].

During the last few decades, stable isotopes have emerged as a useful tool in plant physiology studies. Discrimination against ^{13}C during photosynthesis is a well-characterised phenomenon [19]: the heavy stable isotope of carbon (^{13}C) is discriminated in favour of the more abundant and light isotope (^{12}C). In C_3 species, the carbon isotope composition of plant material is primarily caused by the discrimination occurring during carboxylation by the enzyme Rubisco (*ca*. 29‰) and during the diffusion of CO_2 from the atmosphere to the chloroplast (*ca*. 4.4‰) [20], providing an integrated measure of C isotope discrimination that averages -22‰ for most C_3 species [21]. In relation to C_4 plants, this metabolic pathway is characterized by a CO_2 concentrating mechanism across the mesophyll cell and bundle sheath cell [22, 23]. According to Farquhar [24], carbon isotope discrimination in C_4 species reflects biochemical fractionation of Rubisco and PEPc as well as their interconnectivity, which leads the carbon isotope composition of C_4 species to fall into a narrow range between -12‰ and -15‰ [19, 25–27].

Nitrogen (N) is an essential element for life, and plant growth and development depend upon its supply and assimilation. N can be absorbed as nitrate (NO_3^-) (the dominant form) and ammonium (NH_4^+). After root uptake by transporters, the first step in nitrate assimilation is reduction to nitrite (NO_2^-) by nitrate reductase (NR). While a significant portion is reduced in the leaves, a fraction of this nitrate is nevertheless reduced in roots [28]. Subsequently NO_2^- is reduced to NH_4^+ by nitrite reductase, which is further incorporated into amino acids. The use of stable isotopes ($^{12}C/^{13}C$ and $^{14}N/^{15}N$) has made a significant contribution towards understanding N assimilation and the interactions with C allocation [28]. Similar to C, plant enzymes discriminate between heavy (^{15}N) and light (^{14}N) isotopes of N as a consequence of the faster reactivity of the latter. If nitrate availability is high and the resistance associated with nitrate uptake is low, nitrate reduction consumes fewer of the available nitrate molecules and $^{14}N/^{15}N$ discrimination is produced during reduction. On the other hand, if nitrate availability is low, no $^{14}N/^{15}N$ discrimination is possible during N reduction [28].

The aim of this study was to determine the effect of *WS* on plant performance (i.e. photosynthesis, physiological parameters and biomass production) of two of the main perennial rhizomatous grass species used for biomass production: *Arundo donax* L. and *Panicum virgatum* L. The $^{13}CO_2$ isotope labelling technique was used to study the partitioning of recently fixed C in each organ (i.e. leaves, stems, roots and rhizomes) and the respiratory metabolism after labelling under *WS* conditions. Moreover, a $^{15}NH_4$-$^{15}NO_3$ isotope labelling technique was used to study the N cycle in these plants under *WS*.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Arundo donax L. (giant reed) seedlings were obtained from a private company (Piccoplant, Oldenburg, Germany). *Panicum virgatum* L. (switchgrass) cv. Alamo plants were obtained from seeds that were germinated on a moist filter in a Petri dish, placed in a long day chamber (16h of photoperiod) at day/night temperatures of 22/18 ºC, respectively, and 70% HR. Giant reed and switchgrass were grown for six months in a greenhouse at the Experimental Field Service of Barcelona University (Barcelona, Spain) in 5-L plastic pots filled with peat:perlite:vermiculite (3:1:1) and were irrigated with a complete Hoagland solution (Hoagland and Arnon, 1950). The average temperatures and vapour pressure deficit during the growth period were 25/15ºC (day/night) and 0.75 kPa, respectively. Relative humidity ranged from 40 to 65% and the maximum photosynthetic photon flux density (PPFD) was ~1000 µmol $m^{-2}s^{-1}$. The $\delta^{13}C$ of CO_2 in the greenhouse air ($\delta^{13}C_{greenhouse}$) was – 11.8‰ ± 0.1‰.

In order to study the response of these two species to different *WS* levels, after six months of growth the plants were subjected to three water treatments for 21 days. One third of the plants were fully irrigated (*C*; n=9) and two thirds of the plants were subjected to *WS* by withholding water until 50% of pot capacity (*MS*, mild stress; n=9) or until 25% of pot capacity (*SS*, severe stress; n=9). Afterwards, the same plants were used to study resource acquisition through application of ^{13}C and ^{15}N labelling procedures.

2.2. Measurements

2.2.1. Gas Exchange Parameters

Leaf-level gas exchange was measured using a portable photosynthesis system (Li6400, Li-Cor Inc., Lincoln, NE,

USA) provided with a Leaf Chamber Fluorometer (6400-40) of 2 cm^2 and a 10% blue light source.

At the beginning of the experiment (Ti), A/C_i curves with chlorophyll fluorescence determinations were conducted between 10:00 and 18:00 h in fully expanded leaves (n=3) from each species at 25 ºC with a light rate saturated at 1200 µmol m^{-2} s^{-1} of PPFD and an airflow rate of 500 ml min^{-1}. The response of A to Ci was constructed by measuring these values at a range of CO_2 concentrations from 70 to 1500 ppm (µmol mol^{-1}). Net CO_2 assimilation rate (A_{sat}, mol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and transpiration (T, mmol H_2O m^{-2} s^{-1}) were measured directly with the Li-Cor. Instantaneous water use efficiency ($_i$WUE) was calculated as = A_{sat}/T. Modulated chlorophyll fluorescence measurements were done at the same time with the Leaf Chamber Fluorometer to estimate the relative quantum yield of photosystem II (ϕ_{PSII}), the efficiency of excitation energy capture by open PSII reaction centres (F'_v/F'_m), the maximum quantum yield of PSII (F_v/F_m) and photochemical quenching (q_p) determined in a totally expanded leaf after 30 min of dark adaptation [29].

After 21 days (Tf) of *WS* treatments, A/C_i curves with chlorophyll fluorescence were also measured in each treatment (n=3) and for both species.

2.2.2. RWC Measurements and Chlorophy ll Content Determinations

Relative water content (RWC, %) of the leaves was determined as (*FW-DW*)/(*TW-DW*) x 100, where *FW* is the fresh weight, *DW* is the dry weight after being dried in an oven at 60ºC until constant weight and *TW* is the turgid weight of the leaf after equilibration in distilled water for 24 h at 4ºC. RWC was calculated as the mean of three leaves for each species and for each treatment at the end of the experiment (Tf).

Chlorophyll content was measured in the youngest fully expanded leaves using a portable meter (Minolta SPAD 502 Meter, Plainfield, IL, USA) at the beginning (Ti) and at the end of the experiment (Tf). Nine plants per treatment were measured (each measurement is the mean value of five measurements that were performed in the middle of each leaf).

2.2.3. ^{13}C and ^{15}N Labelling Procedures

A double labelling treatment with ^{13}C and ^{15}N was conducted in both species (n=9) after the *WS* period in a controlled environment chamber (Conviron E15, Controlled Environments Ltd., Winnipeg, Manitoba, Canada). Plants were kept inside the chamber for an acclimation period of 3 days at a CO_2 concentration of 400 ppm, for a 16-hour light period at 400 µmol m^{-2} s^{-1} of PPFD, and a relative humidity of 70-80% with a temperature regime of 25/18 ºC (light/dark).

Air samples were taken before labelling and the $\delta^{13}C$ of CO_2 of the air inside the Conviron chamber was measured ($\delta^{13}C$ = -11.44 ± 0.12‰). Commercial $^{13}CO_2$ was used to enrich the air inside the Conviron chamber with 99.9% ^{13}C (Euriso-top, Saint-Aubin, France). Air was introduced into the chamber with a 50ml syringe (SGE, Ringwood, Australia) placed on a syringe pump (IV Perfusor, Spritze) at a rate of 12.5 $^{13}CO_2$ ml h^{-1}. This system allowed homogenous labelling throughout the day. The $\delta^{13}C$ inside the chamber during labelling was 360 ± 31‰ for giant reed and 180 ± 22‰ for switchgrass. The ^{13}C labelling time was calculated according to the net assimilation rates of both species in each treatment, assuming that all species assimilate the same amount of labelled CO_2 (ca. 3000 ± 85 and 4500 ± 203 mmol C m^{-2} for giant reed and switchgrass, respectively) [30]. The $\delta^{13}C$ of air samples were determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), as described by Nogués et al. [31], and were carried out at the Scientific and Technological Centres (CCiT) of the University of Barcelona.

The ^{15}N labelling was also applied during the same ^{13}C labelling period by replacing the ^{14}N in the Hoagland solution by $^{15}NH_4{}^{15}NO_3$ enriched with ^{15}N 5.31% (Cortec Net, France). The $\delta^{15}N$ of the solution was 1.4 ± 1.7‰.

Stress conditions were maintained during the labelling period (i.e. *C* plants were fully irrigated whereas *MS* and *SS* plants were kept under 50 and 25% of PC, respectively). After labelling, ^{15}N was removed by washing the substrate with distilled water. Plants were then irrigated with normal Hoagland solution.

Isotopic analysis samples were taken at different times: T0, pre-labelling; T1: 24 h after the end of the labelling and T2: 7 days after the end of the labelling.

(i) $\delta^{13}C$ and $\delta^{15}N$ of total organic matter ($\delta^{13}C_{TOM}$ and $\delta^{15}N_{TOM}$) and total C and N content (C_c and N_c).

Leaf, shoot, rhizome and root samples were collected at T0, T1 and T2, dried at 60ºC until constant weight, and analysed for the C and N isotopic composition of total organic matter ($\delta^{13}C_{TOM}$; $\delta^{15}N_{TOM}$). A ground sample of 0.8 mg was weighed in a tin capsule and used for each determination, and three replicates were analysed for each treatment. The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios (R) of plant material and C_c and N_c (% C g^{-1} dry matter; % N g^{-1} dry matter) were determined using and elemental analyser (EA1108, Series I; Carlo Erba Instrumentazione, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta C; Finnigan, Mat., Bremen, Germany) operating in continuous flow mode at the CCiT of the University of Barcelona. The C/N ratio was estimated in each organ on a dry mass basis.

(ii) $\delta^{13}C$ determination of total soluble sugars (TSS).

The extraction procedures for TSS were similar to those described by Nogués et al. [32]. Leaf powder (50 mg) was suspended with 1 mL of distilled water in an Eppendorf tube (Eppendorf Scientific, Hamburg, Germany). After centrifugation, starch was removed from the pellet by HCl solubilisation. Soluble proteins of the supernatant were heat denatured and precipitated. The TSS of the proteinless extracts were collected and transferred to tin capsules for isotope analysis and dried in an oven at 60 °C. Isotope analysis of TSS was conducted using the same EA-IRMS described above.

(iii) R_d and $\delta^{13}C$ of dark-respired CO_2 determination

($\delta^{13}C_R$).

Prior to the dark respiration determinations, the plants were dark-adapted for 45 min in a dark room. Plant organs (i.e. leaf, shoot, rhizome and root) were placed separately in a plastic gas analysis chamber to determine the $\delta^{13}C$ of dark respired CO_2 ($\delta^{13}C_R$). In the case of rhizome and root respiration analysis, the tissues were cleaned and immediately dried on paper before putting inside the chamber. Determinations of dark respiration (R_d; mol gDW^{-1} s^{-1}) were conducted in a plastic chamber (20 x 12 x 6 x 10^{-6} m^3) with two fans connected to a Li-6400 photosynthesis system (Li-Cor, Lincoln, NE, USA). Ingoing air was passed through the chamber at a rate of 500 ml min^{-1} and the temperature in the chamber was maintained at 25 ºC. The PPFD inside the chamber was maintained at 0 µmol photon m^{-2} s^{-1} by covering the entire system with a black cover. The gas analysis chamber, included in the closed system, was first flushed with CO_2-free air to ensure that only the CO_2 respired in the chamber was accumulated. The CO_2 concentration inside the chamber was measured using the Li-6400. After 5 minutes, CO_2 samples were collected with a 50 ml syringe (SGE, Ringwood, Australia) and immediately injected into a 10 ml vacutainer (BD Vacutainers, Plymouth, UK). The vacutainers were previously over-pressurised with N_2 to avoid retro-diffusion of ambient CO_2 into the syringe. The $\delta^{13}C$ of air samples was also determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), as described by Nogués et al. [31] at the CCiT of the University of Barcelona.

(iv) Calculations.

The $^{13}C/^{12}C$ ratios (R) of the total organic matter (TOM) and air samples and the $^{15}N/^{14}N$ ratios (R) of TOM were expressed as $\delta^{13}C$ and $\delta^{15}N$ values using international secondary standards of known $^{13}C/^{12}C$ ratios (IAEA CH7 polyethylene foil, IAEA CH6 sucrose and USGS 40l-glutamic acid) calibrated against Vienna Pee Dee Belemnite calcium carbonate (VPDB) with and analytical precision of 0.1‰ and $^{15}N/^{14}N$ ratios (IAEA N_1 and IAEA N_2 ammonium sulfate and IAEA NO_3 potassium nitrate) referred to N_2 in air with an analytical precision of 0.2‰.

$$\delta^{13}C \text{ or } \delta^{15}N = \left(\frac{R_{sample}}{R_{standard}} \right) - 1 \quad (1)$$

The carbon ($\Delta^{13}C$) and nitrogen ($\Delta^{15}C$) isotope discrimination was calculated in the TOM from the isotope compositions δ_a and δ_p [21] as:

$$\Delta^{13}C \text{ or } \Delta^{15}N = \frac{\delta_a - \delta_p}{\delta_p + 1} \quad (2)$$

where subscripts "*a*" and "*p*" refer to air and TOM, respectively.

