

## Article

# Maize and Wheat Response to Drought Stress under Varied Sulphur Fertilisation

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**Abstract:** This study aimed to examine the influence of long-lasting moderate (45% field water capacity—FWC) and severe (30% FWC) water stress and application of sulphur (elemental sulphur or sulphate) on the growth, yield and mineral composition of wheat and maize. Concentrations of macro- and micronutrients were determined in the aboveground parts of the plants. Drought stress caused a marked decrease in the growth parameters of both plants. Under both optimal water conditions (60% FWC) and moderate water stress (45% FWC), grain yields of wheat grown without sulphur application were not significantly different. Applying elemental sulphur caused an increase in grain yield under moderate stress, whereas sulphate was more effective in wheat grown under adequate water supply. Severe water stress significantly lowered wheat yield, regardless of sulphur fertilisation. Increasing water stress resulted in a greater reduction in maize growth, with an average 50% decrease in dry mass under severe water stress. Both crops maintained relatively high levels of macro- (N, P, K, Mg, Ca, S) and microelements (Mn, Fe, Cu, Zn) and did not suffer noticeably from deficiencies in such. Sulphur application did not modify these relationships. In conclusion, sulphur fertilisation may be recommended in wheat cultivation when plants are exposed to moderate water stress.

**Keywords:** drought; water stress; elemental sulphur; sulphate; macroelements; microelements



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## 1. Introduction

Drought is currently the most important environmental factor that has a huge impact on the growth of plants and their productivity [1,2]. Suboptimal water supply affects plants in a number of interacting manners, and the plant response is dependent on the plant species and stage of plant growth, severity and duration of stress and other environmental factors [1]. Under drought conditions, plants show numerous morphological, physiological, and biochemical changes [2]. Drought disrupts water relations, mineral uptake, photosynthesis efficiency and partitioning of assimilates and ultimately causes a significant reduction in crop yields [1,3,4]. Fahad et al. [1] show that yield losses in maize and wheat caused by drought reach 63–87% and 57%, respectively. Hence, to guarantee successful crop production, it is necessary to find effective ways to mitigate the negative effects of this stress. To achieve this goal, it is very important to use appropriate breeding programs to obtain crop genotypes resistant to suboptimal water supply. Applying a specific mineral fertilisation may help plants to cope with drought stress [2]. Hence, knowledge concerning uptake and accumulation of nutrients in plant tissues is very important from both an agricultural and an ecological perspective. The macroelement sulphur (S) is present in plant tissues in the smallest amount, in comparison to all other essential macronutrients, and yet is considered a limiting element in high-yielding agriculture [5,6]. Research concerning sulphur application in agricultural plant production is therefore very necessary. To ensure

proper growth and development, plants require sulphur at a level of 0.1–1.0% on a dry weight basis, and the average concentration of S in plant tissues ranges from 0.2 to 0.5% [7]. Plants take sulphur mainly as sulphate ( $\text{SO}_4^{2-}$ ), but elemental sulphur that is oxidised to sulphate in the soil is another good source of this element [8].

Sulphur plays a crucial role not only in the growth and development of higher plants, but also in stress tolerance and drought tolerance. Data concerning the effect of drought on sulphur nutrition are scant, although some studies have indicated that S nutrition plays a role in stress tolerance and defence mechanisms [1,9,10]. Sulphur, among other things, is a component of glutathione that is an important non-enzymatic antioxidant, being a crucial element in antioxidative mechanisms in plant cells [11]. Sulpholipids containing sulphur are present in chloroplastic membranes where they might protect photosynthetic apparatus under stress conditions. Usmani et al. [12] examined maize grown under drought stress and fertilised with different S fertilisers ( $\text{K}_2\text{SO}_4$ ,  $\text{FeSO}_4$ ,  $\text{CuSO}_4$  and  $\text{Na}_2\text{SO}_4$ ). They demonstrated that sulphur availability positively influenced some physiological parameters in water-stressed maize and among various S sources,  $\text{K}_2\text{SO}_4$  application resulted in the maximum increase in plant yield. Lee et al. [10] showed that drought stress induced by PEG (polyethylene glycol) resulted in a reduction in S uptake and significantly decreased the amount of sulphur assimilated into amino acids and proteins. Our earlier study demonstrated that applying elemental sulphur to the soil alleviated the negative effects of stress caused by chromium pollution [13]. Hence, we wished to further investigate if sulphur fertilisation improves plant functioning under drought soil conditions. It is very important to know the effects of S fertilisers on the uptake of other nutrients, particularly nitrogen (N), potassium (K) and phosphorus (P). Relationships between S fertilisers and other minerals under water scarcity conditions are still not clear, and getting to know them will allow for more effective management of crops grown under drought stress.

This study aimed to investigate the reactions of wheat and maize to long-lasting moderate (45% field water capacity—FWC) and severe (30% FWC) drought stress and examine the influence of sulphur fertilisation on yield and mineral composition in the plants.

## 2. Materials and Methods

### 2.1. Materials, Setup and Procedure

Research was conducted in the vegetation facilities of the Department of Plant Nutrition of the Wrocław University of Environmental and Life Sciences in Poland. Experiments were set up in four replicates in Wagner-type pots containing 5 kg of soil. The physical and chemical properties of the soil are described in Table 1. Temperature and light conditions during plant vegetation were natural, while soil moisture was controlled by watering with distilled water and soil moisture was maintained throughout the entire vegetation period of the cultivated plants at 30%, 45% and 60% field capacity (Table 2).

**Table 1.** Physico-chemical properties of the soil before the experiment.

Agronomic Category of Soil	pH	C <sub>organic</sub>	S <sub>total</sub>	P	K	Mg	S-SO <sub>4</sub>	Zn	Mn	Fe	Cu
	1 M KCl dm <sup>3</sup>	g kg <sup>-1</sup> Soil			mg kg <sup>-1</sup> Soil Soluble Forms						
Medium	4.80	6.32	0.178	64.0	88.0	48.0	9.26	39.0	110	577	2.94

Initially, the soil had an acidic pH (1 mol dm<sup>-3</sup> KCl), a medium level of phosphorus according the Egner-Riehm method [14] and low levels of potassium [14] and magnesium according the Schachtschabel method [15]. The amount of overall S and S-SO<sub>4</sub> in the soil classified it as low-fertility soil. There were low levels of the microelement iron present, as well as medium levels of copper and manganese and high levels of zinc according the Rinkis method [16]. Before sowing, calcium was added to the soil (liming) by applying calcium carbonate at a dose calculated for 1Hh (5 g CaCO<sub>3</sub>). The agricultural plants studied

were spring wheat (Tybalt variety) and maize (Mosso variety). Twenty-five grains of wheat were sown into pots and 10 evenly spaced plants were left after thinning, while 12 grains of maize were sown, leaving 6 plants after thinning. The vegetation period of wheat was 115 days, while that of maize was 99 days. Wheat was collected at the full maturity stage and maize was collected at the full bloom stage (BBCH 67). Overall, the experimental design included nine treatments in order to study the interaction of applied sulphur fertilisation and FWC (Table 2).

