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# Source-sink limitations for grain weight in wheat and barley under waterlogging conditions during pre-anthesis

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## Abstract

In wheat and barley, grain yield is strongly affected by waterlogging, especially during the period immediately previous to anthesis. Although waterlogging reduces grain yield mainly by reductions in grain number, mean grain weight (MGW) is also frequently reduced. The aim of this work was to determine whether increases in the source-sink ratio produces smaller reductions in MGW due to waterlogging in wheat and barley. Two experiments were carried out combining a waterlogging condition 20 days pre-anthesis, two soil N conditions at sowing and source-sink manipulations during grain-filling (untrimmed or 50% trimmed spikes). Waterlogging reduced MGW in both species up to 35% in wheat and 44% in barley. The negative effect of waterlogging on MGW was intensified with warmer temperatures and higher atmospheric demand. Increases in the source-sink ratio during the grain-filling period (by trimming treatments) showed a positive impact on MGW in wheat (up to 40%) and barley (up to 20%) under waterlogging conditions. For both species, grain weight response to increases in source-sink ratio was higher at lower grain weight and/or spike hierarchies (i.e. spikes from tillers). Our results showed that waterlogging during pre-anthesis affected grain weight through a reduction in the source during grain-filling and a possible impact on potential grain weight, depending on the intensity of the stress.

KEYWORDS
grain hierarchies, *Hordeum vulgare L. ssp. distichum*, potential grain weight, radiation interception, *Triticum aestivum L.*, water-soluble carbohydrates

## 1 | INTRODUCTION

Waterlogging is a global constraint on cropping systems due to the increase in the frequency of extreme climate events (Wollenweber et al., 2003) affecting most of the grains crops including wheat and barley. Wheat and barley are the most important small grains representing ca. 30% and 6% of world winter cereals production (https://www.fao.org). The severity of the effects of waterlogging depends on the susceptibility of the species (or genotype) to the stress, the growth stage of the plant (de San Celedonio et al., 2014; Ploschuk et al., 2020; Ploschuk et al., 2021; Setter & Waters, 2003; Watson et al., 1976), and the duration (Marti et al., 2015; Olgun et al., 2008) and intensity (Malik et al., 2001) of the waterlogging event. Interactions with the environment can also modulate plant responses to stress, as a waterlogging event combined with high evaporative demand leads to a higher yield reduction in grain crops (Grassini et al., 2007; Ploschuk et al., 2020; Ploschuk et al., 2021).

Grain yield is strongly affected in wheat and barley when waterlogging occurs during the period immediately previous to anthesis (Sayre et al., 1994), coinciding with the critical period for potential yield determination in both crops (Arisnabarreta & Miralles, 2008; Fischer, 1985). It was determined for both species that the most harmful period in terms of yield reduction for the occurrence of a waterlogging event was during the stem elongation period (i.e. 20 days immediately prior to anthesis), known as the "critical waterlogging period" for yield definition in wheat and barley (de San Celedonio et al., 2014; Ploschuk et al., 2020). Waterlogging accelerates leaf senescence and causes reductions in the rate of photosynthesis of the remaining leaves (de San Celedonio et al., 2017; Ploschuk et al., 2018). Although yield reductions are mainly related to decreases in grain number (de San Celedonio et al., 2018; Ploschuk et al., 2020), grain weight is also reduced by waterlogging events before anthesis (Araki et al., 2012; Ciancio et al., 2021).

During the stem elongation period, floret primordia in the spike are differentiated, but a proportion of them degenerate and die coinciding with the active competition for photo-assimilates between spike and stem (González et al., 2005). The level of assimilate supply to the growing spike determines the final number of grains per spike (Arisnabarreta & Miralles, 2010) as grain setting is, in general, a non-limiting factor (Miralles & Slafer, 1999). At the beginning of the grain-filling period, the grain number is already set whilst grain weight is being determined. After anthesis, sink strength is exclusively driven by the grain growth in both species. Under no water or nutritional deficiencies, the number of grains per plant is the main component that explains variations in yield in wheat and barley, whilst grain weight remains practically unchanged. However, with a waterlogging condition, grain yield penalizations are explained by reductions in both the number of grains per plant and grain weight (de San Celedonio et al., 2014).

Grain weight in wheat and barley differs according to the position of each grain along with the spike. In both species, grains located in apical or basal spikelets within a spike tend to be lighter than those in central spikelets (Slafer & Savin, 1994). Additionally, in the plurifloral spikelets of wheat, grains located in distal positions of the spikelet have lower potential grain weight than those located near the rachis (Miralles & Slafer, 1995). Likewise, grains from tillers are usually lighter than grains from main stems. Differences in weight between grain categories could be explained by either the supply of photo-assimilates (source limitation) and/or the capacity of the grains to accumulate available carbohydrates (sink limitation). During the grain-filling period, actual photosynthesis from the remaining green leaves and remobilization of reserves from the stems and leaf sheaths determine the capacity of the crop to supply assimilates to the grains (source capacity), whilst the number of grains per unit area and their potential size determines the sink capacity.

Source-sink manipulations during post-anthesis in wheat have shown that the crop is mainly sink-limited (Borrás et al., 2004), whilst barley can be either sink- or source-limited (Alvarez Prado et al., 2013; Bingham et al., 2007; Dreccer et al., 1997). These studies have traditionally been performed under potential growing conditions (i.e. without water or nutritional restrictions) and with treatments usually applied after anthesis. However, growing conditions during pre-anthesis have a predominant role in grain growth and final yield. It was observed that waterlogging during pre-anthesis reduced grain weight 15%–20% in wheat and ca. 10% in barley (de San Celedonio et al., 2014; Marti et al., 2015). Grain weight penalization due to waterlogging during pre-anthesis could be related to its negative effect on potential grain weight, which is determined during the pre-anthesis phase (Calderini et al., 1999), and/or by the delayed effect of waterlogging on leaf senescence that takes place once the waterlogging is released (de San Celedonio et al., 2017) reducing the magnitude of actual photosynthesis during the grain-filling period. Supporting the second speculation, it has been determined that the detrimental effect of waterlogging on aerial biomass is not evident during the event but appears once waterlogging is removed (de San Celedonio et al., 2017) being plant resources crucial for recovery after waterlogging remotion (Ciancio et al., 2021; Zahra et al., 2021).

The evidences described above allow us to hypothesize that (i) waterlogging, even though it occurred before the grain-filling period, reduces grain weight, but (ii) if the source-sink ratio is recomposed during grain filling after the waterlogging episode, it is possible a partial compensation in grain weight. The aim of this work was to determine whether increases in the source-sink ratio during the grain-filling period lessen reductions in mean grain weight due to waterlogging during the critical period in wheat and barley.

## 2 | MATERIALS AND METHODS

### 2.1 | Growing conditions and treatments

Two experiments were carried out in 1 $m^3$ (1 m × 1 m × 1 m) containers under natural weather conditions at the experimental field of the School of Agriculture, University of Buenos Aires, Argentina (34° 35´S, 58° 29´W) in the 2011 and 2013 growing seasons. Experiments were sown on 29 July 2011 (EX1) and 10 June 2013 (EX2) within the optimum range of sowing dates for the location.

