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Shoot Yield and Mineral Nutrient Concentrations of Six Microgreens in the Brassicaceae Family Affected by Fertigation Rate

Tongyin Li *, Jacob D. Arthur and Guihong Bi

Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS 39762, USA; jda360@msstate.edu (J.D.A.); gbi@pss.msstate.edu (G.B.)
* Correspondence: tl665@msstate.edu

Abstract: Microgreens have become an important specialty crop valued by their varying texture, vibrant colors, and nutrient-dense features. As the number of species and cultivars rapidly increases for microgreen production, fertigation requirements in relation to shoot production and nutrient compositions remain unclear. This study aimed to investigate the shoot yield, visual quality, and mineral nutrient concentrations of six microgreens in the Brassicaceae family including the 'Waltham' broccoli, 'Red Acre' cabbage, Daikon radish, 'Red Russian' kale, pea, and Rambo radish in two experiments in December 2020 and January 2021. Each microgreen was fertigated with 120 mL of fertilizer solution daily for five consecutive days with a rate of 0, 70, 140, 210, or 280 mg·L^{-1} N from a general-purpose fertilizer. Broccoli, Daikon radish, and kale similarly produced the highest fresh shoot weights of 916.5 to 984 g·m^{-2} in December 2020, while pea produced the highest fresh shoot weight of 2471 g·m^{-2} in January 2021 among cultivars. The fertigation rates of 140, 210, and 280 mg·L^{-1} N resulted in similar fresh and dry shoot weights of selected microgreens, suggesting 140 mg·L^{-1} N should be sufficient for microgreen fertilization. Mineral nutrients in microgreens varied among cultivars: pea microgreens had the highest nitrogen (N) concentrations of 70.6 to 75.2 mg·g^{-1} in December 2020 and 72.1 to 75.4 mg·g^{-1} in January 2021; and cabbage microgreens were rich in calcium (Ca) in both experiments. The kale, pea, and Rambo radish microgreens contained the highest concentrations of iron (Fe) and manganese (Mn) in December 2020. The fertigation rate affected macronutrient concentrations but did not affect micronutrient concentrations including Fe, Mn, or zinc (Zn).

Keywords: broccoli; kale; cabbage; pea; radish; microshoot production; fertilization; macronutrient; micronutrient

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1. Introduction

Microgreens are young immature seedlings that are typically harvested 7–21 days after germination, with a shoot height of 2.5 to 10 cm, and are consumed raw with the expanding cotyledons and/or the first pair of true leaves [1–4]. Microgreens are used in various foods to add flavor, texture, and vibrant colors [5]. Microgreens are most valued for their nutrient-dense properties and are reported to sometimes have higher concentrations of nutrients than baby leaf or mature plants [6–9]. The rapidly expanding microgreen industry has been driven by both growers, seeking high-value and easy-to-produce specialty crops to diversify their production, and consumers, constantly searching for nutrient-dense functional food choices [6,10].

A wide range of species have been produced as microgreens including vegetable, herb, grain, or wild species. Popular microgreen species belong to families including Asteraceae, Alliaceae, Apiaceae, Amaranthaceae, Lamiaceae, Brassicaceae, Fabaceae, etc. [10–14]. Alternative and underutilized species such as purslane (*Portulaca olearacea*), borage (*Borage officinalis*), and small-seed legume species were also explored to be grown as microgreens to increase the diversity and sustainability of the production system [15,16]. Consumer acceptance for microgreens was mainly determined by visual appearance, texture, and flavor, in particular with lower astringency, bitterness, and sourness [5,17].

Species in the Brassicaceae family are the most popular choices to be produced as microgreens due to the ease of production, fast shoot growth, and high nutritional values [10,18,19]. An assessment of mineral nutrient compositions in microgreens from the Brassicaceae family comprising 30 varieties within 10 species from 6 genera revealed that *Brassica* microgreens are most rich in macronutrients potassium (K) and Ca and micronutrients Fe and Zn [20]. They are also good sources of antioxidant phytochemicals with a substantial variation within and among species, particularly in ascorbic acid, α-tocopherol, phylloquinone, β-carotene, lutein/zeaxanthin, total glucosinolates, and total phenolics, as well as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [21,22].

Nutrient supplementation was generally found to increase the fresh shoot yield of microgreens [13,23,24]. Palmitessa et al. [25] recommended 50%-strength Hoagland nutrient solution for high yield and desirable shoot height compared to 25% or 12.5% Hoagland solution for three *Brassica* genotypes including *Brassica oleracea* var. *italic*, *Brassica oleracea* var. *botrytis*, and *Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort. Considering both nutritional and sensory quality, Keutgen et al. [17] recommended tap water without fertilizer for garden cress (*Lepidium sativum* L.) and 25% modified Hoagland solution for radish cress (*Raphanus sativus* L.) microgreens in household production compared with 50% and 100% strengths. The sensory quality seemed to increase in tested cress microgreens with increasing fertilizer concentration as described by Keutgen et al. [17]. The effects of fertilizer application on health-beneficial secondary metabolites varied among reports and species. The absence of nutrient supplementation presented an abiotic stress and resulted in an extensive increase in lutein, β-carotene, total ascorbic acid, and total anthocyanins in rocket (*Diplotaxis tenuifolia*) microgreens, while it did not affect secondary metabolites in Brussels sprout (*Brassica oleracea* var. germmifera) microgreens [18]. The optimal nutrient management of microgreens should consider fresh yield, visual quality, nutritional values, health-beneficial compounds, as well as postharvest quality.

Microgreen yield, mineral nutrients, and phytochemical concentrations in response to fertigation rate vary among species and cultivars. The objective of this study was to investigate the effect of five fertigation rates ranging from 0 to 280 mg·L^{-1} N on the shoot growth and mineral nutrients of six microgreen species/cultivars in the Brassicaceae family when grown with a peat-based soilless substrate.

