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Influence of Sink Size on ^{15}N and ^{13}C Allocation during Different Phenological Phases of Spring Wheat Cultivars

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Abstract: The scientific objective of this study was to answer the question of whether sink limitation is also true for high quality wheat varieties. We examined the incorporation of ^{15}N and ^{13}C during phenological phases into vegetative parts and grains of Elite wheat Triso (E) and Quality wheat Naxos (A) when the spike is halved. Three splits of fertilizer were applied at EC 11, EC 30, EC 59, whereby 10% at EC 30 and EC 59 was ^{15}N, and plants were also labelled with $^{13}CO2$. The application of only the third split as ^{15}N, combined with spike-halving, resulted in a significantly higher ^{15}N-content (+11%) of 0.486 mg ^{15}N/g DM, compared to the control (0.437 mg^{15}N/g DM). Labelling whole plants with $^{13}CO_2$ at EC 59 resulted in a significantly higher ^{13}C-content—40%—(0.223 mg ^{13}C/g DM) of the grains of the control for Triso at the fully-ripe stage (EC 89), compared to Naxos (0.160 mg ^{13}C/g DM). This superiority was reduced to 34%, and was also demonstrated by spike-halving (0.226 mg ^{13}C/g DM, 0.169 mg ^{13}C/g DM). Remobilization of ^{15}N for control and spike-halving treatments were 68.2% and 61.1%, respectively. This clearly demonstrates that the reduction of the sink size by spike-halving leads to a 7% reduction in the remobilization of ^{15}N from vegetative to reproductive tissues.

Keywords: *Triticum aestivum* L.; ^{15}N; ^{13}C pulse labelling; isotopic dilution method; spike-halving; phenological phases

1. Introduction

Wheat is the most widely grown cereal globally, being adapted to a broad range of temperatures, water regimes, and fertilization levels. Protein is the major nitrogen-containing component of cereal grains, and most protein data are based on nitrogen determination, followed by multiplication by nitrogen-to-protein conversion factors, which range from 5.7 to 6.31 for cereal products [1]. However, the protein content of cereals can vary substantially, and greater than two-fold ranges in protein content are found between crops of the same species. This variation is due partly to genetic differences, but agronomic and climatic factors are of greater importance. This variation may be of little significance with bulk crops encountered in industrialized operations, but may be important in less developed regions. Although not usually considered as a good protein source, many cereals provide an adequate amount, relative to energy, for adults [1]. The understanding and assessment of grain yield responses to assimilate availability, especially during different phenological phases, is of considerable interest in the area of crop physiology. Abundant evidence exists that crops experience periods during the growing life cycle when yield is mainly limited by source strength, sink capacity, or is co-limited by both. As crop yield is more strongly related to seed number per unit land area than to mean seed dry weight, it is not surprising that critical growth periods for yield determination have been identified to be during the crop phenological phase when the final seed number is determined [2]. The pre-anthesis, 'source' development time frame (assimilation, accumulation, and translocation of carbohydrate and nitrogenous compounds) is followed by the period from anthesis

to grain maturity, when the growth and potential size of the grains acting as 'sinks' for nutrients such as N and C are determined. It is known that the N requirement for protein synthesis in the developing wheat kernel is met by 50–70% of the mobilization of previously-assimilated N present in vegetative tissues, and also by the direct uptake and assimilation of N during grain filling. The mobilization and recycling ability, however, can differ among wheat varieties, and is also influenced by early or late maturity [3,4]. Furthermore, it is postulated by Barneix [5] that there are two main regulatory points during grain-filling when plant N status may, or may not, be ample. The N uptake transporters in the roots are depressed due to the high amino acid concentration in the tissues, resulting in low N uptake. Alternatively, a high amino acid concentration keeps the cytokinin level high, resulting in the repression of leaf protein degradation by proteolysis of the main protein of green plant parts, Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39). Rubisco is a key reservoir of nitrogen which is remobilized during senescence-related conditions. One of the factors affecting the harvest index in relatively favourable wheat-growing environments relates to the optimizing partitioning of assimilates, which influences the distribution of assimilates to different plant organs, and also affects source:sink ratios, and ultimately the determination of potential seed number and size [6]. The most common approach regarding the determination of whether there is source- or sink-limitation has been the imposition of treatments in which either source or sink strengths are manipulated after flowering. Source manipulations are commonly defoliations or shadings, while sink manipulations involve the removal of grains (e.g., partially trimming the spikes), determining reductions or increases in the source-sink relationship, respectively [7].