**Table 2.** Treatments in the pot experiment.

Field Water Capacity	Form of Sulphur	Dose of S mg kg <sup>-1</sup>
30%	Without S	0
	S-S <sup>0</sup> —elemental	60
	S-SO <sub>4</sub> —sulphate (VI)	60
45%	Without S	0
	S-S <sup>0</sup> —elemental	60
	S-SO <sub>4</sub> —sulphate (VI)	60
60%	Without S	0
	S-S <sup>0</sup> —elemental	60
	S-SO <sub>4</sub> —sulphate (VI)	60

Sulphur was applied before seeds were sown. Elemental S was ground to an average grain size of less than 0.1 mm to increase the rate of S oxidation in the soil [17]. For both plants, the same dose of nitrogen was applied (1.6 g per pot; NH<sub>4</sub>NO<sub>3</sub> in an aqueous solution). Half of the dose was applied before sowing and half during the topdressing stage (spring wheat BBCH 30 and maize BBCH 19). The size of the dose for the remaining macroelements depended on the soil properties. To each 5 kg pot of soil was added 0.6 g phosphorus, 1.5 g potassium and 0.3 g of magnesium. Fertilisation with microelements was applied in standard quantities for pot experiments in compounds that did not contain sulphur. Macro- and microelements were applied before sowing (in an aqueous solution or in solids) and mixed into the entire amount of soil in the pot.

## 2.2. Methods for Chemical Analysis

Before and after the vegetation experiments, representative soil and plant samples were collected for agricultural and chemical analysis. After conducting the preparations for the soil material, we determined the soil pH of 1 mol dm<sup>-3</sup> KCl using the potentiometric method, the overall S content (S total) via the Butters–Chenery method [18] and the content of S sulphates (VI) with the Bardsley and Lancaster method [19]. In plant material collected during the study, we determined the overall level of nitrogen (N organic) using the Kjeldahl method and the S total via the Butters–Chenery method. To determine levels of other elements, the plant material was dry mineralised, and then the ash was taken up with nitric acid and measured in solution: phosphorus via the vanadic–molybdate method, potassium and calcium with flame photometry, and magnesium and microelements via atomic absorption spectrophotometry.

## 2.3. Statistical Methods

The yield sizes and results of the chemical analysis were subjected to a two-way variance analysis. Prior to performing the analysis of variance, tests for homogeneity of variance within groups were performed using the Levene’s test and the Shapiro–Wilk test of the correspondence of variables to the normal distribution. The relevance of mean

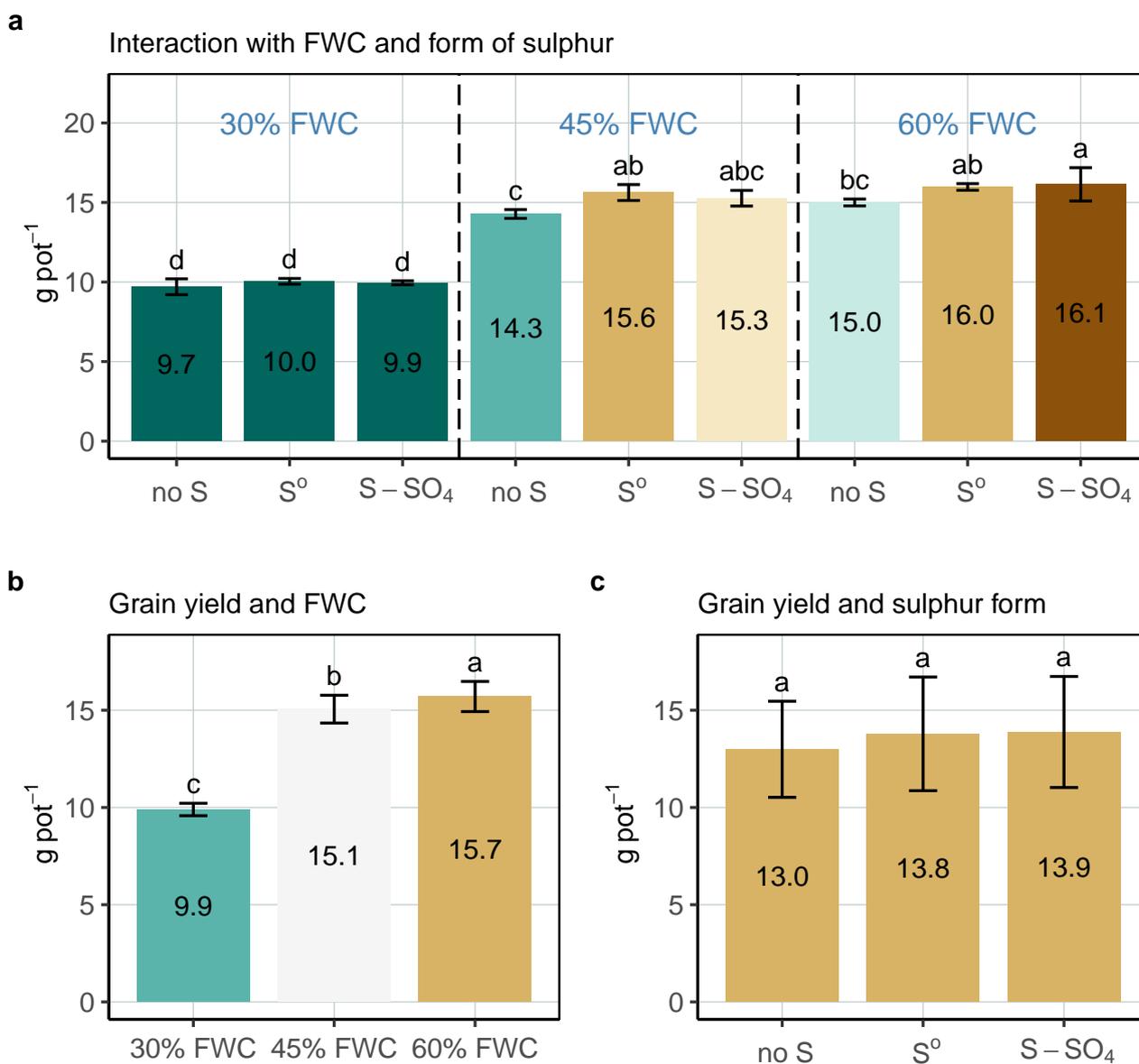
differences was evaluated using the Tukey post hoc test with a significance level of  $p = 0.05$ . The statistical program R [20] was used for all statistical analyses.