2.2.4. Biomass Parameters

Biomass parameters including height (H, cm), number of stems (NS) and number of green leaves (NGL) were measured at Ti and Tf in both species. Plant organs were separated into leaves, stems, roots and rhizomes and fresh weight (g) was measured as well as leaf and stem area (LA and SA, cm^2) at Tf in each treatment and in both species. Stem area was calculated from the formula for calculating the area of a cylinder, where the height was the stem height and average (n=3) of the diameter was measured with a calliper. Leaf areas were estimated prior to drying using a flat-bed scanner (Hewlett-Packard ScanJet model Iicx, San Diego, USA) and analysed with an image processing program (Image, University of Sheffield, 2003). Dry weight was obtained after drying organs in an oven at 60 ºC until constant weight was reached.

Leaf Dry Weight (LDW, g), Shoot Dry Weight (SDW, g), Total Dry Weight (TDW, g), Shoot/Root ratio (S/R), Leaf Mass Area (LMA; Kg m^{-2}), Specific Leaf Area (SLA, m^2 Kg^{-1}), Leaf Area Ratio (LAR; m^2 Kg^{-1}) and Leaf Weight Ratio (LWR; Kg Kg^{-1}) were calculated.

2.3. Statistical Analysis

The *WS* effect on plant development in both species was tested by two factor (*WS* and time) analyses of variance (ANOVA). The statistical analysis was conducted with the SPSS 21.0 software package (SPSS Inc, Chicago, IL, USA). The means ± standard errors (SE) were calculated for each parameter. When a particular F-test was significant, we compared the means using a Tukey multiple comparison. The results were accepted as significant at $P < 0.05$.

3. Results

3.1. Isotopic Composition

3.1.1. The $\delta^{13}C$ of Total Organic Matter ($\delta^{13}C_{TOM}$) and Total C Content (C_c)

No significant differences were found in the $^{13}C_{TOM}$ between giant reed organs before labelling (T0) in *C* conditions (Figure 1). A ^{13}C-enrichment was observed in each organ with increasing *WS*, and significant differences between *C* and *SS* treatments were found in each organ, except in leaves (P = 0.184). Moreover, no significant differences were found between switchgrass organs before labelling (T0) in *C* conditions or between treatments for the same organ. In general, roots were the most ^{13}C enriched organs in each treatment in giant reed, whereas no significant differences between organs were found in switchgrass at T0. $A^{13}C$ enrichment was observed after labelling (T1) in both species. In general, ^{13}C enrichment was observed in both species and in each organ with increasing levels of *WS* (Figures 1a and 1b), except in stems and roots in giant reed, where the greatest increase was observed in the *MS* treatment (P = 0.034 and 0.004, respectively). Stems had the highest $^{13}C_{TOM}$ at T1 in each treatment in both species. The $^{13}C_{TOM}$ values in *C* conditions at T2 were depleted relative to T1 (Figure 1b) in leaves, stems, roots and rhizomes in both giant reed and switchgrass. The *C* condition was more ^{13}C depleted

than stress conditions (*MS* and *SS*) in each organ. In general, stems had the highest $^{13}C_{TOM}$ values in each treatment, except in the *C* and *SS* treatments in giant reed, where no significant differences between organs were found ($P = 0.264$ and 0.410, respectively).

Significant differences were found in most of the organs between T1 and T2 in each treatment in giant reed, except for leaves and rhizomes under *MS* ($P = 0.689$ and 0.129, respectively). On the other hand, no significant differences were found in most of the organs between T1 and T2 in switchgrass (except in leaves in *C* conditions and roots and rhizomes in the *SS* treatment).

No significant differences in *C* content (C_c; % mg^{-1}; Table 2) were found in roots and rhizomes between treatments in both species. However, a different pattern in leaves and stems was found between species: significant differences were found between treatments in giant reed leaves ($P = 0.045$) whereas C_c in stems remained constant ($P = 0.082$). On the other hand, significant differences were found between treatments in switchgrass stems ($P = 0.014$) whereas C_c in leaves remained constant ($P = 0.860$). In general, a similar C_c was found in both species, except in control conditions, where a lower amount of C was found in leaves and stems of giant reed than in switchgrass (Table 2). Leaves and stems had low and high C/N ratios, respectively, in both species. In general, a higher C/N ratio was found in switchgrass than in giant reed, and mainly in these two organs (Table 2).

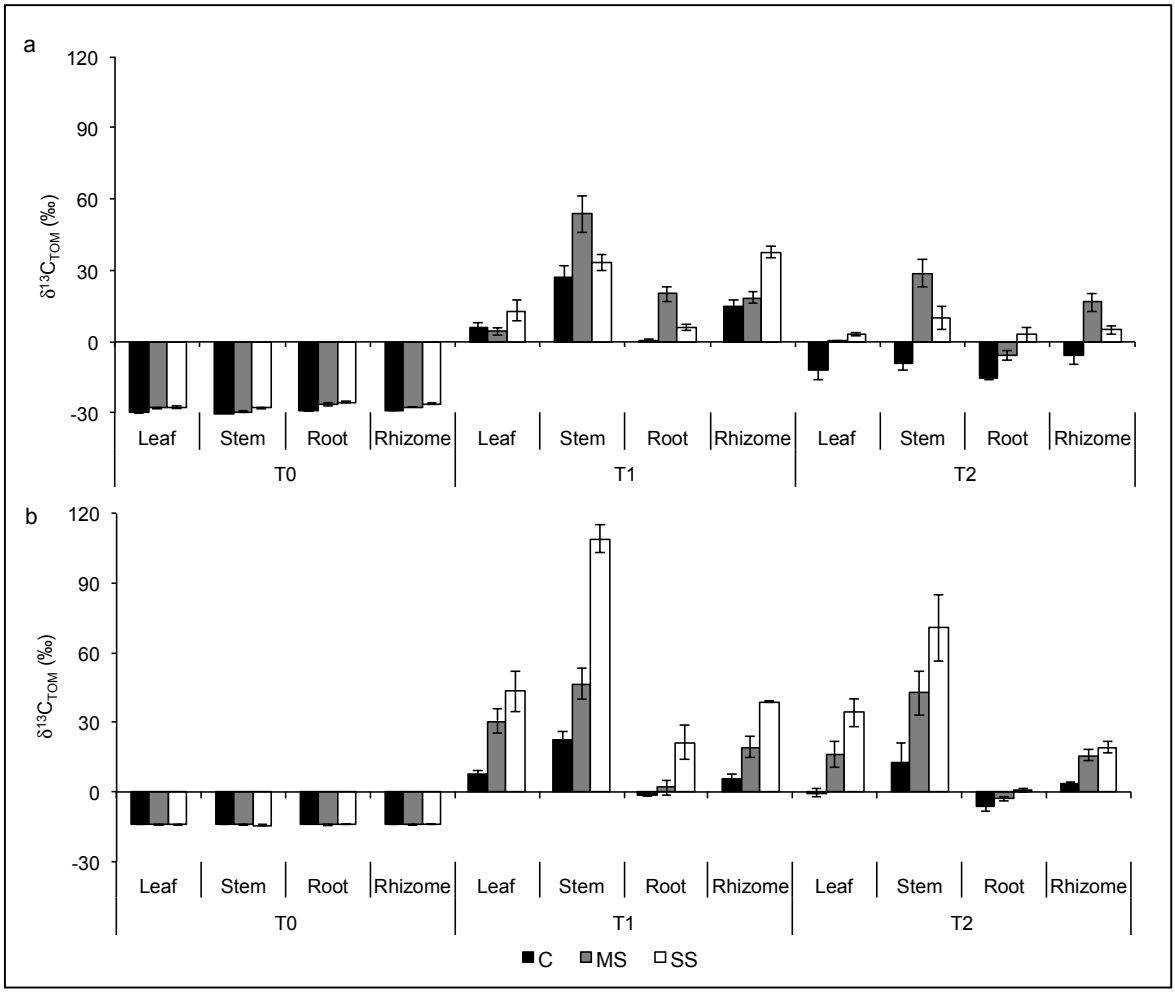

Figure 1. *Water stress effects (Control (C, 100% PC), Mild Stress (MS, 50% PC) and Severe Stress (SS, 25% PC) on ^{13} values (‰) of CO_2 in total organic matter ($^{13}C_{TOM}$) in leaves, stems, roots and rhizomes in both species (A. donax L. (A) and P. virgatum L. (B)) before labelling (T0), 1 day after labelling (T1) and 7 days after labelling (T2). Statistical analysis is presented in Table 6.*

3.1.2. The $\delta^{13}C$ of Total Soluble Sugars ($\delta^{13}C_{TSS}$)

A ^{13}C-enrichment in $^{13}C_{TSS}$ was observed in each organ with increasing *WS* (Figure 2) and significant differences between *C* and *SS* treatments were found in most organs, except in the stems ($P = 0.087$). In relation to switchgrass, only significant differences between *C* and *SS* treatments were found in leaves ($P = 0.016$). In general, roots and rhizomes had the lowest $^{13}C_{TSS}$ in each treatment in both species.

An enrichment in ^{13}C was observed in both species after labelling (T1). ^{13}C-enrichment was also observed in both species and in each organ with increasing degrees of stress, although significant differences were only found in stems and roots in giant reed ($P = 0.020$ and 0.005, respectively). In the case of switchgrass, significant differences between

treatments were found in each organ, except in roots (P = 0.057). Stems had the highest $^{13}C_{TSS}$ at T1 in each organ and in both species.

A ^{13}C-depletion between T1 and T2 was observed in both species under *C* conditions, and was greater in giant reed than in switchgrass. Significant differences were found in each organ except in leaves in giant reed, whereas no significant differences in any organ were found in switchgrass. Depletion in the $^{13}C_{TSS}$ between T1 and T2 was also observed in both species under *MS* and *SS* conditions. These decreases were significant in each of giant reed's organs and in most organs in switchgrass (except for roots and rhizomes in the *MS* treatment and rhizomes in the *SS* treatment).

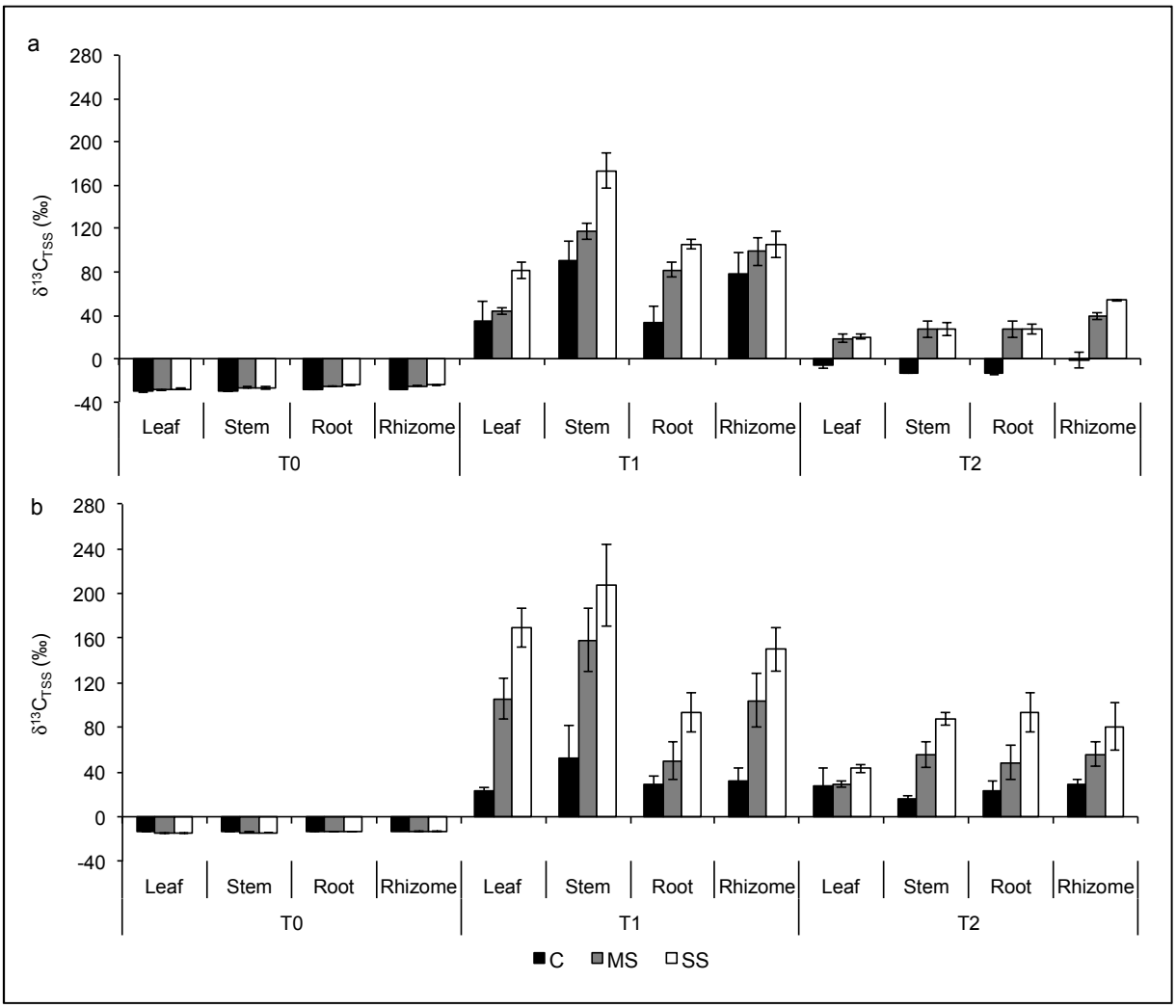

Figure 2. *Water stress effects (Control (C, 100% PC), Mild stress (MS, 50% PC) and Severe Stress (SS, 25% PC) on ^{13}C values (‰) of sugars ($^{13}C_{TSS}$) in leaves, stems, roots and rhizomes in both species (A. donax L. (A) and P. virgatum L. (B)) before labelling (T0), 1 day after labelling (T1) and 7 days after labelling (T2). Statistical analysis is presented in Table 6.*

3.1.3. $\delta^{13}C$ of Dark-Respired CO_2 Determination ($^{13}C_R$) and R_d.