Treatments in both experiments consisted of a combination of: (i) Species: Baguette 13 as wheat cultivar (*Triticum aestivum* L.) and Scarlett as barley cultivar (*Hordeum vulgare* L. ssp. *distichum*) (hereafter wheat and barley). The selected genotypes are commercial cultivars with similar phenology, widely adopted by farmers in Argentina. (ii) Water condition: a control treatment, well-drained during the entire phenological cycle (Ctl) and a waterlogging treatment applied from 20 days pre-anthesis to anthesis. The moment of the waterlogging treatment was selected based on de San Celedonio et al. (2014), this work determined that the period immediately previous to anthesis was the most sensitive to waterlogging in terms of yield penalization in both species. Waterlogging treatment was imposed by artificial irrigation, where each container was equipped with an individual drip irrigation system and a one-inch valve at the bottom, that allowed rapid drainage of water at the end of the treatment. (iii) Soil nitrogen level: A low nitrogen (N) level of 60 kg $ha^{-1}$ (N0) and a high N level of 160 kg $ha^{-1}$ (N1). Nitrogen level was reached by fertilization with urea in a divided dose (30% at sowing, 30% at the beginning of tillering and 40% at the beginning of stem elongation). (iv) Trimming: seven days after anthesis 50% of the spikelets of the spikes contained in 50 lineal cm rows were trimmed, by removing all the spikelets by hand from one lateral of the spike (trimmed spike, TS), in order to increase the source-sink ratio. Spikes in another 50 cm row remained untrimmed as control (entire spike, ES) (Figure 1).

The experimental design was a split-plot with three replicates per treatment. The main plot was the random combination of (i) species, (ii) waterlogging and (iii) N fertilization, and the sub-plot was assigned to the source-sink treatments.

The containers were filled with a 3:1 mixture of sand and soil and a 5 cm layer of gravel was placed at the bottom for drainage once the waterlogging event ended. Plant density was 250 pl $m^{-2}$ in both experiments, with an inter-row spacing of 10 cm. Sowing was carried out by placing seeds on a biodegradable adhesive paper tape, 4.5 cm apart. Biotic adversities were controlled in both experiments.

## 2.2 | Measurements

Time to anthesis in wheat, defined as the moment in which 50% of the spikes from each plot showed anthers, was determined following the decimal code of Zadoks (Zadoks et al., 1974). As in barley, true anthesis (when pollen of anthers is released over the stigma of the ovary) occurs when the spike is in the sheath of the flag leaf, time to anthesis was determined by opening the spikelets and visualizing pollen release. All the plants contained in two rows 40 cm long were harvested at anthesis and the green leaf blades were separated for measuring leaf green area (Li-Cor 3100). Stomatal conductance was determined 12 days after anthesis in the flag leaf in wheat and in the leaf immediately below the flag leaf in barley in two plants per experimental unit (container) using a leaf porometer (Decagon SC-1).

From anthesis to physiological maturity (PM), photosynthetically active radiation interception (iPAR) of the canopy was determined with a linear ceptometer (CAVARAD, Cavadevises, Argentina) between 12:00 PM and 02:00 PM on clear days. Two measurements were made at each container, one above the canopy, to determine the incident PAR (I0), and another below, following the senescence profile, representing transmitted PAR (It). The fraction of intercepted PAR (iPAR %) was calculated as (I0−It)/I0. The dynamics of intercepted radiation for each treatment during postanthesis was fitted with a sigmoid function (Miralles & Slafer, 1997). Photosynthetically active radiation intercepted by the crop (iPAR; MJ $m^{-2}$ $day^{-1}$) was calculated each day as the product of iPAR %, incident global radiation (MJ $m^{-2}$ $day^{-1}$) and 0.48 (i.e. ratio of photosynthetically active to total radiation (Szeicz, 1974).

At maturity, all the plants contained in two 50 cm rows (one control and the other with trimmed spikes) in each container were harvested. The spikes were separated from the stems plus sheath. The spikes harvested at maturity were threshed characterizing different categories of grains. Individual grain weight of central (CS) and basal spikelets (BS) was determined considering the proximal (grains 1 and 2) or distal positions (grain 3) within each spikelet (Figure 1), in spikes from the main stem (EX1 and EX2) and tillers (EX1). Also, mean grain weight (MGW) in the main stem and tillers was registered. To estimate the effect of the source-sink treatments on the weight of the grains, the results of the trimmed spike were relativized compared to the untrimmed spike (i.e. entire spike) as:

Percentage change in grain weight = (grain weight of entire spike−grain weight of trimmed spike) × 100 grain $weight^{-1}$ of entire spikes.

In EX1, water-soluble carbohydrates (WSC) concentration in stems were determined in the samples harvested at anthesis and at maturity

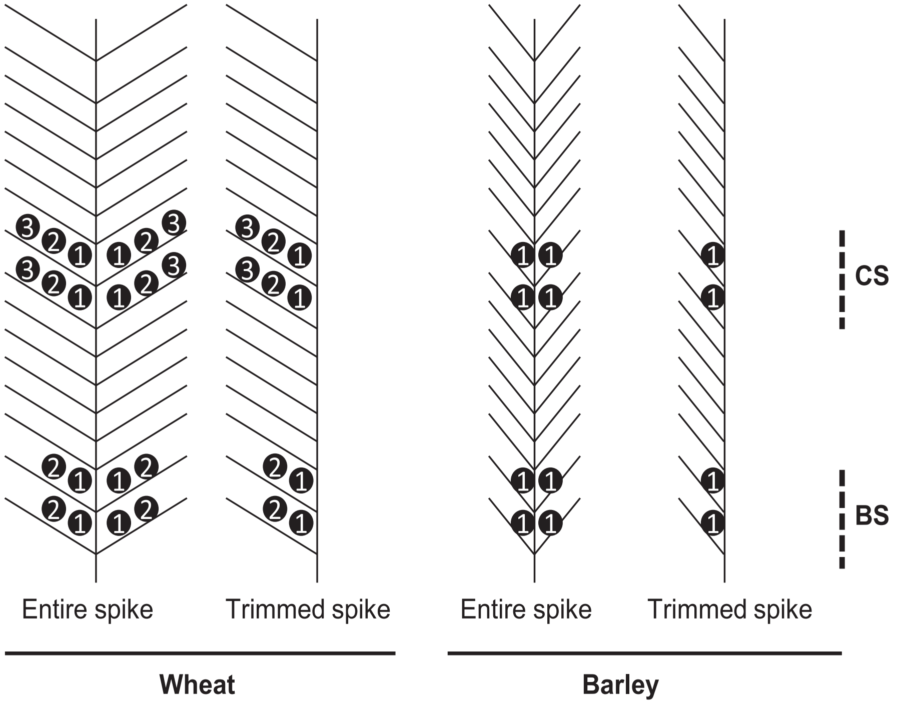

**FIGURE 1** Diagram of the source-sink treatments carried out in wheat and barley spikes. An example of the untrimmed (entire spike) and trimmed spikes with the grain positions sampled across the spike and within the spikelets in each species is detailed. BS: basal spikelet, CS: central spikelet; 1: The first grain nearest the rachis; 2: The second grain nearest the rachis; 3: The third grain nearest the rachis

using the anthrone method (Yemm & Willis, 1954). The content of WSC per unit area available at anthesis and maturity was estimated as:

WSC (g $m^{-2}$) = dry weight of stems (g $m^{-2}$) × WSC concentration (glucose (g) × 0.1 g dry weight of stems) × $100^{-1}$.

The source-sink ratio during grain filling (kJ $grain^{-1}$) was determined considering the ratio between the cumulative iPAR between anthesis and physiological maturity (source) and the grain number per plant at maturity (sink). This analysis allows establishing whether the stress modified the quantity of source per grain during the grain-filling period.

Meteorological data (air minimum and maximum temperature, global radiation and reference evapotranspiration) were recorded every hour throughout the crop cycle in both experiments by an automatic meteorological station (Vantage Pro 2, Davis Instruments Co. Inc., EE.UU) located in the experimental site.