### 3. Results and Discussion

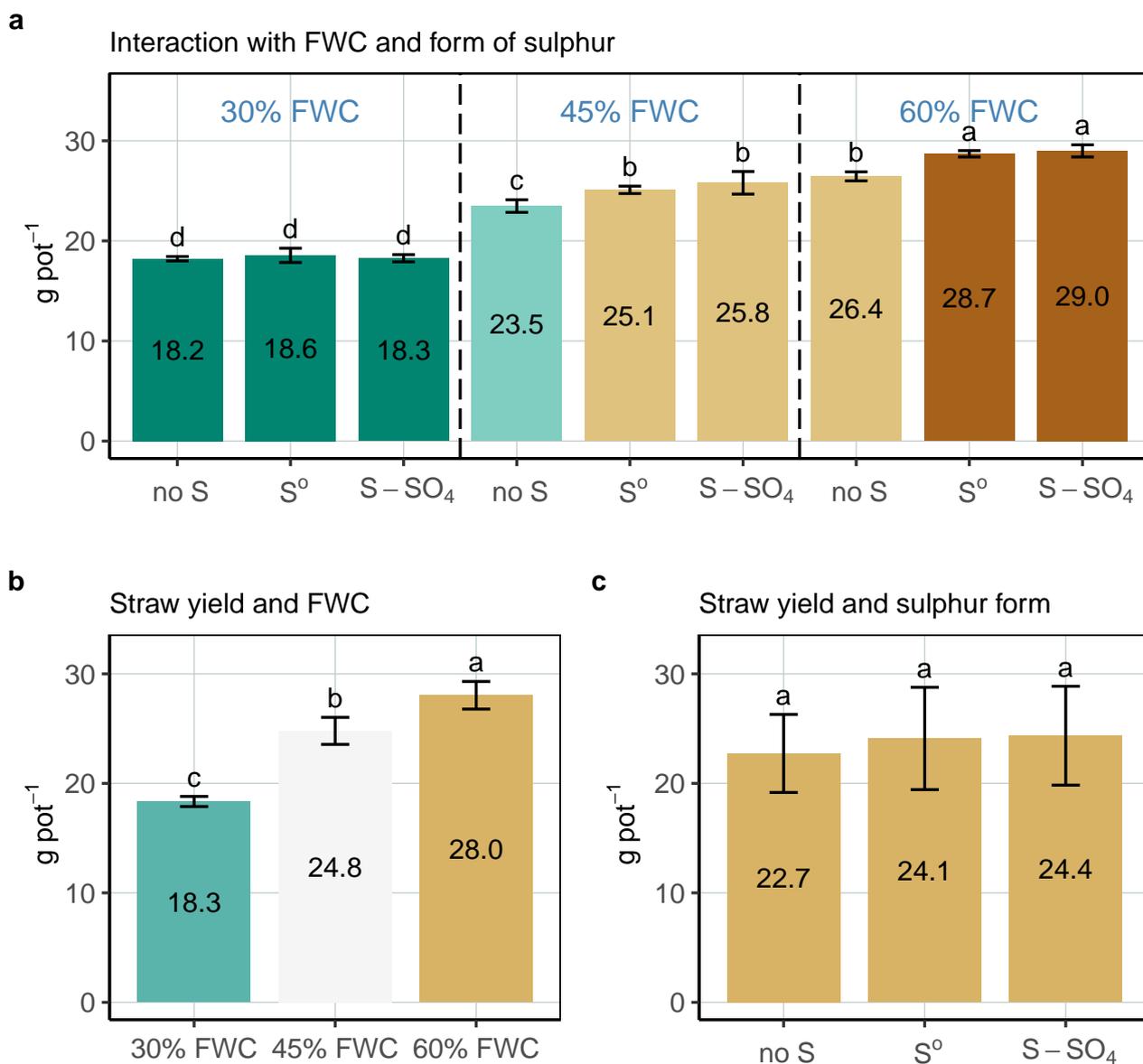
#### 3.1. Plant Growth and Yield

Drought affects many aspects of plant growth and development, diminishes the germination and establishment of seedlings, reduces cell division and differentiation rates, decreases biomass accumulation and consequently causes dramatically lower crop yields. For major crop plants, average yields can be reduced by more than 50% [1,21].

Our results show that the growth of both wheat and maize markedly dropped under drought conditions (Figures 1a,b, 2a,b, 3a,b and 4a,b), and wheat reacted better than maize to sulphur fertilisation under optimal conditions (60% FWC) and moderate water stress (45% FWC) (Figure 2a).

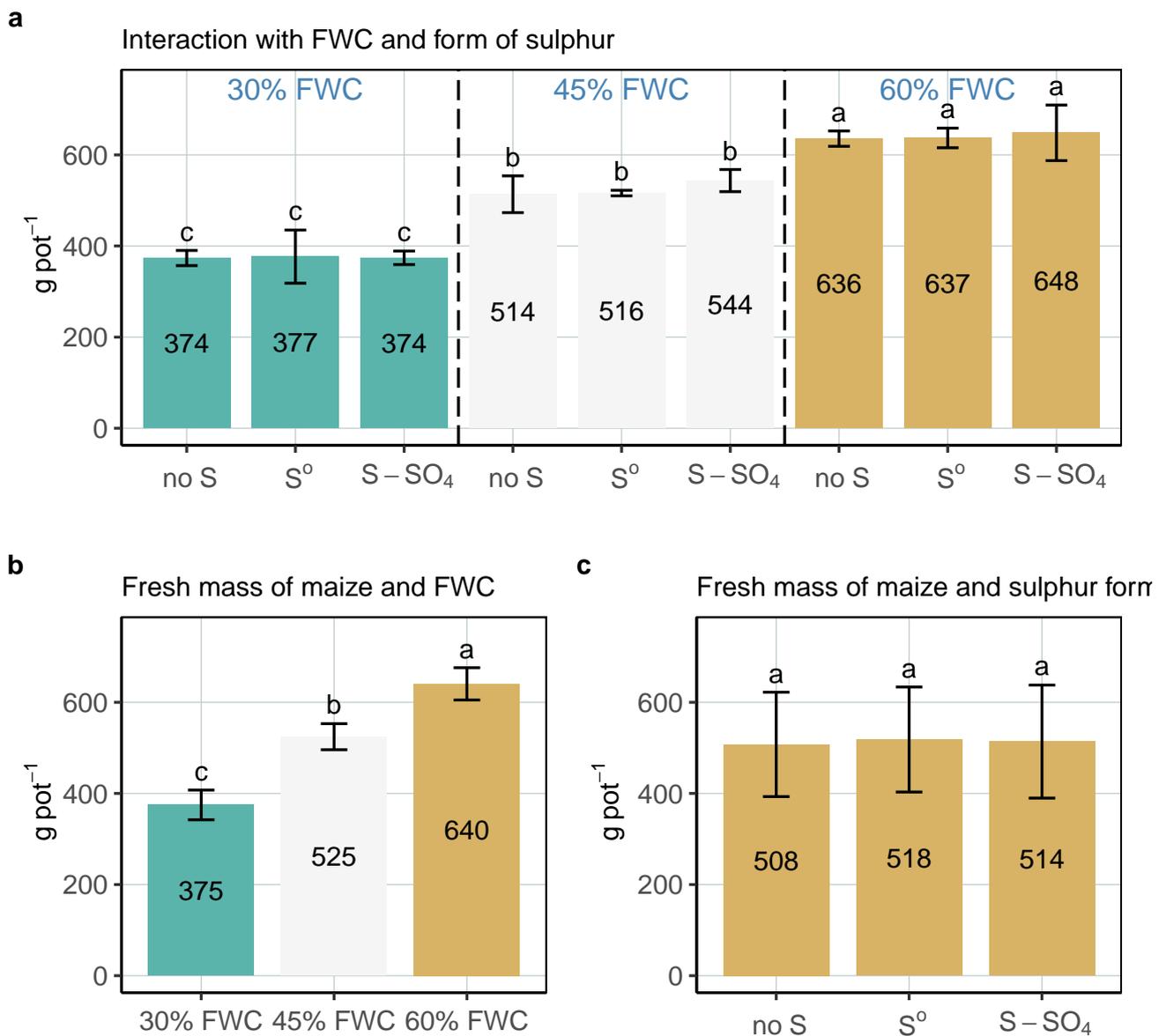


**Figure 1.** Grain yield of spring wheat. Values indicated by the same letter are not significantly different ( $\alpha = 0.05$ ).