Enrichment of ^{13}C was observed in each organ with increases in the degree of stress (Figure 3a). However, significant differences between the *C* and *SS* treatments were only found in stems and roots (P = 0.041 and 0.029, respectively). In relation to switchgrass, no significant differences between treatments were found in each organ at T0 under *C* conditions. In general, leaves and rhizomes were more ^{13}C depleted than the other organs in each treatment in giant reed, whereas no significant differences between organs were found in switchgrass at T0.

A ^{13}C enrichment after labelling (T1) was observed in both species (Figure 3). In general, a trend towards ^{13}C enrichment was observed in both species and in each organ with increasing degrees of stress, except in giant reed leaves, although no significant differences were observed between treatments (P = 0.229). Moreover, no significant differences were found in giant reed roots or rhizomes (P = 0.630 and 0.082, respectively). However, significant differences were found in giant reed stems and in each switchgrass organ. In general, the giant reed rhizome was the most ^{13}C enriched organ, whereas more similar values between organs were found in switchgrass in each treatment. A ^{13}C depletion was observed in *C* conditions between T1 and T2 (Figure 3). Depleted $^{13}C_R$ values were observed in *C* conditions relative

to stress conditions (*MS* and *SS*) in each organ except in giant reed leaves, where no significant differences were found (P = 0.132) and in switchgrass stems, where the *MS* treatment was more ^{13}C enriched than *C* and *SS*. In general, rhizomes in both species were the most ^{13}C-enriched organs, except in giant reed under *C* conditions, where no significant differences between organs were found (P = 0.129).

Decreases in R_d (Table 2) were found in both species during the stress treatment in roots and rhizomes, whereas no significant differences were found between treatments in leaves in both species (P = 0.072 and 0.407, respectively) and in stems in switchgrass (P = 0.052). In general, roots presented the highest R_d rates whereas rhizomes had the lowest R_d rates in giant reed. On the other hand, roots and rhizomes had the highest R_d rate in switchgrass (Table 2).

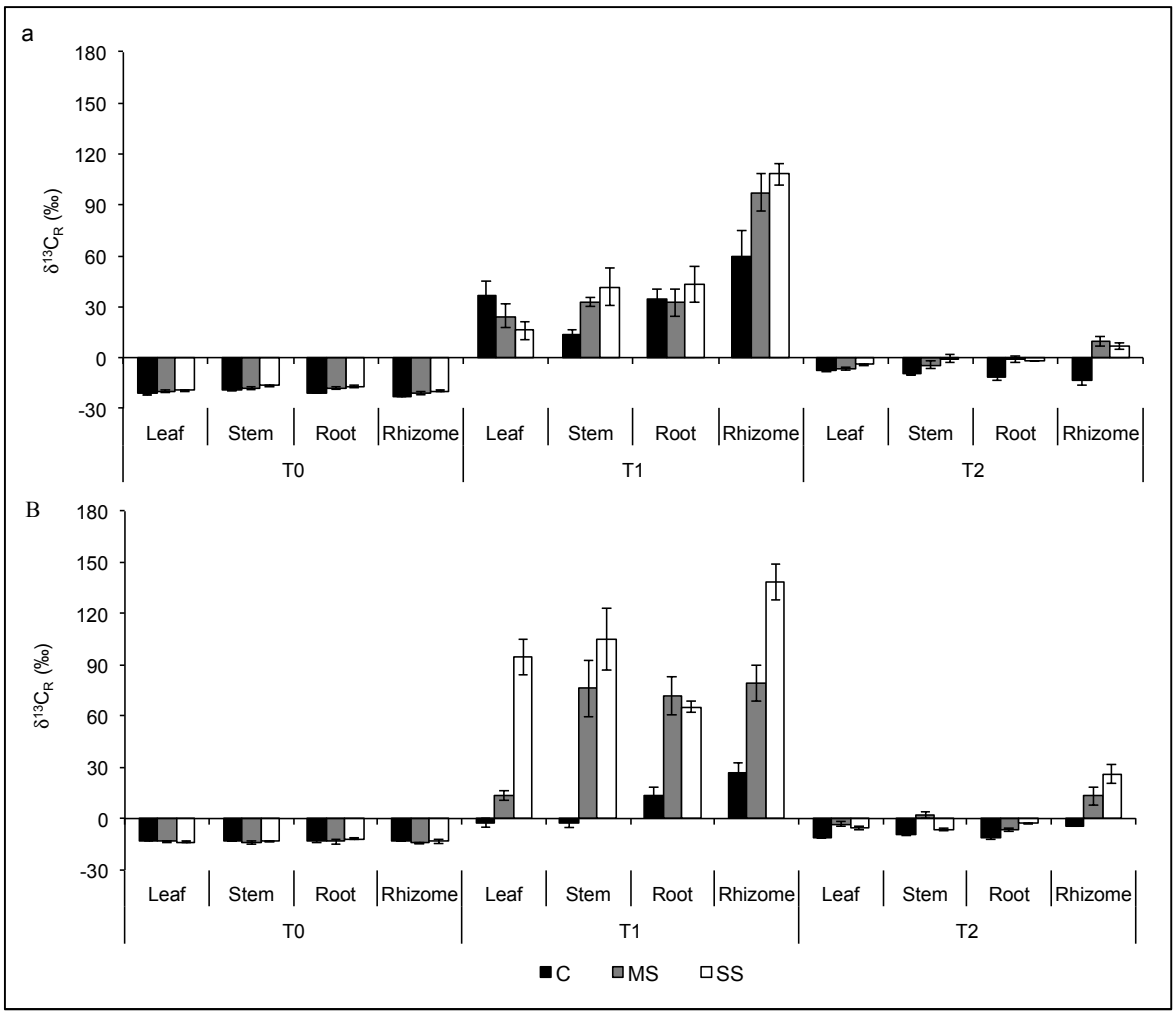

Figure 3. *Water stress effects (Control (C, 100% PC), Mild Stress (MS, 50% PC) and Severe Stress (SS, 25% PC) on ^{13} values (‰) of respired CO_2 ($^{13}C_R$) in leaves, stems, roots and rhizomes in both species (A. donax L. (A) and P. virgatum L. (B)) before labelling (T0), 1 day after labelling (T1) and 7 days after labelling (T2). Statistical analysis is presented in Table 6.*

3.1.4. The $\delta^{15}N$ of Total Organic Matter ($\delta\ ^{15}N_{TOM}$) and Total N Content (N_c)

The pattern of $\delta^{15}N_{TOM}$ observed (Figure 4) was similar pattern to $^{13}C_{TOM}$ (Figure 1). No significant differences between treatments were observed in each organ in either species at T0 in *C* conditions, except in giant reed roots, where ^{15}N depletion was observed relative to *C* conditions (P = 0.044). In general, ^{15}N enrichment was observed in stems compared to the other organs in both species, especially rhizomes, which showed the lowest $\delta^{15}N_{TOM}$ values in each treatment. Large ^{15}N enrichment was observed after labelling (T1) in both species, especially in giant reed (Figure 4a). In general, ^{15}N depletion was observed in both species and in each organ with increasing degrees of stress, and significant differences were found between *C* and *SS* in each organ in both species. Moreover, leaves had the lowest $^{15}N_{TOM}$ values in both species followed by roots in switchgrass. *MS* treatment was surprisingly more ^{15}N enriched than *C* conditions at T2, although significant differences between treatments were only found in stems and rhizomes (P = 0.005 and 0.000, respectively). However, a ^{15}N depletion between *C* and *SS* was observed. In the case of switchgrass, a greater ^{15}N depletion was observed in *MS* in comparison to *C* conditions, although no significant differences were found in leaves (P = 0.063) and stems (P = 0.278). No significant

differences between organs were found in any treatment in either species (except in the *SS* treatment in switchgrass, where roots were more ^{15}N depleted than the other organs). Moreover, no significant differences between T1 and T2 were noticed in most of the organs in each treatment in either species.

The highest N content (N_c; % mg^{-1}) between organs was noted in leaves in both species, but was higher overall in giant reed leaves than in switchgrass in each treatment (P = 0.000). Decreases in N_c were found in giant reed's leaves as the level of stress increased, whereas a different pattern was observed in switchgrass leaves. No significant differences were found between treatments in stems and roots of giant reed and in roots in switchgrass. However, an increase in N_c was observed in stems and rhizomes of switchgrass when the stress increased (Table 2).

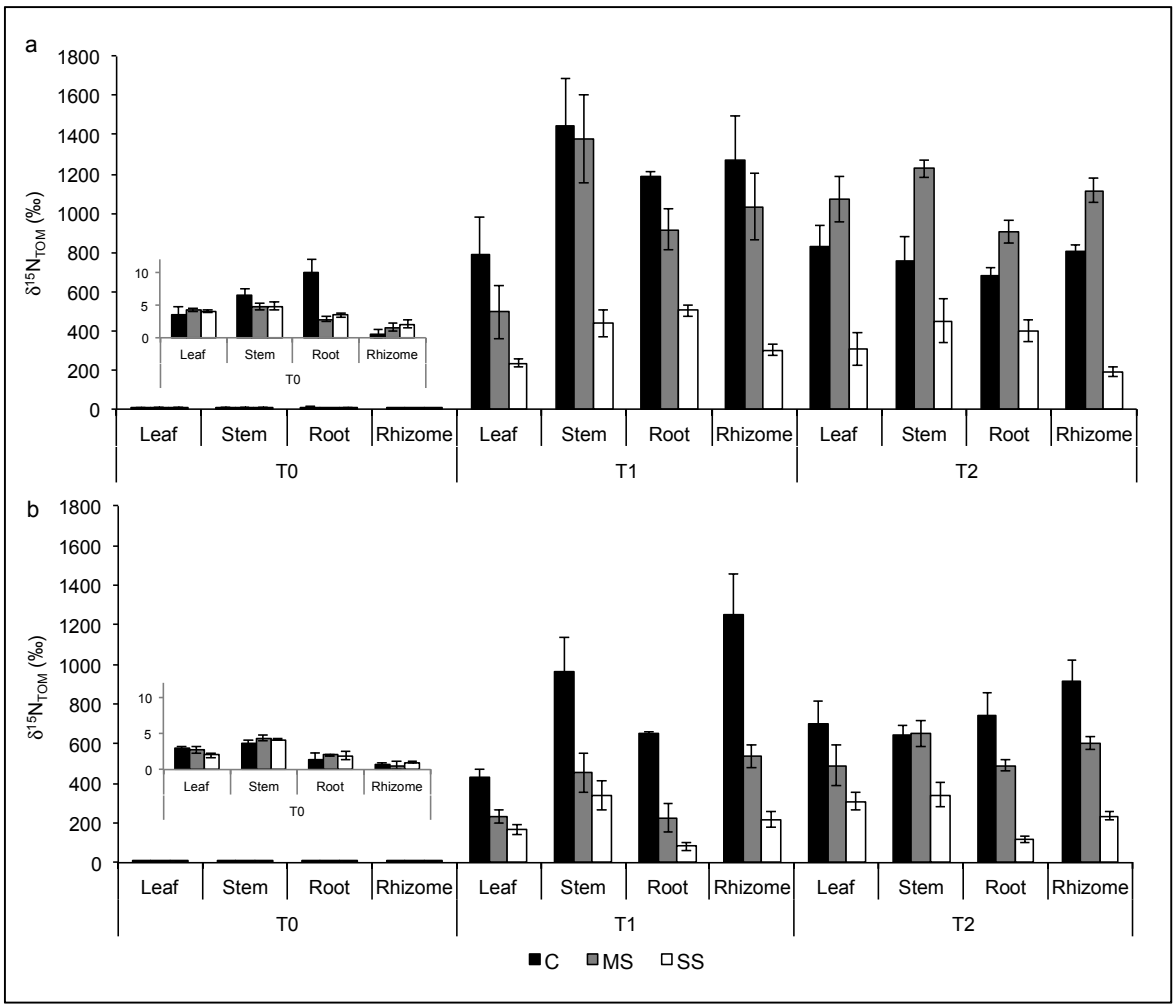

Figure 4. *Water stress effects (Control (C, 100% PC), Mild Stress (MS, 50% PC) and Severe Stress (SS, 25% PC) on ^{15} values (‰) of N_2 in total organic matter ($^{15}N_{TOM}$) in leaves, stems, roots and rhizomes in both species (A. donax L. (A) and P. virgatum L. (B)) before labelling (T0), 1 day after labelling (T1) and 7 days after labelling (T2). Statistical analysis is presented in Table 6.*

3.2. Gas Exchange and Fluorescence

Significant differences were found in most of the photosynthesis parameters between species at the beginning of the experiment (Ti), whereas other parameters like A_{sat} had similar values (P > 0.05) (Table 3). Higher values of photosynthesis parameters were found in giant reed than in switchgrass at Ti except for $_i$WUE, where an increase of 68% was found in switchgrass relative to giant reed. Furthermore, significant differences were found in fluorescence parameters between species at Ti, with the values being higher in giant reed than in switchgrass (Table 3), although other parameters such as q_P and NPQ were unchanged (P > 0.05).

