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Combined Effects of Drought and Soil Fertility on the Synthesis of Vitamins in Green Leafy Vegetables

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Abstract: Green leafy vegetables, such as *Vigna unguiculata*, *Brassica oleraceae*, and *Solanum scabrum*, are important sources of vitamins A, B1, and C. Although vitamin deficiencies considerably affect human health, not much is known about the effects of changing soil and climate conditions on vegetable vitamin concentrations. The effects of high or low soil fertility and three drought intensities (75%, 50%, and 25% pot capacity) on three plant species were analysed (n = 48 pots) in a greenhouse trial. The fresh yield was reduced in all the vegetables as a result of lower soil fertility during a severe drought. The vitamin concentrations increased with increasing drought stress in some species. Regardless, the total vitamin yields showed a net decrease due to the significant biomass loss. Changes in vitamin concentrations as a result of a degrading environment and increasing climate change events are an important factor to be considered for food composition calculations and nutrient balances, particularly due to the consequences on human health, and should therefore be considered in agricultural trials.

Keywords: drought; environment; food composition; food security; human health; nutrition security; plant nutrition; soil fertility; vitamin A; vitamin B1; vitamin C

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1. Introduction

Changes in crop growth conditions do not only affect yields, they also influence the nutrient content of the crop. In areas with a high prevalence of nutritional deficiencies in the human population, even small differences in the amounts of vitamins due to less favorable growing conditions may influence the deficiency rate. Low (pro-)vitamin A (including pro-vitamin A carotenoids, such as β-carotene) intake is one of the most significant threats to health in Sub-Saharan Africa (SSA). In this region, up to 20% of children below five years of age suffered from a vitamin A deficiency in 2016 [1]. Deficient intake of vitamin A impairs visual functions and growth, and increases the risk for respiratory infections, which increases the risk of morbidity and mortality [2]. Vitamin B1 (thiamine) is a common deficiency in staple food-dominated diets, exacerbated by practices such as milling grains [3]. A vitamin B1 deficiency can lead to severe neurological and cardiovascular effects and even death [3]. Vitamin C (ascorbic acid) deficiency is relatively common in middle- to low-income countries. In Kampala, Uganda, for example, 70% of pregnant women were deficient in vitamin C [4]. Vitamin C deficiencies in the diet, in addition to the negative effects of an actual vitamin C deficiency, may contribute to a lower bioavailability of essential minerals from diets, such as Fe and Zn [5].

Green leafy vegetables are rich in micronutrients and other phytochemicals, such as vitamins, which are vital for human health. Many studies show the benefits of increasing green leafy vegetable consumption on human health. An increased and regular intake of green leafy vegetables is, for example, associated with a lower risk for cardiovascular diseases (CVDs) [6]. The authors of a nutrition study in Tanzania confirmed that an increased consumption of green leafy vegetables can contribute to a lower prevalence of anemia and micronutrient deficiencies, particularly in resource-poor communities [7].

Plants contain a wide range of metabolites that have antioxidant potential [8,9] and are pre-cursors of vitamins, essential to human health. Plants produce co-enzymes and antioxidants, such as ascorbic acid, thiamine, and β-carotene, for a specific purpose [10], e.g., to protect themselves from environmental stressors, such as drought or poor soil fertility. Drought causes the production of reactive oxygen species (ROS), which, in turn, can lead to oxidative damage to the photosynthesis apparatus, and, hence, to reduced primary production [11]. Different carotenes and ascorbic acid are the main ROS detoxifying compounds [10,11]. Thiamine is also an important component of plant stress response, in addition to its other functions [12]. For lack of a unifying term in plant nutrition, the vitamin pre-cursors will be referred to as vitamins in this study.

Soil fertility provides the basis of plant production by providing plants with sufficient nutrients and water. Varying levels of soil fertility affect food yields, as well as food quality in terms of mineral nutrient concentrations [13]. Furthermore, vitamin concentrations were found to be inversely affected by nitrogen (N) fertilizer application [14,15], thereby suggesting the possibility that soil fertility could also affect vitamin concentrations.

Climate change has led to an increase in extreme weather events, such as prolonged and more frequently occurring droughts [16]. While the effects of different climate change variables (increasing temperatures, drought, increasing CO_2 and O_3) on mineral and macronutrient concentrations in different plant parts have been measured [17–19], the effects on vitamin concentrations have not been covered thus far.

Both climate change and soil degradation represent two of the biggest challenges for the sustained production of high quantity and quality foods [20], while concurrently being partially responsible for both occurrences. This paper will focus on the following research question: (i) Does soil fertility, (ii) water stress (drought), and the combination of drought stress and varying soil fertility significantly affect the contents of β-carotene, ascorbic acid, and thiamine in the leaves of three green leafy vegetables (*Brassica oleraceae* L., *Solanum scabrum* L., *Vigna unguiculata* L.)?

As the production of vitamins is a stress reaction, especially to stressors such as drought, an increase in vitamin concentrations in all green leafy vegetables with increasing drought is expected. Soil fertility is key for plant nutrient intake, and largely provides the nutrients needed to produce vitamins. Therefore, it is expected that in high soil fertility, the vitamin concentrations will be higher in all the drought severities than in low soil fertility of the same drought severity. The fresh weight is expected to decrease with increasing drought and decreasing soil fertility.

2. Materials and Methods

This study was part of the project "Education and Training for Sustainable Agriculture and Nutrition in East Africa (EaTSANE)". Since the main research area for the EaTSANE project was East Africa, namely the areas of Teso South, Kenya (a region with low soil fertility) and Kapchorwa, Uganda (a region with high soil fertility), the trial was set up to mimic the local conditions. Three local green leafy vegetables were analysed: sukuma wiki (*Brassica oleraceae* L.), black nightshade (*Solanum scabrum* L.), and cowpea (*Vigna unguiculata* L.), using soils with properties similar to the soils found in the East African research areas.

The temperature in a greenhouse of the University of Hohenheim, Germany was adjusted to 22 °C, which is the mean of the average temperature during the growing season in Kapchorwa and Teso South (Table S1). Daylight was allowed from 6 am to 6 pm, as in the target areas. The greenhouse temperature and humidity were monitored during the entire trial using a TGP-4500 Tiny Tag Plus 2 (Gemini Data Loggers, Ltd., Chichester, UK). Two soils were selected to represent the soils of Kapchorwa, Uganda and Teso South, Kenya [21]. The first soil collected showed similarities to the soils with lower fertility ferralsols in Teso South and was classified as an endostagnic alisol collected in Tauchenweiler, Germany (48°47′13.3″ N and 10°02′18.9″ E). In this paper, the soil with lower fertility will be referred to as “infertile”. The second soil, comparable to a higher fertility nitisol, such as from Kapchorwa, Uganda, was an endoleptic cambisol, and was collected in Höwenegg, Germany (47°54′55.7″ N and 8°44′25.7″ E) (Table S3). In this paper, the soil with higher fertility will be referred to as “fertile”.

The soils gathered in Germany show similar properties to the soils of Teso South, Kenya and Kapchorwa, Uganda. The low fertility soils both feature sandy soils, whereas the high fertility soils feature loamy clays. While the pH of both of the low fertility soils is acidic (German alisol: 4.0; Kenyan ferralsol: 4.94), the pH of the high fertility soil was 5.6 in both cases [13]. Both of the low fertility soils showed a very low amount of soil organic matter, whereas the soils of higher fertility showed a higher amount.

The three green leafy vegetables were planted into pots on 17 September 2019. The plastic pots (37.5 cm height and 16 cm diameter) were filled with 1.5 kg gravel as drainage, with a depth of 5 cm. The two soil types were dried and sieved (0.9 cm^2 sieve type), and 5.5 kg of each soil was placed into the different pots. After sowing, the soil was covered by a thin layer of sand (~10 mm, 80 g) to prevent excessive evaporation and soil cracking. Each pot was sown with four seeds of each green leafy vegetable, and then later thinned out, leaving one seedling per pot.