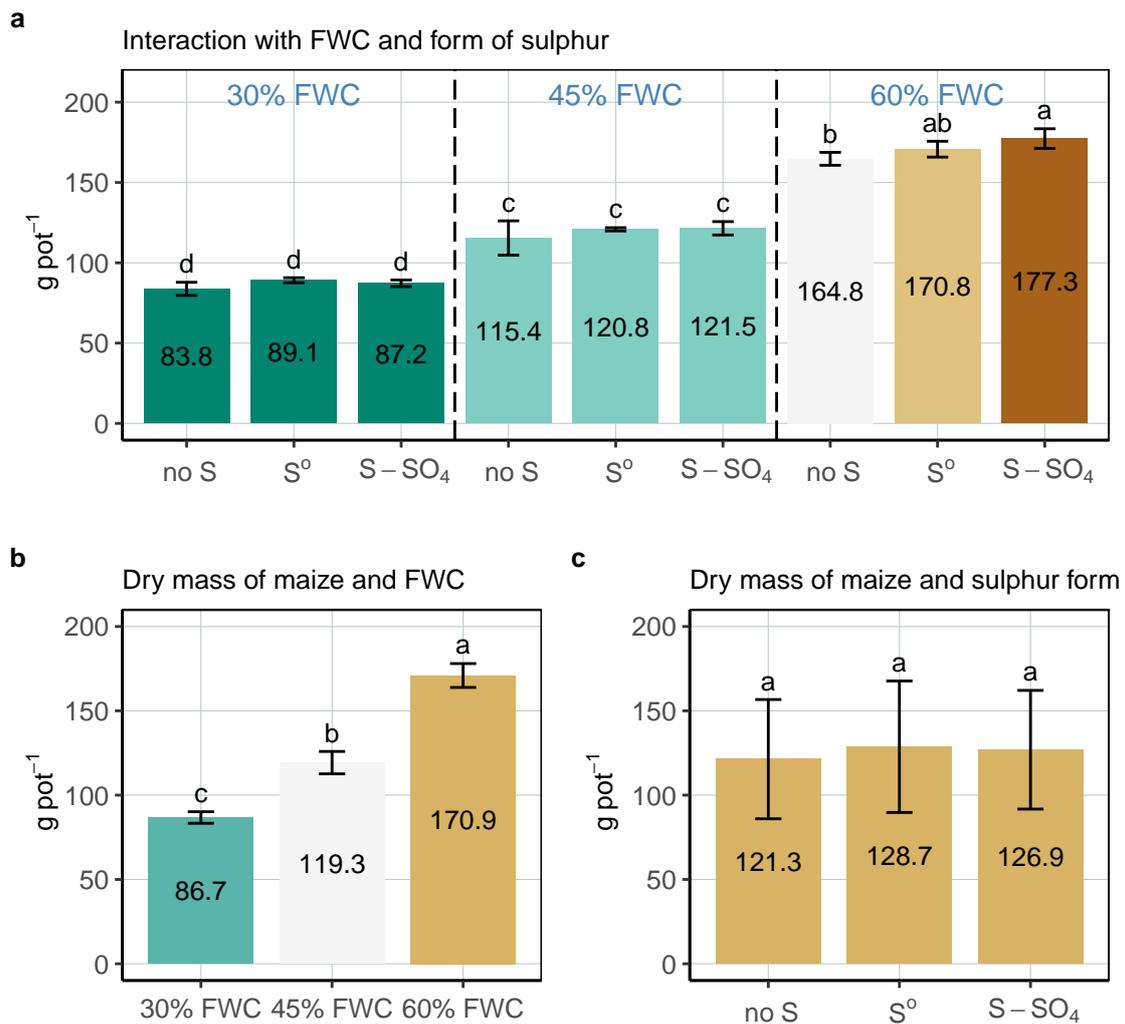
**Figure 2.** Straw yield of spring wheat. Values indicated by the same letter are not significantly different ( $\alpha = 0.05$ ).

Under both optimal water conditions (60% FWC) and moderate water stress, the grain yields of wheat grown without sulphur application were not significantly different (Figure 1a). Applying elemental sulphur caused an increase in grain yield under moderate stress, whereas sulphate was more effective in wheat grown under adequate water supply (Figure 1a). Sulphur application (Figure 2a)—both elemental and sulphate—significantly improved the yield of wheat straw grown under optimal water conditions and moderate stress, but observed increases did not exceed 10% in comparison to plants grown without sulphur. The severe water stress significantly lowered the yield of grain and straw, regardless of sulphur fertilisation (Figures 1a,b and 2a,b). Under this condition, wheat biomass production (grain, straw) was greatly reduced (Figures 1a,b and 2a,b), but not by more than 40% in comparison to well-watered plants (60% FWC).



**Figure 3.** Fresh mass yield of maize. Values indicated by the same letter are not significantly different ( $\alpha = 0.05$ ).

In maize, both stress levels caused a significant reduction in fresh and dry weights of plants (Figures 3a,b and 4a,b). The severe drought stress caused, on average, a 50% reduction in plant dry mass. As a C4 plant, maize uses water very efficiently, but it remains sensitive to water availability. Applying sulphur did not modify maize growth under stress conditions, but it did slightly improve dry matter production in well-watered plants (Figure 4a). Previous studies have shown that drought negatively impacts the yield of crop plants, with the decrease in yield being dependent on the severity of the drought stress and plant growth stage [22–27]. A few studies of sulphur fertilisation indicate that application of this element may help plants better tolerate limited water availability [9,28].



**Figure 4.** Dry mass yield of maize. Values indicated by the same letter are not significantly different ( $\alpha = 0.05$ ).

Overall, both maize and wheat were able to survive long-lasting, high-intensity water shortage (30% FWC), and their biomass production was not reduced by more than 50% when compared to well-watered plants. Under severe water stress, applying sulphur did not affect the growth of plants.

### 3.2. Mineral Nutrition

#### 3.2.1. Macroelements

Plant ability to uptake minerals is a very important factor in determining the quality and quantity of crop yield. Drought stress usually restricts absorption of minerals due to a decreased rate of nutrient diffusion from the soil to the absorbing root surface and lowered translocation within the plant [29,30]. Various studies have shown a decrease in the accumulation of some minerals in plant tissues under water stress, but this response varies across crop species [4,25,29,30]. A lower concentration of particular elements in plant tissues might indicate that mineral uptake is disrupted. Fahad et al. [1] presented the generalisation that under drought conditions, N uptake increases, P uptake declines and K remains unaffected. Our study showed that the concentrations of particular macroelements (S, P, K, Mg, Ca) in wheat grain did not change considerably, either in response to drought conditions or with the addition of sulphur (Tables 3–5).

**Table 3.** Nitrogen and sulphur content and uptake in cultivated plants. Values indicated by the same letter are not significantly different ( $\alpha = 0.05$ ).

Treatments		Spring Wheat			Maize	
		Grain	Straw	Grain + Straw	Content	Uptake
FWC	Sulphur	Content g kg <sup>-1</sup> d.m.		Uptake mg pot <sup>-1</sup>	g kg <sup>-1</sup> d.m.	mg pot <sup>-1</sup>
Nitrogen						
30%	Without S	20.5 abc	6.64 a	319 c	15.1 a	1 270 c
	S-S <sup>0</sup>	18.6 bcd	6.97 a	316 c	15.5 a	1 380 abc
	S-SO <sub>4</sub>	19.0 abcd	6.89 a	315 c	15.6 a	1 360 bc
45%	Without S	19.2 abcd	6.88 a	436 b	12.3 b	1 420 abc
	S-S <sup>0</sup>	17.8 cd	6.37 a	442 b	12.5 b	1 510 ab
	S-SO <sub>4</sub>	17.3 d	6.63 a	431 b	11.6 b	1 410 abc
60%	Without S	21.2 ab	6.65 a	494 a	8.99 c	1 480 abc
	S-S <sup>0</sup>	21.9 a	6.48 a	536 a	8.80 c	1 500 ab
	S-SO <sub>4</sub>	21.6 ab	6.54 a	537 a	9.02 c	1 600 a
FWC	30%	19.4 b	6.83 a	317 c	15.4 a	1 330 b
	45%	18.1 b	6.63 a	436 b	12.1 b	1 450 a
	60%	21.6 a	6.56 a	522 a	8.94 c	1 530 a
Sulphur	Without S	20.3 a	6.72 a	417 a	12.1 a	1 390 a
	S-S <sup>0</sup>	19.4 a	6.60 a	431 a	12.3 a	1 460 a
	S-SO <sub>4</sub>	19.3 a	6.69 a	428 a	12.1 a	1 460 a
Sulphur						
30%	Without S	1.54 b	1.28 e	38.3 e	0.420 d	35.2 f
	S-S <sup>0</sup>	1.60 b	2.08 c	54.7 d	0.640 a	57.1 cd
	S-SO <sub>4</sub>	1.64 b	1.86 d	50.3 d	0.629 a	54.9 de
45%	Without S	1.50 b	1.47 e	55.9 d	0.396 d	45.9 ef
	S-S <sup>0</sup>	1.61 b	2.05 cd	78.1 b	0.550 b	66.5 bc
	S-SO <sub>4</sub>	1.63 b	2.25 bc	81.2 b	0.519 bc	63.0 cd
60%	Without S	1.64 b	1.44 e	62.5 c	0.316 e	52.1 de
	S-S <sup>0</sup>	1.92 a	2.41 ab	99.7 a	0.445 d	76.0 ab
	S-SO <sub>4</sub>	1.90 a	2.48 a	103 a	0.465 cd	82.4 a
FWC	30%	1.59 b	1.74 a	47.8 c	0.563 a	49.1 b
	45%	1.58 b	1.92 a	71.7 b	0.488 ab	58.5 ab
	60%	1.82 a	2.11 a	88.3 a	0.409 b	70.2 a
Sulphur	Without S	1.56 b	1.40 b	52.2 b	0.378 b	44.4 b
	S-S <sup>0</sup>	1.71 a	2.18 a	77.5 a	0.545 a	66.5 a
	S-SO <sub>4</sub>	1.72 a	2.20 a	78.1 a	0.538 a	66.7 a