At the end of the experiment (Tf; Figure 5), significant differences were found between species in most of the photosynthesis parameters under *C* conditions. In particular, A_{sat} and g_s were higher in giant than switchgrass, whereas switchgrass had greater $_i$WUE values. Moreover, significant differences were found between treatments (Figure 5). Decreases in A_{sat}, g_s and T were clearly found in both species subjected to increased *WS* levels (from *C* to *SS*). Furthermore, increases in $_i$WUE were found in both species in *SS* with respect to *C*. Positive correlations were found in both species between g_s and A_{sat} (R^2 = 0.916 and 0.901 in giant reed and switchgrass, respectively) than between RWC and A_{sat} (R^2 = 0.694 and 0.469 in giant reed and switchgrass, respectively).

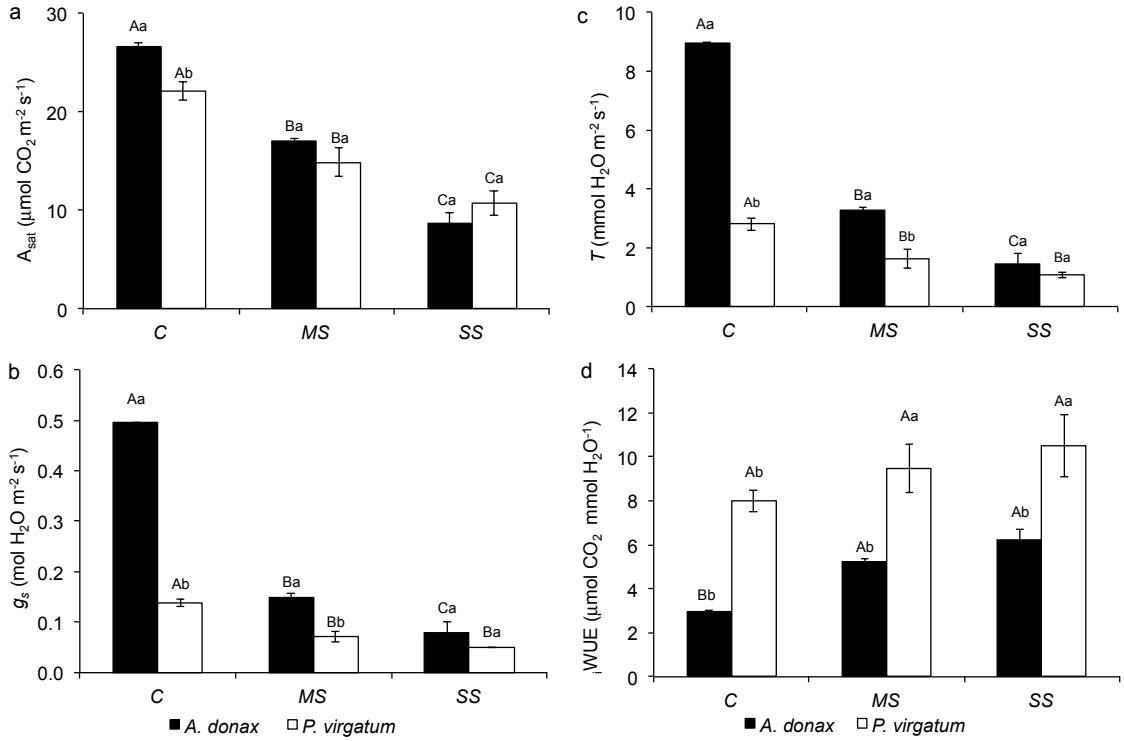

Figure 5. *Photosynthesis parameters (A_{sat}, assimilation rate at light saturation (a); g_s, stomatal conductance (b); T, transpiration rate (c); WUE, water use efficiency (d)) in both species (A. donax L. and P. virgatum L.) for each treatment: i) Control (C, 100% PC), ii) Mild stress (MS, 50% PC) and iii) Severe Stress (SS, 25% P) at the end of the experiment (Tf). Data are the means of three replicates and the standard error is shown. Data were analysed with an ANOVA Tukey analysis. Different capital letters indicate significant differences (P < 0.05) between treatments for the same specie and different small letters indicate significant differences (P < 0.05) between species for the same treatment. Photosynthesis parameters at the beginning of the experiment (Ti) can be found in Table 2.*

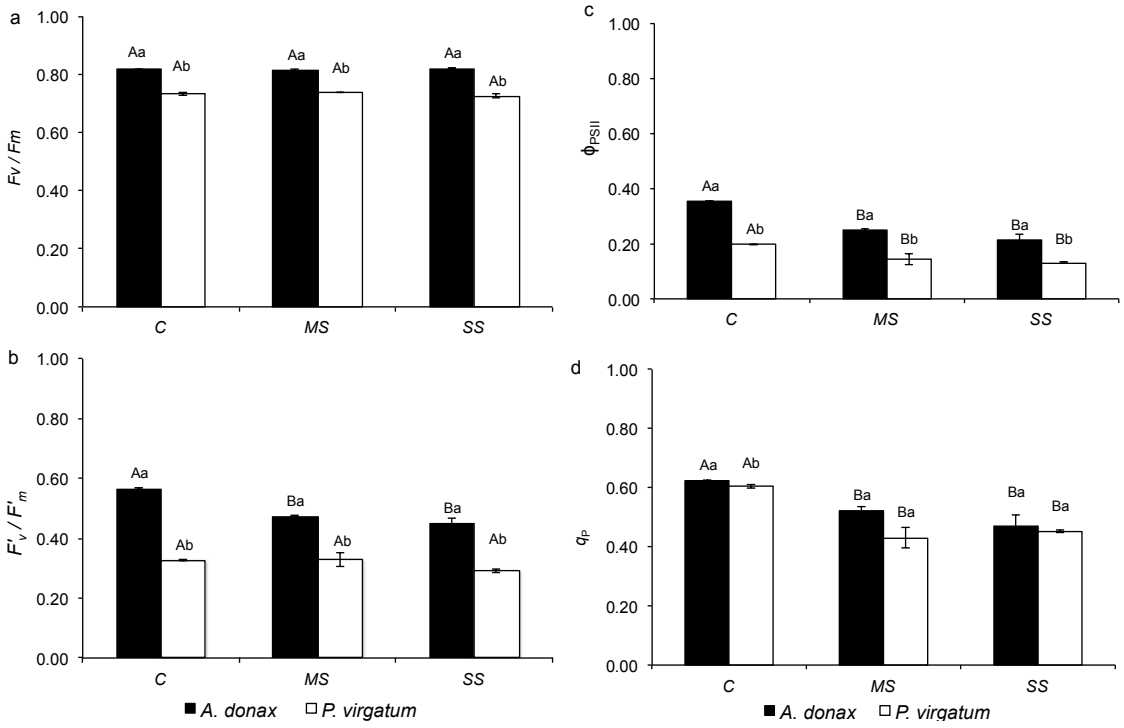

Figure 6. *Fluorescence parameters (F_v/F_m, maximal photochemical efficiency in the dark –adapted stage (a); F'_v/F'_m, maximal photochemical efficiency in light (b); ϕ_{PSII}, relative quantum yield of Photosystem II electron transport (c); q_P, photochemical quenching (d)) in both species (A. donax L. and P. virgatum L.) for each treatment: i) Control (C, 100% PC), ii) Mild Stress (MS, 50% PC) and iii) Severe Stress (SS, 25% PC) at the end of the experiment (Tf). Data are the means of three replicates and the standard error is shown. Data were analysed with an ANOVA Tukey analysis. Different capital letters indicate significant differences (P < 0.05) between treatments for the same specie. Different small letters indicate significant differences (P < 0.05) between species for the same treatment. Fluorescence parameters at the beginning of the experiment (Ti) can be found in Table 2.*

Decreases in fluorescence parameters were also observed between *C* and *SS* treatments at Tf in both species (Figure 6). The F'_v/F'_m of giant reed and switchgrass decreased by 20% and 11%, respectively, and the ϕ_{PSII} by 40% and 33%, respectively. A similar decrease was observed in q_P in both species, while F_v/F_m did not change with increasing *WS* levels. NPQ increased from 1.351 ± 0.042 in *C* conditions to 2.198 ± 0.126 in *SS* conditions in giant reed and from 1.672 ± 0.009 in *C* conditions to 2.143 ± 0.149 in *SS* conditions in switchgrass (data not shown).

3.3. RWC and Chlorophyll Content

No significant differences were found in *C* and *MS* treatments in either species at the end of the experiment (Tf). As expected, RWC values were lower in the *SS* treatment, where significant differences were found with respect to *C* and *MS*. Giant reed showed small decreases in the *MS* and *SS* treatments (3% and 18%, respectively) relative to *C*, whereas switchgrass declined by 6% and 49% in *MS* and *SS* relative to *C* (Figure 7).

Higher chlorophyll content values were found in giant reed than in switchgrass at Ti, whereas no significant differences were found at Tf (Table 4). No significant differences were found in giant reed between Ti and Tf for each treatment (P = 0.944 (*C*), 0.297 (*MS*) and 0.697 (*SS*)) nor were there significant differences between treatments at Ti and Tf (P = 0.678 and 0.839, respectively; Table 4). Nevertheless, an increase in SPAD values between Ti and Tf in switchgrass was found in the *C* and *SS* treatments. Moreover, no significant differences were found between treatments at Ti (P = 0.005), although an increase in SPAD values was noted in *SS* with respect to the other treatments at Tf.

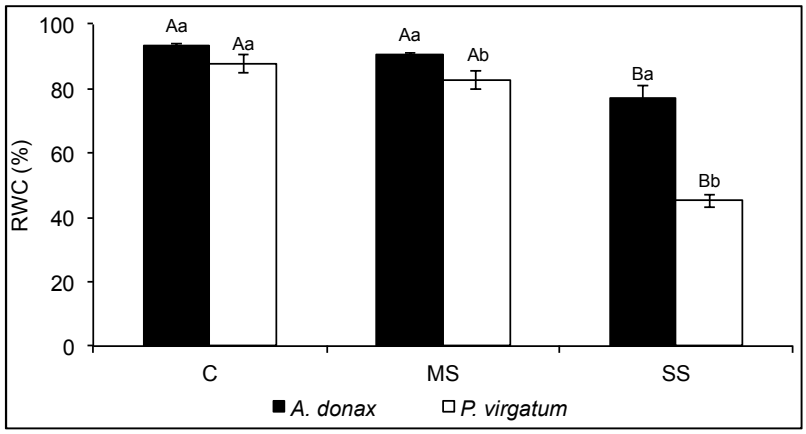

Figure 7. *Changes in Relative Water Content (RWC, %) in both species (A. donax L. and P. virgatum L.) for each treatment: i) Control (C, 100% PC), ii) Mild Stress (MS, 50% PC) and iii) Severe Stress (SS, 25% PC) at the end of the experiment (Tf). Data are the means of three replicates and SE is shown. Data were analysed with an ANOVA Tukey analysis. Different capital letters indicate significant differences (P < 0.05) between treatments for the same species. Different small letters indicate significant differences (P < 0.05) between species for the same treatment*

3.4. Biomass Parameters

In both species, significant differences were found for most of the biomass parameters in each treatment and time (Table 1). A higher H was found in giant reed than in switchgrass at Ti in each treatment, whereas at Tf similar values were recorded except in the *SS* treatment, where the values for switchgrass were smaller than giant reed. A greater NS and NGL were observed in switchgrass than in giant reed in each treatment and time.

An increase in height (H), number of stems (NS) and number of green leaves (NGL) was observed in all treatments in both species (except for NGL in the *SS* treatment of switchgrass) at Tf with respect to Ti. In the case of giant reed, an increase in H was observed in all treatments at Tf with respect to Ti, although only significant differences were observed in *C* (P = 0.000). In switchgrass, significant differences between Ti and Tf were found in all treatments due to remarkable increases in H (Table 1). Regarding NS, significant differences between species were found at Ti and Tf in each treatment. Moreover, an increase in the NS value between Ti and Tf was also observed in all treatments in both species, although only significant differences were found in *C* in giant reed, which had a 60% increase. The increase in NGL between Ti and Tf was higher in giant reed than in switchgrass, with significant differences found in *C* and *MS* (P = 0.000 and 0.006, respectively), whereas in switchgrass significant differences were only found in the *C* treatment (P = 0.012) and a substantial decrease was observed in the *SS* treatment (-43%; P = 0.000).

Decreases in H and NGL were observed in both species as *WS* increased at Tf with respect to *C*, but were higher in switchgrass. Furthermore, similar decreases in NS were also observed in both species throughout the treatments at Tf with respect to *C* (Table 1).