Trimming spikes after anthesis has been found to increase grain protein concentration, indicating that the potential for grain protein deposition has not been reached. However, it is still not clear how the post-anthesis N uptake and N mobilization on the source side, or the protein synthesis on the sink side, limit(s) the realization of this potential. It has been found [8], that a wheat cultivar with large spikes and numerous kernels also had a relatively high potential for N uptake after anthesis. Additional N application at anthesis could increase the plant's post-anthesis N uptake and also the grain N content. By contrast, a cultivar with a different phenotype, including small spikes, demonstrated relatively low N uptake after anthesis, and additional N supplied at anthesis had only a small effect on its post-anthesis N uptake. When the sink size of the large spike was reduced (by removing the upper one-third of the spike), post-anthesis N uptake was markedly reduced, indicating a feedback regulation of sink size on the post-anthesis N uptake, possibly involving the root function [8]. This study also showed that the rapid senescence of a small size spike limited its capacity to take up more N after anthesis. Wheat harvests always have yearly-specific characteristics. Yield and quality are the result of a combination of the variety and cultivation practices, including fertilization, and the weather, mainly air temperature and rainfall. Regarding the comparison of wheat genotypes, quality criteria, including N- and protein content, respectively, must be considered. The crude protein content of wheat grain is strongly influenced by the amount and timing of nitrogen fertilization, and there also are variety-specific differences. Increasing grain protein content tends to have a positive effect on the baking behaviour of 'good' varieties. 'Quality wheat', which has high protein and sedimentation values, is in Germany assigned to the 'A' group. Historically, the designation 'A' comes from the German term 'Aufmischweizen', which was able (and still is) to compensate for the deficits of other varieties with high protein qualities. The 'E' group with very high 'internal values' is referred to as 'Elite wheat', which is almost 'too high' for most domestic bread and pastry recipes. However, they can be used to specifically compensate for the baking weaknesses of other varieties. The scientific question of this study was to assess if a sink limitation is also true for spring wheat varieties of high-quality levels, such as Elite and Quality wheat. To achieve this goal, the isotopic dilution technique with ^{15}N and $^{13}CO_2$ was combined, and the three phenological phases [9] of stem elongation to the end of heading, end of heading to fully ripe, and from stem elongation

to fully ripe, of the Elite spring wheat Triso and the Quality wheat Naxos (both *Triticum aestivum* L.) were examined.

2. Materials and Methods

A pot experiment (March to July 2018) was conducted in an open wire house with a Perspex rain shelter at Humboldt University of Berlin located in Berlin-Dahlem (52.47° N, 13.30° E, h = 51 m). The experimental layout was a Completely Randomized Design (CRD), widely used in agriculture research. Plant culture vessels (Mitscherlich pots) were filled with 6.0 kg soil (Albic Luvisol, poor silty to medium loamy sand), pH 6.0, which contained 0.11% N, 1.22% C, 400.4 mg P kg^{-1}, 279.4 mg K kg^{-1}. Fifteen seeds were sown per pot, and at emergence thinned to ten plants, and then supplied with 82 mg P and 313 mg K pot^{-1}. Three splits of fertilizer as NH_4NO_3 in a 200 mL solution, representative of 90 + 45 + 60 kg N ha^{-1}, 283, 141, 188 mg N pot^{-1}, respectively, were applied when the first-leaf unfolded (EC 11), at the beginning of stem elongation (EC 30), and at the end of heading: inflorescence fully emerged (EC 59), respectively (Table 1). Ten percent of the N splits at EC 30, EC 59 or both developmental stages, was applied as ^{15}N ($^{15}NH_4{}^{14}NO_3$, 96.4 atom %, Campro Scientific GmbH, Germany), resulting in three different treatments A–C, with three replications (pots) (A) and five replications (B, C) each (Table 1). Compared to the control group (whole spike) in treatments B and C, spike-halving was done at EC 59, the end of heading, of three main stems in each Mitscherlich pot. The water content of the soil was monitored by Time Domain Reflectometry (TDR) beginning at about 10 a.m. (Monday, Wednesday, and Friday). The maximum soil moisture was ~20 vol%, whereby ~18 vol% was considered as optimum for the well-watered plants.