Some significant differences were observed in the case of nitrogen (Table 3), which saw a decrease in content in the grains under drought conditions and was not significantly affected by sulphur application. Both sulphur forms applied (S elemental and sulphate) caused a 17% increase in S concentration in the grains of plants grown under optimal conditions (Table 3).

In wheat straw, particular macroelement quantities were more changeable than in grain, with only N levels remaining stable under all conditions (Tables 3–5). Wheat fertilised with sulphur contained, on average, 56% more S than did unfertilised plants. This effect was observed in plants grown under both optimal conditions and drought stress (Table 3).

Water stress promoted S accumulation in maize shoots, with levels increasing with increasing stress intensity (Table 3). Sulphur-fertilised maize accumulated considerably more S than did non-fertilised plants. Under severe water stress, maize shoots contained approximately 50% more S than did non-fertilised plants. A few reports concerning the effect of drought on sulphur nutrition indicate a positive role of sulphur in alleviating the effects of drought stress [9,11]. Fatma et al. [31] demonstrated that an excess S supply improved photosynthesis and growth of mustard grown under salt stress condition.

As a vital constituent of many cellular compounds, sulphur not only plays an important role in the normal functioning of plants, but is also involved in defence mechanisms in stimulating the antioxidative system in cells. Some researchers claim that under stressful conditions, the demand for S is greater and plants increase sulphate uptake compared to other ions [32,33]. Applying sulphur might enhance the efficiency of other essential macronutrients such as N and P [28]. Usmani et al. [12] showed that S availability positively influenced leaf water status, gas exchange characteristics and antioxidative machinery in water-stressed maize plants. In summary, a plant's capacity to acquire S and carry on high sulphur use efficiency plays a significant role in the alleviation of the negative effects of drought stress [10].

In maize tissues, increasing water stress resulted in a greater accumulation of N and P (Tables 3 and 4). Nitrogen concentrations increased by, on average, 36% and 73% for moderate and severe water stress, respectively. Plants require N in large amounts, as it is a constituent of many essential cell compounds and its deficiency rapidly inhibits plant growth. Neither maize nor wheat suffered from nitrogen deficiency, and N concentration in the aboveground parts of stressed plants was similar to or higher than that of well-watered plants.

As N plays a fundamental role in plant growth and productivity, adequate concentration of this element in plant tissues is particularly important to their functioning under stressful conditions. In leaves, most N content is involved in photosynthesis as either enzymes or chlorophyll. Ding et al. [3] contend that photosynthesis and water uptake are the two key traits that enhance crop tolerance to drought. Conversely, however, they also maintain that a high nitrate supply may decrease plant drought tolerance. Nitrogen is also necessary for antioxidative protection as a component of enzymes and osmoprotectants that protects cells from the harmful effects of different abiotic stresses. In contrast, other plant studies have shown that drought affects N metabolism and significantly reduces N concentration [22,34].

Changes in P concentration were relatively small in both plant species (Table 4). Phosphorus is essential in processes connected with the storage and transfer of energy, photosynthesis, regulation of enzyme activity and transport of carbohydrates. Hence, an adequate level of P promotes metabolic processes such as respiration, photosynthesis, cell division and expansion, and the uptake and assimilation of other minerals [35,36]. Several studies indicate that drought stress reduces P uptake, as well as its subsequent transport to the stem, resulting in P deficiency in plant tissues [4,23,37–39]. Despite a slight decrease in P concentration, the examined plants were well supplied with this nutrient (Table 4).

Potassium plays a vital role in the regulation of water status, osmotic adjustment and charge balance in plants. In addition to osmoregulation and stomatal movements, K also regulates enzyme activity and the stability of membranes [7,40,41]. In this study, K content in

maize shoots increased by 17% and 48% under moderate and severe water stress, respectively (Table 4). Applying sulphur had no effect on these parameters. Tadayyon et al. [42] obtained similar results with castor bean (*Ricinus communis*), in which K concentration in plant tissue increased with increasing severity of drought stress. Accumulation of K in plant tissues may help plants adjust osmotically and maintain activity of aquaporins involved in water uptake, thus improving drought stress tolerance [43]. The straw of wheat grown under drought stress accumulated significantly less potassium than control plants. This indicates that drought conditions limited potassium uptake and transport within the plant. According to Anschutz et al. [40], in addition to its well-established role as an essential macronutrient, K is also an important signalling agent mediating a wide range of plant adaptive responses to the environment. A disruption of K homeostasis in wheat may impair many biochemical processes and increase a plant's sensitivity to water stress, suggesting that increased K fertilisation could possibly help plants cope better with drought stress. Urbina et al. [44] also demonstrated that severe drought stress decreases K concentration in plants.

Subsequent macroelement calcium regulates any physiological processes, including movement of water and solutes, cell division, cell-wall synthesis, membrane and stomatal functions, and signal transduction. Straw from wheat grown under drought stress contained approximately 70% more calcium than did plants grown under optimal conditions (Table 5). Maize grown under drought stress also accumulated calcium, but the observed increase was lower (41% on average). Alternatively, Nahar and Gretzmacher [45] indicated that a reduction in soil water potential results in reduced calcium uptake. Our results showed that despite the very low mobility of this element, the uptake and distribution of calcium were not disrupted and did not limit plant functioning under stressful conditions. Tadayyon et al. [42] also stated that in *R. communis*, calcium concentration increased as drought stress increased and was lower in control plants.

Little information is available concerning the effect of drought on Mg nutrition in plants. Magnesium plays a vital role in photosynthesis as an essential component of chlorophyll and is also a cofactor for many enzymes and an important agent in protein synthesis. In *R. communis* [42], only very severe drought stress (75% moisture depletion) resulted in a significant decrease in Mg concentration in plant leaves. Nahar and Gretzmacher [45] also found a decrease in Mg concentration in tomato plants under drought stress. Our results showed that the plants were able to take up a sufficient amount of this element under drought conditions (Table 5). Magnesium content in wheat grain was not affected by different treatments, but in wheat straw grown under optimal water conditions, sulphur application resulted in a higher Mg concentration. Sulphate fertilisation was more effective than application of elemental S, with observed increases of 21% and 11% for sulphate and S application, respectively (Table 5). Under drought conditions, applying S did not significantly change Mg levels in wheat straw. In maize shoots, sulphur fertilisation did not modify Mg concentration, although severe water stress caused a considerable increase in Mg content. Under this condition, irrespective of sulphur fertilisation, the mean increase in Mg concentration in maize shoots amounted to 30% more than that of plants grown under optimal water conditions. It can be assumed that photosynthesis was not disrupted by Mg deficiency [46].