The pot water capacity (PC) was analysed using the gravimetric methods provided by [22]. Three treatments in the watering regime were used: 75% PC as control, 50% PC as mild stress, and 25% as severe drought stress. A total of 144 pots were used, 48 per plant species, with two soil fertilities (fertile and low fertility soil), and three drought intensities (control 1, control 2, mild, and severe) (Table S2). The double control group was used to improve the statistical power [23]. Each treatment had six replicates organised into a randomized complete block design (Figure S1). After plant germination, all the pots were weighed and watered every two days to maintain the assigned drought conditions.

Fifty-one days after sowing, flower buds of the control plants became visible. The entire aboveground part of all the pots was harvested and frozen after recording their fresh weight. The belowground biomass (roots) were washed, then dried with paper towels, and the fresh weight recorded. The fresh leaves were stored at −80 °C and then lyophilized using a freeze dryer (LyoQuest laboratory freeze dryer, Telstar, Spain) for 24 h, and the dry weight was recorded. The samples were then ground, homogenized, and stored in the dark at −20 °C.

The plant water use efficiency (WUE) was calculated as the ratio between the above-ground biomass (yield) and water use (evapotranspiration). WUE is generally high when the plants are exposed to drought conditions or are drought tolerant [24], and is, therefore, used to evaluate drought resistance [25]. The WUE of the yield was determined by the division of the fresh yield by water consumption [24].

The concentrations of thiamine, β-carotene, and ascorbic acid were measured in the leaf samples using high performance liquid chromatography (HPLC) at the Institute of Nutritional Sciences, University of Hohenheim. The HPLC data were recorded and analysed using the Shimadzu LabSolutions Software (Version 5.54, Shimadzu Deutschland GmbH, Duisburg, Germany). The chromatographic analysis of the vitamins was conducted using the Shimadzu HPLC system. The Shimadzu HPLC system consisted of a DGU 20A3 Degassing Unit, an LC-20AT Pump, a SIL-20AC HT AutoSampler, and a CBM-20-A Communication Module. The method for thiamine analysis was based on the European Standard (DIN EN 14122:2014) of vitamin B1 determination [26]; however, minor adaptations were made. β-carotene was measured using the method of [27]. The retinol equivalents (REs) of β-carotene were calculated using a factor 1 µg RE = 6 µg β-carotene [28]. Ascorbic acid was measured using the method of [29].

Since C_3 plants close their stomata to reduce water loss during water-limiting conditions, $^{13}CO_2$ fixation is decreased and $\delta^{13}C$ is discriminated due to reduced CO_2 diffusion in and out of the leaves [30]. Therefore, $\delta^{13}C$ isotope discrimination has been used to measure water stress in C_3 plants [31]. The $\delta^{13}C$ contents of green leafy vegetables were analysed by comparing the control (75% PC) to the drought treatments (50% and 25% PC) using randomly selected subsamples (n = 4 per PC of each species) of the green leafy vegetable samples. The selected subsamples were measured with a Euro EA Elemental Analyser (Euro Vector, Pavia, Italy) coupled to a Finnigan Delta IRMS (Thermo Fischer Scientific, Waltham, MA, USA) at the core facility of the University of Hohenheim.

The fulfilment of the recommended nutrient intake (RNI) of vitamin A, ascorbic acid, and thiamine by consuming 150 g of green leafy vegetables (fresh weight) as an average serving size was calculated for female adults (19–50 years) [28].

SAS (SAS® University Edition, SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis. The fresh yield, belowground biomass, number of nodules, irrigation water added, water use efficiency (WUE), pot capacity (PC), thiamine, β-carotene, and ascorbic acid were subjected to analysis of variance (ANOVA) for each treatment. ANOVA using PROC GLIMMIX was used to compare the treatments between the plant species and the significance of factors by an F-test at $\alpha = 0.05$. A two-factorial model was fitted as an equation (SUPPL MAT). The interaction of the treatment and species was significant; therefore, the cell means were compared using a SLICE statement with the SLICEBY options, i.e., SLICEBY = treatments and SLICEBY = species, in the GLIMMIX procedure. The means of yield and WUE differences between the different soil fertility and drought conditions were compared. The vitamin concentrations were expressed on a fresh weight basis. The absolute vitamin amounts (mg per pot) were calculated for thiamine, β-carotene, and ascorbic acid by the multiplication of the fresh leaf yield (g/pot) by the vitamin content (mg/100 g FW) and divided by 100 [32]. The vitamin data were used to analyse whether the changed vitamin contents compensate for the treatments' changed yield. An analysis of covariance (ANCOVA) using PROC GLM was used to compare the treatment means adjusted for a covariate soil fertility within the plant species.

3. Results

3.1. Plant Yield Analysis

All the fresh leaf yields of the three green leafy vegetables were significantly higher (*B. oleraceae*: $p < 0.01$, *V. unguiculata*: $p < 0.001$, *S. scabrum*: $p < 0.0001$) in fertile soil than in low fertility soil with the same watering regime, with the exception of *B. oleraceae* with a 75% pot capacity (PC), where the difference was not significant (Table 1). Regardless of the soil fertility, the fresh leaf yields of the green leafy vegetables decreased with an increasing level of drought (Table 1 and Figure S1). However, the yield losses caused by drought did not differ significantly between the fertile and low fertility soils (Figure 1).

Table 1. Fresh leaf yield (g/pot), belowground biomass (g/pot FW), nodule number per pot, total irrigation water added (ml/pot), and water use efficiency (WUE: fresh yield/water use, g/L) in *Vigna unguiculata*, *Brassica oleraceae*, and *Solanum scabrum* under two soil fertilities with three watering regimes (75% pot capacity (PC), 50% PC, and 25% PC).

			Fresh Yield (g/pot)	**Belowground (g/pot FW)**	**No. of Nodules**	**Irrigation Water Added (mL/pot)**	**WUE (g/L)**
Vigna unguiculata							
	Fertile soil						
		75% PC	25.2 ± 1.3 [c]	12.0 ± 1.1 [bc]	11.2 ± 3.4 [a]	2882 ± 115 [a]	8.8 ± 2.0 [def]
		50% PC	18.6 ± 1.9 [d]	9.0 ± 1.5 [cd]	6.3 ± 2.1 [b]	1871 ± 162 [bc]	10.1 ± 2.9 [cdef]
		25% PC	12.2 ± 1.9 [ef]	5.2 ± 1.5 [de]	1.2 ± 0.5 [bc]	832 ± 162 [de]	15.3 ± 2.9 [bcd]
	Infertile soil						
		75% PC	13.8 ± 1.3 [e]	2.0 ± 1.1 [ef]	0.0 [c]	2192 ± 165 [b]	6.3 ± 2.0 [ef]
		50% PC	7.9 ± 1.9 [fg]	1.0 ± 1.5 [ef]	0.0 [c]	1239 ± 163 [d]	6.2 ± 2.9 [ef]
		25% PC	2.5 ± 1.9 [h]	0.9 ± 1.5 [f]	0.0 [c]	405 ± 162 [ef]	8.8 ± 2.9 [cdef]