Table 1. Growth-stages related N/^{15}N and $^{13}CO_2$ applications and samplings (2018).

Treatment (Day of Year)	EC Stage	N-Fertilization (mg N pot^{-1})	^{15}N-Fertilization (mg N pot^{-1})	N Total (mg N pot^{-1})
A (116)	11	283	0	283
A (151)	30 [+ $^{13}CO_2$]	127	14	141
A (166)	59	Sampling	-	-
B (116)	11	283	0	283
B (151)	30	141	0	141
B (166)	59 [+ $^{13}CO_2$]	169	19	188
B (204)	89	Sampling	-	-
C (116)	11	283	0	283
C (151)	30 [+ $^{13}CO_2$]	141	14	141
C (166)	59	169	19	188
C (204)	89	Sampling	-	-

At the sampling plant, the organs (leaves, stems, spikes, and grains) of these three main stems of each pot were pooled for analysis. The 'E' variety *Triso* (DSV Saaten), Europe's proven spring wheat, characterized by very good climate and yield stability, and the Quality variety *Naxos* (A) (Dr. Hermann Strube, Söllingen) were examined. At EC 30 (treatment A, C) or EC 59 (treatment B), the wheat plants were pulse-labelled with $^{13}CO_2$ [10], generated by adding 5 mL 2M perchloric acid to 0.9 g barium [^{13}C] carbonate (99 atom%, Campro Scientific GmbH, Germany). Labelling was conducted during one hour between 10:00 and 11:00 a.m., placing five Mitscherlich pots in a transparent 'round bottom bag' ('Roundliner'), diameter: 59.5 cm, height: 150 cm, LDPE-foil: 150 μm (Roundliner GmbH, Forst, Germany) (Table 1). Roundliner were then removed and plants cultivated until the end of heading (EC 59, treatment A) or until the fully-ripe stage (EC 89, treatment B, C). All ^{13}C measurements were performed using the mass spectrometer Tracer mass 20-20; SerCon, Crewe, UK, and the ^{13}C-content was calculated [11]. ^{15}N measurements were performed using the emission spectrometer NOI-6PC, Leipzig, Germany, and the ^{15}N-content was calculated [11]. Nitrogen and carbon quantities were measured using an elemental analyser (vario MAX CNS, Elementar-Analysensysteme GmbH, Hanau, Germany). At each of the

sampling dates, the total dry matter (DM) of plants was measured by drying at 60 °C to constant weight. Plants of treatments B and C, sampled at the fully-ripe (EC 89) growth stage, were subdivided into the grains (generated by the ear thresher, Walter-Wintersteiger, Obernberg/Inn, Austria), and the rest of the plants (leaves, stems, spikes, glumes = 'vegetative plant parts') and ground to pass through a 1-mm screen for N/^{15}N and C/^{13}C analysis. The data (mean, standard deviation (SD), Student's *t*-test, which compares the mean values of a maximum of two groups) were analyzed using IBM SPSS Statistic 25.0 statistical software.

3. Results and Discussion

The mean monthly air temperature (T_{mean}) (Table S1) from March to July was 2.2, 19.9, 19.0, 20.0 and 22.5 °C, respectively, and in March was 3.0 °C lower, and 3.4, 4.1, 1.8, 2.4 °C higher from April to July, respectively, than the temperatures during the reference period of 1991–2020. The coldest temperatures (T_{min}) from March to July was in the range between −1.5 and 14.9 °C, whereas the range for the warmest temperatures (T_{max}) varied between 6.9 and 29.8 °C. From March to July the relative humidity was 75.7, 67.3, 53.7, 60.5, 56.6%, respectively, compared to the reference period with 73.7, 65.0, 64.8, 64.7, and 66.0%, respectively.