2. Materials and Methods

2.1. Plant Materials and Microgreen Cultivation

This study was conducted in a greenhouse at Mississippi State University in Starkville, MI, USA (33.4552° N, 88.7944° W). Six genotypes including 'Waltham' broccoli, 'Red Acre' cabbage, 'Red Russian' kale, speckled pea, 'Daikon' Radish, and 'Rambo' Radish were evaluated for shoot growth and mineral nutrient concentrations. Microgreen seeds of all selected cultivars were purchased from True Leaf Market (Salt Lake City, Utah). Seed sowing rate for each microgreen was determined according to supplier recommendation and summarized in Table 1. Hundred-seed weight of each cultivar was measured with three replications. This study consisted of two experiments with the first being conducted on 30 November 2020 and then repeated on 5 January 2021.

Microgreens were grown with a peat-based soilless substrate (PRO-MIX BX general purpose; Premier Tech Horticulture, Quebec, Canada) in black plastic trays with drainage holes (width 25.72 cm, length 25.72 cm, depth 6.03 cm; T.O. Plastics, Clearwater, MN, USA). Seeds of appropriate weight for each tray were weighed out and manually sown onto the surface of the growing trays filled with substrate. After sowing, an additional thin layer of substrate was added on top to cover the seeds and provide a dark environment beneficial for germination. The temperature in the greenhouse was set to 25.6 °C/23.9 °C day/night with natural light. Microgreens were manually watered as needed approximately once per day until harvest.

Table 1. Common name, scientific name, seeding rate, 100-seed weight, and harvest date of six microgreens.

Common Name	Scientific Name	Seeding Rate (g·m^{-2})	100-Seed Wt. (g)	Harvest Date [1] (DAP)
Broccoli	*Brassica oleracea* var. *italica* cv. 'Waltham'	98.3	0.33 ± 0.015	10
Cabbage	*Brassica oleracea* var. *capitata* cv. 'Red Acre'	83.1	0.39 ± 0.012	10–11
Daikon radish	*Raphanus sativus* var. *longipinnatus* cv. 'Daikon'	173.8	1.36 ± 0.019	10
Kale	*Brassica napus* var. *pabularia* cv. 'Red Russian'	75.6	0.22 ± 0.005	10
Pea	*Pisum sativum*	1285.0	15.3 ± 0.88	10–11
Rambo radish	*Raphanus sativus* cv. 'Rambo'	189.0	1.10 ± 0.03	10–11

[1] Microgreens were harvested with the expanding cotyledons (microgreen stage 1) or with the first pair of true leaves (microgreen stage 2).

Four days after seed sowing, microgreens were fertigated daily with one of five fertilizer rates including 0, 70, 140, 210, or 280 mg·L^{-1} N sourced from a general-purpose water-soluble fertilizer 20N-8.7P-16.6K (Peters® Professional 20-20-20 General Purpose, also containing (wt/wt) 0.05% magnesium (Mg), 0.05% Fe, 0.025% Mn, 0.013% boron (B), 0.013% copper (Cu), 0.005% molybdenum (Mo), and 0.025% Zn; ICL Specialty Fertilizers, Tel-Aviv, Israel) for five consecutive days. At each application, 120 mL of nutrient solution of a given rate or water (as in the 0 mg·L^{-1} N control) was manually applied to each tray by top dressing.

2.2. Shoot Harvest and Data Collection

Prior to shoot harvest, shoot height was measured in each tray from the substrate surface to the highest point of shoot growth. Each tray of microgreen species was given a visual quality rating from 1 to 5, where 1 = seedling growth covers 20% of the growing surface or less; 2 = seedling growth covers 20% to 40% of the growing surface; 3 = seedling growth covers 40% to 60% of the growing surface; 4 = seedling growth covers 60% to 80% of the growing surface; and 5 = seedling growth covers over 80% of the growing surface with healthy plant growth.

Microgreens grown in each tray were then carefully harvested by cutting the shoots closely above the substrate surface using a pair of clean scissors. Microgreens were harvested at either microgreen stage 1 with the expanding cotyledons or at microgreen stage 2 with the first pair of true leaves as described by Waterland et al. [8]. Freshly harvested shoots from each tray were measured for fresh weight (FW). Fresh microgreen shoots were then oven-dried at 60 °C until constant weight and measured for dry shoot weight (DW).

2.3. Mineral Nutrient Analyses

Dry microgreen samples were ground to pass a 1 mm sieve with a grinder (Wiley mini mill, Thomas Scientific, Swedesboro, NJ, USA) for mineral nutrient analyses. Combustion analysis determination of total N concentration with 0.25 g of dry tissue was carried out using an elemental analyzer (vario MAX cube; Elementar Americas Inc., Long Island, NY, USA). A dry tissue sample of 0.5 g was digested with 1 mL of 6 M hydrochloric acid (HCl) and 50 mL of 0.05 M HCl for the concentrations of phosphorus (P), K, Ca, Mg, sulfur (S), Cu, Fe, Mn, Zn, and B using inductively coupled plasma optical emission spectrometry (SPECTROBLUE; SPECTRO Analytical Instruments, Kleve, Germany). Microgreen samples were tested at the Mississippi State University Extension Service Soil Testing Laboratory. Concentrations of macronutrients (mg·g^{-1}) and micronutrients (μg·g^{-1}) in microgreens are presented on a dry weight basis.

2.4. Experimental Design and Statistical Analyses

This experiment was conducted in a randomized complete block design with factorial arrangement of treatments and five replications. Microgreen species/cultivar (6) and fertigation rate (5 rates) were the two main experimental factors, resulting in 30 treatment combinations. Each raised bed in the greenhouse served as a block or replication consisting of all thirty treatment combinations, which were randomly distributed within a block. Each growing tray was considered as one experimental unit. The significance of any main effect or the interaction between the two factors was determined through analysis of variance (ANOVA) using GLMMIX procedure of SAS (version 9.4; SAS Institute, Cary, NC, USA). Where indicated by ANOVA, means were separated through Tukey's honest significant difference at $\alpha = 0.05$. Data from the two experiments were compared as repeated measures, where experiment date was used as a factor to analyze its effect. All statistical analyses were performed using SAS.

3. Results

3.1. Shoot Growth and Visual Rating

Shoot height, fresh and dry shoot weights, and visual rating varied among microgreen cultivars and fertigation rates without interaction in both experiments in December 2020 and January 2021, except that shoot height was not affected by fertigation rate in January 2021 (Tables 2 and 3).