Table 1. *Cont.*

		Fresh Yield (g/pot)	Belowground (g/pot FW)	No. of Nodules	Irrigation Water Added (mL/pot)	WUE (g/L)
Brassica oleraceae						
Fertile soil						
	75% PC	41.5 ± 1.7 [a]	9.4 ± 1.3 [c]	-	2842 ± 142 [a]	14.9 ± 2.5 [bcd]
	50% PC	32.8 ± 2.1 [b]	10.2 ± 1.6 [c]	-	2080 ± 179 [bc]	16.4 ± 3.2 [bc]
	25% PC	12.6 ± 2.1 [ef]	1.4 ± 1.6 [ef]	-	398 ± 179 [ef]	43.2 ± 3.2 [a]
Infertile soil						
	75% PC	40.3 ± 1.4 [a]	3.9 ± 1.1 [ef]	-	2928 ± 120 [a]	13.9 ± 2.1 [bcde]
	50% PC	15.0 ± 1.9 [de]	1.0 ± 1.5 [ef]	-	1204 ± 162 [d]	11.8 ± 2.9 [cdef]
	25% PC	4.7 ± 1.9 [gh]	0.6 ± 1.5 [f]	-	342 ± 162 [f]	14.1 ± 2.9 [bcde]
Solanum scabrum						
Fertile soil						
	75% PC	35.0 ± 1.3 [b]	21.5 ± 1.1 [a]	-	2844 ± 115 [a]	12.4 ± 2.0 [cde]
	50% PC	25.9 ± 1.9 [c]	15.4 ± 1.5 [b]	-	1783 ± 162 [c]	14.8 ± 2.9 [bcd]
	25% PC	17.4 ± 1.9 [de]	10.4 ± 1.5 [c]	-	917 ± 162 [d]	20.6 ± 2.9 [b]
Infertile soil						
	75% PC	16.5 ± 1.3 [de]	3.0 ± 1.1 [ef]	-	1804 ± 115 [c]	9.0 ± 2.0 [cdef]
	50% PC	4.8 ± 1.9 [gh]	0.9 ± 1.5 [f]	-	1013 ± 162 [d]	4.8 ± 2.9 [f]
	25% PC	1.1 ± 1.9 [h]	0.7 ± 1.5 [f]	-	209 ± 162 [f]	14.4 ± 2.9 [bcd]

Analysis of variance (ANOVA) using PROC GLIMMIX was used to compare the treatments between plant species and the significance of factors by an F-test at $\alpha = 0.05$. LS-means ± SE within the column with the same letter are not significantly different ($p < 0.05$).

B. oleraceae showed the highest yield loss with increasing drought regardless of the soil fertility, as evidenced in the steeper slope increase in the linear regression (Figure 1). Nevertheless, *B. oleraceae* obtained a higher total fresh yield under no drought (control, 75% PC) ($p < 0.0001$) and mild drought (50% PC) ($p < 0.0001$) conditions than the other two green leafy vegetables (Table 2). In contrast, *S. scabrum* had the highest yield loss with decreasing soil fertility under all watering regimes, shown by the highest interceptor differences between the regression lines of fertile and low fertility soils, while *B. oleraceae* showed the lowest yield loss by different soil fertility (Figure 1). The belowground biomass was significantly lower in the low fertility soil than in the fertile soil for all the green leafy vegetables (all $p < 0.0001$) (Table 1). Only severe drought (25% PC) significantly ($p < 0.05$) decreased the belowground fresh weight of all the species in the fertile soil (Table 1).

Table 2. Levels of β-carotene contents and retinol activity equivalents (RAE) of *V. unguiculata*, *B. oleraceae*, and *S. scabrum* under two soil fertilities with three watering regimes (75% pot capacity (PC), 50% PC, and 25% PC).

		β-Carotene (mg/100 g FW)	RAE (µg/100 g FW)	β-Carotene (mg/Fresh Yield)	RAE (µg/Fresh Yield)
Vigna unguiculata					
Fertile soil					
	75% PC	7.37 ± 0.26 [bc]	614 ± 21 [bc]	1.86 ± 0.09 [bc]	155 ± 7 [bc]
	50% PC	7.64 ± 0.36 [bc]	636 ± 30 [bc]	1.42 ± 0.13 [de]	118 ± 10 [de]
	25% PC	8.04 ± 0.36 [b]	670 ± 30 [b]	0.97 ± 0.13 [f]	80 ± 10 [f]
Infertile soil					
	75% PC	6.89 ± 0.26 [cd]	574 ± 21 [cd]	0.97 ± 0.09 [f]	81 ± 7 [f]
	50% PC	6.41 ± 0.36 [de]	534 ± 30 [de]	0.57 ± 0.13 [g]	47 ± 10 [g]
	25% PC	4.92 ± 0.36 [gh]	410 ± 30 [gh]	0.12 ± 0.13 [h]	10 ± 10 [h]
Brassica oleraceae					
Fertile soil					
	75% PC	4.02 ± 0.32 [hi]	335 ± 26 [hi]	1.66 ± 0.11 [cd]	138 ± 9 [cd]
	50% PC	4.11 ± 0.40 [ghi]	343 ± 33 [ghi]	1.36 ± 0.14 [de]	114 ± 12 [de]
	25% PC	3.66 ± 0.40 [i]	305 ± 33 [i]	0.46 ± 0.14 [gh]	38 ± 12 [gh]

Table 2. *Cont.*

		β-Carotene (mg/100 g FW)	RAE (μg/100 g FW)	β-Carotene (mg/Fresh Yield)	RAE (μg/Fresh Yield)
Brassica oleraceae					
Infertile soil					
	75% PC	4.03 ± 0.27 [hi]	336 ± 22 [hi]	1.62 ± 0.10 [cd]	135 ± 8 [cd]
	50% PC	3.48 ± 0.36 [i]	290 ± 30 [i]	0.55 ± 0.13 [g]	46 ± 10 [g]
	25% PC	3.61 ± 0.36 [i]	301 ± 30 [i]	0.17 ± 0.13 [h]	14 ± 10 [h]
Solanum scabrum					
Fertile soil					
	75% PC	7.59 ± 0.26 [bc]	632 ± 21 [bc]	2.66 ± 0.09 [a]	221 ± 7 [a]
	50% PC	8.09 ± 0.36 [b]	674 ± 30 [b]	2.11 ± 0.13 [b]	176 ± 10 [b]
	25% PC	9.48 ± 0.36 [a]	790 ± 30 [a]	1.62 ± 0.13 [cd]	135 ± 10 [cd]
Infertile soil					
	75% PC	7.08 ± 0.26 [cd]	590 ± 21 [cd]	1.17 ± 0.09 [ef]	97 ± 7 [ef]
	50% PC	5.99 ± 0.36 [ef]	499 ± 30 [ef]	0.31 ± 0.13 [gh]	26 ± 10 [gh]
	25% PC	5.13 ± 0.45 [fg]	428 ± 37 [fg]	0.06 ± 0.16 [h]	5 ± 13 [h]

Analysis of variance (ANOVA) using PROC GLIMMIX was used to compare the treatments between plant species and the significance of factors by an F-test at $\alpha = 0.05$. LS-means ± SE within the column with the same letter are not significantly different ($p < 0.05$).

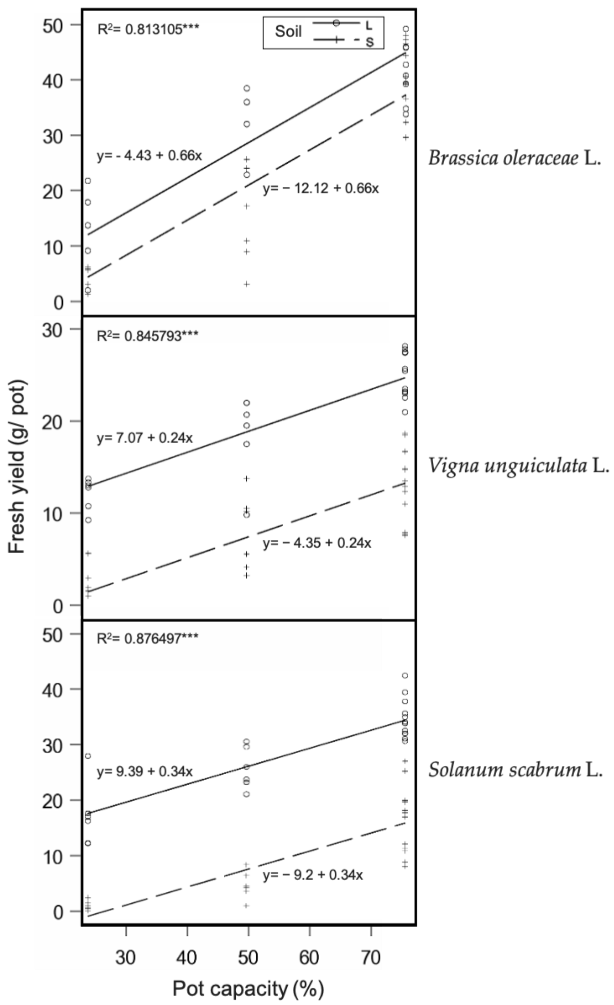

Figure 1. Fresh yield (g/pot) of the green leafy vegetables *B. oleraceae*, *V. unguiculata*, and *S. scabrum*, along with the pot capacity (%) for the fertile (L) and infertile (S) soil. Analysis of covariate (ANCOVA) was performed. The regression lines in each plant species are parallel. Regression formulas and R^2 are given. The triple asterisk indicates significance at $p < 0.001$.