3.1. Treatment A: Sampling at End of Heading (EC 59), N Uptake between EC 11 and EC 59, ^{15}N Uptake between Stem Elongation (EC 30) and End of Heading (EC 59), C Accumulation between EC 11 and End of Heading (EC 59), and ^{13}C Accumulation between Stem Elongation (EC 30) and End of Heading (EC 59)

The amount of dry matter of the spikes of Triso and Naxos (Table 2), accumulated between the phenological phase first-leaf unfolded (EC11) and the end of heading (EC 59), was not different, whereas the vegetative parts of Triso (E) demonstrated a marked growth advantage of 26%. The N-content of the spikes was likewise not different between the two cultivars. However, the N-content of the vegetative parts was 28% higher for Naxos (A) compared to Triso (E). The ^{15}N-content of spikes and the vegetative parts reflect the sink intensity between the stem elongation phase (EC 30) and the end of heading (EC 59). At this phenological stage, spikes representing only about 14% dry matter of the vegetative parts clearly demonstrated a higher attraction for ^{15}N of 25% and 28% (0.615 mg^{15}N/g DM, 0.755 mg^{15}N/g DM, for Triso, Naxos, respectively), compared to the vegetative parts (0.491 mg^{15}N/g DM, 0.588 mg^{15}N/g DM, Triso, Naxos, respectively). Naxos (A) demonstrated for spikes as well for vegetative parts about a 22% higher ($p < 0.05$) ^{15}N-content than for Triso (E). The C-content at the end of heading (phenological phase between EC 11 and EC 59) was statistically not different between Triso (E) and Naxos (A), but on average was markedly higher for spikes (4%, $p < 0.05$) compared to the vegetative plant parts. Interestingly, labelling with ^{13}CO$_2$ for one hour at stem elongation (EC 30) resulted in a significantly higher ^{13}C-content (0.142 mg^{13}C/g DM; factor 3.2) of the spike of Naxos (A) at the end of heading (EC 59) by comparison with Triso (E) (0.045 mg^{13}C/g DM). The ^{13}C-content at this growth stage was about 4.7 and 1.8 times as high in vegetative parts as in the spikes of Triso (E) and Naxos (A), respectively.

3.2. Treatment B: Sampling at Fully-Ripe Stage (EC 89), N Uptake between EC 11 and EC 89, ^{15}N Uptake and ^{13}C Accumulation between End of Heading (EC 59) and Fully-Ripe Stage (EC 89)

The single-grain weight (SGW; mean of treatment B and C, ± SE, n = 10) was 30.6 ± 0.54 mg DM and 38.4 ± 0.24 mg DM for the control plants of Triso (E) and Naxos (A), respectively, and was by spike-halving markedly increased ($p < 0.05$) to 34.3 ± 0.64 mg DM and 46.9 ± 0.78 mg DM. The increase of the SGW of the Quality wheat Naxos (A) was nearly twice as high (22%) as for the Elite wheat Triso (E) (12%). Grain DM, however, was reduced by 22% ($p < 0.05$) as a result of spike-halving (data not shown).

Table 2. Treatment A: Sampling at end of heading (EC 59), N uptake between EC 11 and EC 59, ^{15}N uptake between stem elongation (EC 30) and end of heading (EC 59), C accumulation between EC 11 and end of heading (EC 59), and ^{13}C accumulation between stem elongation (EC 30) and end of heading (EC 59).

Organ Cultivar	Dry Matter (g DM/pot *)	N-Content (mg N/g DM)	^{15}N-Content (mg^{15}N/g DM)	C-Content (mg C/g DM)	^{13}C-Content (mg ^{13}C/g DM)
Spike					
Triso (E)	0.390 ± 0.01 [a]	18.20 ± 0.26 [a]	0.615 ± 0.01 [b]	453.7 ± 1.26 [a]	0.045 ± 0.02 [b]
Naxos (A)	0.357 ± 0.01 [a]	19.20 ± 1.00 [a]	0.755 ± 0.01 [a]	451.9 ± 0.42 [a]	0.142 ± 0.01 [a]
Vegetative parts					
Triso (E)	2.883 ± 0.09 [a]	16.33 ± 0.99 [b]	0.491 ± 0.02 [b]	433.9 ± 1.94 [a]	0.212 ± 0.05 [a]
Naxos (A)	2.280 ± 0.10 [b]	20.87 ± 0.60 [a]	0.588 ± 0.01 [a]	435.0 ± 0.35 [a]	0.290 ± 0.01 [a]

(mean ± SD; different letters indicate significant differences of ear and vegetative plant parts between cultivars according to Student's *t*-test; * three main stems per pot pooled; pots n = 3).