Table 1. *Changes in biomass parameters (H, height (cm); NS, number of stems; NGL, number of green leaves) in both species (A. donax L. and P. virgatum L.) for each treatment: i) Control (C, 100% PC), ii) Mild stress (MS, 50% PC) and iii) Severe Stress (SS, 25% PC) between the beginning (Ti) and the end of the experiment (Tf). Data are the means of nine replicates and the standard error is shown. Data were analysed with an ANOVA Tukey analysis. Different capital letters indicate significant differences (P < 0.05) between time for the same specie and treatment. Different small letters indicate significant differences (P < 0.05) between treatments for the same specie and time. Different Greek letters indicate significant differences (P < 0.05) between species for the same time and treatment.*

			H			**NS**			**NGL**		
			Mean	***SE***		**Mean**	***SE***		**Mean**	***SE***	
A. donax	C	Ti	51.3	*3.7*	Ba	2.4	*0.2*	Ba	11.6	*1.4*	Ba
		Tf	75.6	*3.9*	Aa	3.8	*0.3*	Aa	26.1	*1.9*	Aa
	MS	Ti	61.9	*3.6*	Aa	2.4	*0.2*	Aa	11.9	*1.1*	Ba
		Tf	70.2	*2.4*	Aab	3.0	*0.2*	Aa	18.3	*1.8*	Ab
	SS	Ti	55.4	*4.6*	Aa	1.2	*0.1*	Ab	8.3	*0.9*	Aa
		Tf	62.2	*4.6*	Ab	1.8	*0.2*	Ab	9.0	*1.1*	Ac
P. virgatum	C	Ti	26.7	*1.2*	Bb	26.4	*3.0*	Aa	48.0	*5.0*	Ba
		Tf	74.4	*4.1*	Aa	31.2	*3.9*	Aa	82.0	*11*	Aa
	MS	Ti	27.3	*0.9*	Bb	19.3	*2.5*	Aab	40.4	*4.1*	Aa
		Tf	60.3	*4.3*	Ab	22.4	*2.6*	Aab	42.1	*3.5*	Ab
	SS	Ti	32.9	*1.1*	Ba	16.7	*2.5*	Ab	41.1	*4.0*	Aa
		Tf	49.2	*2.6*	Ab	17.2	*1.8*	Ab	21.0	*0.9*	Bb

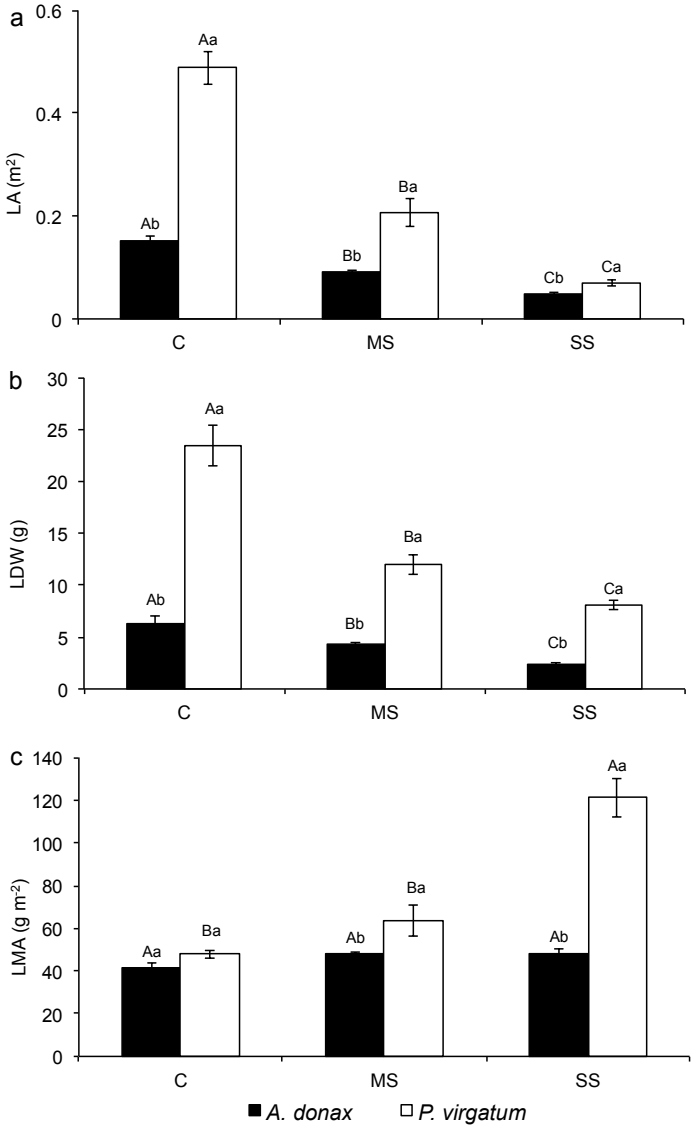

Figure 8. *Effect of stress in biomass parameters (LA, Leaf Area (a); LDW, Leaf Dry Weight (b); LMA, Leaf Mass Area (c) in both species (A. donax L. and P. virgatum L.) for each treatment: i) Control (C, 100% PC), ii) Mild Stress (MS, 50% PC) and iii) Severe Stress (SS, 25% PC) at the end of the experiment (Tf). Data are the means of nine replicates and the standard error is shown. Data were analysed with an ANOVA Tukey analysis. Different capital letters indicate significant differences (P < 0.05) between treatments for the same specie and time. Different small letters indicate significant differences (P < 0.05) between species for the same time and treatment*

An important and similar decrease in Leaf Area (LA) and Stem Area (SA) was noticed in both species at Tf with increasing *WS* (Figure 8, Table 5). LA decreased by 40% and 58% between *C – MS* and by 69% and 86% between *MS – SS* in giant reed and switchgrass, respectively, whereas SA decreased by 32% and 75% between *C – MS* and 65% and 75% between *MS – SS* in giant reed and switchgrass, respectively. Although decreases in LA and SA as a consequence of *WS* were greater in switchgrass than in giant reed, the LA and SA values were greater in switchgrass in each treatment, as also occurred with Leaf Dry Weight (LDW) and Shoot Dry Weight (SDW), which were approximately 70% higher in switchgrass than in giant reed (Figure 8, Table 5). In addition, a decrease in LDW and SDW with increasing levels of *WS* was also observed in both species.

Leaf Mass Area (LMA) was the only biomass parameter that increased with increasing *WS* (Figure 8). LMA values in the *C* treatment were similar in both species and remained constant in giant reed in *MS* and *SS* ($P = 0.059$), whereas a substantial increase was observed in switchgrass, reaching a value of 121.2 ± 8.9 in *SS*.

Total Dry Weight (TDW, Table 5) also decreased in both species, although different patterns were observed. Giant reed had a greater decrease in more advanced stages of stress, whereas switchgrass showed a greater decrease in the first stages of stress. In addition, the decreases under the *SS* treatment relative to *C* were more or less similar in both species, although TDW values in switchgrass were significantly higher than in giant reed. Shoot/Root ratio (S/R, Table 5) values were significantly higher in giant reed than in switchgrass (Table 5). Root dry mass in switchgrass was significantly higher than in giant reed and almost double its shoot dry mass. In contrast, giant reed root dry mass was significantly lower than its shoot dry mass. A decrease was also observed in S/R due to *WS* in giant reed but not in switchgrass, where no significant differences were found ($P = 0.825$).

Specific Leaf Area (SLA) had a similar pattern to LMA but it was reversed. SLA values in the *C* treatment were similar in both species, and a slight decrease was observed in giant reed between *C* and *MS* that remained constant in *SS* (Table 5). On the other hand, a significant decrease was observed in switchgrass.

Significant differences in the Leaf Area Ratio (LAR) were found between *C* and *MS* in giant reed, whereas in switchgrass the LARs were significantly different between *MS* and *SS*. A decrease was observed in LAR under *SS* relative to *C* in both species, but it was greater in switchgrass. On the other hand, significant differences in the Leaf Weight Ratio (LWR) were only found between *C* and *SS* in giant reed whereas no significant differences were found between treatments in switchgrass. Higher LAR and LWR values were recorded in giant reed than in switchgrass (Table 5).

4. Discussion

4.1. δ^{13}C and δ^{15}N before and after Labelling

Our data confirmed that $\delta^{13}C_{TOM}$, $\delta^{13}C_{TSS}$ and $\delta^{13}C_R$ in control conditions (and before the labelling) are largely dependent on the photosynthetic pathway [25] and that C_3 plants are more ^{13}C depleted than C_4 plants [25–27, 33]. The lower ^{13}C depletion in C_4 plants is due to PEPc exhibiting a different intrinsic kinetic isotope effect and utilizing a species of inorganic carbon with an isotopic composition at equilibrium that is different from the substrate used by Rubisco in C_3 plants [21]. Moreover, the negative $^{13}C_R$ values in C_4 plants is caused by a slow leak of enriched CO_2 from the bundle sheath. The leaking CO_2 pool is enriched in ^{13}C by the preference of Rubisco for the light isotope (^{12}C), so as the enriched CO_2 leaks out it depletes the ^{13}C of the CO_2 left behind [34]. ^{13}C variations also occur within a single plant between organs due to a possible fractionation during export of assimilates, which is named post-photosynthetic discrimination [33], and/or during dark respiration [25]. Although differences in bulk ^{13}C between different plant parts (leaves and roots) have been reported by Hobbie and Werner [26] and Badeck et al. [33], our results indicated no clear post-photosynthetic discrimination or dark respiration discrimination under *C* conditions in these species (Figures 1 and 3, respectively). Sucrose has been classified by some authors [25, 32] as the most ^{13}C-enriched respiratory substrate in intact leaves of some C_3 plants. However, our data do not indicate greater ^{13}C enrichment in sucrose in comparison to $^{13}C_{TOM}$ at T0 in either species under *C* conditions (Figures 1 and 2). A lower ^{13}C discrimination was observed during respiration than in TOM [25, 32, 35, 36], and was greater in giant reed than in switchgrass, with similar differences having been observed between other C_3 and C_4 plants by Ghashghaie et al. [25]. In relation to TSS, the -5‰ to -10‰ ^{13}C-enrichment in $^{13}C_R$ observed at T0 (Figures 2 and 3) in giant reed was similar to values observed in other C_3 species (-6‰) [32, 37], however, a lower enrichment was observed in switchgrass (Figures 2 and 3). Differences in the metabolic pathways between C_3 and C_4, such as photorespiration, sugar metabolism and fluxes during the reductive pentose phosphate pathway could be the causes of the isotopic differences [26].

The large ^{13}C enrichment in TSS, TOM and CO_2 respired in most of the organs at T1 in *C* conditions is observed as a consequence of ^{13}C labelling (Figures 1, 2, 3), indicating that $^{13}CO_2$ was assimilated by photosynthesis and was incorporated into translocation, storage and respiration processes. In this case, post-photosynthetic discrimination was observed as expected [19, 25]. The greater $^{13}C_{TOM}$ and $^{13}C_{TSS}$ values in stems in both species would imply that the assimilated C was transported along the stem during the sampling period or that this organ could act as a sink for C assimilates. In this regard, this accumulation of sugars

(especially sucrose) has also been observed in other C_4 species belonging to the *Panicoideae* subfamily, for example sorghum (*Sorghum bicolor*) and sugar cane (*Saccharum officinarum*) [38–40]. In relation to the rhizome, its high ^{13}C enrichment would confirm that this organ is being used as a C sink [41].

The lower $\delta^{13}C_{TOM}$, $\delta^{13}C_{TSS}$ and $\delta^{13}C_R$ values in *C* conditions at T2 compared to T1 (although still more ^{13}C-enriched than values before labelling (T0) (Figures 1, 2, 3)), would suggest that leaves and the other organs have not used all of the labelled C substrate during their metabolism [32]. The fact that stems and rhizomes were the most ^{13}C-enriched organs would confirm their role as permanent C sinks. The notion that the stem is a C sink is due to it being photosynthetically active and thus capable of assimilating ^{13}C directly, incorporating it into the tissues [42, 43].

Our results clearly indicate that *WS* modulates both the C and N isotopic composition (Figures 1, 2, 3 and 4). A ^{13}C enrichment (or a decrease in photosynthetic discrimination) in TSS, TOM and CO_2 respired due to *WS* was observed due to decreases in g_s and therefore in the intercellular partial pressure of CO_2 (c_i) [21, 25]. As c_i decreases, the ^{13}C of the CO_2 inside the leaf is progressively enriched and the photosynthate produced is likewise enriched [34]. The tendency for $^{13}C_{TOM,}$ $^{13}C_{TSS}$ and $^{13}C_R$ to increase in both species at T1 and T2 under *WS* conditions (Figures 1, 2, 3) would confirm the use of stable isotopes as an appropriate tool to measure stress. According to Hobbie and Werner [26], *WS* may result in altered intramolecular isotopic patterns because of additional isotopic fractionation during photorespiration and concurrent changes in the flux patterns of C from trioses to glucose. As in *C* conditions, rhizomes and stems had the greatest ^{13}C enrichment, indicating that both organs were also C sinks, even under *WS* conditions. In general, *WS* accelerated plant phenology and, consequently, the accumulation of storage compounds in the rhizome occurred in response to stress. This effect was more clearly visible in switchgrass than in giant reed.