### 2.3 | Data analysis

To analyse the species, waterlogging, N and their interaction effects, an ANOVA was used by applying the ´*aov*´ function in R (R Core Team, 2019). Significant differences were evaluated with Tukey´s test ($p < .05$). Then, sp.plot function from package ´agricolae´ was also used to test the effects of source-sink manipulation on spikes.

## 3 | RESULTS

### 3.1 | Environmental conditions and phenology

Mean values of temperature, incident global radiation, photoperiod and reference evapotranspiration ($ET_0$) of EX1 and EX2 are shown in Figure 2. The average temperature during the crop cycle (from emergence to physiological maturity) was 18 and 15°C for EX1 and EX2, respectively. Incident-accumulated global radiation during the crop cycle was 1997 MJ $m^{-2}$ in EX1, and 1954 MJ $m^{-2}$ in EX2. The warmer temperature and higher radiation in EX1 led to a higher $ET_0$, principally during the 20 day-waterlogging treatment (Figure 2). Thus, during the waterlogging period, wheat and barley experienced a higher atmospheric demand in EX1 compared to EX2. EX1 also experienced longer photoperiod than EX2.

In terms of phenology, the duration of the emergence-anthesis phase was in average 79 days in EX1 and 113 days in EX2, without significant differences between species ($p \geq .05$). Thus, both cultivars explored similar environmental conditions during pre and postanthesis periods. Waterlogging did not affect the duration of the phases in any experiment ($p \geq .05$).

### 3.2 | Differential impact of waterlogging on mean grain weight in wheat and barley

An ample phenotypic variability was observed in mean grain weight (MGW) in the whole plant, ranging in EX1 from 20.7 to 37.6 mg for wheat and from 11.7 to 46.1 mg for barley and in EX2 from 25.9 to 36.6 mg for wheat and from 32.3 to 43.3 mg for barley (Figure 3). At the tiller level, the MGW differed between species with heavier grains in barley than in wheat ($p < .01$; Table 1; Figure 3). Regarding the main stems, no differences in MGW between species were observed in EX1 (36.2 vs. 32.5 mg for barley and wheat) whilst in EX2 barley showed higher MGW (41.2 mg) than wheat (36.0 mg) (Table 1; Figure 3). There was no relationship between grain number per plant and MGW in any case ($p \geq .05$).

Waterlogging significantly reduced MGW of wheat and barley in EX1 whilst in EX2, the reductions were only significant in barley MS (Table 1; Figure 3). Reductions in MGW for the whole plant due to waterlogging in EX1 showed decreases from 32% to 56% for barley and from 32% to 39% for wheat. In EX2, the reduction in MGW for the main stem in response to waterlogging was 5% in wheat and 10% in barley (Figure 3). No clear effect of N application was observed on MGW in any species or grain hierarchy, nor interactions with the species or waterlogging treatment (Table 1; Figure 3).

### 3.3 | Source availability under waterlogging conditions

Radiation interception at anthesis was similar ($p \geq .05$) between the control and the waterlogging plants with no interactions with the species. The mean values of radiation interception were 94% and 89% for the control and the waterlogged plants in EX1, respectively, and 94% and 93% in EX2. Cumulative iPAR was recorded to establish whether the waterlogging stress modified source availability during grain filling. In EX1, waterlogging had a negative impact on cumulative iPAR with a differential effect associated with N level ($p < .05$): under high N levels, the reduction in iPAR by the effect of waterlogging was 61% (−119 MJ $m^{-2}$) in comparison to the control, whilst under low N conditions the reduction was 45% (−200 MJ $m^{-2}$), on average for both species. In EX1, the cumulative iPAR throughout the grain-filling period was on average higher in wheat (238 MJ $m^{-2}$) than in barley (198 MJ $m^{-2}$; $p < .05$) due to the detrimental effect of waterlogging on barley. In EX2, iPAR was similar between species ($p \geq .05$), and waterlogging reduced this value by 13% (−28 MJ $m^{-2}$) on average for both species and N conditions (Table 2; $p < .05$). N fertilization increased iPAR from 191.2 to 223.6 MJ $m^{-2}$ ($p < .05$) in EX2.

There was a positive association between iPAR and grain weight during grain filling in both species (Figure 4a). This response was more marked in barley under waterlogging conditions where grain weight varied from 10 to 40 mg with increases in iPAR from 50 to 200 MJ $m^{-2}$. On the contrary, a milder response was observed in wheat where grain weight varied between 20 and 35 mg with a variation of the iPAR from 100 to 220 MJ $m^{-2}$. Under control conditions, there was a slight increase in barley grain weight, whilst no response was observed in wheat.

The source-sink ratio (kJ $grain^{-1}$) was determined to establish whether the stress modified the amount of source per grain during the grain-filling period (Table 2). In EX1, the source-sink ratio varied depending on the species and water condition (S*WC interaction; $p < .05$), and there was no effect of the N level. Under waterlogging conditions, wheat showed the highest source-sink values (20.53 kJ grain$^{-1}$ on average for both N conditions), whilst the opposite was observed in barley (7.4 kJ grain$^{-1}$ on average for both N conditions). In EX2, the source-sink ratio was, in general, lower than in EX1, and similar between species with no waterlogging effect. In the case of wheat, grain weight was independent of the source-sink ratio reached during grain filling, and for a similar value of source-sink, the waterlogged treatments showed a lower grain weight than controls (Figure 4b). In barley, grain weight appeared to be reduced with source-sink ratios below 10 kJ per grain, which occurred only under waterlogging conditions (Figure 4c).

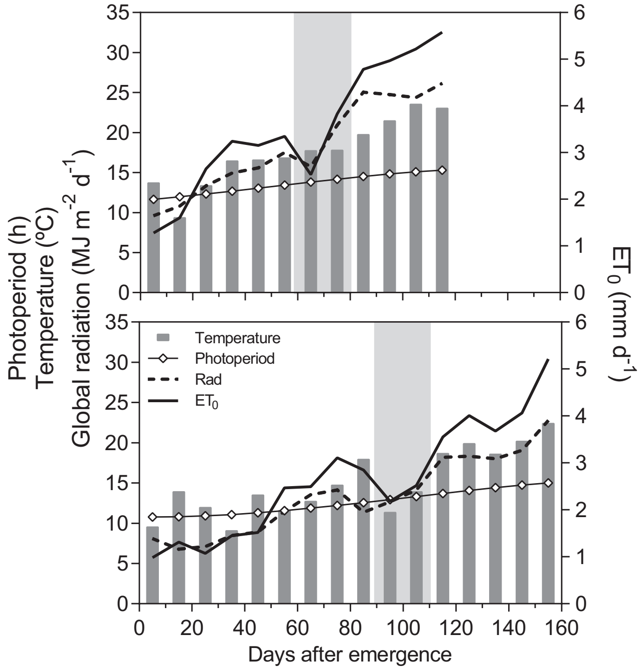

**FIGURE 2** Meteorological conditions during the crop cycle in EX1 (2011, late sowing date; upper panel) and EX2 (2013, early sowing date; bottom panel). Values are average of daily mean temperature, daily global incident radiation, daily photoperiod and potential evapotranspiration (ET$_0$). The shadow bar in the graph indicates the waterlogging period

WSC stored in stems were determined at anthesis and at maturity to evaluate the accumulation and consumption of reserves during grain filling. Stem WSC concentration was similar for wheat in both water conditions and N levels. In contrast, the WSC concentration at anthesis in barley was reduced by 60% by waterlogging under low N levels ($p < .05$). The concentration of WSC in the stems at PM differed between species depending on the N and water conditions ($p < .01$; Table 2). In wheat, no differences in WSC at PM were observed between waterlogging and control conditions following the trend observed at anthesis. However, barley showed a higher WSC concentration at maturity under waterlogging compared to controls in high N conditions, but the opposite was observed under low N. For the fertilized conditions, under waterlogging, between 13% (wheat) and 28% (barley) of the WSC concentration that was available at anthesis remained at maturity, whilst for the control it was 23% in wheat and 10% in barley, suggesting a lower capacity of barley to consume the reserves under waterlogging. Regarding the content of WSC available at anthesis per unit area, it was higher in wheat than in barley, with a significant reduction under waterlogging conditions in both species (Table 2). In wheat, 52% of WSC per unit area at anthesis was reduced due to waterlogging and low nitrogen level, whilst in barley waterlogging provoked an 18% reduction in total WSC per unit area at maturity. In the rest of the situations, there were no differences in the WSC content neither wheat nor barley (Table 2).