With regard to dry matter, N-content, (with one exception, grain—spike-halving), and C-content, no differences between Triso (E) and Naxos (A) were recognizable for treatment B (Table 3) and treatment C (Table 4). Therefore, the data for both treatments were pooled (n = 20). The DM of grains of the entire spike (control) (of three main stems of each replication) was on average 2.00 ± 0.11 g DM, which was clearly reduced ($p < 0.05$) by spike-halving, by 22%, to 1.55 ± 0.14 g DM. Although vegetative plant parts are also present on the spike (e.g., glumes), spike-halving had no influence on the DM of vegetative plant parts, yielding 1.67 ± 0.13 g DM and 1.58 ± 0.18 g DM, for control and spike-halving, respectively. The N-content in grains and vegetative parts reflects the N uptake between the early phenological stage first-leaf unfolded (EC 11) and fully-ripe (EC 89). Spike-halving at the end of heading (EC 59) led to a significantly higher N-content (plus 7%) of 21.32, 22.30 mg N/g DM in Triso (E) (Tables 3 and 4), compared to Naxos (A), with 19.84, 20.68 mg N/g DM. The average N-content in grains for the control treatment was 18.85 ± 0.96 mg N/g DM, and for the vegetative parts at maturity was 3.92 ± 0.62, 3.62 ± 0.65 mg N/g DM, respectively, which is relatively low. This indicates an efficient remobilization. The C-content of the grains and the vegetative parts for the control and the spike-halving treatment was on average 434, 437, 442, and 445 mg C/g DM, respectively, and statistically not different.

Table 3. Treatment B: Sampling at fully-ripe stage (EC 89), N uptake between EC 11 and EC 89, ^{15}N uptake and ^{13}C accumulation between end of heading (EC 59) and fully-ripe stage (EC 89).

Organ Cultivar	Dry Matter (g DM/pot *)	N-Content (mg N/g DM)	^{15}N-Content (mg^{15}N/g DM)	C-Content (mg C/g DM)	^{13}C-Content (mg ^{13}C/g DM)
Grain—Control					
Triso (E)	1.98 ± 0.07 [a]	19.14 ± 0.22 [a]	0.439 ± 0.03 [a]	436.6 ± 0.94 [a]	0.223 ± 0.03 [a]
Naxos (A)	2.00 ± 0.12 [a]	17.98 ± 0.70 [a]	0.436 ± 0.03 [a]	432.9 ± 4.48 [a]	0.160 ± 0.01 [b]
Grain—Spike-halving					
Triso (E)	1.58 ± 0.23 [a]	21.32 ± 0.98 [a]	0.503 ± 0.04 [a]	436.8 ± 0.93 [a]	0.226 ± 0.01 [a]
Naxos (A)	1.49 ± 0.12 [a]	19.84 ± 0.22 [b]	0.469 ± 0.02 [a]	436.4 ± 1.01 [a]	0.169 ± 0.01 [b]
Vegetative parts—Control					
Triso (E)	1.78 ± 0.13 [a]	3.34 ± 0.60 [a]	0.036 ± 0.01 [a]	441.3 ± 9.05 [a]	0.422 ± 0.13 [a]
Naxos (A)	1.60 ± 0.09 [a]	4.22 ± 0.63 [a]	0.050 ± 0.02 [a]	439.2 ± 5.41 [a]	0.396 ± 0.10 [a]
Vegetative parts—Spike-halving					
Triso (E)	1.76 ± 0.10 [a]	3.38 ± 0.71 [a]	0.034 ± 0.01 [a]	443.6 ± 4.99 [a]	0.404 ± 0.12 [a]
Naxos (A)	1.42 ± 0.07 [a]	3.48 ± 0.52 [a]	0.048 ± 0.01 [a]	446.1 ± 3.46 [a]	0.382 ± 0.11 [a]

(mean ± SD; different letters indicate significant differences of ear and vegetative plant parts between cultivars according to Student's *t*-test; * three main stems per pot pooled; pots n = 5).

Table 4. Treatment C: Sampling at fully-ripe stage (EC 89), N uptake between EC 11 and EC 89, ^{15}N uptake and ^{13}C accumulation between stem elongation (EC 30) and fully-ripe stage (EC 89).