Leaves are the major N storage organs in both species. The lower N_c observed in C_4 leaves relative to C_3 leaves (Table 2) for the same photosynthetic activity in C conditions (Figure 5) was expected due to a higher Rubisco carboxylation activity in C_4 as a consequence of a lower photorespiration [44, 45]. Moreover, from 15% to 30% of the total N_c of leaves in C_3 plants is contained in Rubisco, whereas this value decreases to 6-9% in C_4 plants, according to Sage et al. [44]. Decreases in N_c in leaves due to *WS* observed in both species (Table 2) might be a consequence of a decrease in Rubisco content [46] or a decrease in *T* (Figure 5c). Indeed, in the latter case the low reduction in N_c in the leaves (lower than 17%) with respect to the reduction in g_s (84%) would suggest that the N_c reduction was a consequence of low water absorption by the roots as a result of reduced *T* [47]. The fact that switchgrass stems have the lowest N_c but maintain a high C/N ratio (Table 2) and a high ^{13}C labelling (Figure 1b), even in *WS* conditions, would confirm that this organ is an important C sink.

The $^{15}N_{TOM}$ values at T0 in both species lay within a similar range as the natural abundance [28]. Leaves and stems were the main N sinks in both species, whereas rhizomes were N sources, due to the high and low $\delta^{15}N_{TOM}$ values observed between the aboveground and belowground organs under *C* conditions, respectively (Figure 4), indicating bottom-up translocation of N. ^{15}N was absorbed and integrated into metabolism by roots, being later translocated to the other organs as evidenced by the ^{15}N enrichment observed in both species at T1 under *C* conditions (Figure 4). The fact that leaves were the organs with the lowest $\delta^{15}N_{TOM}$ would indicate that the translocation was slow. The higher $\delta^{15}N_{TOM}$ values observed in giant reed than in switchgrass after labelling (T1) confirmed the greater need for N in C_3 plants than in C_4 plants [44]. In theory, species with a high N demand will have lower discrimination against ^{15}N. The high $\delta^{15}N_{TOM}$ values at T2 relative to natural abundance and the increase in $\delta^{15}N_{TOM}$ values of leaves between T1 and T2 would confirm the slow ^{15}N metabolism as well as the continuous bottom-up translocation of ^{15}N.

The reduction in $\delta^{15}N_{TOM}$ due to *WS* (Figure 4) in both species was expected following the reports of Robinson et al. and Yousfi et al. [48, 49]. According to Farquhar et al. [21], a reduction in g_s due to stress (Figure 5b) should lead to a reduction in the loss of ammonia and nitrous oxide, decreasing the $\delta^{15}N_{TOM}$. Isotope fractionation of N may occur during uptake from the medium into root cells or during subsequent enzymatic assimilation into other N forms.

4.2. Photosynthetic Capacity

The high photosynthetic capacity of giant reed (C_3) has been demonstrated [5, 50], as A_{sat} was similar in both species at Ti (Table 3) or even greater in giant reed than in switchgrass at Tf in *C* conditions (Figure 5). However, the lower values of g_s and *T* and the higher $_i$WUE found in switchgrass compared to giant reed in *C* conditions (Figure 5) are a consequence of lower intercellular [CO_2] requirements to saturate Rubisco carboxylation in switchgrass. Therefore, the operation of a CO_2-concentrating mechanism enhances the efficiency of C_4 relative to C_3 photosynthesis and the ability to limit water loss while maintaining net carbon uptake in the leaves [51].

Many studies on photosynthesis under *WS* have found decreases in A_{sat} or g_s in giant reed [41, 52, 53] and switchgrass [6, 54] as well as other species [55, 56]. In our experiment, these decreases are significantly higher in giant reed than in switchgrass (Figure 5), indicating a higher *WS* effect in the C_3 species than in the C_4. Not only do C_4 species have a higher WUE than C_3 species, as mentioned above, but NAD-ME grasses (such as switchgrass) also increase their whole-plant WUE to a greater extent than NADP-ME grasses under *WS* [22]. The lack of change in F_v/F_m values under stress in both species (Figure 6) indicates that there was no damage to the PSII reaction centres. This means that photoinhibition is not observed in either species under *WS*, and this is contrary to expectations from reports of other

plants exposed to abiotic and biotic stress [57]. Chlorophyll fluorescence parameters in switchgrass are lower than giant reed. However, decreases (or increases in the case of NPQ) due to stress are also lower in switchgrass, confirming a higher *WS* effect in the C_3 species than in the C_4.

The greater correlation between A_{sat} and g_s than between A_{sat} and RWC shows how some parameters related to photosynthesis seem more dependent on stomatal conductance than on leaf water status. Our data indicate that down-regulation of different photosynthetic processes under *WS* depends more on CO_2 availability in the mesophyll (i.e. on stomatal closure) than on leaf water content, even in different plant species, as mentioned by Sharkey [58] and Medrano et al. [14]. Therefore, our data indicates how g_s can be used as an integrative parameter reflecting the *WS* experienced by the plant. The rapid stomatal response to decreased water content allows plants to delay water loss, which according to this feature indicates that, they could be considered as species that avoid dehydration [47, 59].

Moreover, the similar RWC values observed between *C* and *MS* in both species (Figure 7) indicate that *WS* was moderated. For example, the RWC values of switchgrass in the *SS* treatment (Figure 7) did not correlate with the higher $_i$WUE observed in Figure 5d for the same treatment. The lower RWC values might be due to the leaf curling observed in switchgrass leaves, which could have made leaf hydration more difficult during the RWC measurement. Moreover, the difficulty in leaf hydration in switchgrass could have been aggravated by the appreciable increase in the LMA observed between treatments (Figure 8). Therefore, the increase in leaf thickness (and curling) in switchgrass might have influenced the RWC measurements.

4.3. Plant Morphology Parameters

In general, giant reed tends to be a tall plant with few stems and wide leaves, whereas switchgrass is smaller with a large number of stems and long narrow leaves. Although both species had considerable biomass yields in control conditions, the larger LA, SDW and TDW values (Figure 8, Table 5), as well as the more substantial root growth of switchgrass, indicate features present in this species that are desirable in bioenergy crops because they reduce erosion and enhance carbon capture in the soil [1, 60, 61]. Cell growth has been reported among the primary processes affected by *WS* [17]. According to Blum [62], a decrease in LA is one mechanism that limits plant water use and reduces damage due to *WS*. Our results showed how *WS* had a considerable effect on biomass production in both species due to a lower C assimilation but also a decrease in biomass parameters such as H, NS, NGL, LA, SDW and TDW, among others (Figures 5 and 8, Table 5). Furthermore, the increase in LMA is another mechanism used by plants to improve efficiency versus water loss and it leads to smaller and thicker leaves [63].

A range of dehydration avoiding mechanisms have been reported in plants including stomatal closure, leaf abscission and leaf rolling [64]. In our case, all three of these parameters have been observed in switchgrass (Figure 5 and Table 1). This could mean that switchgrass has physiological advantages against *WS* due to its C_4 photosynthetic pathway. The lower S/R ratio in switchgrass (Table 5) indicates an extensive root development relative to the shoot dry mass. This should enable switchgrass to have a better adaptation to drought than giant reed. However, due to the fact that the S/R ratios in switchgrass are constant under control and stress conditions (Table 5) there is no correlation with a dehydration avoidance mechanism, where an increase in root dry mass would be expected in *WS*.

Although both species have been identified as drought tolerant [1], our results obtained in greenhouse conditions would indicate a greater tolerance and a better adaptation to *WS* in switchgrass than in giant reed, according to Quinn et al. [65], as well as a greater biomass production (LDW), even in *WS* conditions.

5. Conclusions

Our data show that, before labelling, giant reed is more ^{13}C depleted in TOM and in dark respiration than switchgrass as a consequence of the different photosynthetic pathways. The increase in ^{13}C after labelling (T1) indicates that $^{13}CO_2$ is assimilated by photosynthesis and is incorporated into translocation, storage and respiration processes. Moreover, our data indicate that not all the labelled C substrate has been used during the subsequent 7 days of metabolism.

Interestingly, our results showed how stems are sinks for ^{13}C and confirmed that the rhizome is a permanent sink for labelled C. In addition, our results also confirm that stems and rhizomes could be classified as nitrogen sinks and that there is a slow turnover of N in both species due to the high $\delta^{15}N_{TOM}$ values at T2 compared to T1.

In addition, both species modulate the assimilation of ^{13}C in accordance with the *WS* level: plants are more ^{13}C enriched as the *WS* increases. Changes in plant phenology and accumulation of storage compounds in the rhizome might exist as a response against WS.

Decreases in photosynthesis parameters such as A_{sat}, g_s, and *T* were observed as a consequence of the *WS* increase in both species, being more important in giant reed than in switchgrass. No change in the F_v/F_m values under stress in either species was an indication of a lack of damage to the PSII reaction centres. Smaller decreases in fluorescence parameters (or increases in the case of NPQ) in switchgrass than in giant reed due to stress confirmed a higher *WS* effect in the C_3 species than in the C_4. In both species, the assimilation of the C through the stomata and the carboxylation efficiencies of the plants were more affected by *WS* than the electron transport of PSII. The photosynthesis parameters seem to be more dependent on stomatal conductance than on leaf water status according to the greater correlation between A_{sat} and g_s than between A_{sat} and RWC. A slight decrease in plant growth was observed in relation to the smaller increase in height and the lower number of stems and green leaves, as well as the decrease

in leaf area, stem area and leaf area index over the stress treatments. Despite the fact that plant morphology in giant reed and switchgrass is significantly different, a similar decrease in total dry weight due to stress was observed in both species.

Acknowledgements

The present work was supported by the European project OPTIMA (Optimization of Perennial Grasses for Biomass Production, Grant Agreement n 289642) and a CONICYT BecasChile Grant (Grant 72120250) as financial support to ES.

Appendix

Table 2. *Values of dark respiration (R_d; □mol m^{-2} s^{-1}), C content (C_c; %C mg^{-1}), N content (N_c; %N mg^{-1}), and C/N Ratio in both species (A. donax L. and P. virgatum L.) for each treatment: Control (C, 100% PC), Mild stress (MS, 50% PC) and Severe Stress (SS, 25% PC) at the end of the experiment (Tf). Data are the means of nine replicates and the standard error is shown. Data were analysed with an ANOVA Tukey analysis. Different capital letters indicate significant differences (P < 0.05) between treatments for the same organ and specie. Different small letters indicate significant differences (P < 0.05) between organs for the same treatment and specie. Different Greek letters indicate significant differences (P < 0.05) between species for the same organ and treatment.*

			R_d			C_c			N_c			C/N		
			mol gDW^{-1} s^{-1}			%C mg^{-1}			%N mg^{-1}					
			Mean	SE		Mean	SE		Mean	SE		Mean	SE	
A. donax L.	C	L	-12.8	1.0	Ab	50.7	1.6	Ba	4.5	0.3	Aa	11.6	0.5	Bc
		S	-23.1	1.5	Aa	50.7	0.6	Aa	1.6	0.2	Ab	34.8	4.0	Aa
		R	-24.5	1.8	Aa	52.3	1.5	Aa	1.8	0.1	Ab	28.8	1.5	Aab
		Rz	-10.5	1.3	Ab	53.5	0.6	Aa	2.1	0.1	ABb	26.2	1.3	Bb
	MS	L	-9.9	0.8	Ab	54.4	0.6	Aab	4.0	0.1	ABa	13.5	0.3	Ab
		S	-9.9	1.0	Bb	52.7	0.7	Ab	1.8	0.3	Ab	35.4	4.6	Aa
		R	-16.9	1.2	Ba	54.2	1.5	Aab	1.8	0.1	Ab	36.2	2.8	Aa
		Rz	-5.4	0.9	Bc	54.9	0.9	Aa	1.8	0.1	Bb	31.1	1.0	Aa
	SS	L	-8.2	0.8	Ab	53.2	0.4	ABa	3.7	0.1	Ba	14.6	0.4	Ac
		S	-9.7	1.0	Bb	54.1	1.5	Aa	1.7	0.1	Ac	34.2	2.6	Aa
		R	-15.3	1.6	Ba	54.8	0.2	Aa	1.7	0.1	Ac	32.8	2.0	Aa
		Rz	-3.8	0.8	Bc	53.4	0.6	Aa	2.2	0.1	Ab	25.1	1.8	Bb
P. virgatum L.	C	L	-10.5	0.9	Ab	54.3	0.6	Aa	2.8	0.1	Aa	19.1	0.3	Bc
		S	-9.1	1.2	Ab	53.1	0.5	ABa	1.0	0.1	Bd	60.6	7.0	Aa
		R	-32.5	3.5	Aa	52.3	1.5	Aa	1.9	0.1	Ab	29.0	1.2	Abc
		Rz	-23.7	4.0	Aa	54.8	0.7	Aa	1.5	0.1	Bc	36.3	1.3	Ab
	MS	L	-8.5	1.2	Ab	54.4	0.5	Aab	2.5	0.1	Ba	21.7	0.6	Ac
		S	-9.0	1.9	Ab	53.7	0.4	Ab	1.2	0.1	Bc	50.4	4.9	Aa
		R	-19.9	2.0	Ba	53.7	0.4	Ab	1.9	0.1	Ab	28.5	1.8	Abc
		Rz	-17.5	2.8	ABa	55.3	0.4	Aa	1.8	0.1	Ab	32.9	2.4	Ab
	SS	L	-10.7	1.6	Aa	54.6	0.4	Aa	2.6	0.1	ABa	21.1	0.8	ABa
		S	-12.2	0.6	Aa	52.1	0.2	Bb	1.8	0.1	Ab	29.1	1.6	Ba
		R	-11.5	2.1	Ba	53.8	0.9	Aab	2.1	0.2	Aab	27.8	2.9	Aa
		Rz	-12.4	1.7	Ba	53.8	0.9	Aab	2.0	0.2	Ab	28.5	2.7	Aa