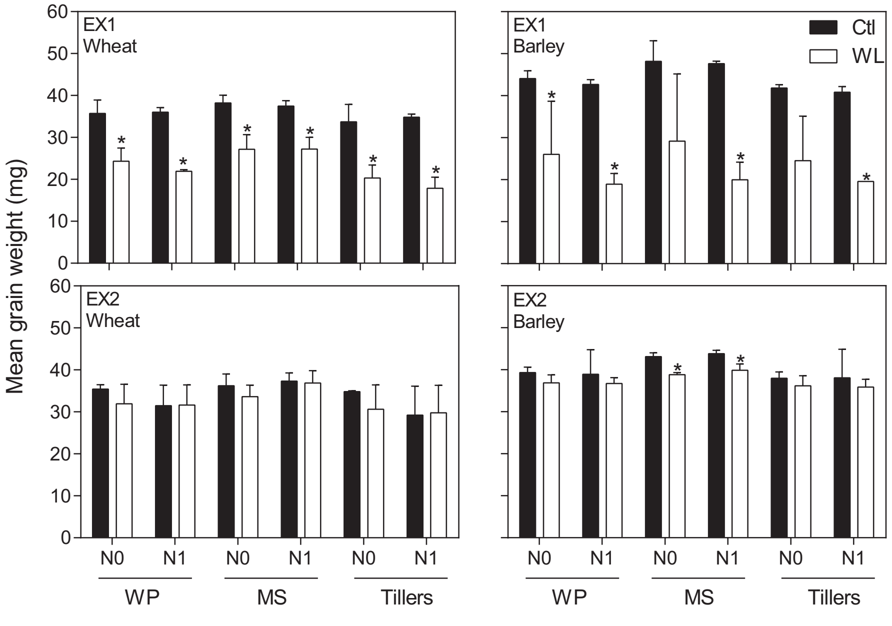

**FIGURE 3** Mean grain weight at maturity for waterlogging (open bars: waterlogged 20 days before anthesis -WL) and control (close bars: control without waterlogging -Ctl) treatments for the whole plant (WP), main stems (MS) and tillers for wheat (left panels) and barley (right panel) under high (N1) or low (N0) availabilities of nitrogen at sowing for EX1 (upper panels) and EX2 (bottom panels). Vertical segments indicate one standard error ($n = 3$). The asterisk indicates a significant difference ($p < .05$, Tukey´s test) between Ctl and the WL

**TABLE 1** Mean squares and significance for the main factor species (S), water conditions (WC) and nitrogen (N), and their interactions on mean grain weight (MGW) for the whole plant (WP), main stems (MS) and tillers for EX1 (late sowing date) and EX2 (early sowing date)

| Variation source | MGW (mg) | | | | | |
|---|---|---|---|---|---|---|
| | EX1 | | | EX2 | | |
| | WP | MS | Tillers | WP | MS | Tillers |
| *S* | 129.1 ns | 83.6 ns | 299.9** | 170.7** | 175.5*** | 209.4** |
| *WC* | 1496.3*** | 1730.6*** | 1469.9*** | 24.0 ns | 47.9** | 21.8 ns |
| *N* | 81.0 ns | 40.6 ns | 7.0 ns | 8.6 ns | 14.3 ns | 16.5 ns |
| *S*WC* | 56.1 ns | 249.7* | 13.6 ns | 0.7 ns | 10.0 ns | 0.1 ns |
| *S*N* | 40.8 ns | 31.3 ns | 3.4 ns | 5.4 ns | 2.6 ns | 14.9 ns |
| *WC*N* | 57.9 ns | 24.0 ns | 17.5 ns | 5.6 ns | 2.2 ns | 7.1 ns |
| *S*WC*N* | 18.9 ns | 33.1 ns | 0.1 ns | 4.3 ns | 1.1 ns | 9.7 ns |

Abbreviation: ns, non-significant ($p \geq .05$).

*$p < .05$; **$p < .01$; ***$p < .001$.

Accessibility to the source was evaluated through stomatal conductance at the beginning of the grain-filling period (Table 2). Stomatal conductance was differentially affected depending on species and water conditions in EX1 ($p < .05$). In wheat, stomatal conductance was reduced by waterlogging from 190 to 75 mol $m^{-2}$ $s^{-1}$ (−60%) on average for both N conditions. In barley, the stomatal conductance under low N conditions was reduced by waterlogging from 194 to 48 mmol $m^{-2}$ $s^{-1}$ (−75%), and from 255 mmol $m^{-2}$ $s^{-1}$ to nil under high N availability (Table 2). In EX2, stomatal conductance was not affected by any treatment ($p \geq .10$).

### 3.4 | Source-sink ratio increases during grain filling reduced the negative impact of waterlogging on grain weight

As expected, grain weight differed according to the position of each grain within the spike (Table 3). Grains from central spikelets were heavier than those from basal spikelets (20% in EX1 and 10% in EX2) and grains from main stems were heavier than those from tillers, in both species and experiments (EX1: 19% and 27% for wheat and barley, respectively; EX2: 13% and 11% for wheat and barley, respectively; Table 3). Waterlogging significantly reduced grain weight in wheat and barley ($p < .01$) at all evaluated positions in EX1 and in lower hierarchy grains in EX2 (grains from basal spikelets, and distal grains from central spikelets) (Table 3). Additionally, in EX2 water condition and N interaction was significant for grain weight of the basal position at main stems in barley ($p < .05$; Table 3), as higher N increased grain weight under well-watered conditions, whilst the opposite occurred under waterlogging. By the effect of the waterlogging treatments, wheat grain weight tended to be reduced more markedly in the lighter grains whilst in barley it was observed a general decrease for all the grain categories (Figure 5).

Trimming showed a positive impact on MGW in wheat under waterlogging conditions in EX1 (40% on average; Table 3), whilst in EX2 the response, although positive (ca 20% on average), was no significant. Thus, this positive response partially compensated reductions in MGW caused by waterlogging in the entire spikes (Table 3). There was no relationship between grain number per plant and grain weight by the effect of the trimming treatments. The positive effect of increases in the source-sink ratio (by trimming) on MGW under waterlogging was different between experiments when considering different positions. In EX1, grains from central and distal spikelets of tillers were the positions with the highest responses, especially when N was applied (Table 3). In EX2, all wheat grain positions showed significant positive responses to trimming when plants grew under waterlogging in both N levels (Table 3). In barley, a positive effect of trimming over MGW was observed, ranging from 17% to 21%, in EX2, and regardless of the water condition (Table 3). Such increases were explained by a positive response in all evaluated grain positions (Table 3). Grains from trimmed spikes under waterlogging reached similar or even higher weights compared to those from entire spikes under control conditions, suggesting that potential grain weight was not affected in EX2 (Table 3). There was no remarkable effect of the trimming treatment on barley MGW in EX1 (Table 3).