Organ Cultivar	Dry Matter (g DM/pot *)	N-Content (mg N/g DM)	^{15}N-Content (mg ^{15}N/g DM)	C-Content (mg C/g DM)	^{13}C-Content (mg ^{13}C/g DM)
Grain—Control					
Triso (E)	2.10 ± 0.11 [a]	19.76 ± 0.70 [a]	0.839 ± 0.08 [a]	435.7 ±1.00 [a]	0.108 ± 0.01 [a]
Naxos (A)	1.94 ± 0.11 [a]	18.50 ± 1.04 [a]	0.826 ± 0.12 [a]	432.7 ± 5.77 [a]	0.112 ± 0.01 [a]
Grain—Spike-halving					
Triso (E)	1.58 ± 0.08 [a]	22.30 ± 1.26 [b]	0.892 ± 0.06 [a]	437.2 ± 0.88 [a]	0.114 ± 0.01 [a]
Naxos (A)	1.55 ± 0.11 [a]	20.68 ± 0.85 [a]	0.884 0.13 [a]	437.3 ± 2.82 [a]	0.118 ± 0.01 [a]
Vegetative parts—Control					
Triso (E)	1.73 ± 0.11 [a]	3.66 ± 0.47 [a]	0.111 ± 0.02 [a]	439.6 ± 5.91 [a]	0.300 ± 0.15 [a]
Naxos (A)	1.60 ± 0.09 [a]	4.44 ± 0.39 [a]	0.147 ± 0.02 [a]	449.0 ± 1.84 [a]	0.370 ± 0.09 [a]
Vegetative parts—Spike-halving					
Triso (E)	1.65 ± 0.17 [a]	3.32 ± 0.30 [a]	0.091 ± 0.01 [a]	442.7 ± 5.37 [a]	0.278 ± 0.12 [a]
Naxos (A)	1.48 ± 0.13 [a]	4.30 ± 0.58 [a]	0.128 ± 0.04 [a]	448.9 ± 1.08 [a]	0.311 ± 0.10 [a]

(mean ± SD; different letters indicate significant differences of ear and vegetative plant parts between cultivars according to Student's *t*-test; * three main stems per pot pooled; pots n = 5).

For assessment of the importance of N uptake and allocation between the end of heading (EC 59) and fully-ripe, which represents the grain filling period, 10% of the third N split was applied as ^{15}N, namely 19 mg, representing 60 kg N (Table 1). Interestingly, there were no statistically significant differences between Triso (E) and Naxos (A) regarding ^{15}N-content. Therefore, data for both varieties were pooled (n = 10). The ^{15}N-content in grains and vegetative parts reflects the ^{15}N uptake from fertilizer between the end of heading (EC 59) and fully-ripe (EC 89). Remarkably, applying only the third split as ^{15}N, combined with spike-halving, led in this case to a significantly higher ^{15}N-content (plus 11%) of 0.486 ± 0.036 mg ^{15}N/g DM, compared to the control, with 0.437 ± 0.025 mg ^{15}N/g DM. The ^{15}N-content of the vegetative plant parts was not influenced by spike-halving, and was low, with 0.041 ± 0.009 mg ^{15}N/g DM and 0.043 ± 0.019 mg ^{15}N/g DM, representing about 10% of ^{15}N accumulation in the grains.

Labelling the whole plants with ^{13}CO$_2$ for one hour at the end of heading (EC 59) resulted in a significantly higher ^{13}C-content of 40% (0.223 mg ^{13}C/g DM) of the spike of the control of Triso (E) at the fully-ripe stage (EC 89) in comparison to Naxos (A) (0.160 mg ^{13}C/g DM) (Table 3). This superiority was slightly reduced to 34%, but also was demonstrated markedly in the treatment spike-halving (0.226 mg ^{13}C/g DM, 0.169 mg ^{13}C/g DM), respectively. During the grain-filling period, the assimilated supply to fill the grains is the current photosynthesis, of which spike, especially of glumes' photosynthesis [12], might be a major contributor. Additionally, the translocation of non-structural reserves stored before the onset of grain filling [4,7] is most likely involved. It seems that the Elite wheat Triso (E) has in the grains a clearly higher capacity, or 'sink strength', for the accumulation of ^{13}C native-carbonaceous compounds, which can be direct assimilates from photosynthesis, but/and also transient-stored ^{13}C-compounds, e.g., fructans, which are important storage polysaccharides in stems. A source-related ^{13}C limitation cannot be assumed, because in the vegetative parts the ^{13}C-content was about twice as high as in spikes at fully-ripe stage, and this pool cannot be 100% fixed in encrusted compounds, such as in the cellulose-lignin complex. However, the reason lies in the higher C-assimilate accumulation in Naxos (A), by a similar C-content, which leads to a significantly higher SGW of 38.4 and 46.9 mg. The lower ^{13}C-enrichment (above natural abundance) of the grains of Naxos (A), 0.0340 ^{13}C-excess, compared to 0.0475 ^{13}C-excess for Triso (E), confirms the dilution effect through increased storage of natural, unlabeled carbon between end of heading (EC 59) and fully-ripe (EC 89).