Table 2. Shoot height, fresh and dry shoot weights, and visual rating varied among six microgreens.

Microgreens	December 2020				January 2021			
	Shoot Height [1,2] (cm)	Fresh Shoot Weight ($g \cdot m^{-2}$)	Dry Shoot Weight ($g \cdot m^{-2}$)	Visual Rating (1–5)	Shoot Height (cm)	Fresh Shoot Weight ($g \cdot m^{-2}$)	Dry Shoot Weight ($g \cdot m^{-2}$)	Visual Rating (1–5)
Broccoli	8.16 bc	984.0 a	69.8 b	4.84 a	7.37 c	1131.0 b	77.0 c	5.00 a
Cabbage	6.73 d	849.0 b	58.6 cd	4.65 ab	6.27 d	943.6 c	63.3 d	4.94 a
Daikon radish	8.54 b	982.7 a	78.5 a	4.30 b	9.24 b	1156.0 b	88.6 b	4.12 b
Kale	6.73 d	916.5 ab	54.5 d	4.78 a	5.83 d	1014.0 c	59.1 d	5.00 a
Pea	12.84 a	359.5 d	34.1 e	3.28 d	14.94 a	2471.0 a	179.5 a	5.00 a
Rambo radish	7.78 c	722.1 c	63.0 c	3.74 c	7.78 c	824.2 d	72.8 c	3.51 c
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1] Different lower case letters within a column suggest significant difference among means as indicated by Tukey's HSD test at $p < 0.05$. [2] The means of each microgreen were obtained by averaging means from all five fertigation rates because there was no interaction between cultivar/species and fertigation rate.

Table 3. Shoot height, fresh and dry shoot weights, and visual rating varied among five fertigation rates.

Fertilizer Rate ($mg \cdot L^{-1}$ N)	December 2020				January 2021			
	Shoot Height [1,2] (cm)	Fresh Shoot Weight ($g \cdot m^{-2}$)	Dry Shoot Weight ($g \cdot m^{-2}$)	Visual Rating (1–5)	Shoot Height (cm)	Fresh Shoot Weight ($g \cdot m^{-2}$)	Dry Shoot Weight ($g \cdot m^{-2}$)	Visual Rating (1–5)
0	7.84 b	666.1 c	53.0 b	3.96 a	8.24	1082 c	81.9 c	4.49 b
70	8.39 ab	779.0 b	58.3 ab	4.16 a	8.49	1203 b	87.7 b	4.48 b
140	8.55 ab	825.0 ab	61.2 a	4.34 a	8.73	1316 a	93.1 a	4.74 a
210	8.77 a	880.2 a	64.4 a	4.48 ab	8.59	1312 a	93.3 a	4.55 ab
280	8.77 a	861.3 ab	61.9 a	4.39 b	8.78	1371 a	94.3 a	4.71 ab
p-value	0.0052	<0.0001	<0.0001	0.0014	0.50	<0.0001	<0.0001	0.0028

[1] Different lower case letters within a column suggest significant difference among means as indicated by Tukey's HSD test at $p < 0.05$. [2] The means of each fertigation rate were obtained by averaging means from all six microgreens because there was no interaction between cultivar/species and fertigation rate.

Among tested species/cultivars, pea produced the largest shoot height of 12.84 cm in December 2020 and 14.94 cm in January 2021 (Table 2). Cabbage and kale produced the lowest shoot height in both experiments, with broccoli, Daikon radish, and Rambo radish producing intermediate shoot heights. When affected by fertigation rate, 210 and 280 $mg \cdot L^{-1}$ N increased shoot height compared with the no-fertilizer control in December 2020 (Table 3). However, shoot height was not affected by fertigation rate in January 2021.

The trends of fresh and dry shoot weights among species varied in two experiments. In December 2020, broccoli, Daikon radish, and kale similarly produced the highest fresh shoot weight ranging from 916.5 to 984 g·m^{-2}, higher than that of pea or Rambo radish, with pea producing the lowest fresh shoot weight of 359.5 g·m^{-2}. The separation of dry shoot weight followed a similar trend to fresh shoot weight among microgreens: Daikon radish produced the highest dry shoot weight of 78.5 g·m^{-2}, higher than that of any other cultivar, with pea producing the lowest dry shoot weight of 34.1 g·m^{-2} in December 2020.

In January 2021, pea produced the highest fresh and dry shoot weights of 2471 g·m^{-2} and 179.5 g·m^{-2}, respectively (Table 2). The ranking in fresh shoot weight among cultivars was pea > broccoli (1131 g·m^{-2}), or Daikon radish (1156 g·m^{-2}) > cabbage (943.6 g·m^{-2}), or kale (1014 g·m^{-2}) > Rambo radish (824.2 g·m^{-2}). By comparison, the ranking of dry shoot weight among cultivars was pea > Daikon radish (88.6 g·m^{-2}) > broccoli (77.0 g·m^{-2}), or Rambo radish (72.8 g·m^{-2}) > cabbage (63.3 g·m^{-2}), or kale (59.1 g·m^{-2}). When affected by fertigation rate, 140, 210, and 280 mg·L^{-1} N resulted in similar fresh and dry shoot weights in both experiments, higher than that of the no-fertilizer control (Table 3).

For visual rating, broccoli, cabbage, and kale similarly produced the highest visual rating scores of 4.65 to 4.84 in December 2020, and 4.94 to 5.0 in January 2021, respectively (Tables 2 and 3). Pea microgreens had the lowest visual rating of 3.28 in December 2020, but had the comparable highest visual rating of 5.0 with broccoli, cabbage, and kale in January 2021. This difference in pea shoot yield was mainly due to the poor germination of pea microgreens in the December experiment. Rambo radish had visual ratings of 3.74 in December 2020 and 3.51 in January 2021, lower than that of broccoli, cabbage, Daikon radish, or kale in both experiments. This was also due to the poor germination of Rambo radish seeds.