A negative relationship was observed between the response of grain weight to increases in source-sink ratio and grain weight at maturity (Figure 6). Thus, the lighter the grains the larger the response to increases in source-sink ratio, evidencing that the response in grain weight was higher at lower grain weight within the spikelets (e.g. distal grains) and/or grains from lower spike hierarchies (i.e. tillers). Additionally, the highest responses were observed in plants under waterlogging conditions, in which grain weight was strongly affected (Figure 6). Although this trend was observed in both species, the effects in wheat were more evident, showing grain weight responses from −10% to 100%, whilst in barley the response ranged from −5% to 60% (Figure 6).

## 4 | DISCUSSION

It is widely accepted that waterlogging throughout the wheat and barley crop cycle imposes yield penalties, especially if the stress occurs during the period immediately previous to anthesis (de San Celedonio et al., 2014). Negative effects of waterlogging on grain yield being mainly related to reductions in grain number, although

**TABLE 2** Waterlogging effects on wheat and barley under two nitrogen availabilities on source-sink ratio during grain filling (i.e. ratio between photosynthetically active radiation intercepted by the crop -iPAR- between anthesis (Anth) and physiological maturity (PM), and the number of grains at maturity), iPAR between anthesis and physiological maturity, water-soluble carbohydrates (WSC, only in EX1) concentration and absolute value per unit area in stems at anthesis and at physiological maturity, and the stomatal conductance ($g_s$) at the beginning of grain filling in two experiments (EX) for wheat and barley grown under well-watered conditions or waterlogged 20 days before anthesis and two soil N levels. The significance of the effect of the species (S), water conditions (WC) nitrogen fertilization (N), and their interactions is detailed. Within each column and experiment, different letters indicate significant differences ($p$ < .05) for the effect of the treatments

| EX | Species | Nitrogen | Water condition | Source-sink (kJ grain$^{-1}$) | iPAR Anth-PM (MJ m$^{-2}$) | WSC Anth [mg Glu (100 gDW$^{-1}$)] | WSC PM [mg Glu (100 gDW$^{-1}$)] | WSC Anth (g m$^{-2}$) | WSC PM (g m$^{-2}$) | $g_s$ (mmol m$^{-2}$ s$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| EX1 | Wheat | $N_0$ | Control | $14.57^{ab}$ | $266.4^{ab}$ | $20.51^{ab}$ | $1.45^{c}$ | $141.9^{a}$ | $8.7^{bc}$ | $192.9^{a}$ |
| | | | Waterlogging | $14.37^{ab}$ | $159.0^{bc}$ | $19.32^{abc}$ | $1.41^{c}$ | $74.2^{bc}$ | $8.6^{bc}$ | $75.1^{b}$ |
| | | $N_1$ | Control | $13.41^{ab}$ | $341.3^{a}$ | $13.82^{bc}$ | $3.16^{abc}$ | $131.6^{ab}$ | $8.9^{bc}$ | $188.6^{a}$ |
| | | | Waterlogging | $26.69^{a}$ | $184.3^{bc}$ | $15.39^{bc}$ | $1.96^{c}$ | $92.7^{abc}$ | $8.4^{bc}$ | $74.7^{b}$ |
| | Barley | $N_0$ | Control | $11.52^{ab}$ | $264.5^{ab}$ | $23.37^{a}$ | $5.25^{a}$ | $103.4^{abc}$ | $20.7^{a}$ | $194.0^{a}$ |
| | | | Waterlogging | $9.71^{ab}$ | $133.9^{c}$ | $14.15^{bc}$ | $2.08^{bc}$ | $53.5^{c}$ | $3.5^{c}$ | $48.0^{b}$,(¶) |
| | | $N_1$ | Control | $15.26^{ab}$ | $319.5^{a}$ | $12.47^{c}$ | $1.16^{c}$ | $86.2^{abc}$ | $14.8^{ab}$ | $255.5^{a}$ |
| | | | Waterlogging | $5.09^{b}$ | $75.3^{c}$ | $17.63^{abc}$ | $4.84^{ab}$ | $90.7^{abc}$ | $6.5^{bc}$ | --((¶¶)) |
| | | | *S* | * | * | *ns* | ** | ** | *ns* | *ns* |
| | | | *WC* | *ns* | *** | *ns* | *ns* | *** | *ns* | *** |
| | | | *N* | *ns* | *ns* | *** | *ns* | *ns* | *ns* | *ns* |
| | | | *S*WC* | * | *ns* | *ns* | *ns* | *ns* | *ns* | * |
| | | | *S*N* | *ns* | *ns* | *ns* | * | *ns* | *ns* | *ns* |
| | | | *WC*N* | *ns* | * | *** | ** | * | ** | *ns* |
| | | | *S*WC*N* | *ns* | *ns* | * | *** | *ns* | *** | *ns* |
| EX2 | Wheat | $N_0$ | Control | $9.16^{a}$ | $211.2^{a}$ | – | – | – | – | 177.1 |
| | | | Waterlogging | $7.55^{a}$ | $179.2^{a}$ | – | – | – | – | 181.6 |
| | | $N_1$ | Control | $6.78^{a}$ | $257.5^{a}$ | – | – | – | – | 193.6 |
| | | | Waterlogging | $11.04^{a}$ | $195.4^{a}$ | – | – | – | – | 155.3 |
| | Barley | $N_0$ | Control | $8.06^{a}$ | $187.7^{a}$ | – | – | – | – | 200.9 |
| | | | Waterlogging | $9.06^{a}$ | $186.9^{a}$ | – | – | – | – | 188.4 |
| | | $N_1$ | Control | $8.35^{a}$ | $230.4^{a}$ | – | – | – | – | 202.8 |
| | | | Waterlogging | $8.10^{a}$ | $211.2^{a}$ | – | – | – | – | 124.6 |
| | | | *S* | *ns* | *ns* | – | – | – | – | *ns* |
| | | | *WC* | *ns* | * | – | – | – | – | *ns* |
| | | | *N* | *ns* | * | – | – | – | – | *ns* |
| | | | *S*WC* | *ns* | *ns* | – | – | – | – | *ns* |
| | | | *S*N* | *ns* | *ns* | – | – | – | – | *ns* |
| | | | *WC*N* | *ns* | *ns* | – | – | – | – | *ns* |
| | | | *S*WC*N* | * | *ns* | – | – | – | – | *ns* |

Abbreviations: *$p$ < .05; **$p$ < .01; ***$p$ < .001ns: non-significant ($p$ ≥ .05).

(¶)One of the replicates had a conductance below the sensitivity level of the device and was considered as 0 (zero).

(¶¶)The three replicates had a conductance below the sensitivity level of the device.