3.3. Treatment C: Sampling at Fully-Ripe Stage (EC 89), N Uptake between EC 11 and EC 89, ^{15}N Uptake and ^{13}C Accumulation between Stem Elongation (EC 30) and Fully-Ripe Stage (EC 89)

For treatment C, the two-time ^{15}N fertilization (Table 1), at stem elongation (EC 30) and at the end of heading (EC 59), showed no differences in the ^{15}N-content of the grains and vegetative parts between Triso (E) and Naxos (A), and was also not influenced by spike-halving (Table 4, grains, average n = 10: 0.833 ± 0.094 mg ^{15}N/g DM, 0.888 ± 0.098 mg ^{15}N/g DM; vegetative plant parts, average n = 10: 0.129 ± 0.027 mg ^{15}N/g DM, 0.110 ± 0.033 mg ^{15}N/g DM). However, about 14% of the in grains accumulated ^{15}N remains in the vegetative plant parts when ^{15}N was applied at stem elongation (EC 30) plus at the end of heading. For treatment B, ^{15}N application only at the end of heading (EC 59), this value amounted to 9% (Table 3, grains, average n = 10: 0.437 ± 0.025 mg ^{15}N/g DM, 0.486 ± 0.035 mg ^{15}N/g DM; vegetative plant parts, average n = 10: 0.043 ± 0.019 mg ^{15}N/g DM, 0.041 ± 0.009 mg ^{15}N/g DM). These results show that the N/^{15}N accumulation in wheat grains of these two cultivars,—independent of quality class—is apparently more sink-than source-limited, as shown in most conditions [2], because the ^{15}N-content in vegetative organs is obviously not a limiting factor for N/^{15}N, and therefore for the protein accumulation. In a field experiment with three wheat (*Triticum aestivum* L.) cultivars used in western Turkey, Anapo, Negev, and Sagittario, the uptake of ^{15}N into mature grains was not influenced by cultivar, sowing rate, or water supply treatment when ^{15}N was applied at either stem elongation or at flowering. The single fertilizer split, with each portion applied at a different growth stage (stem elongation/flowering), was translocated at the same level into the grains. This suggests that soil moisture under rain-fed conditions may not be a limiting factor for ^{15}N-uptake and, therefore, an additional water supply would not have an influence on the ^{15}N content of grains. [13] On the other hand, for winter wheat it was shown that the maximum proportion of ^{15}N fertilizer recovered was higher for the application at stem elongation (59–68%) than for the application at tillering (39–46%) [14]. The placement method (Split application (SA) of N fertilizer or a one-time band application (BA) of solid ^{15}N-urea) and the N application rate significantly affected nitrogen derived from fertilizer (Ndff) in grain, straw and total wheat plant [15]. The N uptake in straw derived from fertilizer was in the range of 3.1–22.1 kg ha^{-1}, which accounted for 16–29% of total Ndff, and, correspondingly, 71–84% of total Ndff was found in grain. Effah [16] reported on wheat plant organs (sampled 14 days after anthesis (DAA): ear, leaves, stem; at maturity: biomass was divided into shoots (with chaff) and grains) that increasing N fertilizer rates resulted in a considerable rise in percentage of ^{15}N content. The amount of ^{15}N in plant organs increased at an increasing rate at 14 DAA. However, this trend changed at maturity, where there was no significant difference between N fertilization of 105, 157, and 210 kg N^{-ha} in terms of ^{15}N content of the same organs.