The separation of visual rating among fertigation rates was not as much (Table 3). In December 2020, 280 mg·L^{-1} N resulted in a lower visual rating of 4.39 compared with 0, 70, or 140 mg·L^{-1} N. In January, the four fertigation rates 0, 70, 210, and 280 mg·L^{-1} N resulted in similar visual ratings of 4.48 to 4.71, with 140 mg·L^{-1} N resulting in the highest visual rating of 4.74 among the five fertigation rates.

3.2. Nitrogen Concentration

Nitrogen concentrations in the tested microgreens were affected by the interaction between cultivar and fertigation rate in both experiments (Table 4). In December 2020, pea microgreens had the highest N concentrations ranging from 70.6 to 75.2 mg·g^{-1}, higher than any other cultivar at each given fertigation rate. The highest fertigation rate of 280 mg·L^{-1} N generally resulted in a higher N concentration than 0 to 140 mg·L^{-1} N in broccoli, cabbage, daikon radish, and kale microgreens, and higher than 0 and 70 mg·L^{-1} N in pea and Rambo radish microgreens. Rambo radish had the lowest N concentration among cultivars when fertigated with 280 mg·L^{-1} N.

In January 2021, the fertigation rate of 280 mg·L^{-1} N also resulted in a higher N concentration than 0 to 140 mg·L^{-1} in broccoli, cabbage, and Daikon radish, and higher than 70 mg·L^{-1} in kale and Rambo radish microgreens. Nitrogen concentrations in pea microgreens were similar among all five fertigation rates. Pea microgreens also had the highest N concentration than any other cultivar at each fertigation rate in January 2021.

3.3. Phosphorus Concentration

In December 2020, phosphorus concentrations were affected by the interaction between cultivar and fertigation rate (Table 4). The two radish microgreens were generally high in P among tested cultivars regardless of fertigation rate. Rambo radish had a higher P concentration, ranging from 11.9 to 12.9 mg·g^{-1}, than broccoli, cabbage, kale, or pea at each fertigation rate. The three microgreens including cabbage, kale, and pea had similar P concentrations of 8.34 to 9.57 mg·g^{-1} regardless of fertigation rate. Phosphorus concentrations in microgreens did not respond much to fertigation rate, where five fertigation rates resulted in similar P concentrations in broccoli, cabbage, kale, pea, and Rambo radish.

Table 4. Macronutrient concentrations of six microgreens affected by the interaction between cultivar and fertigation rate in December 2020 and January 2021.

Microgreens	Fertigation Rate ($mg{\cdot}L^{-1}$ N)	December 2020						January 2021	
		Nitrogen [1] ($mg{\cdot}g^{-1}$)	Phosphorus ($mg{\cdot}g^{-1}$)	Potassium ($mg{\cdot}g^{-1}$)	Calcium ($mg{\cdot}g^{-1}$)	Magnesium ($mg{\cdot}g^{-1}$)	Sulfur ($mg{\cdot}g^{-1}$)	Nitrogen ($mg{\cdot}g^{-1}$)	Sulfur ($mg{\cdot}g^{-1}$)
Broccoli	0	42.4 m	9.54 e–i	35.5 ab	14.50 cde	4.18 a–f	13.74 c–f	36.7 m	13.62 e–h
	70	50.8 hij	10.72 c–f	37.9 ab	14.29 de	4.48 abc	14.09 cde	43.7 jk	15.33 d–g
	140	51.2 ghij	11.02 b–e	40.1 ab	14.40 de	4.31 a–f	14.22 cd	47.8 hi	13.22 fgh
	210	54.5 efg	10.17 e–h	34.3 ab	13.41 edf	3.94 c–g	12.49 d–i	51.6 e–h	12.80 gh
	280	57.0 cde	9.98 e–i	33.8 b	13.09 ef	3.82 e–h	12.14 d–j	54.1 c–f	13.01 fgh
Cabbage	0	41.4 m	8.84 ghi	39.7 ab	17.05 ab	4.42 a–d	18.81 a	42.8 kl	20.47 a
	70	49.5 ijk	9.15 f–i	39.8 ab	17.28 a	4.58 ab	18.78 a	48.8 ghi	17.27 bcd
	140	51.6 ghi	9.57 e–i	38.4 ab	17.71 a	4.61 a	17.90 ab	54.0 c–f	19.06 abc
	210	54.2 efg	9.19 f–i	36.3 ab	16.53 abc	4.36 a–e	15.61 bc	57.6 bc	19.31 ab
	280	58.0 cd	9.06 f–i	34.6 ab	15.17 bcd	4.30 a–f	15.01 bcd	60.0 b	18.94 abc
Daikon radish	0	47.2 kl	10.21 d–g	35.7 ab	9.60 h	3.75 f–i	11.12 e–k	46.6 ijk	13.58 e–h
	70	51.8 ghi	11.21 a–e	35.5 ab	10.0 gh	3.81 e–h	9.66 ijk	52.3 d–g	12.80 gh
	140	53.2 fgh	12.19 abc	39.2 ab	9.51 h	3.90 c–g	10.97 f–k	53.1 def	13.33 fgh
	210	55.3 def	12.70 ab	39.2 ab	8.99 h	4.10 a–g	10.62 g–k	53.8 c–f	11.97 hi
	280	57.5 cde	12.18 abc	37.1 ab	9.38 h	3.86 d–g	10.38 h–k	57.5 bc	12.71 fg
Kale	0	42.6 m	9.23 f–i	40.1 ab	11.71 g	3.88 d–g	14.98 bcd	39.4 ml	17.73 a–d
	70	48.9 ijk	9.26 f–i	39.3 ab	10.90 gh	3.73 ghi	14.45 cd	46.7 jkl	17.19 bcd
	140	53.4 fgh	8.86 ghi	35.8 ab	10.09 gh	3.53 ghi	13.47 c–g	53.3 def	15.37 d–g
	210	55.4 def	8.38 i	33.8 b	9.65 gh	3.26 hi	12.82 c–h	54.8 cde	16.69 b–e
	280	59.4 c	8.34 i	34.2 ab	8.86 h	3.19 i	12.02 d–j	56.0 bcd	16.0 c–f
Pea	0	70.6 b	8.48 i	37.5 ab	5.36 i	2.34 j	6.63 lmn	72.1 a	6.05 jk
	70	71.1 b	8.54 ghi	37.2 ab	5.16 i	2.27 j	5.92 mn	75.2 a	6.29 jk
	140	73.5 ab	8.82 ghi	37.0 ab	5.00 i	2.27 j	5.50 n	74.2 a	5.84 k
	210	73.9 ab	8.35 i	34.6 ab	5.08 i	2.16 j	4.94 n	74.1 a	6.01 jk
	280	75.2 a	8.40 i	35.0 ab	4.79 i	2.08 j	4.57 n	75.4 a	5.88 k
Rambo radish	0	44.1 lm	11.93 a–d	37.6 ab	10.72 gh	4.27 a–f	9.39 jkl	47.0 ij	9.16 ij
	70	48.1 jk	12.26 abc	36.5 ab	9.94 gh	4.16 a–f	9.00 kl	50.1 f–i	8.38 jk
	140	50.2 hijk	12.27 abc	36.7 ab	9.68 gh	4.04 a–g	9.32 jkl	51.1 e–h	8.94 ijk
	210	51.7 ghi	12.36 abc	36.6 ab	9.11 h	4.00 b–g	8.54 klm	52.0 d–g	8.61 jk
	280	53.2 fgh	12.93 a	41.1 a	9.76 gh	4.08 a–g	9.73 ijk	53.4 def	8.45 jk
p-value	Cultivar	<0.0001	<0.0001	0.2616	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Fertigation rate	<0.0001	0.0025	0.0088	<0.0001	<0.0001	<0.0001	<0.0001	0.04
	Interaction	<0.0001	0.0007	0.0006	0.043	0.0062	0.01	<0.0001	0.046