Within all the vegetables, the WUE changed more drastically in the drought treatments in fertile soil than in the drought treatments on infertile soil with increasing drought intensity. The exception to this is *S. scabrum*, where the changes in fertile and infertile soil with increasing drought intensity were similar.

The WUE of *B. oleraceae* was higher than *V. unguiculata* and *S. scabrum* under severe drought (25% PC) in the fertile soil, which was the highest WUE at 43.2 g/L (F-test, $p < 0.0001$) among the three green leafy vegetables under all the treatments, while the yield was not significantly different ($p < 0.05$) (Table 1). In the low fertility soil, *B. oleraceae* had the highest water demand under control (75%), and the yield was higher than the other green leafy vegetables (Table 1).

3.2. $\Delta^{13}C$ Measurement as an Indicator for Water Stress

All three green leafy vegetables showed an increase in their $\delta^{13}C$ signature, i.e., less negative $\delta^{13}C$ values, with the decreasing irrigated water amount, i.e., increasing drought intensities (Figure S2). Under drought conditions, *V. unguiculata* had the highest changes of $\delta^{13}C$ values, ranging between −31.76‰ and −26.67‰ (R^2 = 0.611, p = 0.0027) for leaves under both soil fertilities, followed by *B. oleraceae* with ranges between −34.48‰ and −32.62‰ (R^2 = 0.619, p = 0.0024). *S. scabrum* also showed an increase of $\delta^{13}C$ values, but the change was not significant (Figure S2).

3.3. Effects of Drought and Soil Fertility on Vitamin Concentrations

3.3.1. Thiamine

The thiamine concentrations of *B. oleraceae* (R^2 = 0.47; $p < 0.05$) and *V. unguiculata* (R^2 = 0.54; $p < 0.01$) nearly doubled in response to increasing drought intensities in the low fertility soil (Figure 2). In *V. unguiculata*, the thiamine concentrations were two times higher than *B. oleraceae* with the combination of low fertility soil and drought, as the regression line's slope was double (Figure 2). However, in the fertile soil, the thiamine concentrations did not respond to drought in *B. oleraceae* or only slightly increased in *V. unguiculata* (R^2 = 0.46; $p < 0.05$) (Figure 2). In *S. scabrum*, the thiamine concentration was not affected by drought, but was significantly higher (p = 0.005) in the low fertility soil compared to the fertile soil (Figure 2). Despite the increase in the thiamine concentration, the total thiamine yields (fresh leaf yield (g/pot) x thiamine concentration as mg/100 g fresh weight (FW)) of *V. unguiculata* (R^2 = 0.60; $p < 0.001$) and *S. scabrum* (R^2 = 0.64; $p < 0.001$) were significantly lower in the low fertility soil than in the fertile soil and fell with increasing drought (Figure 3).

There was no interaction between drought and soil fertility in the thiamine yield (Figure 3). In *B. oleraceae*, an increased thiamine concentration through the drought and low soil fertility compensated for the decline in fresh yield in the lower soil fertility, as the thiamine yield of *B. oleraceae* showed no differences between soil fertilities (Figures 1–3).

3.3.2. Beta-Carotene (Pro-Vitamin A)

Interactions between soil fertility and drought were found in the β-carotene concentrations of *V. unguiculata* (p = 0.0038) and *S. scabrum* ($p < 0.0001$) (Figure 2). The β-carotene concentrations in *V. unguiculata* (R^2 = 0.24; $p < 0.05$) and *S. scabrum* (R^2 = 0.40; $p < 0.01$) fell significantly due to the combination of low soil fertility and increasing drought, while the concentrations rose in the fertile soil under drought conditions, but only significantly in *S. scabrum* (R^2 = 0.40; $p < 0.001$) (Figure 2). When comparing the β-carotene concentration changes in the low fertility soil, the β-carotene concentrations of *V. unguiculata* and *S. scabrum* decreased significantly from the control (75% PC) to severe drought (25% PC) (Table 2). In the fertile soil (S), the β-carotene concentration of *S. scabrum* increased during the drought treatment (Table 2). *B. oleraceae* β-carotene concentrations were not significantly affected by soil fertility and drought treatments (Figure 2 and Table 2). Despite the inverse effects of the soil fertility by drought on the β-carotene concentrations of *V. unguiculata* and *S. scabrum* (Figure 2), there was no interaction between the soil fertility and drought on the β-carotene yields (Figure 3). Furthermore, the β-carotene yields of all three green leafy vegetables were higher in the fertile soil than in the low fertility soil (S) (Figure 3).

3.3.3. Ascorbic Acid

In *B. oleraceae,* the ascorbic acid content significantly decreased with increasing drought (R^2 = 0.39; p < 0.01), and the concentration was consistently higher in the fertile soil regardless of the drought intensity (Figure 2). In contrast, in *V. unguiculata*, the ascorbic acid concentration increased with severe drought (25% PC) in the low fertility soil (R^2 = 0.45; p < 0.01), while the concentration was not significantly affected by drought in the fertile soil (Figure 2). The ascorbic acid concentration of *S. scabrum* rose with drought (R^2 = 0.13; p < 0.05) but was not affected by soil fertility (Figure 2). The ascorbic acid yield was higher in the fertile soil than in the low fertility soil and decreased with drought in each green leafy vegetable (Figure 3). The three green leafy vegetables showed no interaction between the soil and drought on the ascorbic acid yields (Figure 3).