In a dual-labelling (^{15}N/^{13}C) pot experiment [17] on winter wheat, the δ^{13}C values (in atom%) for ear, leaves and stems were 107.2 ± 18.5, 375.4 ± 25.0 and 198.8 ± 20.1 at stem elongation, and 285.3 ± 11.4, 103.7 ± 6.2 and 68.5 ± 4.1, respectively, during grain filling. This implies that at the initial phenological stage of stem elongation the leaves are more strongly enriched by ^{13}C than are ears, and this constellation is reversed during grain filling. This statement also applies to ^{15}N, since the ^{15}N excess (in atom %) in ear, leaves and stems amounted to 3.8 ± 0.2, 3.71 ± 0.2 3.6 ± 0.2 at stem elongation, and 5.4 ± 0.3 4.8 ± 0.1, 4.9 ± 0.1 during grain filling.

Calculations based on these measurements revealed an average ^{15}N remobilization for the control = [(^{15}N-content vegetative parts fully-ripe$_{control}$, treatment C- ^{15}N-content vegetative parts fully-ripe$_{control}$ treatment B/^{15}N-content vegetative parts fully-ripe$_{control}$, treatment C) × 100] and the treatment spike-halving = [(^{15}N-content vegetative parts fully-ripe$_{spike-halving}$, treatment C-^{15}N-content vegetative parts fully-ripe$_{spike-halving}$ treatment B/^{15}N-content vegetative parts fully-ripe$_{spike-halving}$, treatment C) × 100] of 68.2% and 61.1% remobilization, respectively. This clearly shows that the reduction of the sink size by spike-halving at the end of the heading leads to a 7% reduction of the mobilization of ^{15}N from vegetative parts.

A source-related limitation for the acquisition of ^{15}N in the grains of the main shoot cannot therefore be determined for the high-quality varieties of Triso (E) and Naxos (A). Rather, the interplay and coordination between generative and vegetative organs can be shown in these spring wheat cultivars. An example for such interplay was shown for the barley Risø16 mutation, which leads to the inactivation of cytosolic ADP-Glc pyrophosphorylase, and results in decreased ADP-Glc and endospermal starch levels [18]. It was shown that this mutation is accompanied by a decrease in storage protein accumulation and seed size, which indicates that alteration of a single enzymatic step can change the network of storage metabolism as a whole. A comparative analysis of genes in Risø16 revealed an overlap between metabolic and hormonal regulation, which leads to a coordinated down-regulation of endosperm-specific and ABA-inducible gene expression (storage proteins) together with repression by sugars. Such co-regulation ensured that decreased carbon fluxes into starch lead to a coordinated inhibition of glycolysis, amino acid and storage proteins biosynthesis, which is useful in the prevention of osmotic imbalances and oxidative stress due to the increased accumulation of sugars. Differences in the remobilization of assimilates temporarily stored in the vegetative plant parts for grain development might be an important aspect for determining grain dry weight response when 'direct' assimilate availability, like fertilizer N, is reduced. For Triso (E), the differences in the ^{13}C-content of grains discernible in the phase-end of heading to fully-ripe (Table 3, EC 59-EC 89) were not discernible in this phase, EC 30-EC 89 (Table 4), when the wheat plants were labelled with ^{13}CO$_2$ for one hour at stem elongation (EC 30). Furthermore, the ^{13}C accumulation in grains corresponds to about 36% of the ^{13}C accumulation in vegetative plant parts.

4. Conclusions

Regarding the assessment of the uptake and accumulation of ^{15}N from fertilizer nitrogen and ^{13}C via the air in grains and vegetative plant parts, it is advisable to accomplish this goal by utilizing phenological development phases. During the phase end-of-heading until fully-ripe stage, it seemed that the Elite wheat Triso (E) had a relatively higher metabolic utilization of ^{13}C native carbon in the grain. This may be due to a higher ability to form assimilate, and also to re-mobilize stored ^{13}C-carbon. Also, for spring wheat varieties of high-quality levels, Triso (E) and Naxos (A), the acquisition of ^{15}N is limited by the sink size of the grains, and is not source-limited. Remarkably, sink size, when reduced by spike-halving, resulted in a decreased remobilization of ^{15}N from vegetative plants parts, indicating a coordinated response of the plant. Therefore, it is more important to adapt the N application rates during the different phenological phases and to consider the cultivar specific requirements to reach the optimal crude protein content of spring wheat.

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