Table 3. *Photosynthesis parameters (A_{sat}, assimilation rate at light saturation; g_s, stomatal conductance; T, transpiration rate and $_i$WUE, water use efficiency) and fluorescence parameters (F_v/F_m, maximal photochemical efficiency in the dark –adapted stage; F'_v/F'_m, maximal photochemical efficiency in light; ϕ_{PSII}, relative quantum yield of Photosystem II electron transport; q_P, photochemical quenching and NPQ, non-photochemical quenching) at the beginning of the experiment (Ti) in both species (A. donax L. and P. virgatum L.). Data are the means of three replicates and SE is shown. Data were analysed with an ANOVA Tukey analysis (ns: non-significant; * P < 0.05; ** P < 0.01 and *** P < 0.001).*

		A_{sat}	g_s	T	$_i$WUE	F_v/F_m	F'_v/F'_m	ϕ_{PSII}	q_P	NPQ
A. donax	Mean	22.9	0.3	5.7	4.1	0.8	0.5	0.3	0.6	1.7
	SE	2.4	0.0	0.3	0.6	0.0	0.0	0.0	0.0	0.2
P. virgatum	Mean	22.0	0.1	3.2	6.9	0.7	0.3	0.2	0.6	1.7
	SE	2.0	0.0	0.4	0.3	0.0	0.0	0.0	0.0	0.3
		ns	*	**	*	*	**	**	ns	ns

Table 4. *Changes in chlorophyll content (SPAD units) in both species (A. donax L. and P. virgatum L.) for each treatment: i) Control (C, 100% PC), ii) Mild stress (MS, 50% PC) and iii) Severe Stress (SS, 25% PC) at the beginning (Ti) and at the end of the experiment (Tf). Data are the means of nine replicates and the standard error is shown. Data were analysed with an ANOVA Tukey analysis. Different capital letters indicate significant differences (P < 0.05) between time for the same species and treatment. Different small letters indicate significant differences (P < 0.05) between treatments for the same species and time. Different Greek letters indicate significant differences (P < 0.05) between species for the same time and treatment.*

	C						*MS*						*SS*					
	Ti			Tf			Ti			Tf			Ti			Tf		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
A. donax	42.1	0.7	Aa	42.1	1.0	Aa	41.5	0.5	Aa	42.7	1.0	Aa	41.6	0.5	Aa	42.0	0.9	Aa
P. virgatum	35.9	0.7	Ba	41.0	1.4	Ab	35.7	0.6	Aa	37.4	0.5	Ab	33.4	0.9	Ba	44.9	1.0	Aa

Table 5. *Biomass parameters (SA, Stem Area (m^2); SDW, Shoot Dry Weight (g); TDW, Total Dry Weight (g); S/R, Shoot Root ratio (g g^{-1}); SLA, Specific Leaf Area (m^2 Kg^{-1}); LAR, Leaf Area Ratio (m^2 Kg^{-1}), LWR, Leaf Weight Ratio (Kg Kg^{-1}) in both species (A. donax L. and P. virgatum L.) for each treatment: i) Control (C, 100% PC), ii) Mild stress (MS, 50% PC) and iii) Severe Stress (SS, 25% PC) at the end of the experiment (Tf). Data are the means of nine replicates and the standard error is shown. Data were analysed with an ANOVA Tukey analysis. Different small letters indicate significant differences (P < 0.05) between treatments for the same species and time. Different Greek letters indicate significant differences (P < 0.05) between species for the same time and treatment.*

		C			***MS***			***SS***		
		Mean	***SE***		**Mean**	***SE***		**Mean**	***SE***	
	SA	0.03	0.00	a	0.02	0.00	b	0.01	0.00	c
	SDW	13.2	1.7	a	9.0	0.4	b	5.1	0.6	c
	TDW	16.1	2.1	a	11.9	0.5	a	7.0	0.7	b
A. donax	S/R	4.5	0.2	a	3.3	0.2	b	2.5	0.3	c
	SLA	24.8	1.2	a	21.0	0.6	b	21.1	0.9	b
	LAR	9.3	0.6	a	7.7	0.3	b	7.1	0.4	b
	LWR	0.40	0.01	a	0.37	0.01	ab	0.33	0.02	b
	SA	0.17	0.02	a	0.07	0.01	b	0.04	0.00	b
	SDW	47.9	3.3	a	24.8	1.7	b	16.4	1.0	c
	TDW	130.7	15.0	a	69.0	7.3	b	48.3	3.9	b
P. virgatum	S/R	0.64	0.06	a	0.63	0.06	a	0.58	0.07	a
	SLA	21.1	0.7	a	17.1	1.4	b	8.6	0.6	c
	LAR	3.9	0.3	a	3.1	0.4	a	1.5	0.1	b
	LWR	0.19	0.01	a	0.18	0.01	a	0.17	0.01	a

Table 6. *Statistical analysis of water stress effects on $^{13}C_R$ (‰), $^{13}C_{TOM}$ (‰), $^{15}N_{TOM}$ (‰) and $^{13}C_{TSS}$ (‰).*

	$^{13}C_{TOM}$		$^{13}C_{TSS}$		$^{13}C_R$		$^{15}N_{TOM}$	
	F	***F***	***F***	***F***	***F***	**Sig**	***F***	**Sig**
Model	46.9	***	25.6	***	39.5	***	24.5	***
Species	206.9	***	45.7	***	13.1	***	92.5	***
Time	873.0	***	605.9	***	925.3	***	438.6	***
Treatment	120.8	***	93.5	***	80.1	***	138.6	***
Organ	118.2	***	18.8	***	36.3	***	14.9	***
Species*Time	4.4	*	4.6	*	0.9	ns	28.1	***
Species*Treatment	31.6	***	5.3	**	18.5	***	19.8	***
Species*Organ	24.3	***	2.5	ns	6.6	***	3.4	***
Time*Treatment	29.1	***	30.8	***	37.7	***	45.8	***
Time*Organ	35.8	***	13.4	***	17.9	***	10.0	***
Treatment*Organ	7.5	***	1.3	ns	4.6	***	3.2	**
Species*Time*Treatment	14.0	***	6.3	***	20.3	***	6.5	***
Species*Time*Organ	5.5	***	3.2	**	14.5	***	2.0	ns
Species*Treatment*Organ	11.6	***	1.2	ns	4.9	***	1.4	ns
Time*Treatment*Organ	3.5	***	1.1	ns	4.4	***	1.8	*
Species*Time*Treatment*Organ	4.8	***	1.3	ns	6.6	***	0.9	ns

Abbreviations

A_{sat}, light saturated net CO_2 assimilation rate; *C*, control conditions; C/N, carbon/nitrogen ratio; C_c, total carbon content; F_v/F_m, maximum quantum efficiency of PSII; F_v'/F_m', photochemical efficiency of PSII; g_s, stomatal conductance; H, height; LA, leaf area; LAR, leaf area ratio; LDW, leaf dry weight; LMA, leaf mass area; LWR, leaf weight ratio; *MS*, moderate stress conditions; N_c, total nitrogen content; NGL, number of green leaves; NPQ, non-photochemical quenching; NS, number of stems; PC, pot capacity; PPFD, photosynthetic photon flux density; q_p, photochemical quenching; R_d, dark respiration; RWC, relative water content; SA, stem area; SDW, shoot dry weight; SLA, specific leaf area; S/R, shoot/root ratio; *SS*, severe stress conditions; *T*, transpiration; TDW, total dry weight; *WS*, water stress; WUE_{inst}, instantaneous water use efficiency; ϕ_{PSII}, relative quantum efficiency of PSII; ^{13}C, carbon-13 isotope; ^{15}N, nitrogen-15 isotope; $\delta^{13}C_{TOM}$, ^{13}C isotopic composition of total organic matter; $\delta^{15}N_{TOM}$, ^{15}N isotopic composition of total organic matter; $\delta^{13}C_{TSS}$, ^{13}C isotopic composition of total soluble sugars; $\delta^{13}C_R$, ^{13}C isotopic composition CO_2 respired in dark.

References

[1] I. Lewandowski, J. M. O. Scurlock, E. Lindvall, M. Christou, The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe, Biomass and Bioenergy. 25 (2003) 335–361. doi:10.1016/S0961-9534(03)00030-8.

[2] I. E. Palmer, R. J. Gehl, T. G. Ranney, D. Touchell, N. George, Biomass yield, nitrogen response, and nutrient uptake of perennial bioenergy grasses in North Carolina, Biomass and Bioenergy. 63 (2014) 218–228. doi:10.1016/J.biombioe.2014.02.016.

[3] C. Mariani, R. Cabrini, A. Danin, P. Piffanelli, A. Fricano, S. Gomarasca, M. Dicandilo, F. Grassi, C. Soave, Origin, diffusion and reproduction of the giant reed (*Arundo donax* L.): a promising weedy energy crop, Ann. Appl. Biol. 157 (2010) 191–202. doi:10.1111/j.1744-7348.2010.00419.x.

[4] L. G. Angelini, L. Ceccarini, N. N. o Di Nasso, E. Bonari, Comparison of *Arundo donax* L. and *Miscanthus x giganteus* in a long-term field experiment in Central Italy: Analysis of productive characteristics and energy balance, Biomass and Bioenergy. 33 (2009) 635–643. doi:https://doi.org/10.1016/j.biombioe.2008.10.005.

[5] B. Rossa, A. V Tüffers, G. Naidoo, D. J. Willert, *Arundo donax* L. (Poaceae) — a C_3 species with unusually high photosynthetic capacity, Bot. Acta. 111 (2014) 216–221. doi:10.1111/j.1438-8677.1998.tb00698.x.

[6] J. N. Barney, J. J. Mann, G. B. Kyser, E. Blumwald, A. Van Deynze, J. M. DiTomaso, Tolerance of switchgrass to extreme soil moisture stress: Ecological implications, Plant Sci. 177 (2009) 724–732. doi:10.1016/j.plantsci.2009.09.003.

[7] M. Gutierrez, V. E. Gracen, G. E. Edwards, Biochemical and cytological relationships in C_4 plants, Planta. 119 (1974) 279–300. doi:10.1007/BF00388331.

[8] M. D. Hatch, C_4 photosynthesis: a unique elend of modified biochemistry, anatomy and ultrastructure, Biochim. Biophys. Acta - Rev. Bioenerg. 895 (1987) 81–106. doi:https://doi.org/10.1016/S0304-4173(87)80009-5.

[9] C. L. Porter, An Analysis of Variation Between Upland and Lowland Switchgrass, *Panicum Virgatum* L., in Central Oklahoma, Ecology. 47 (1966) 980–992. doi:10.2307/1935646.

[10] D. A. Warner, M. S. B. Ku, G. E. Edwards, Photosynthesis, leaf anatomy, and cellular constituents in the polyploid C_4 Grass *Panicum virgatum*, Plant Physiol. 84 (1987) 461 LP-466. http://www.plantphysiol.org/content/84/2/461.abstract.

[11] M. Sanderson, R. Reed, S. McLaughlin, S. Wullschleger, B. Conger, D. Parrish, D. Wolf, C. Taliaferro, A. Hopkins, W. Ocumpaugh, M. Hussey, J. Read, C. Tischler, Switchgrass as a sustainable crop, Bioresour. Technol. 56 (1996) 83–93.

[12] M. D. Casler, C. M. Tobias, S. M. Kaeppler, C. R. Buell, Z.-Y. Wang, P. Cao, J. Schmutz, P. Ronald, The switchgrass genome: tools and strategies, 4 (2011). doi:10.3835/plantgenome2011.10.0026.

[13] A. Yokota, K. Takahara, K. Akashi, Water stress, in: K. V Madhava Rao, A. S. Raghavendra, K. Janardhan Reddy (Eds.), Physiology and molecular bioliology of stress tolerance in plants, Springer Netherlands, Dordrecht, 2006: pp. 15–39. doi:10.1007/1-4020-4225-6_2.

[14] H. Medrano, J. M. Escalona, J. Bota, J. Gulías, J. Flexas, Regulation of photosynthesis of C_3 plants in response to progressive drought: Stomatal conductance as a reference parameter, Ann. Bot. 89 (2002) 895–905. doi:10.1093/aob/mcf079.

[15] J. Flexas, J. Bota, F. Loreto, G. Cornic, T. D. Sharkey, Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants, Plant Biol. 6 (2008) 269–279. doi:10.1055/s-2004-820867.

[16] J. Flexas, A. Diaz-Espejo, J. Galmés, R. Kaldenhoff, H. Medrano, M. Ribas-Carbo, Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves, Plant, Cell Environ. 30 (2007) 1284–1298. doi:10.1111/j.1365-3040.2007.01700.x.

[17] M. M. Chaves, J. Flexas, C. Pinheiro, Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell, Ann. Bot. 103 (2009) 551–560. doi:10.1093/aob/mcn125.

[18] P. Kramer, J. Boyer, Cell water relations, in: Water Relations of Plants and Soils, 1st edn, 1995: pp. 42–83.