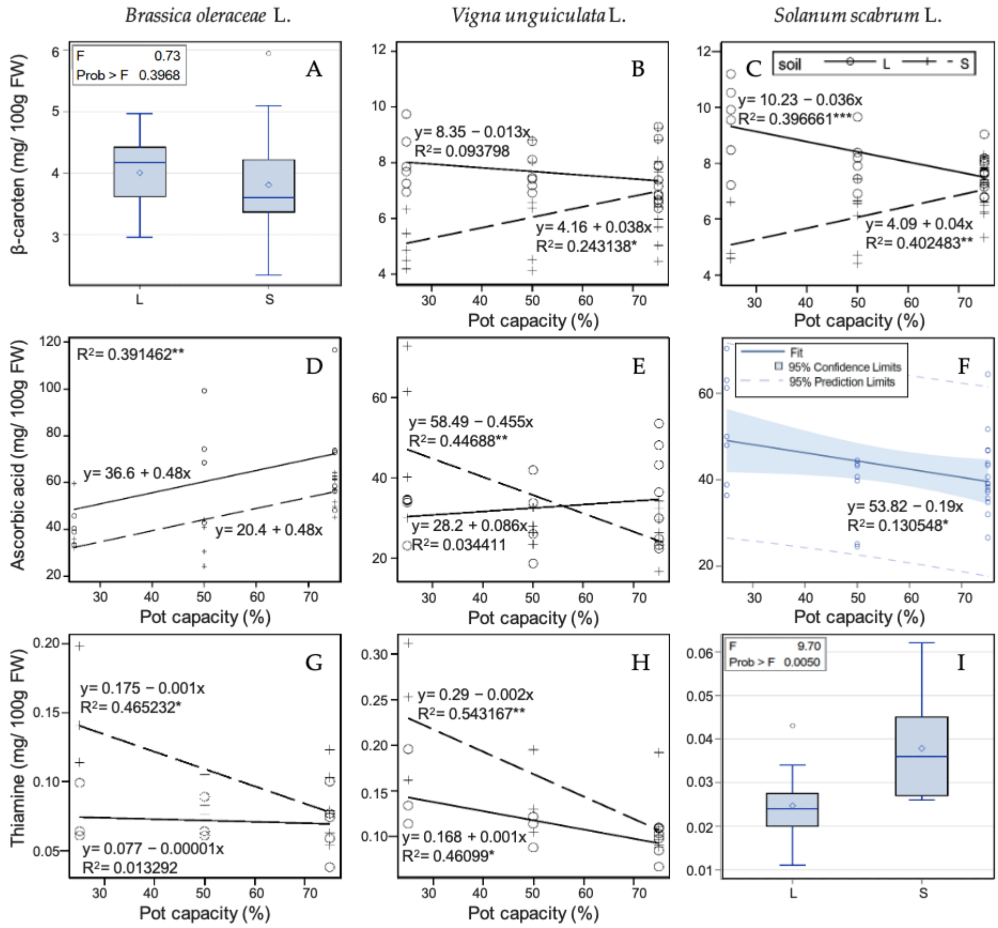

Figure 2. (**A**–**I**) Relationship between vitamin content (mg/100 g FW) of green leafy vegetables *B. oleraceae*, *V. unguiculata*, and *S. scabrum*, and pot capacity (%) in two soils, fertile and infertile soils. Analysis of covariate (ANCOVA) was performed. Boxplots (**A**,**I**) showing the comparison of vitamin content by soil fertility. A fit plot (**F**) was adapted to *S. scabrum* ascorbic acid contents due to no significant effect by the soil fertility. Regression respective formula and R^2 are given. Triple, double, and single asterisks indicate significance at p < 0.001, <0.01, and <0.05, respectively. *L* refers to fertile soil, whereas *S* refers to infertile soil.

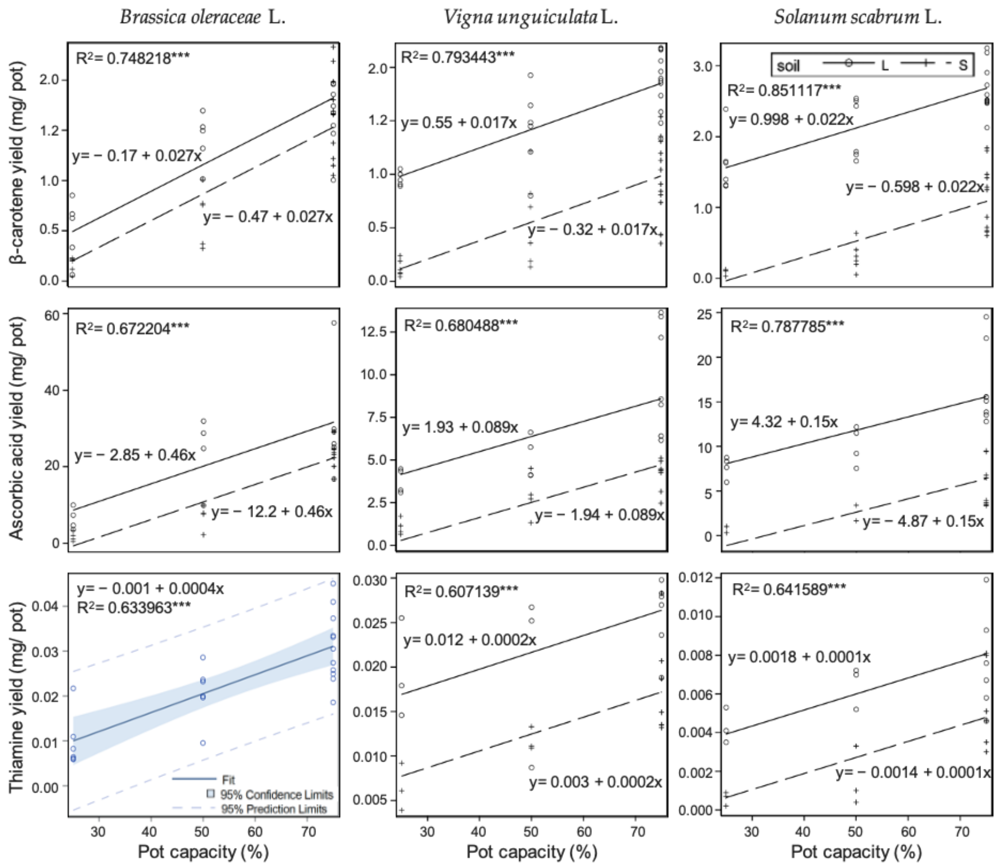

Figure 3. Relationship between vitamin yields (mg/pot) of green leafy vegetables *B. oleraceae*, *V. unguiculata*, and *S. scabrum*, and pot capacity (%) in two soils, fertile and infertile soils. Analysis of covariate (ANCOVA) was performed. The regression lines in each plant species are parallel. A fit plot (bottom-left) was adapted to the thiamine yield of *B. oleraceae* due to no significant effect of soil fertility. Regression respective formula and R^2 are given. The triple asterisk indicates significance at $p < 0.001$.

3.4. Fulfilment of Recommended Nutrient Intake (RNI) by Green Leafy Vegetables under Different Growth Conditions

For β-carotene intake, 150 g of fresh leaves of *V. unguiculata* and *S. scabrum* reached more than 200% of vitamin A's RNI, regardless of the treatments, while *B. oleraceae* reached more than 100% of the RNI for vitamin A in 150 g of fresh leaves (Table 3). The thiamine concentrations of *B. oleraceae* and *V. unguiculata* significantly increased with drought in the low fertility soil (Figure 2). However, *S. scabrum* could only contribute less than 6% of the RNI for vitamin B1 by 150 g fresh leaves (Table 3). For ascorbic acid intake, *B. oleraceae* reached more than 200% of RNI in 150 g of the fresh leaves under a well-watered condition in the fertile soil. Additionally, *B. oleraceae* could provide more than 100% of vitamin C's RNI, regardless of the soil fertility, even when the ascorbic acid concentration was decreased by drought (Table 3). In *V. unguiculata*, the ascorbic acid content in 150 g of fresh leaves reached 170% of RNI in the low fertility soil under severe drought (25% PC) (Table 3). In addition, *S. scabrum* could provide over 160% of RNI for ascorbic acid concentration in 150 g of fresh leaves under severe drought conditions (25% PC), regardless of the soil fertility (Table 3).

Table 3. Percentage of recommended nutrient intakes (RNI) for vitamin content (mg/150 g FW for thiamine and ascorbic acid and µg/150 g FW for RAE) and vitamin yield (mg/plant for thiamine and ascorbic acid and µg/plant for RAE) of *V. unguiculata*, *B. oleracea*, and *S. scabrum* under two soil fertilities (fertile; L and infertile soil; S) with three watering regimes (75% pot capacity (PC), 50% PC, and 25% PC).

	% of RNI [1] for Vitamin Content (mg or µg/150 g FW)			**% of RNI [1] for Vitamin Yield (mg or µg/Plant)**		
	Thiamine	**Ascorbic Acid**	**RAE [2]**	**Thiamine**	**Ascorbic Acid**	**RAE [2]**
Vigna unguiculata						
Fertile soil (L)						
75% PC	13.0	118	184	2.4	21	31
50% PC	14.7	101	191	1.8	11	24
25% PC	20.2	105	201	1.7	9	16
Infertile soil (S)						
75% PC	15.5	75	172	1.6	8	16
50% PC	19.5	92	160	1.1	6	9
25% PC	33.0	170	123	0.5	2	2
Brassica oleraceae						
Fertile soil (L)						
75% PC	9.8	238	101	2.7	68	28
50% PC	9.7	237	103	2.3	53	23
25% PC	10.2	132	92	1.2	51	8
Infertile soil (S)						
75% PC	11.3	189	101	3.0	15	27
50% PC	12.0	117	87	1.5	14	9
25% PC	20.6	135	90	0.6	4	3
Solanum scabrum						
Fertile soil (L)						
75% PC	2.9	152	190	0.7	37	44
50% PC	3.4	127	202	0.6	22	35
25% PC	4.3	180	237	0.4	17	27
Infertile soil (S)						
75% PC	4.9	122	177	0.5	13	19
50% PC	5.3	125	150	0.2	5	5
25% PC	5.5	166	128	0.1	1	1

[1] RNI, recommended nutrient intake; values for female adults (19–50 years) (WHO & FAO, 2004); [2] RAE, retinol activity equivalent, bioconversion of ingested β-carotene to retinol based on equivalency factors, for which 1 µg RAE = 12 µg β-carotene (WHO & FAO, 2004).