In summary, our results show that in plants grown under drought stress, although macronutrient concentrations were somewhat disturbed, relatively high macronutrient levels were maintained overall, and plants did not noticeably suffer from deficiencies.

**Table 4.** Phosphorus and potassium content and uptake in cultivated plants. Values indicated by the same letter are not significantly different ( $\alpha = 0.05$ ).

Treatments		Spring Wheat			Maize						
		Grain	Straw	Grain + Straw	Content	Uptake					
FWC	Sulphur	Content g kg <sup>-1</sup> d.m.		Uptake mg pot <sup>-1</sup>	g kg <sup>-1</sup> d.m.	mg pot <sup>-1</sup>					
Phosphorus											
30%	Without S	2.68	a	1.01	bc	44.4	e	7.92	a	665	c
	S-S <sup>0</sup>	2.57	ab	0.888	c	42.3	e	8.07	a	720	c
	S-SO <sub>4</sub>	2.56	ab	0.922	bc	42.2	e	7.80	a	680	c
45%	Without S	2.27	b	0.970	bc	55.2	d	7.93	a	909	b
	S-S <sup>0</sup>	2.38	ab	1.04	abc	64.2	c	7.72	a	934	b
	S-SO <sub>4</sub>	2.35	ab	1.06	abc	62.6	c	7.62	a	926	b
60%	Without S	2.56	ab	1.14	ab	68.5	bc	8.41	a	1390	a
	S-S <sup>0</sup>	2.61	a	1.25	a	77.6	a	8.30	a	1420	a
	S-SO <sub>4</sub>	2.58	ab	1.16	ab	75.2	ab	8.61	a	1530	a
FWC	30%	2.60	a	0.942	b	43.0	c	7.93	ab	688	c
	45%	2.33	b	1.03	b	60.7	b	7.76	b	923	b
	60%	2.58	a	1.18	a	73.8	a	8.44	a	1440	a
Sulphur	Without S	2.50	a	1.04	a	56.0	a	8.09	a	986	a
	S-S <sup>0</sup>	2.52	a	1.06	a	61.4	a	8.03	a	1020	a
	S-SO <sub>4</sub>	2.50	a	1.05	a	60.0	a	8.01	a	1040	a
Potassium											
30%	Without S	3.94	a	9.56	cd	213	d	68.7	ab	5750	c
	S-S <sup>0</sup>	3.98	a	9.36	d	214	d	74.9	a	6680	bc
	S-SO <sub>4</sub>	3.84	a	9.80	bcd	217	d	69.4	ab	6060	c
45%	Without S	3.64	a	10.2	bcd	292	c	58.3	bc	6710	bc
	S-S <sup>0</sup>	3.50	a	10.8	b	334	b	56.6	bcd	6840	bc
	S-SO <sub>4</sub>	3.57	a	10.7	bc	323	bc	54.7	cd	6640	bc
60%	Without S	3.84	a	12.0	a	375	a	50.2	cd	8280	a
	S-S <sup>0</sup>	4.04	a	10.8	ab	376	a	49.5	cd	8460	a
	S-SO <sub>4</sub>	4.03	a	10.6	bc	371	a	44.9	d	7950	ab
FWC	30%	3.92	a	9.57	b	214	c	71.0	a	6160	b
	45%	3.57	b	10.6	a	316	b	56.6	b	6730	b
	60%	3.97	a	11.1	a	374	a	48.2	c	8230	a
Sulphur	Without S	3.80	a	10.6	a	293	a	59.1	a	6910	a
	S-S <sup>0</sup>	3.84	a	10.3	a	308	a	60.4	a	7330	a
	S-SO <sub>4</sub>	3.81	a	10.4	a	304	a	56.3	a	6880	a

**Table 5.** Magnesium and calcium content and uptake in cultivated plants. Values indicated by the same letter are not significantly different ( $\alpha = 0.05$ ).

Treatments		Spring Wheat			Maize						
		Grain	Straw	Grain + Straw	Content	Uptake					
FWC	Sulphur	Content g kg <sup>-1</sup> d.m.		Uptake mg pot <sup>-1</sup>	g kg <sup>-1</sup> d.m.	mg pot <sup>-1</sup>					
<b>Magnesium</b>											
30%	Without S	0.803	a	1.32	ab	31.8	d	6.17	a	517	d
	S-S <sup>0</sup>	0.794	a	1.30	ab	32.1	d	5.89	a	526	d
	S-SO <sub>4</sub>	0.772	a	1.27	abc	30.9	d	5.65	ab	493	d
45%	Without S	0.767	a	1.37	a	43.2	bc	4.97	ab	573	cd
	S-S <sup>0</sup>	0.765	a	1.16	bc	42.0	c	5.01	ab	605	bcd
	S-SO <sub>4</sub>	0.786	a	1.29	abc	44.4	bc	5.10	ab	622	bcd
60%	Without S	0.825	a	1.08	c	41.0	c	4.42	b	729	abc
	S-S <sup>0</sup>	0.834	a	1.20	abc	47.7	ab	4.47	b	764	ab
	S-SO <sub>4</sub>	0.812	a	1.31	ab	51.0	a	4.79	ab	849	a
FWC	30%	0.790	b	1.30	a	31.6	c	5.90	a	512	c
	45%	0.773	b	1.28	a	43.2	b	5.03	b	600	b
	60%	0.824	a	1.20	a	46.6	a	4.56	b	781	a
Sulphur	Without S	0.798	a	1.26	a	38.7	a	5.19	a	606	a
	S-S <sup>0</sup>	0.798	a	1.22	a	40.6	a	5.12	a	631	a
	S-SO <sub>4</sub>	0.790	a	1.29	a	42.1	a	5.18	a	655	a
<b>Calcium</b>											
30%	Without S	0.177	a	6.84	a	127	abcd	6.21	a	520	b
	S-S <sup>0</sup>	0.180	a	6.70	a	126	abcd	5.88	a	524	b
	S-SO <sub>4</sub>	0.189	a	6.62	a	122	bcd	5.89	a	514	b
45%	Without S	0.166	a	6.76	a	161	ab	5.15	ab	595	b
	S-S <sup>0</sup>	0.179	a	6.04	ab	159	ab	4.88	abc	590	b
	S-SO <sub>4</sub>	0.172	a	6.56	a	167	a	4.92	abc	600	b
60%	Without S	0.183	a	3.38	c	92.1	d	3.69	cd	608	b
	S-S <sup>0</sup>	0.186	a	3.73	c	110	cd	3.52	d	601	b
	S-SO <sub>4</sub>	0.192	a	4.63	bc	137	abc	4.47	bcd	793	a
FWC	30%	0.182	ab	6.72	a	125	b	5.99	a	519	b
	45%	0.172	b	6.45	a	162	a	4.99	b	595	ab
	60%	0.187	a	3.91	b	113	b	3.89	c	667	a
Sulphur	Without S	0.176	a	5.66	a	126	a	5.02	a	574	a
	S-S <sup>0</sup>	0.181	a	5.49	a	132	a	4.76	a	571	a
	S-SO <sub>4</sub>	0.185	a	5.94	a	142	a	5.09	a	636	a