[19] F. W. Badeck, G. Tcherkez, S. Nogues, C. Piel, J. Ghashghaie, Post-photosynthetic fractionation of stable carbon isotopes between plant organs - a widespread phenomenon, Rapid Commun Mass Spectrom. 19 (2005) 11. doi:10.1002/rcm.1912.

[20] G. D. Farquhar, M. H. O'Leary, J. A. Berry, On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves, Funct. Plant Biol. 9 (1982) 121–137. https://doi.org/10.1071/PP9820121.

[21] G. D. Farquhar, K. T. Hubick, A. G. Condon, R. A. Richards, Carbon isotope fractionation and plant water-use efficiency BT - Stable Isotopes in Ecological Research, in: P. W. Rundel, J. R. Ehleringer, K. A. Nagy (Eds.), Springer New York, New York, NY, 1989: pp. 21–40.

[22] O. Ghannoum, S. von Caemmerer, J. P. Conroy, The effect of drought on plant water use efficiency of nine NAD-ME and nine NADP-ME Australian C_4 grasses, Funct. Plant Biol. 29 (2002) 1337–1348. doi:http://dx.doi.org/10.1071/FP02056.

[23] O. Ghannoum, C_4 photosynthesis and water stress, Annu. Bot. 103 (2008) 635-644 doi:10.1093/aob/mcn093.

[24] G. D. Farquhar, On the nature of carbon isotope discrimination in C_4 species, Funct. Plant Biol. 10 (1983) 205–226. https://doi.org/10.1071/PP9830205.

[25] J. Ghashghaie, F.W. Badeck, G. Lanigan, S. Nogués, G. Tcherkez, E. Deléens, G. Cornic, H. Griffiths, Carbon isotope fractionation during dark respiration and photorespiration in C_3 plants, Phytochem. Rev. 2 (2003) 145–161. doi:10.1023/B:PHYT.0000004326.00711.ca.

[26] E. A. Hobbie, R. A. Werner, Intramolecular, compound‐specific, and bulk carbon isotope patterns in C_3 and C_4 plants: a review and synthesis, New Phytol. 161 (2003) 371–385. doi:doi:10.1111/j.1469-8137.2004.00970.x.

[27] L. A. Cernusak, N. Ubierna, K. Winter, J. A. M. Holtum, J. D. Marshall, G. D. Farquhar, Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants, New Phytol. 200 (2013) 950–965. doi:10.1111/nph.12423.

[28] G. Tcherkez, M. Hodges, How stable isotopes may help to elucidate primary nitrogen metabolism and its interaction with (photo)respiration in C_3 leaves, J. Exp. Bot. 59 (2008) 1685–1693. http://dx.doi.org/10.1093/jxb/erm115.

[29] S. Nogués, N. R. Baker, Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV‐B radiation, J. Exp. Bot. 51 (2000) 1309–1317. http://dx.doi.org/10.1093/jxb/51.348.1309.

[30] S. Nogués, S. Aljazairi, C. Arias, E. Sánchez, I. Aranjuelo, Two distinct plant respiratory physiotypes might exist which correspond to fast-growing and slow-growing species, J. Plant Physiol. 171 (2014) 1157–1163. doi:10.1016/J.JPLPH.2014.03.006.

[31] S. Nogués, I. Aranjuelo, A. Pardo, J. Azcón‐Bieto, Assessing the stable carbon isotopic composition of intercellular CO_2 in a CAM plant using gas chromatography‐combustion‐isotope ratio mass spectrometry, Rapid Commun. Mass Spectrom. 22 (2008) 1017–1022. doi:10.1002/rcm.3460.

[32] S. Nogués, G. Tcherkez, G. Cornic, J. Ghashghaie, Respiratory carbon metabolism following illumination in intact french bean leaves using $^{12}C/^{13}$ isotope labeling, Plant Physiol. 136 (2004) 3245 LP-3254. http://www.plantphysiol.org/content/136/2/3245.abstract.

[33] F. W. Badeck, G. Tcherkez, S. Nogués, C. Piel, J. Ghashghaie, Post-photosynthetic fractionation of stable carbon isotopes between plant organs—a widespread phenomenon, Rapid Commun. Mass Spectrom. 19 (2005) 1381–1391. doi:10.1002/rcm.1912.

[34] J. D. Marshall, J. R. Brooks, K. Lajtha, Sources of variation in the stable isotopic composition of plants, Stable Isot. Ecol. Environ. Sci. 2 (2007) 22–60.

[35] J. Ghashghaie, M. Duranceau, F. W. Badeck, G. Cornic, M. T. Adeline, E. Deleens, $\delta^{13}C$ of CO_2 respired in the dark in relation to $\delta^{13}C$ of leaf metabolites: comparison between *Nicotiana sylvestris* and *Helianthus annuus* under drought, Plant. Cell Environ. 24 (2001) 505–515. doi:10.1046/j.1365-3040.2001.00699.x.

[36] G. Tcherkez, S. Nogués, J. Bleton, G. Cornic, F. Badeck, J. Ghashghaie, Metabolic origin of carbon isotope composition of leaf dark-respired CO_2 in french bean, Plant Physiol. 131 (2003) 237 LP-244. http://www.plantphysiol.org/content/131/1/237.abstract.

[37] M. Duranceau, J. Ghashghaie, F. Badeck, E. Deleens, G. Cornic, $\delta^{13}C$ of CO_2 respired in the dark in relation to $\delta^{13}C$ of leaf carbohydrates in *Phaseolus vulgaris* L. under progressive drought, Plant, Cell Environ. 22 (1999) 515–523. doi:10.1046/j.1365-3040.1999.00420.x.

[38] A. A. Jaradat, Genetic resources of energy crops: Biological systems to combat climate change, AJCS. 4 (2010) 309–323. https://naldc.nal.usda.gov/naldc/download.xhtml?id=49241&content=PDF (accessed April 17, 2018).

[39] T. L. Slewinski, Non-structural carbohydrate partitioning in grass stems: a target to increase yield stability, stress tolerance, and biofuel production, J. Exp. Bot. 63 (2012) 4647–4670. http://dx.doi.org/10.1093/jxb/ers124.

[40] S. Bihmidine, C. T. Hunter, C. E. Johns, K. E. Koch, D. M. Braun, Regulation of assimilate import into sink organs: update on molecular drivers of sink strength, Front. Plant Sci. 4 (2013) 177. doi:10.3389/fpls.2013.00177.

[41] J. J. Mann, J. N. Barney, G. B. Kyser, J. M. Di Tomaso, *Miscanthus* × *giganteus* and *Arundo donax* shoot and rhizome tolerance of extreme moisture stress, GCB Bioenergy. 5 (2012) 693–700. doi:10.1111/gcbb.12039.

[42] L. A. Cernusak, J. D. Marshall, J. P. Comstock, N. J. Balster, Carbon isotope discrimination in photosynthetic bark, Oecologia. 128 (2001) 24–35. http://www.jstor.org/stable/4222973.

[43] J. Ghashghaie, F. W. Badeck, Opposite carbon isotope discrimination during dark respiration in leaves versus roots – a review, New Phytol. 201 (2014) 751–769. doi:10.1111/nph.12563.

[44] R. F. Sage, R. W. Pearcy, The nitrogen use efficiency of C_3 and C_4 plants, Plant Physiol. 84 (1987) 959 LP-963. http://www.plantphysiol.org/content/84/3/959.abstract.

[45] O. Ghannoum, J. R. Evans, S. von Caemmerer, Chapter 8 Nitrogen and water use efficiency of C_4 plants BT - C4 Photosynthesis and related CO_2 concentrating mechanisms, in: A. S. Raghavendra, R. F. Sage (Eds.), Springer Netherlands, Dordrecht, 2011: pp. 129–146. doi:10.1007/978-90-481-9407-0_8.

[46] D. W. Lawlor, Limitation to photosynthesis in water‐stressed leaves: stomata vs. metabolism and the role of ATP, Ann. Bot. 89 (2002) 871–885.

[47] J. Gulías, J. Flexas, A. Abadía, H. Madrano, Photosynthetic responses to water deficit in six Mediterranean sclerophyll species: possible factors explaining the declining distribution of *Rhamnus ludovici-salvatoris*, an endemic Balearic species, Tree Physiol. 22 (2002) 687–697. http://dx.doi.org/10.1093/treephys/22.10.687.

[48] D. Robinson, L. L. Handley, C. M. Scrimgeour, D. C. Gordon, B. P. Forster, R. P. Ellis, Using stable isotope natural abundances ($\delta^{15}N$ and $\delta^{13}C$) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes, J. Exp. Bot. 51 (2000) 41–50. http://dx.doi.org/10.1093/jexbot/51.342.41.

[49] S. Yousfi, M. D. Serret, J. Voltas, J. L. Araus, Effect of salinity and water stress during the reproductive stage on growth, ion concentrations, $\Delta^{13}C$, and $\delta^{15}N$ of durum wheat and related amphiploids, J. Exp. Bot. 61 (2010) 3529–3542. http://dx.doi.org/10.1093/jxb/erq184.

[50] R. J. Webster, S. M. Driever, J. Kromdijk, J. McGrath, A. D. B. Leakey, K. Siebke, T. Demetriades-Shah, S. Bonnage, T. Peloe, T. Lawson, S. P. Long, High C_3 photosynthetic capacity and high intrinsic water use efficiency underlies the high productivity of the bioenergy grass *Arundo donax*, Sci. Rep. 6 (2016) 20694. http://dx.doi.org/10.1038/srep20694.

[51] C. B. Osmond, K. Winter, H. Ziegler, Functional significance of different pathways of CO_2 fixation in photosynthesis BT - Physiological Plant Ecology II: Water Relations and Carbon Assimilation, in: O. L. Lange, P. S. Nobel, C. B. Osmond, H. Ziegler (Eds.), Springer Berlin Heidelberg, Berlin, Heidelberg, 1982: pp. 479–547. doi:10.1007/978-3-642-68150-9_16.

[52] L. L. Nackley, K. A. Vogt, S.-H. Kim, *Arundo donax* water use and photosynthetic responses to drought and elevated CO_2, Agric. Water Manag. 136 (2014) 13–22. doi:10.1016/J.AGWAT.2014.01.004.

[53] S. L. Cosentino, D. Scordia, E. Sanzone, G. Testa, V. Copani, Response of giant reed (*Arundo donax* L.) to nitrogen fertilization and soil water availability in semi-arid Mediterranean environment, Eur. J. Agron. 60 (2014) 22–32. doi:https://doi.org/10.1016/j.eja.2014.07.003.

[54] E. Meyer, M. J. Aspinwall, D. B. Lowry, J. D. Palacio-Mejía, T. L. Logan, P. A. Fay, T. E. Juenger, Integrating transcriptional, metabolomic, and physiological responses to drought stress and recovery in switchgrass (*Panicum virgatum* L.), BMC Genomics. 15 (2014) 527.

[55] G. Cornic, A. Massacci, Leaf photosynthesis under drought stress BT - Photosynthesis and the Environment, in: N. R. Baker (Ed.), Springer Netherlands, Dordrecht, 1996: pp. 347–366. doi:10.1007/0-306-48135-9_14.

[56] J. M. Escalona, J. Flexas, H. Medrano, Stomatal and non-stomatal limitations of photosynthesis under water stress in field-grown grapevines, Aust. J. Plant Physiol. 26 (1999) 421. doi:10.1071/PP99019.

[57] N. R. Baker, Chlorophyll Fluorescence: A probe of photosynthesis in vivo, Annu. Rev. Plant Biol. 59 (2008) 89–113. doi:10.1146/annurev.arplant.59.032607.092759.

[58] T. D. Sharkey, Water-stress effects on photosynthesis, (1990).

[59] F. Li, S. Kang, J. Zhang, Interactive effects of elevated CO_2, nitrogen and drought on leaf area, stomatal conductance, and evapotranspiration of wheat, Agric. Water Manag. 67 (2004) 221–233. doi:https://doi.org/10.1016/j.agwat.2004.01.005.

[60] E. Heaton, T. Voigt, S. P. Long, A quantitative review comparing the yields of two candidate C_4 perennial biomass crops in relation to nitrogen, temperature and water, Biomass and Bioenergy. 27 (2004) 21–30. doi:https://doi.org/10.1016/j.biombioe.2003.10.005.

[61] M. Fagnano, A. Impagliazzo, M. Mori, N. Fiorentino, Agronomic and environmental impacts of Giant reed (*Arundo donax* L.): Results from a long-term field experiment in hilly areas subject to soil erosion, BioEnergy Res. 8 (2015) 415–422. doi:10.1007/s12155-014-9532-7.

[62] A. Blum, Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive?, Aust. J. Agric. Res. 56 (2005) 1159–1168. doi:10.1071/AR05069.

[63] G. Erice, S. Louahlia, J. J. Irigoyen, M. Sanchez-Diaz, J.-C. Avice, Biomass partitioning, morphology and water status of four alfalfa genotypes submitted to progressive drought and subsequent recovery, J. Plant Physiol. 167 (2010) 114–120. doi:https://doi.org/10.1016/j.jplph.2009.07.016.

[64] P. J. Kramer, J. S. Boyer, Water relations of plants and soils, Academic press, 1995.

[65] L. D. Quinn, K. C. Straker, J. Guo, S. Kim, S. Thapa, G. Kling, D. K. Lee, T. B. Voigt, Stress-tolerant feedstocks for sustainable bioenergy production on marginal land, Bioenergy Res. 8 (2015) 1081–1100. doi:10.1007/s12155-014-9557-y.