3.5. *Effects of Drought under Two Soil Fertilities on Fresh Yield and Vitamin Contents*

Table 4 shows a summary of the drought's effect on the fresh yield and vitamin contents of three green leafy vegetables under two different soil fertility conditions. For the fresh yield, the three green leafy vegetables were significantly decreased by drought (Figure 1 and Table 4). The β-carotene content was significantly increased in *S. scabrum* in fertile soil, while the content was significantly decreased by drought in *V. unguiculata* and *S. scabrum* in infertile soil (Figure 2 and Table 4). The ascorbic acid content was significantly increased in *S. scabrum* during drought, regardless of the soil condition, and in *V. unguiculata* in interfile soil. However, the ascorbic acid content was significantly decreased in *B. oleraceae* by drought (Figure 2 and Table 4). For thiamine, the content was increased by drought in *V. unguiculata* in both fertile and infertile soils and in *B. oleraceae* in infertile soil (Figure 2 and Table 4).

Table 4. Comparison of effects of increasing drought in two soil fertility conditions (fertile, L and infertile soil, S) on fresh yield and vitamin content (mg/100 g FW) of *V. unguiculata*, *B. oleraceae*, and *S. scabrum*.

		Brassica oleraceae	*Vigna unguiculata*	*Solanum scabrum*
Fresh yield	Fertile soil (L)	↓	↓	↓
	Infertile soil (S)	↓	↓	↓
β-carotene	Fertile soil (L)	-	-	↑
	Infertile soil (S)	-	↓	↓
Ascorbic acid	Fertile soil (L)	↓	-	↑
	Infertile soil (S)	↓	↑	↑
Thiamine	Fertile soil (L)	-	↑	-
	Infertile soil (S)	↑	↑	-

The arrows show trends of interaction (upwards blue arrow: positive, downwards red arrow: negative). Hyphen sign indicates no interaction.

4. Discussion

Our research demonstrates that green leafy vegetables respond differently to soil fertility, drought stress, or its combination in regard to the yield and vitamin concentrations. The observed increase in the $\delta^{13}C$ values, i.e., less negative $\delta^{13}C$ values, in the three green leafy vegetables with a decreasing irrigated water amount typically indicate the experienced water stress for C_3 plants [17,30,31].

The yields of the three green leafy vegetables most strongly decreased in the low fertility soil (endostagnic alisol) compared to the fertile soil (endoleptic cambisol) under all watering regimes due to the lower total soil fertility in the low fertility soil. *B. oleraceae* had the highest fresh biomass yield among all the three green leafy vegetables in both soil treatments under control and mild drought, therefore indicating its lower sensitivity to soil fertility compared to the other two vegetables. However, the decreasing yield rate by drought was higher in *B. oleraceae* than *V. unguiculata* and *S. scabrum*, confirming that *B. oleraceae* had the highest susceptibility to water stress among the three vegetables measured. Likewise, *Brassica* spp., such as kale (*B. oleracea*) [33] and Chinese cabbage (*B. rapa*) [34], are sensitive to drought stress.

Among the three green leafy vegetables, *S. scabrum* was the most vulnerable to low soil fertility regarding the fresh biomass yield. However, in the fertile soil, the *S. scabrum* yield was higher than *V. unguiculata* under mild drought and in the control. Although the fresh yield of *V. unguiculata* was lower than that of *B. oleraceae* and *S. scabrum* under the control (75% PC), the decreasing yield rate by drought was lower than the others. Therefore, *V. unguiculata* was the most drought-tolerant vegetable among the three green leafy vegetables. High drought tolerance under the severe drought of *V. unguiculata* was also identified in a comparison with two other green leafy vegetables: *Amaranthus* spp. and *Corchorus olitorius* [35].

The thiamine concentration was significantly increased by the combination of severe drought and low soil fertility in the leaves of *B. oleraceae* and *V. unguiculata*, whereas in *S. scabrum*, the thiamine concentration was only increased in low soil fertility, but not by drought. The increased thiamine concentrations were possibly due to the effect of induced oxidative stress through severe drought stress [36] and/or proton (H+) rhizotoxicity (lower pH in the low fertility soil) [37]. Oxidative stress induces the precursors of thiamine; hence, thiamine is upregulated [12,36,38].

The authors of [14] observed that β-carotene concentrations of kale responded positively to increasing soil N content, which was also observed in this trial (33% higher mineral N level in the fertile soil than in the low fertility soil). However, there were no significant β-carotene concentration changes in the leaves of the investigated green leafy vegetables by soil fertility under well-watered conditions in our trial. The different results compared to the literature might be explained by the N-fertilizer dose in the experiment by [14] being 217% higher between treatments, therefore much larger than the 33% difference in the present trial. In addition, as the soil materials of this study were under acidic conditions, the availability of soil nitrogen (N) might also be restricted by soil acidity, the form of nitrogen (NH4 vs. NO3) thereby not allowing for the full uptake of N present in the soil [39].

In contrast to thiamine, β-carotene showed a different reaction to the drought treatments between the fertile and interfile soils. The β-carotene concentrations were significantly decreased under the combination of drought and low soil fertility treatments in the leaves of *V. unguiculata* and *S. scabrum*. In the fertile soil, the β-carotene concentration was increased significantly in *S. scabrum* under severe drought. The latter result is consistent with the effect of severe drought (30% field capacity) on β-carotene concentrations in the leaves of amaranth, where the total antioxidant capacity was increased with induced drought stress [40]. The opposite effect that was observed in *S. scabrum* in low soil fertility can be attributed to the fact that *S. scabrum* is most affected by lower soil fertility, implying that it may be less susceptible to drought than to nutrient stress.

The ascorbic acid concentration increased with more intensive drought conditions in *S. scabrum*, regardless of the soil fertility, and in *V. unguiculata* in low fertility soil. A higher concentration of ascorbic acid during drought was also found in amaranth leaves, with an elevation of 163% during a severe drought treatment [40].

The ascorbic acid concentration was significantly reduced in the leaves of *B. oleraceae* by increasing drought intensity in both soil types. Similar results of lower ascorbic acid concentrations by drought were identified in rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), lemon balm (*Melissa officinalis* L.) [41], and soybean (*Glycine max* L. Merr) [42]. Our observed highly variable species response of ascorbic acid content to drought stress confirms results by [42]; however, this does not allow us to make general recommendations.

Regardless of the soil fertility, the absolute amounts of vitamins decreased with lower yields, although the concentrations of the analysed vitamins were mostly higher. The reason for this was the loss of total plant biomass as a result of drought severity. A similar drought reaction was observed regarding the mineral nutrient concentrations in maize (*Zea mays* L.) grains and cassava (*Manihot esculenta* Crantz) tubers during a mild drought in Kenya [17], i.e., while the nutrient concentrations of minerals increased as a result of mild drought, only the total mineral amount (yield x concentration) of calcium was significantly higher compared to a normal season. Therefore, higher vitamin concentrations under drought stress, observed particularly for the thiamine and ascorbic acid levels of *V. unguiculata* and *S. scabrum*, could not compensate for the loss of the total biomass.

Droughts are known to affect human health, particularly through the lower production of foods [17,43]. Droughts, often caused by El Niño–southern oscillation (ENSO) events, occur with increasing frequency in the southern hemisphere [43], which unfortunately also coincides with areas with high nutrient deficiencies [44]. The results of this study show that drought would also affect the total production of the vitamins A, B1, and C in both areas of high and low soil fertility, thereby reducing vitamin levels in many already nutritional deficient geographic areas. In areas with lower soil fertility, the negative impact on diets worsens as they show both lower mineral nutrient concentrations [13] and predominantly (vegetable-dependent) significantly lower vitamin concentrations and yields.