### 3.2.2. Microelements

Because plants require much smaller amounts of microelements than macronutrients, little attention has been given to studying the effects of drought on micronutrient requirements. Indeed, low moisture in the soil could disturb their uptake and induce deficiency in plant tissues. In wheat grain, levels of the examined microelements (Cu, Fe, Mn, Zn) were relatively stable under the tested conditions (Tables 6 and 7). Statistically significant differences were observed for Fe and Mn. Greater differences in Fe concentration were found in wheat straw, with an observed decrease of up to 25% in comparison to control plants. Tadayyon et al. [42] also demonstrated that drought stress decreased Fe content in *R. communis*, although the maximum decline under severe water stress (75% moisture depletion) was only 11%. In this study, the observed reduction in Fe in maize shoots was not statistically significant (Table 6), and sulphur fertilisation had no effect on these parameters. In wheat straw, drought stress decreased not only Fe, but also Cu concentration, although it did not affect Zn or Mn content (Table 7). Applying elemental sulphur caused an increase in Mn content in wheat straw, but no positive effect of sulphur application was observed in maize. Nevertheless, increasing water stress resulted in a higher content of this element in plant tissues. Manganese plays a crucial role in photosynthesis, respiration, antioxidative metabolism and the activation of some enzymes, so a high Mn concentration in plant tissues may be crucial for protecting cells against the harmful effects of reactive oxygen species generated under drought stress.

Samarah et al. [27] found that drought stress increased concentrations of Zn and Cu in soybean seeds and that the increase in mineral concentration was not due to the reduction in dry matter accumulation.

Drought stress in maize shoots caused an increase in the concentration of both Cu and Zn, which was particularly evident under severe water stress (Table 7). Generally, the observed changes exceeded 50% in relation to plants grown in optimal conditions (Table 7). Applying sulphur did not change these relationships. It is possible that higher concentrations of these microelements allow plants to scavenge reactive oxygen species more effectively, ultimately leading to better adaptation to stress conditions. It is noteworthy that the Zn nutritional status of plants is essential for crop productivity and quality worldwide.

### 3.2.3. Total Mineral Uptake

Generally, drought considerably limited the total uptake of macroelements, with a greater reduction observed in plants exposed to severe water stress (Tables 3–5). The significant decrease in total N uptake by wheat did not result in an analogous decline in N content in the straw and grain, although some negative symptoms were observed in the latter. Although the severe drought stress caused an important decline in the total uptake of numerous nutrients (N, S, P, K, Mg), they remained present in aboveground tissues at high levels (Tables 3–5). Engels and Marschner [47] claim that translocation of minerals is dependent on external factors and is also internally regulated according to the growth-related demand of shoots. Drought-induced inhibition of plant growth (Figures 1–4) reduced plants' mineral nutrient requirements, allowing the plants to maintain an adequate nutritional status. Total mineral uptake was reduced by more than 40% in comparison to well-watered plants in the case of N and P in both plant species and in the case of S and K in wheat. As expected, sulphur fertilisation had an effect on total S uptake by plants grown under both optimal and stressful conditions (Table 3), although the impact was only statistically significant for P, K and Mg in wheat. Reduction in the uptake of various macroelements under stress has been reported in numerous plant species [4,23,29,39,45]. Similar changes were observed for total uptake of microelements and, in general, sulphur fertilisation did not change these relationships (Table 5). Of all the microelements measured here, iron experienced the greatest reduction in both wheat and maize.

**Table 6.** Manganese and iron content and uptake in cultivated plants. Values indicated by the same letter are not significantly different ( $\alpha = 0.05$ ).

Treatments		Spring Wheat			Maize	
		Grain	Straw	Grain + Straw	Content	Uptake
FWC	Sulphur	Content mg kg <sup>-1</sup> d.m.		Uptake mg pot <sup>-1</sup>	mg kg <sup>-1</sup> d.m.	mg pot <sup>-1</sup>
<b>Manganese</b>						
30%	Without S	37.8 ab	91.8 ab	2.04 e	64.8 ab	5.44 b
	S-S <sup>0</sup>	37.6 ab	101 ab	2.25 de	69.8 a	6.23 b
	S-SO <sub>4</sub>	35.5 b	81.3 b	1.84 e	60.8 abc	5.31 b
45%	Without S	37.3 b	93.9 ab	2.74 cd	57.6 abcd	6.66 ab
	S-S <sup>0</sup>	37.6 ab	115 a	3.57 ab	56.7 abcd	6.85 ab
	S-SO <sub>4</sub>	35.9 b	97.2 ab	2.99 bc	53.8 bcde	6.54 ab
60%	Without S	36.1 b	92.3 ab	2.98 bc	43.2 de	7.12 ab
	S-S <sup>0</sup>	42.8 a	118 a	4.07 a	40.4 e	6.89 ab
	S-SO <sub>4</sub>	39.9 ab	89.9 ab	3.25 bc	46.2 cde	8.16 a
FWC	30%	37.0 a	91.4 a	2.04 b	65.2 a	5.66 b
	45%	36.9 a	102 a	3.10 a	56.1 b	6.68 a
	60%	39.6 a	100 a	3.43 a	43.3 c	7.39 a
Sulphur	Without S	37.1 a	92.7 b	2.59 b	55.2 a	6.41 a
	S-S <sup>0</sup>	39.3 a	111 a	3.30 a	55.7 a	6.66 a
	S-SO <sub>4</sub>	37.1 a	89.4 b	2.69 ab	53.6 a	6.67 a
<b>Iron</b>						
30%	Without S	48.4 ab	65.4 c	1.66 c	57.9 a	4.86 c
	S-S <sup>0</sup>	46.8 ab	65.4 c	1.69 c	69.6 a	6.21 bc
	S-SO <sub>4</sub>	44.7 b	61.9 c	1.57 c	64.6 a	5.63 bc
45%	Without S	46.0 ab	78.3 abc	2.50 b	67.6 a	7.80 b
	S-S <sup>0</sup>	45.3 b	69.9 bc	2.51 b	61.8 a	7.47 b
	S-SO <sub>4</sub>	51.3 ab	69.5 bc	2.53 b	65.9 a	8.01 b
60%	Without S	51.4 ab	85.2 ab	3.02 a	69.9 a	11.5 a
	S-S <sup>0</sup>	53.3 a	89.1 a	3.41 a	68.3 a	11.6 a
	S-SO <sub>4</sub>	51.6 ab	84.7 ab	3.29 a	73.8 a	13.0 a
FWC	30%	46.6 b	64.3 c	1.64 c	64.0 a	5.57 c
	45%	47.5 b	72.6 b	2.51 b	65.1 a	7.76 b
	60%	52.1 a	86.3 a	3.24 a	70.6 a	12.1 a
Sulphur	Without S	48.6 a	76.3 a	2.39 a	65.1 a	8.06 a
	S-S <sup>0</sup>	48.4 a	74.8 a	2.54 a	66.6 a	8.44 a
	S-SO <sub>4</sub>	49.2 a	72.1 a	2.46 a	68.1 a	8.90 a

**Table 7.** Copper and zinc content and uptake in cultivated plants. Values indicated by the same letter are not significantly different ( $\alpha = 0.05$ ).