[1] Different lower case letters within a column suggest significant difference among means as indicated by Tukey's HSD test at $p < 0.05$.

In January 2021, phosphorus concentrations varied among microgreen cultivars and were separately affected by fertigation rate without interaction (Tables 5 and 6). Rambo radish had the highest P concentration of 13.1 $mg{\cdot}g^{-1}$ among tested microgreens and pea microgreens had the lowest P concentration of 8.92 $mg{\cdot}g^{-1}$. The separation of P concentrations among cultivars was Rambo radish > Daikon radish > broccoli, or kale > cabbage > pea (Table 5). The five fertigation rates generally resulted in similar P concentrations in tested microgreens except that 280 $mg{\cdot}L^{-1}$ N resulted in higher P concentrations than the no-fertilizer control (Table 6).

3.4. Potassium Concentration

In December 2020, potassium concentrations were affected by the interaction between cultivar and fertigation rate (Table 4). Potassium concentrations were generally similar among all treatment combinations, except that Rambo radish fertigated with 280 $mg{\cdot}L^{-1}$ N had a higher K concentration of 41.1 $mg{\cdot}g^{-1}$ than broccoli (33.8 $mg{\cdot}g^{-1}$) fertigated with 280 $mg{\cdot}L^{-1}$ N or kale (33.8 $mg{\cdot}g^{-1}$) fertigated with 210 $mg{\cdot}L^{-1}$ N.

Table 5. Macronutrients including phosphorus, potassium, calcium, and magnesium varied among six microgreens in January 2021.

Microgreens	January 2021 Phosphorus [1,2] (mg·g^{-1})	Potassium (mg·g^{-1})	Calcium (mg·g^{-1})	Magnesium (mg·g^{-1})
Broccoli	10.59 c	39.9 b	13.10 b	4.19 b
Cabbage	9.77 d	42.6 a	15.14 a	4.60 a
Daikon radish	11.62 b	39.6 b	8.86 d	4.13 b
Kale	10.36 c	44.4 a	9.79 c	3.81 c
Pea	8.92 e	32.8 c	5.33 e	2.59 d
Rambo radish	13.05 a	37.8 b	10.04 c	4.29 b
p-value	<0.0001	<0.0001	<0.0001	<0.0001

[1] Different lower case letters within a column suggest significant difference among means as indicated by Tukey's HSD test at $p < 0.05$. [2] The means of each microgreen were obtained by averaging means from all five fertigation rates because there was no interaction between cultivar/species and fertigation rate.

Table 6. Macronutrients including phosphorus, calcium, and magnesium varied among five fertigation rates in January 2021.

Fertigation Rate [1,2] (mg·L^{-1} N)	January 2021 Phosphorus (mg·g^{-1})	Potassium (mg·g^{-1})	Calcium (mg·g^{-1})	Magnesium (mg·g^{-1})
0	10.4 b	40.7	11.0 a	4.03 a
70	10.6 ab	39.3	10.3 ab	4.02 ab
140	10.8 ab	38.9	10.1 b	3.93 ab
210	10.9 ab	39.1	10.1 b	3.87 ab
280	11.0 a	39.5	10.3 ab	3.83 b
p-value	0.022	0.25	0.019	0.013

[1] Different lower case letters within a column suggest significant difference among means as indicated by Tukey's HSD test at $p < 0.05$. [2] The means of each microgreen were obtained by averaging means from all six microgreens because there was no interaction between cultivar/species and fertigation rate.

In January 2021, potassium concentrations varied among cultivars and were not affected by fertigation rate (Tables 5 and 6). Cabbage and kale had the highest K concentration of 42.6 mg·g^{-1} and 44.4 mg·g^{-1}, respectively, higher than that of any other microgreen cultivar. Broccoli, Daikon radish, and Rambo radish similarly had the second highest K concentrations of 37.8 mg·g^{-1} to 39.9 mg·g^{-1}, with pea having the lowest K concentration of 32.8 mg·g^{-1}.

3.5. Calcium Concentration

In December 2020, calcium concentrations were affected by the interaction between cultivar and fertigation rate (Table 4). Cabbage microgreens had the highest Ca concentrations of 15.2 to 17.7 mg·g^{-1}, and broccoli had the second highest Ca concentrations of 13.1 to 14.5 mg·g^{-1}, with pea having the lowest Ca concentrations of 4.79 to 5.36 mg·g^{-1} at each fertigation rate. The three microgreens Daikon radish, kale, and Rambo radish had similar Ca concentrations of 8.86 to 11.7 mg·g^{-1} regardless of fertigation rate.