Increasing agrobiodiversity by including different species, even of the same food group (in this case, green leafy vegetables), can, however, increase food and nutrition security. This study shows that green leafy vegetables are differently adapted to stress, while producing one or two vitamins in a higher concentration than the others. *B. oleraceae*, for example, is less susceptible to low fertility soils; however, it is more susceptible to drought. Therefore, to optimally cover all nutrient needs, the focus must be placed on rehabilitation of soil fertility linked with intelligent agrobiodiversity and dietary diversity to enhance the probability of adequate nutrient uptake and to minimize the risk for malnutrition.

5. Conclusions

The research conducted in this study covers a new area of research, in that it not only studies the effects of the combination of drought and soil fertility on the quantity of plant production, but also observes the resulting vitamin production.

The results of the trial show that both drought and varying soil fertility can strongly affect vitamin concentrations and final amounts in plants. The varying results observed during the trials show the importance of consuming a variety of foods, particularly when consuming foods from regions with environmental stressors, to fulfill nutritional needs.

The co-existence of increasing drought events with increased soil degradation in areas with high levels of malnutrition makes it vital to include research on food quality into agricultural trials. The results of such an inclusion could be used to form recommendations for the plants that are best to cultivate in different geographic regions, and could allow particularly rural areas to fulfill their nutritional needs.

The results of this study can be used to derive recommendations as to which green leafy vegetables can be used under which conditions to maximize vitamin production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13050984/s1, Table S1: Mean temperature and humidity of the greenhouse and the first growing season (the data recorded from 21 April to 2 August in 2019) of the project areas in Uganda and Kenya.; Table S2: The gravimetric amount of 100% pot capacity (PC), 75% PC, 50% PC, 25% PC, and a single pot with two soil types (fertile soil; cambisol and infertile soil; alisol).; Table S3: Summary of chemical properties of collected soil samples in Tauchenweiler and Höwenegg, Germany (Stahr & Böcker, 2014).; Figure S1: A sample layout of a 4 * 6 factorial experiment involving three species (SW: *B. oleracea*, CP: *V. unguiculata*, and BN: *S. scabrum*), two soil fertility (L: fertile soil and S: unfertile soil), and two drought treatments with a double number of the control (DC: drought control, DM: drought mild, and DS: drought severe) in a randomized complete block design with six replications. The layout was designed using the SAS program.; Figure S2: $\delta^{13}C$ measurements of samples of *B. oleracea* (SW, •), *V. unguiculata* (CP, +), and *S. scabrum* (BN, △) according to the mean of irrigated water amount for each treatment in the greenhouse trial ($n = 4$ per pot capacity of species). Regression respective formula and R^2 are given. The triple asterisk indicates significance at $p < 0.001$.

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References

1. Ugandan Bureau of Statistics (UBOS); ICF. *Ugandan Demographic and Health Survey 2016*; UBOS: Kampala, Uganda; ICF: Rockville, MD, USA, 2018.
2. Yang, R.Y.; Fischer, S.; Hanson, P.M.; Keatinge, J.D.H. Increasing micronutrient availability from food in Sub-saharan Africa with indigenous vegetables. *ACS Symp. Ser.* **2013**, *1127*, 231–254. [CrossRef]
3. Whitfield, K.C.; Bourassa, M.W.; Adamolekun, B.; Bergeron, G.; Bettendorff, L.; Brown, K.H.; Cox, L.; Fattal-Valevski, A.; Fischer, P.R.; Frank, E.L.; et al. Thiamine deficiency disorders: Diagnosis, prevalence, and a roadmap for global control programs. *Ann. N. Y. Acad. Sci.* **2018**, *1430*, 3–43. [CrossRef] [PubMed]
4. Rowe, S.; Carr, A.C. Global vitamin c status and prevalence of deficiency: A cause for concern? *Nutrients* **2020**, *12*, 2008. [CrossRef] [PubMed]
5. Lane, D.J.R.; Richardson, D.R. The active role of vitamin C in mammalian iron metabolism: Much more than just enhanced iron absorption! *Free Radic. Biol. Med.* **2014**, *75*, 69–83. [CrossRef]
6. Ojagbemi, A.; Okekunle, A.; Olowoyo, P.; Akpa, O.; Akinyemi, R.; Ovbiagele, B.; Owolabi, M. Dietary intakes of green leafy vegetables and incidence of cardiovascular diseases. *Cardiovasc. J. Afr.* **2021**, *32*, 4. [CrossRef]
7. Stuetz, W.; Gowele, V.; Kinabo, J.; Bundala, N.; Mbwana, H.; Rybak, C.; Eleraky, L.; Lambert, C.; Biesalski, K. Consumption of Dark Green Leafy Vegetables Predicts Vitamin A and Iron Intake and Status among Female Small-Scale Farmers in Tanzania. *Nutrients* **2019**, *11*, 1025. [CrossRef]
8. Yang, R.Y.; Keding, G.B. Nutritional contributions of important African indigenous vegetables. In *African Indigenous Vegetables in Urban Agriculture*, 1st ed.; Shackleton, C.M., Pasquini, M.W., Drescher, A.W., Eds.; Earthscan: London, UK, 2009; pp. 105–143.
9. Moyo, S.M.; Serem, J.C.; Bester, M.J.; Mavumengwana, V.; Kayitesi, E. (2020) African green leafy vegetables health benefits beyond nutrition. *Food Rev. Int.* **2020**, *37*, 601–618. [CrossRef]
10. Asensi-Fabado, M.A.; Munné-Bosch, S. Vitamins in plants: Occurrence, biosynthesis and antioxidant function. *Trends Plant Sci.* **2010**, *15*, 582–592. [CrossRef]
11. Mattos, L.M.; Moretti, C.L. Oxidative Stress in Plants Under Drought Conditions and the Role of Different Enzymes. *Enzym. Eng.* **2016**, *5*, 136. [CrossRef]
12. Fitzpatrick, T.B.; Chapman, L.M. The importance of thiamine (vitamin B1) in plant health: From crop yield to biofortification. *J. Biol. Chem.* **2020**, *295*, 12002–12013. [CrossRef]
13. Fischer, S.; Hilger, T.; Piepho, H.-P.; Jordan, I.; Karungi, J.; Towett, E.; Shepherd, K.; Cadisch, G. Soil and farm management effects on yields and nutrient concentrations of food crops in East Africa. *Sci. Total Environ.* **2020**, *716*, 137078. [CrossRef]
14. Kopsell, D.A.; Kopsell, D.E.; Curran-Celentano, J. Carotenoid pigments in kale are influenced by nitrogen concentration and form. *J. Sci Food. Agric.* **2007**, *87*, 900–907. [CrossRef]
15. Onyango, C.M.; Harbinson, J.; Imungi, J.K.; Van Kooten, O. Influence of maturity at harvest, N fertiliser and postharvest storage on dry matter, ascorbic acid and ß-carotene contents of vegetable amaranth (*Amaranthus hypochondriacus*). *Int. J. Postharvest. Technol. Innov.* **2011**, *2*, 180–196. [CrossRef]
16. Cai, W.; Borlace, S.; Lengaigne, M.; van Rensch, P.; Collins, M.; Vecchi, G.; Timmermann, A.; Santoso, A.; McPhaden, M.J.; Wu, L.; et al. Increasing frequency of extreme El Niño events due to greenhouse warming. *Nat. Clim. Change* **2014**, *4*, 111–116. [CrossRef]
17. Fischer, S.; Hilger, T.; Piepho, H.-P.; Jordan, I.; Cadisch, G. Do we need more drought for better nutrition? The effect of precipitation on nutrient concentration in East African food crops. *Sci. Total Environ.* **2019**, *658*, 405–415. [CrossRef]
18. Soares, J.C.; Santos, C.S.; Carvalho, S.M.P.; Pintado, M.M.; Vasconcelos, M.W. Preserving the nutritional quality of crop plants under a changing climate: Importance and strategies. *Plant Soil* **2019**, *443*, 1–26. [CrossRef]
19. D'Oria, A.; Courbet, G.; Billiot, B.; Jing, L.; Pluchon, S.; Arkoun, M.; Maillard, A.; Roux, C.P.; Trouverie, J.; Etienne, P.; et al. Drought specifically downregulates mineral nutrition: Plant ionomic content and associated gene expression. *Plant Direct* **2022**, *6*, e402. [CrossRef]
20. Borrelli, P.; Robinson, D.A.; Panagos, P.; Lugato, E.; Yang, J.E.; Alewell, C.; Wuepper, D.; Montanarella, L.; Ballabio, C. Land use and climate change impacts on global soil erosion by water (2015–2070). *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 21994–22001. [CrossRef]
21. Fischer, S.; Hilger, T.; Piepho, H.-P.; Jordan, I.; Cadisch, G. Missing association between nutrient concentrations in leaves and edible parts of food crops—A neglected food security issue. *J. Food Chem.* **2021**, *345*, 128723. [CrossRef]
22. Turner, N.C. Imposing and maintaining soil water deficits in drought studies in pots. *Plant Soil* **2019**, *439*, 45–55. [CrossRef]
23. Pérez-Llorca, M.; Fenollosa, E.; Salguero-Gómez, R.; Munné-Bosch, S. What is the minimal optimal sample size for plant ecophysiological studies? *Plant Physiol.* **2018**, *178*, 953–955. [CrossRef] [PubMed]