Treatments		Spring Wheat			Maize						
		Grain	Straw	Grain + Straw	Content	Uptake					
FWC	Sulphur	Content mg kg <sup>-1</sup> d.m.		Uptake mg pot <sup>-1</sup>	mg kg <sup>-1</sup> d.m.	mg pot <sup>-1</sup>					
<b>Copper</b>											
30%	Without S	4.16	a	5.15	b	0.134	c	5.28	abc	0.441	c
	S-S <sup>0</sup>	4.27	a	5.28	b	0.141	c	5.62	ab	0.501	bc
	S-SO <sub>4</sub>	3.62	a	5.26	b	0.132	c	5.62	ab	0.491	bc
45%	Without S	3.26	a	5.71	ab	0.181	b	6.23	a	0.711	a
	S-S <sup>0</sup>	3.86	a	5.73	ab	0.208	a	5.13	abc	0.620	abc
	S-SO <sub>4</sub>	4.37	a	5.44	b	0.203	a	5.27	abc	0.639	abc
60%	Without S	3.42	a	6.08	a	0.212	a	3.21	c	0.529	abc
	S-S <sup>0</sup>	3.52	a	5.76	ab	0.222	a	3.35	c	0.572	abc
	S-SO <sub>4</sub>	3.60	a	5.75	ab	0.225	a	3.80	bc	0.672	ab
FWC	30%	4.02	a	5.23	b	0.136	c	5.51	a	0.478	b
	45%	3.83	a	5.62	a	0.197	b	5.54	a	0.656	a
	60%	3.51	a	5.87	a	0.220	a	3.45	b	0.591	a
Sulphur	Without S	3.62	a	5.65	a	0.176	a	4.91	a	0.560	a
	S-S <sup>0</sup>	3.88	a	5.59	a	0.190	a	4.70	a	0.564	a
	S-SO <sub>4</sub>	3.86	a	5.48	a	0.187	a	4.89	a	0.600	a
<b>Zinc</b>											
30%	Without S	41.4	a	24.4	a	0.846	b	11.5	a	0.962	bc
	S-S <sup>0</sup>	42.3	a	28.8	a	0.960	b	10.8	ab	0.962	bc
	S-SO <sub>4</sub>	41.5	a	27.7	a	0.918	b	11.6	a	1.02	bc
45%	Without S	44.3	a	31.3	a	1.37	a	9.41	abc	1.09	abc
	S-S <sup>0</sup>	41.0	a	27.6	a	1.35	a	6.91	c	0.836	c
	S-SO <sub>4</sub>	42.5	a	29.1	a	1.38	a	7.70	bc	0.936	bc
60%	Without S	43.7	a	27.6	a	1.38	a	7.03	c	1.16	abc
	S-S <sup>0</sup>	44.5	a	24.9	a	1.43	a	7.50	c	1.28	ab
	S-SO <sub>4</sub>	47.1	a	25.1	a	1.49	a	7.90	bc	1.40	a
FWC	30%	41.7	a	27.0	a	0.908	b	11.3	a	0.980	b
	45%	42.6	a	29.3	a	1.37	a	8.01	b	0.954	b
	60%	45.1	a	25.9	a	1.43	a	7.48	b	1.28	a
Sulphur	Without S	43.1	a	27.8	a	1.20	a	9.31	a	1.07	a
	S-S <sup>0</sup>	42.6	a	27.1	a	1.25	a	8.40	a	1.03	a
	S-SO <sub>4</sub>	43.7	a	27.3	a	1.26	a	9.08	a	1.12	a

### 3.3. Soil Parameters after Cultivation

The soil reaction (pH) after the cultivation of both crops significantly increased (in comparison to the initial value), although to a greater extent following wheat cultivation (Tables 1 and 8). The examined treatments (sulphur fertilisation and drought intensities) had relatively little effect on the magnitude of the observed pH increases.

The results of this study are in agreement with our earlier research [13]. The smallest change in pH was observed in the soil after maize cultivation, fertilised by sulphates and well watered (60% FWC). In soil that was not fertilised by sulphur, sulphate concentrations were low (5.81–9.0 mg kg<sup>-1</sup> soil). As expected, sulphur application resulted in a significant increase in sulphate concentration in the soil, and differences between the two examined forms of sulphur were relatively small, indicating that despite the decrease in soil moisture, oxidation of elemental sulphur was efficient. Moreover, in line with our earlier research [13], the concentration of sulphates in the soil was considerably higher after wheat cultivation than after maize cultivation.

**Table 8.** Soil pH and the content of S total, sulphates (VI). Values indicated by the same letter are not significantly different ( $\alpha = 0.05$ ).

Treatments		pH	S Total		S-SO <sub>4</sub>		S-SO <sub>4</sub> in S Total		
		KCl 1M dm <sup>-3</sup>	mg kg <sup>-1</sup>				%		
Spring wheat									
30%	Without S	6.41	ab	172	d	12.3	c	7.18	c
	S-S <sup>0</sup>	6.12	c	184	cd	79.3	a	43.1	a
	S-SO <sub>4</sub>	6.19	bc	198	bc	73.4	a	37.1	ab
45%	Without S	6.49	a	183	cd	11.5	c	6.28	c
	S-S <sup>0</sup>	6.14	bc	216	ab	68.5	ab	31.7	ab
	S-SO <sub>4</sub>	6.32	abc	217	a	63.1	ab	29.0	b
60%	Without S	6.54	a	149	e	10.5	c	7.05	c
	S-S <sup>0</sup>	6.39	abc	176	d	48.5	b	27.6	b
	S-SO <sub>4</sub>	6.55	a	171	d	48.4	b	28.3	b
FWC	30%	6.24	b	185	b	55.0	a	29.1	a
	45%	6.32	b	205	a	47.7	a	22.3	a
	60%	6.49	a	165	c	35.8	a	21.0	a
Sulphur	Without S	6.48	a	168	b	11.4	b	6.84	b
	S-S <sup>0</sup>	6.22	b	192	a	65.4	a	34.1	a
	S-SO <sub>4</sub>	6.35	ab	196	a	61.6	a	31.5	a

Table 8. Cont.

Treatments		pH		S Total		S-SO <sub>4</sub>		S-SO <sub>4</sub> in S Total	
		KCl 1M dm <sup>-3</sup>		mg kg <sup>-1</sup>				%	
Maize									
30%	Without S	5.97	ab	149	d	6.79	d	4.57	c
	S-S <sup>0</sup>	5.58	abc	222	ab	11.8	c	5.32	c
	S-SO <sub>4</sub>	6.07	a	216	b	25.0	a	11.6	a
45%	Without S	6.06	a	150	d	7.62	cd	5.08	c
	S-S <sup>0</sup>	6.04	a	235	a	19.9	b	8.49	b
	S-SO <sub>4</sub>	5.90	ab	230	ab	24.5	a	10.7	a
60%	Without S	5.47	abc	124	e	5.81	d	4.70	c
	S-S <sup>0</sup>	5.34	bc	172	c	10.0	cd	5.83	c
	S-SO <sub>4</sub>	4.97	c	164	cd	10.2	cd	6.21	c
FWC	30%	5.87	a	196	a	14.6	ab	7.16	a
	45%	6.00	a	205	a	17.4	a	8.09	a
	60%	5.26	b	153	b	8.67	b	5.58	a
Sulphur	Without S	5.83	a	141	b	6.74	c	4.78	b
	S-S <sup>0</sup>	5.65	a	210	a	13.9	b	6.55	b
	S-SO <sub>4</sub>	5.65	a	204	a	19.9	a	9.50	a

#### 4. Conclusions

The results of this study show that drought stress can cause a significant reduction in productivity for both maize and wheat, although both plants are able to sustain their vigour and growth despite long-lasting water shortage. Drought-induced changes in mineral composition (macro- and microelements) indicated that minerals were still effectively acquired from the soil and transported throughout the whole plant. Sulphur application did not modify these relationships. Applying sulphur did, however, improve wheat biomass production in plants that were well-watered and grown under moderate drought stress, indicating that sulphur fertilisation may be recommended in wheat cultivation when plants are exposed to moderate water stress.

Finally, on the basis of our data and other studies, we think that further research should focus on other aspects of plant reaction to sulphur supplementation and drought stress, particularly photosynthesis, stress metabolites that improve plant tolerance to water scarcity, and systems involved in nutrient uptake and transport within a plant [11,23,48].

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