In January 2021, calcium concentrations separately varied among cultivars and fertigation rates without interaction (Tables 5 and 6). The ranking of Ca concentration among microgreens was cabbage > broccoli > kale, and Rambo radish > Daikon radish > pea, similar to the trend in December 2020. Microgreens fertigated with no fertilizer had a higher Ca concentration than 140 or 210 mg·L^{-1} N. Microgreens fertilized with 70 to 280 mg·L^{-1} N had similar Ca concentrations ranging from 10.1 to 10.3 mg·g^{-1}.

3.6. Magnesium Concentration

In December 2020, magnesium concentrations were affected by the interaction between cultivar and fertigation rate (Table 4). The five fertigation rates generally resulted in similar Mg concentrations within a microgreen except that 70 mg·L^{-1} N increased the Mg concentration in broccoli compared with 280 mg·L^{-1} N. Pea microgreens had the lowest Mg concentrations of 2.08 to 2.34 mg·g^{-1} among microgreens at any given fertigation rate. Cabbage fertigated with any N rate, broccoli fertigated with 0 to 140 mg·L^{-1} N, Daikon radish fertigated with 120 mg·L^{-1} N, and Rambo radish fertigated with 0, 70, 140, and 280 mg·L^{-1} N similarly had the highest Mg concentration of 4.04 to 4.61 mg·g^{-1} among all treatment combinations.

In January 2021, magnesium concentrations were separately affected by the main effects of cultivars and fertigation rate without interaction (Tables 5 and 6). The ranking of Mg concentration among cultivars followed cabbage (4.60 mg·g^{-1}) > broccoli, Daikon radish, or Rambo radish (4.13 to 4.29 mg·g^{-1}) > kale (3.81 mg·g^{-1}) > pea (2.59 mg·g^{-1}). The five fertigation rates generally resulted in similar Mg concentrations in tested microgreens except that 0 mg·L^{-1} N resulted in a higher Mg concentration than 280 mg·L^{-1} N.

3.7. Sulfur Concentration

Sulfur concentrations were affected by the interaction between cultivar and fertigation rate in December 2020 and January 2021 (Table 4).

The five fertigation rates resulted in similar S concentrations within a cultivar in broccoli, pea, kale, and Rambo radish in both experiments and in Daikon radish in December 2020. Among microgreens, cabbage generally had the highest S concentration at any given fertigation rate, 15.0 to 18.8 mg·g^{-1} in December 2020 and 17.3 to 20.5 mg·g^{-1} in January 2021. In December 2020, pea had the lowest S concentrations of 4.57 to 6.63 mg·g^{-1}. In January 2021, pea and Rambo radish similarly had the lowest S concentrations of 5.84 to 9.16 mg·g^{-1} regardless of fertigation rate.

3.8. Copper Concentration

Copper concentrations were affected by the interaction between cultivar and fertigation rate in December 2020 and January 2021 (Table 7).

In December 2020, three species including Daikon radish, kale, and pea had similar Cu concentrations ranging from 1.79 to 4.63 µg·g^{-1}, regardless of fertigation rate. Broccoli and cabbage had higher Cu concentrations than Daikon radish, pea, or Rambo radish when fertigated with 140 mg·L^{-1} N. The fertigation rate of 140 mg·L^{-1} N resulted in higher Cu concentrations than 210 or 280 mg·L^{-1} N in broccoli and cabbage.

In January 2021, cabbage and Daikon radish had higher Cu concentrations, ranging from 11.2 to 15.9 µg·g^{-1}, than any other cultivar, regardless of fertigation rate. The four microgreens including broccoli, kale, pea, and Rambo radish had generally similar Cu concentrations ranging from 2.64 to 5.96 µg·g^{-1}. The five fertigation rates generally resulted in similar Cu concentrations within a microgreen cultivar.

3.9. Iron Concentration

Iron concentrations varied among microgreen cultivars in December 2020 and January 2021 and were not affected by fertigation rate in any experiment (Table 8). In December 2020, kale, pea, and Rambo radish had similar Fe concentrations of 138.3 to 141.8 µg·g^{-1}, higher than that of broccoli, cabbage, or Daikon radish. Broccoli microgreens had a higher Fe concentration than cabbage or Daikon radish, with Daikon radish having the lowest Fe concentration of 91.7 µg·g^{-1}. In January 2021, Daikon radish had the highest Fe concentration of 149.6 µg·g^{-1} among microgreens. Broccoli and cabbage had the second highest Fe concentrations of 114.4 µg·g^{-1} and 120.1 µg·g^{-1}, respectively. Pea and Rambo radish had the lowest Fe concentrations of 78.7 µg·g^{-1} and 81.1 µg·g^{-1}, respectively.

3.10. Manganese Concentration

Manganese concentrations varied among microgreen cultivars in December 2020 and January 2021 and were not affected by fertigation rate (Table 8). In December 2020, kale, pea, and Rambo radish microgreens comparably had the highest Mn concentrations of 44.1 to 46.8 µg·g^{-1}, higher than that of cabbage or Daikon radish. Daikon radish had the lowest

Mn concentration of 23.1 μg·g^{-1} among tested microgreens. In January 2021, broccoli had the highest Mn concentration of 44.3 μg·g^{-1}, and daikon radish had the second highest Mn concentration of 27.2 μg·g^{-1}. The pea, Rambo radish, and cabbage microgreens similarly had the lowest Mn concentrations of 20.5 to 21.3 μg·g^{-1}.

Table 7. Micronutrient concentrations of six microgreens affected by the interaction between cultivar and fertigation rate in December 2020 or January 2021.