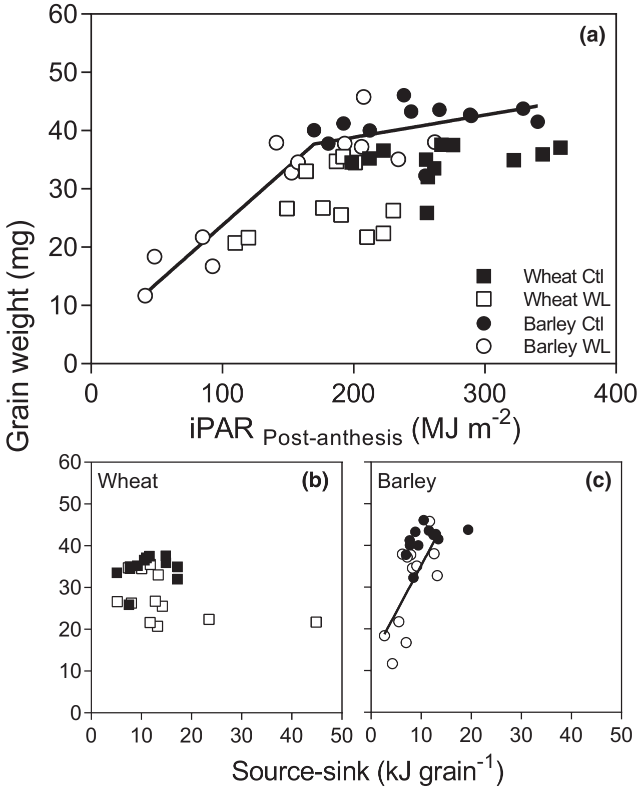

**FIGURE 4** Relationship between grain weight at maturity and (a) iPAR between anthesis and physiological maturity and source-sink ratio during grain filling (kJ grain$^{-1}$) for (b) wheat and (c) barley under well-watered conditions or waterlogged 20 days before anthesis in EX1 and EX2. In (a), the line represents the adjustment for barley using a bilinear function ($R^2 = 0.85$, $p < .05$). In c), the solid line represents the linear regression adjustment for the waterlogging treatments ($y = 2.25\ x + 12.69$; $R^2 = 0.47$; $p < .05$)

grain weight is also negatively affected (Araki et al., 2012; de San Celedonio et al., 2014; Marti et al., 2015; Ploschuk et al., 2020). In this study, we showed, based on our hypothesis, that waterlogging before the grain-filling period (i) reduced grain weight; (ii) the negative effect on grain weight was higher in barley than in wheat, especially under higher environmental demand and (iii) increases in source-sink ratio during grain filling, and after the waterlogging episode, allowed partial compensations of grain weight in wheat but not in barley.

Even though the waterlogging treatments were removed at anthesis, there was a significant reduction in mean grain weight (MGW) of 35% and 44% in wheat and barley, respectively (EX1). Thus, the first hypothesis that drove our work was not rejected. Reductions in MGW had a differential impact on spike hierarchies according to the species, being barley more affected in main stems than in tillers, whilst the opposite was observed in wheat. However, these results are opposed to other published results in the literature, where reductions in grain weight associated with waterlogging were consistent between species (de San Celedonio et al., 2014; Marti et al., 2015; Ploschuk et al., 2020). On the one way, contrasting results across studies are probably due to the experimental setup used in each case. Whilst in previous studies, experiments were conducted in 12 L pots (de San Celedonio et al., 2014), 7.5 L (Marti et al., 2015) and 5 L tubes (Ploschuk et al., 2020), our experiments were conducted in 1000 L (1 m$^3$) containers, emulating micro canopies and implying a different root exploration capacity in deep. Small pots cause a decrease in biomass, with stronger reductions in aerial compared to root biomass, leading to an increased root:shoot ratio (Dambreville et al., 2017; Poorter et al., 2012). Moreover, tillering capacity is very different compared to the plant growing in a crop structure. Regarding stress response, different pot sizes can modify the genotypic ranking of response (Dambreville et al., 2017). Growing plants in 1000 L containers was an attempt to mimic a crop canopy under field conditions, considering that plants growing in pots are regularly smaller than those growing in soil, even when they have no deficiencies of water and nutrients, due to non-hydraulic signals (Passioura, 1988,2002). For instance, in the experiment of Marti et al. (2015) carried out in tubes, plants were grown close simulating a crop structure, however, in our experiments plants grew competing with each other (as occurs in a crop) not only for aboveground resources (e.g. incident solar radiation), but also for underground resources (e.g. space, nitrogen) avoiding non-hydraulic signals (as was described by Passioura, 2002). It is important to highlight that other factors could also explain the contrasting results on MGW due to waterlogging described in the literature. Timing differences at the moment of release of waterlogging conditions and/or that smaller pot size could impose a higher/longer waterlogging stress, preventing a differential penalty in the younger parts of the plant.

On the contrary, the higher negative effect on MGW observed in tillers of wheat than of barley, as was described above, and taking into account the multifloral structures of the wheat spikelets, suggests that waterlogging could have affected the potential grain weight in distal grains, which are younger than proximal grains into the spikelets within the tiller spikes (Bingham et al., 2007; Calderini et al., 1999; Ugarte et al., 2007). However, this particular point is discussed below in this section associated with the trimming treatment.

Despite the above-mentioned impact of waterlogging on MGW, its effect was inconsistent across experiments, mainly due to variations in the environment explored by the crop. The intensity of the waterlogging stress on biomass production is directly related to the evaporative demand, which modifies transpiration rate and probably plant hydric status (Grassini et al., 2007). Moreover, high temperature can modulate the response to waterlogging by accelerating oxygen depletion through the respiration rate of plant roots (Herzog et al., 2016; Setter & Waters, 2003; Trought & Drew, 1982). The lack of waterlogging effect in EX2 (early sowing date) was probably explained by the lower environmental demand (ET$_0$) due to lower temperatures and radiation during waterlogging treatment compared to EX1 (late sowing date). In contrast, when both waterlogging and control treatments were combined with different nitrogen levels, no differences were observed in any situation. Although there is some evidence that waterlogging tends to limit nutrient uptake (Sharma & Swarup, 1988), there was no nitrogen effect in EX1 even under waterlogging.

**TABLE 3** Individual grain weight from different positions (C1-2 are proximal grains from central spikelets; C3 distal grains from central spikelets; B1-2 proximal grains from basal spikelets) in entire and trimmed spikes of wheat and barley under well-watered conditions or waterlogged 20 days before anthesis, under low (N0) or high (N1) N availabilities at sowing in EX1 and EX2. MS stands for Main Stem, T for Tiller and MGW for Mean Grain Weight of the whole plant. Within each column and experiment, different capital letters indicate significant differences (Tukey´s test, $p < .05$) for the effect of the treatments (waterlogging, nitrogen level and spike treatment). Within the water condition and N levels of each column and experiment, the mean values of the bold font style are significantly different for the spike treatment (Tukey´s test, $p < .05$). Within each row, different lowercase letters indicate significant differences (Tukey´s test, $p < .05$) between grains of different positions from entire and trimmed spikes for each particular species and within each water condition and nitrogen level