24. Bramley, H.; Turner, N.C.; Siddique, K.H.M. Water use efficiency. In *Genomics and Breeding for Climate-Resilient Crops*, 1st ed.; Kole, C., Ed.; Springer: Berlin/Heidelberg, Germany, 2013; Volume 2, pp. 225–268. [CrossRef]
25. Akhtar, S.S.; Amby, D.B.; Hegelund, J.N.; Fimognari, L.; Großkinsky, D.K.; Westergaard, J.C.; Müller, R.; Moelbak, L.; Liu, F.; Roitsch, T. Bacillus licheniformis FMCH001 Increases Water Use Efficiency via Growth Stimulation in Both Normal and Drought Conditions. *Front. Plant Sci.* **2020**, *11*, 297. [CrossRef] [PubMed]
26. *BSI Standards: 2014: BS EN 14122:2014*; Foodstuffs—Determination of Vitamin B1 by High Performance Liquid Chromatography. BSI: London, UK, 2014.
27. Wald, J.P.; Nohr, D.; Biesalski, H.K. Rapid and easy carotenoid quantification in Ghanaian starchy staples using RP-HPLC-PDA. *J. Food Comp. Anal.* **2018**, *67*, 119–127. [CrossRef]
28. World Health Organization; Food and Agricultural Organization of the United Nations. *Vitamin and Mineral Requirements in Human Nutrition*, 2nd ed.; World Health Organization: Geeneve, Switzerlnd, 2004; 341p.
29. Hill-Mündel, K.; Schlegl, J.; Biesalski, H.K.; Ehnert, S.; Schröter, S.; Bahrs, C.; Nohr, D.; Nüssler, A.K.; Ihle, C. Preoperative Ascorbic Acid Levels in Proximal Femur Fracture Patients Have No Postoperative Clinical Impact, While Ascorbic Acid Levels upon Discharge Have a Major Effect on Postoperative Outcome. *J. Clin. Med.* **2019**, *9*, 66. [CrossRef]
30. Clay, D.E.; Engel, R.E.; Long, D.S.; Liu, Z. Nitrogen and water stress interact to influence carbon-13 discrimination in wheat. *Soil Sci. Soc. Am. J.* **2001**, *65*, 1823–1828. [CrossRef]
31. Schmitter, P.; Dercon, G.; Hilger, T.; Hertel, M.; Treffner, J.; Lam, N.; Duc Vien, T.; Cadisch, G. Linking spatio-temporal variation of crop response with sediment deposition along paddy rice terraces. *Agric. Ecosyst. Environ.* **2011**, *140*, 34–45. [CrossRef]
32. Hanson, P.; Yang, R.Y.; Chang, L.C.; Ledesma, L.; Ledesma, D. Carotenoids, ascorbic acid, minerals, and total glucosinolates in choysum (*Brassica rapa* cvg. parachinensis) and kailaan (*B. oleraceae* Alboglabra group) as affected by variety and wet and dry season production. *J. Food Compos. Anal.* **2011**, *24*, 950–962. [CrossRef]
33. Pathirana, I.; Thavarajah, P.; Siva, N.; Wickramasingh, A.N.K.; Smith, P.; Thavarajah, D. Moisture deficit effects on kale (*Brassica oleracea* L. var. *acephala*) biomass, mineral, and low molecular weight carbohydrate concentrations. *Sci. Hortic.* **2017**, *226*, 216–222. [CrossRef]
34. van Averbeke, W.; Netshithuthuni, C. Effect of irrigation scheduling on leaf yield of non-heading chinese cabbage (*Brassica rapa* L. subsp. *chinensis*). *South Afr. J. Plant Soil* **2010**, *27*, 322–327. [CrossRef]
35. Maseko, I.; Ncube, B.; Tesfay, S.; Fessehazion, M.; Modi, A.T.; Mabhaudhi, T. Productivity of selected african leafy vegetables under varying water regimes. *Agronomy* **2020**, *10*, 916. [CrossRef]
36. Rapala-Kozik, M.; Kowalska, E.; Ostrowska, K. Modulation of thiamine metabolism in Zea mays seedlings under conditions of abiotic stress. *J. Exp. Bot.* **2008**, *59*, 4133–4143. [CrossRef]
37. Borhannuddin Bhuyan MH, M.; Hasanuzzaman, M.; Nahar, K.; Mahmud, J.A.; Parvin, K.; Bhuiyan, T.F.; Fujita, M. Plants behavior under soil acidity stress: Insight into morphophysiological, biochemical, and molecular responses. In *Plant Abiotic Stress Tolerance: Agronomic, Molecular and Biotechnological Approaches*; Hasanuzzaman, M., Hakeem, K.R., Nahar, K., Alharby, H.F., Eds.; Springer Nature: Basel, Switzerland, 2019; pp. 35–82.
38. Hanson, A.D.; Beaudoin, G.A.; McCarty, D.R.; Gregory, J.F. Does abiotic stress cause functional B vitamin deficiency in plants? *Plant Physiol.* **2016**, *172*, 2082–2097. [CrossRef]
39. Latifah, O.; Ahmed, O.H.; Majid, N.M.A. Soil pH buffering capacity and nitrogen availability following compost application in a tropical acid soil. *Compost. Sci. Util.* **2018**, *26*, 1–15. [CrossRef]
40. Sarker, U.; Oba, S. Drought stress enhances nutritional and bioactive compounds, phenolic acids and antioxidant capacity of Amaranthus leafy vegetable. *BMC Plant Biol.* **2018**, *18*, 258. [CrossRef]
41. Munné-Bosch, S.; Alegre, L. Drought-induced changes in the redox state of α-tocopherol, ascorbate, and the diterpene carnosic acid in chloroplasts of Labiatae species differing in carnosic acid contents. *Plant Physiol.* **2003**, *131*, 1816–1825. [CrossRef]
42. Seminario, A.; Song, L.; Zulet, A.; Nguyen, H.T.; González, E.M.; Larrainzar, E. Drought stress causes a reduction in the biosynthesis of ascorbic acid in soybean plants. *Front Plant Sci.* **2017**, *8*, 1042. [CrossRef]
43. Masih, I.; Maskey, S.; Mussá, F.E.F.; Trambauer, P. A review of droughts on the African continent: A geospatial and long-term perspective. *Hydrol. Earth Syst. Sci.* **2014**, *18*, 3635–3649. [CrossRef]
44. von Grebmer, K.; Bernstein, J.; Wiemers, M.; Schiffer, T.; Hanano, A.; Towey, O.; Chéilleachair, R.N.; Foley, C.; Gitter, S.; Ekstrom, K.; et al. *Global Hunger Index*, 16th ed.; Welthungerhilfe: Bonn, Germany, 2021; 60p. Available online: https://www.globalhungerindex.org/ (accessed on 3 August 2022).