Microgreens	Fertigation Rate [1] (mg·L^{-1} N)	December 2020 Copper (μg·g^{-1})	January 2021 Copper (μg·g^{-1})	January 2021 Boron (μg·g^{-1})
Broccoli	0	6.54 a–d	5.29 cd	17.4 cde
	70	7.64 a	4.97 cd	17.8 cde
	140	7.16 ab	4.56 cd	19.5 b–e
	210	3.85 c–h	6.03 c	23.2 ab
	280	2.80 fgh	5.99 c	24.8 a
Cabbage	0	5.01 a–g	11.56 b	15.2 e
	70	6.93 abc	12.72 ab	18.4 cde
	140	7.57 a	13.85 ab	19.3 b–d
	210	3.76 c–h	14.08 ab	18.3 cde
	280	2.89 e–h	13.61 ab	18.8 b–d
Daikon radish	0	3.44 d–h	14.17 ab	15.1 e
	70	2.31 fgh	11.22 b	15.5 de
	140	1.79 h	13.38 ab	14.7 e
	210	2.00 gh	15.55 a	16.0 cde
	280	2.23 gh	15.93 a	16.0 cde
Kale	0	4.58 a–h	5.96 c	20.8 abc
	70	4.02 b–h	3.31 cd	20.3 a–d
	140	4.83 a–h	3.08 cd	20.7 abc
	210	1.79 h	3.11 cd	18.9 b–e
	280	2.32 fgh	2.94 cd	19.0 b–e
Pea	0	4.54 a–h	3.23 cd	16.8 cde
	70	4.63 a–h	3.67 cd	18.2 cde
	140	3.49 d–h	3.68 cd	18.3 cde
	210	2.36 fgh	3.16 cd	15.7 de
	280	2.93 e–h	2.98 cd	16.5 cde
Rambo radish	0	6.89 abc	3.03 cd	16.8 cde
	70	3.39 e–h	2.64 d	17.3 cde
	140	3.39 e–h	3.21 cd	20.2 a–d
	210	5.49 a–f	2.77 cd	18.2 cde
	280	6.10 a–e	2.90 cd	19.4 b–e
p-value	Cultivar	<0.0001	<0.0001	<0.0001
	Fertigation rate	<0.0001	0.038	<0.0001
	Interaction	<0.0001	0.0002	<0.0001

[1] Different lower case letters within a column suggest significant difference among means as indicated by Tukey's HSD test at $p < 0.05$.

3.11. *Zinc Concentration*

Zinc concentrations varied among microgreen cultivars in December 2020 and January 2021 and were not affected by fertigation rate in either experiment (Table 8). In December 2020, pea and broccoli microgreens had the highest Zn concentrations of 89.6 μg·g^{-1} and 82.7 μg·g^{-1}, higher than that of Daikon radish or Rambo radish. Daikon radish, kale, and Rambo radish similarly had the lowest Zn concentrations of 65.3 to 74.9 μg·g^{-1}. Cabbage had an intermediate Zn concentration of 78.8 μg·g^{-1}. In January, the separation of Zn concentration among microgreens followed Daikon radish (89.2 μg·g^{-1}) > broccoli (75.0 μg·g^{-1}), or cabbage (73.5 μg·g^{-1}) > kale (65.7 μg·g^{-1}) > pea (48.9 μg·g^{-1}), or Rambo radish (47.9 μg·g^{-1}).

Table 8. Micronutrients including iron, manganese, zinc, and boron varied among six microgreens.

Microgreens	December 2020				January 2021		
	Iron [1,2] (µg·g^{-1})	Manganese (µg·g^{-1})	Zinc (µg·g^{-1})	Boron (µg·g^{-1})	Iron (µg·g^{-1})	Manganese (µg·g^{-1})	Zinc (µg·g^{-1})
Broccoli	127.5 b	41.0 b	82.7 ab	24.5 a	114.4 b	44.3 a	75.0 b
Cabbage	110.6 c	36.1 c	78.8 bc	22.7 ab	120.1 b	20.5 d	73.5 b
Daikon radish	91.7 d	23.1 d	65.3 d	21.1 bcd	149.6 a	27.2 b	89.2 a
Kale	138.3 a	46.6 a	74.9 bcd	19.5 d	89.6 c	22.5 c	65.7 c
Pea	141.8 a	46.8 a	89.6 a	21.6 bc	78.7 d	21.3 cd	48.9 d
Rambo radish	141.0 a	44.1 ab	72.3 cd	20.5 cd	81.1 d	22.1 cd	47.9 d
p-value							
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Fertigation	0.80	0.12	0.23	0.18	0.43	0.89	0.11
Interaction	0.09	0.06	0.1	0.06	0.063	0.065	0.25

[1] Different lower case letters within a column suggest significant difference among means as indicated by Tukey's HSD test at $p < 0.05$. [2] The means of each microgreen were obtained by averaging means from all five fertigation rates because there was no interaction between cultivar/species and fertigation rate. The main effect of fertigation rate was not significant for all micronutrients listed in this table.

3.12. Boron Concentration

In December 2020, boron concentrations varied among microgreens and were not affected by fertigation rate (Table 8). Broccoli and cabbage microgreens had the highest B concentrations of 24.5 µg·g^{-1} and 22.7 µg·g^{-1}, respectively. Daikon radish, kale, and Rambo radish similarly had the lowest B concentrations of 19.5 to 21.1 µg·g^{-1}.

In January 2021, boron concentrations were affected by the interaction between cultivar and fertigation rate (Table 7). The five fertigation rates resulted in similar B concentrations in Daikon radish, kale, pea, and Rambo radish. Higher fertigation rates of 210 mg·L^{-1} N and 280 mg·L^{-1} N resulted in a higher B concentration than 0 or 70 mg·L^{-1} N in broccoli. Broccoli also had higher B concentrations of 23.2 µg·g^{-1} and 24.8 µg·g^{-1} than any other cultivar at fertigation rates of 210 mg·L^{-1} N and 280 mg·L^{-1} N, respectively.