| | | | | Wheat | | | | | | Barley | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Central spikelet | | | Basal spikelet | | Whole plant | Central spikelet | | Basal spikelet | | Whole plant |
| Exp | Water condition (w) | Nitrogen level (N) | Spike treatment (ST) | MS.C 1–2 | MS.C–3 | T.C 1–2 | MS.B1–2 | T.B1–2 | MGW | MS.C | T.C | MS.B | T.B | MGW |
| **EX1** | Control | $N_0$ | Entire | 41.6$^{aAB}$ | 32.5$^{cdAB}$ | 38.6$^{abcA}$ | 32.0$^{cdAB}$ | 29.9$^{dA}$ | 35.7$^{A}$ | 48.7$^{abA}$ | 43.0$^{cA}$ | 44.2$^{abcA}$ | 34.3$^{eA}$ | 44.1$^{A}$ |
| | | | Trimmed | 40.0$^{abAB}$ | 29.3$^{dAB}$ | 35.3$^{bcdAB}$ | 34.5$^{abcdA}$ | 33.5$^{bcdA}$ | 34.9$^{A}$ | 49.5$^{aA}$ | 40.4$^{cdA}$ | 44.1$^{bcAB}$ | 37.3$^{deA}$ | 44.4$^{A}$ |
| | | $N_1$ | Entire | **45.4**$^{aA}$ | 35.4$^{cdA}$ | 39.0$^{bcA}$ | 35.6$^{cdA}$ | 33.5$^{cdA}$ | 36.0$^{A}$ | 47.9$^{aA}$ | 41.3$^{bA}$ | **38.0**$^{cABC}$ | 35.8$^{cA}$ | 42.7$^{A}$ |
| | | | Trimmed | **38.8**$^{bcABC}$ | 30.0$^{dAB}$ | 41.9$^{abA}$ | 35.9$^{cA}$ | 37.3$^{bcA}$ | 37.8$^{A}$ | 48.8$^{aA}$ | 41.4$^{bA}$ | **42.3**$^{bAB}$ | 35.5$^{cA}$ | 44.1$^{A}$ |
| | Waterlogging | $N_0$ | Entire | 35.8$^{aBC}$ | 22.5$^{deB}$ | 23.6$^{deBC}$ | **20.6**$^{eB}$ | **14.9**$^{fB}$ | **24.3**$^{BC}$ | 31.3$^{abcAB}$ | 24.4$^{bcB}$ | 23.6$^{bcBCD}$ | 21.8$^{cA}$ | 30.1$^{AB}$ |
| | | | Trimmed | 35.4$^{abBC}$ | 25.5$^{cdeAB}$ | 30.4$^{bcABC}$ | **29.7**$^{cAB}$ | **27.2**$^{cdA}$ | **32.0**$^{AB}$ | 39.6$^{aAB}$ | 35.5$^{abAB}$ | 35.3$^{abABC}$ | 33.2$^{abcA}$ | 32.2$^{AB}$ |
| | | $N_1$ | Entire | 30.1$^{abC}$ | 20.2$^{defB}$ | **19.1**$^{efC}$ | 22.1$^{cdeB}$ | **13.8**$^{fB}$ | **21.9**$^{C}$ | 22.4$^{aB}$ | 22.2$^{aB}$ | 16.9$^{bcD}$ | 15.7$^{cA}$ | 18.9$^{B}$ |
| | | | Trimmed | 36.7$^{aABC}$ | 28.4$^{bcAB}$ | **32.6**$^{abABC}$ | 26.2$^{bcdAB}$ | **27.9**$^{bcA}$ | **32.4**$^{A}$ | 24.3$^{aB}$ | 25.3$^{aB}$ | 21.8$^{abCD}$ | 26.5$^{aA}$ | 22.9$^{AB}$ |
| | | *W* | | * | * | ** | * | * | * | *ns* | *ns* | * | *ns* | * |
| | | *N* | | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |
| | | *W*N* | | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | * | *ns* | *ns* | *ns* |
| | | *ST* | | *ns* | *ns* | * | ** | *** | *** | *ns* | *ns* | *ns* | *ns* | *ns* |
| | | *ST*W* | | ** | * | * | * | * | *** | *ns* | *ns* | *ns* | *ns* | *ns* |
| | | *ST*N* | | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |
| | | *ST*N*W* | | * | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |
| **EX2** | Control | $N_0$ | Entire | **41.3**$^{bBC}$ | 35.6$^{cdB}$ | – | 39.9$^{bcAB}$ | – | 35.4$^{A}$ | **42.4**$^{bC}$ | – | 41.3$^{bBCD}$ | – | **39.3**$^{A}$ |
| | | | Trimmed | **47.1**$^{aA}$ | 35.1$^{dB}$ | – | 41.8$^{bA}$ | – | 36.7$^{A}$ | **51.2**$^{aAB}$ | – | 43.4$^{bABC}$ | – | **46.1**$^{A}$ |
| | | $N_1$ | Entire | **42.4**$^{abBC}$ | 38.8$^{cA}$ | – | 40.7$^{bcAB}$ | – | 31.5$^{A}$ | **45.8**$^{bBC}$ | – | **45.7**$^{bABC}$ | – | 39.9$^{A}$ |
| | | | Trimmed | **45.4**$^{aAB}$ | 39.2$^{cA}$ | – | 40.1$^{bcAB}$ | – | 36.1$^{A}$ | **50.9**$^{aAB}$ | – | **50.3**$^{aA}$ | – | 46.8$^{A}$ |
| | Waterlogging | $N_0$ | Entire | **39.6**$^{bC}$ | **30.1**$^{dC}$ | – | **35.8**$^{cAB}$ | – | 31.9$^{A}$ | **43.5**$^{bC}$ | – | **36.7**$^{cCD}$ | – | **36.8**$^{A}$ |
| | | | Trimmed | **45.9**$^{aAB}$ | **38.5**$^{bcA}$ | – | **41.5**$^{dA}$ | – | 37.3$^{A}$ | **52.2**$^{aA}$ | – | **48.6**$^{aAB}$ | – | **44.0**$^{A}$ |
| | | $N_1$ | Entire | **42.3**$^{bBC}$ | **29.9**$^{fC}$ | – | **33.2**$^{eB}$ | – | 31.6$^{A}$ | 43.7$^{aC}$ | – | 33.4$^{bD}$ | – | **36.7**$^{A}$ |
| | | | Trimmed | **45.4**$^{aAB}$ | **37.2**$^{dAB}$ | – | **40.1**$^{cAB}$ | – | 36.2$^{A}$ | 47.0$^{aABC}$ | – | 41.7$^{aABCD}$ | – | **44.3**$^{A}$ |
| | | *W* | | *ns* | *** | – | *ns* | – | *ns* | *ns* | – | * | – | *ns* |
| | | *N* | | *ns* | ** | – | *ns* | – | *ns* | *ns* | – | *ns* | – | *ns* |
| | | *W*N* | | *ns* | *** | – | *ns* | – | *ns* | *ns* | – | * | – | *ns* |
| | | *ST* | | *** | *** | – | * | – | * | *** | – | *** | – | *** |
| | | *ST*W* | | *ns* | *** | – | * | – | *ns* | *ns* | – | * | – | *ns* |
| | | *ST*N* | | * | *ns* | – | *ns* | – | *ns* | * | – | *ns* | – | *ns* |
| | | *ST*N*W* | | *ns* | *ns* | – | *ns* | – | *ns* | *ns* | – | *ns* | - | *ns* |

Abbreviation: ns, No significant difference ($p \geq .05$)

$^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$

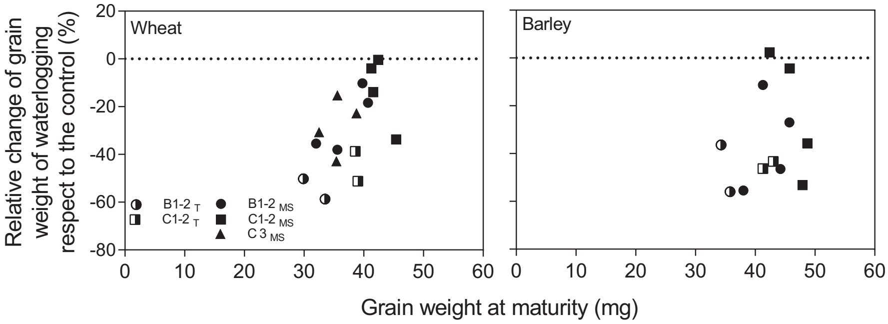

**FIGURE 5** Relationship between the relative change in grain weight at maturity of waterlogged plants 20 days before anthesis with respect to the control without waterlogging for grains located at different positions across the spike and within each spikelet in wheat and barley (C1-2 proximal grains from central spikelets; C3 distal grains from central spikelets; B1-2 proximal grains from basal spikelets; MS and T are grains from main stems for EX1 and EX2 and tillers for EX1, respectively)

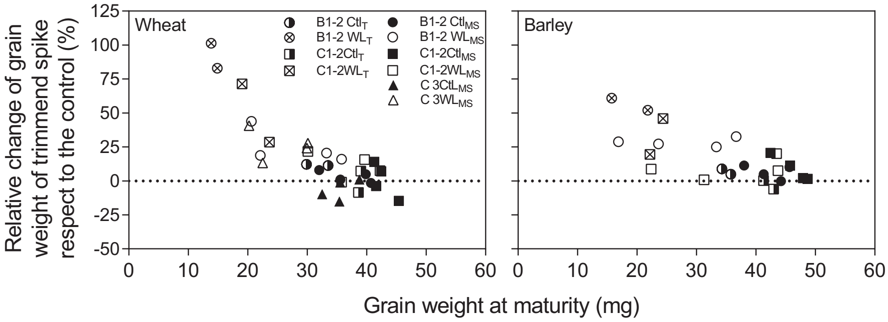

**FIGURE 6** Relationship between the relative change in grain weight at maturity for grains from trimmed spikes compared to grains from the entire spikes and grain weight at maturity for grains located at different positions across the spike and within each spikelet in wheat and barley (C1-2 proximal grains from central spikelets; C3 distal grains from central spikelets; B1-2 proximal grains from basal spikelets; MS and T are grains from main stems for EX1 and EX2 and tillers for EX1, respectively)

Waterlogging partially reduced the available source (cumulated iPAR) during the grain-filling period. Depending on the intensity of the stress, source reductions could have a negative or null impact on MGW. In the present study, reductions in cumulated iPAR were driven by the delayed and negative effect of waterlogging on aerial biomass previously reported in wheat (Hossain et al., 2011; Malik et al., 2001) and barley (de San Celedonio et al., 2017; Pang et al., 2004) and accelerated leaf senescence during grain filling (Ploschuk et al., 2020). Underwater stress conditions, not only radiation interception by plants is reduced, but also leaf photosynthetic rate (de San Celedonio et al., 2017). In our work, stomatal conductance was taken as an indicator at the leaf level. Stomatal closure is a common response mechanism to water stress; in fact, Hasanuzzaman et al. (2019) found that tolerant barley genotypes to drought have lower stomatal conductance under control conditions than more susceptible genotypes, with values for stomatal conductance ranging from ≈75 to 275 mmol $m^{-2}$ $s^{-1}$. The stomatal conductance levels measured in our study for the control condition fell within the medium to low values of this range, which is expected taking into account the advanced ontogenetic stage of the leaves under study (Ploschuk et al., 2018). Barley decreased stomatal conductance and grain weight under waterlogging more than wheat (until reaching null values). Noteworthy, in an experiment carried out with the same wheat and barley cultivars and the same waterlogging duration (20 days) of our work, de San Celedonio et al. (2017) showed that leaf photosynthetic rate at anthesis was reduced by half as a consequence of waterlogging. Thus, the tendency to decrease both stomatal conductance and grain weight under waterlogging is not an indicator by itself of a cause-effect relationship between both variables, but it is an indicator of a reduction in the available source from the beginning of the grain-filling period that persists up to the end of the plant cycle.

Instantaneous iPAR at anthesis (around 90% data not shown) and cumulated iPAR from anthesis to maturity and source-sink ratio (kJ $grain^{-1}$) were similar between wheat and barley under the control conditions. Thus, waterlogging reduced the source-sink ratio as a consequence of drastic leaf senescence during grain filling. Additionally, with the same magnitude of the source, barley established a higher grain weight than wheat.

Reductions in MGW, due to waterlogging were partially compensated by increases in the source-sink ratio (generated by the trimming treatment) during grain filling in wheat and barley, respectively. Trimming treatments during grain filling did not affect grain weight in non-stressed conditions, except for barley under low environmental demand conditions, in line with the previous evidence (Alvarez Prado et al., 2013; Serrago & Miralles, 2014; Zhang et al., 2010). In contrast, trimming treatments significantly increased grain weight under waterlogging in both species, reaching similar values to control conditions, with the exception of barley under more stressful conditions (i.e. EX1). It is important to highlight that trimming treatments were applied when grain dry weight represented around 10% of the final grain weight reached at physiological maturity coinciding with the end of lag phase (Loss et al., 1989). Thus, differences in grain weight between trimmed and entire spikes when the crop was affected by waterlogging can be attributed to differences in grain growth that happened after the setting of grain weight potential. Similar results were observed in wheat when comparing healthy and diseased crops (Serrago & Miralles, 2014). Thus, it is possible to speculate that in the case of barley under more stressful conditions, the slight response to the trimming treatment under waterlogging conditions suggests that, in addition to source reduction, potential grain weight could have been affected even when most of the lag phase was complete (Bingham et al., 2007; Calderini et al., 1999; Ugarte et al., 2007). Our results demonstrate that the second hypothesis of our work is not rejected.

Grains in different positions within the spike showed MGW reductions of different magnitude. One interesting point in our results is that lighter grains showed the highest responses to increases in the source-sink ratio, in line with results presented by other authors with crops subjected to a variety of stresses (Acreche & Slafer, 2006; Maydup et al., 2010; Serrago & Miralles, 2014), suggesting that the waterlogging effect on grain weight changed with grain hierarchy. One hypothesis to explain the differences between grains in basal, central and distal positions could be associated with the fact that not all grains reach anthesis at the same moment, as the florets at distal positions usually reach anthesis a few days later than those at central and basal positions. In barley, grain weight variability is much lower than in wheat (Alvarez Prado et al., 2013; Serrago & Miralles, 2014) suggesting that differences in anthesis time for different floret positions are lower, which would explain the lack of response under more stressful conditions. As in wheat differences in time to anthesis among different floret positions are more important than in barley, restrictions to grain growth caused by waterlogging could affect potential grain size, especially in grains placed in distal positions, since the occurrence of the waterlogging event coincides with less advanced stages of grain development (e.g. during the carpel growth or during lag phase).

Thus, in those grains, the greater responses to the trimming treatments, with respect to the basal grains, could be not only related to more supply of carbohydrates as a consequence of greater source-sink ratio but also due to an increase in the potential grain size.

In summary, wheat and barley grain weight is a numerical yield component that is defined during post-anthesis. However, a waterlogging event previous to anthesis provoked losses in grain weight, which is in accordance with the delay effect of waterlogging on growth observed in both species. Increases in the source-sink ratio partially mitigate the damaging effect of waterlogging on grain weight, especially in grains with low growth potential.

## 5 | CONCLUSIONS

Waterlogging is a current problem in wheat and barley production. The combination of timing and duration with environmental demand defines the intensity of the stress and the recovery capacity of the crop. The question that guided our work was: Is grain weight in wheat and barley plants exposed to a pre-anthesis waterlogging event restricted by the magnitude of the source available per grain during grain filling? Waterlogging reduced grain weight by ≈40% in wheat and barley. Manipulative treatments of increases in the source-sink ratio during the grain-filling period (by trimming spikes one week after anthesis) attempered the decrease in grain weight by waterlogging in wheat (up to 40%) and barley (up to 20%), without a well-defined effect of soil nitrogen level as a regulatory mechanism of the response Our results showed that waterlogging events during stem elongation phase affected grain weight through a reduction in the source during grain-filling as well as by possible impact on potential grain weight, depending on the intensity of the stress. The negative impact of waterlogging was more severe on lower hierarchy grains with lower grain weight. Nevertheless, these grain positions showed the highest recovery capacity with increases in resource availability. Thus, the grain hierarchy conditioned the response to the source-ratio treatments as the lower the grain weight of the control, the higher the response to trimming in both species.

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## DATA AVAILABILITY STATEMENT

Data available on request from the authors

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