4. Discussion

Cultural practice like fertigation rate not only affects shoot yield, but also alters mineral nutrient profiles in microgreens. The fertigation rates of 140, 210, and 280 mg·L^{-1} N resulted in similar fresh and dry shoot weights in tested microgreens, higher than the no-fertilizer control. This result suggested that a medium fertigation rate of 140 mg·L^{-1} should be sufficient for fresh shoot yield in tested microgreen production. This agrees with Murphy et al. [26] that increasing the N concentration in fertigation treatment increased shoot dry weight and leaf N concentration. Daily fertigation with 150 mg·L^{-1} was considered beneficial for shoot yield and economical in the production of beet (*Beta vulgaris* L.) and arugula (*Eruca vesicaria* subsp. *sativa*) microgreens [24,26]. In our previous studies, one-time fertigation with 100 mg·L^{-1} N increased the overall fresh and dry shoot weight of ten microgreens grown with a peat-based substrate and of five microgreens grown with hydroponic fiber mats [13,23]. However, substrate types varied in their compositions and water holding capacities. Such variations make recommendations for optimal fertilization difficult [26,27].

The concentrations of macronutrients including Ca, P, and K and micronutrients including Fe, Mn, Cu, and Zn in the six microgreens tested in the current study are in general agreement with reported ranges by Xiao et al. [20] who analyzed mineral nutrient profiles of 30 microgreen varieties in the Brassicaceae family. Concentrations of macronutrients including N, P, K, Ca, and Mg are also in agreement with our previous study [13], when similar species were grown as microgreens on a peat-based substrate. When compared with sufficient mineral nutrient levels reported in the *Plant Analysis Handbook III* by Bryson et al. [28], the kale, broccoli, and pea microgreens had higher P concentrations than the reported ranges in mature leaves on a dry weight basis; radish microgreens had higher

Ca concentrations; cabbage and radish microgreens had higher S concentrations; and pea and broccoli microgreens had higher Fe concentrations than the reported ranges. Such results agreed with the common perception that microgreens are nutrient-dense compared with their mature counterparts. Waterland et al. [8] reported that most mineral nutrient concentrations were higher in four kale microgreens when measured on a dry weight basis. However, when measured on a fresh weight basis, fresh kale microgreens had lower concentrations of K, Ca, Mg, Fe, and Zn than fresh baby leaves.

Variations were found between experiments. Repeated measured showed that fresh and dry shoot weights, visual ratings, and concentrations of P, K, Mg, S, and Zn were higher in the January 2021 experiment than in the December 2020 experiment (data not shown). Other nutrient concentrations including N and Ca were higher in December 2020 than January 2021. Shoot height and most tested micronutrients including Fe, Mn, Cu, and B were similar between the two experiments (data not shown). Such variations may have resulted from the varying germination quality and the fluctuating microenvironment in a greenhouse. The average air temperature and relative humidity in the greenhouse were 73.4 °C and 42.6% in the December experiment, and 72.1 °C and 39.7% in the January experiment, respectively. Certain substrates may serve as a source of some minerals and affect nutrient compositions in microgreens [23,27]. Cultural practices also affect mineral nutrients as pre-sowing seed soaking consistently reduced fresh and dry shoot weights and multiple mineral nutrients in ten microgreens [13].

When the N concentration increased in the fertilizer solution, there was a general increasing trend of N concentration in the tested microgreens except for pea. However, increasing fertigation rate did not cause as much separation of other nutrient concentrations including both macro- and micronutrients. Therefore, the results suggested that variations in mineral nutrient compositions were more subject to microgreen species or cultivars, and that increasing fertigation rate may not improve mineral concentrations in microgreens. This agrees with Kyriacou et al. [19] that genotype was considered as the major source of variation when it comes to the compositional analyses including mineral nutrients and phytochemicals in four brassicaceous microgreens including Komatsuna (*Brassica rapa* L. var. *perviridis*), Mibuna (*Brassica rapa* L. subsp. *nipposinica*), Mizuna (*Brassica rapa* L. var. *japonica* cv. Greens), and Pak Choi (*Brassica rapa* L. subsp. *chinensis*). To some degree, decisions regarding the optimal fertigation rate and method should probably be prioritized toward fresh shoot production than nutrient profiles. Petropoulos et al. [29] reported that a less frequent nutrient feeding of 10 days resulted in a lower antioxidant activity, total chlorophylls, lutein, and β-carotene than a more frequent fertilization of 20 days, but a higher ascorbic acid and certain phenolic compounds than 20 days of feeding in spinach (*Spinacia oleracea* L.) microgreens. The effect of fertigation rate on health-beneficial bioactive compounds in selected microgreen species grown in a given growing system requires further investigation.

High fertigation rates raise food safety concerns in microgreen production. Petropoulos et al. [29] reported that more frequent fertigation up to 20 days resulted in the highest nitrate concentrations but lowest mineral concentrations in spinach microgreens. In comparison, 10 days of nutrient feeding was considered the cost-effective choice for spinach microgreen cultivation that resulted in high yields, high mineral nutrient, and low nitrate concentrations without compromising bioactive compounds like polyphenols. Nutrient deprivation was reported to be effective in reducing nitrate concentration in microgreens, with the optimal duration of treatment varying among species [30]. Manipulating the molar ratio of NH_4:NO_3 also affected nitrate concentration in *Brassica* microgreens [25].

We also observed that the high fertigation rate combined with high shoot density in microgreen production often causes rotting problems in various species. Rotting can very much affect visual quality and marketability of the entire tray of microgreens. Therefore, caution is required when a high fertigation rate is applied. The optimal fertigation rate should consider factors including species/cultivars, seed treatments, substrates, the growing microenvironment, fertigation method, etc. For example, fertigation may be needed more frequently for slow-growing species compared with fast-growing species. An alternative fertigation method through subirrigation may reduce moisture among microshoots and thus reduce potentially rotting problems than top dressing [3]. Peat-based soilless substrates have higher water holding capacities than hydroponic fiber mats [27], and may require less frequent irrigation or fertigation.

Sensory quality and visual appearance both play a key role in consumer preference regarding microgreens and their willingness to consume them [5]. While visual quality like vibrant colors serve as initial attractive factors to customers, the eventual acceptance of microgreens was more attributed to their taste and texture, specifically with low astringency, sourness, and bitterness [5]. The visual rating in this study varied among tested genotypes, but was not separated much by fertigation rate, except for the observation that shoot decay was often associated with higher fertigation rates. In a study investigating the nutritional and sensory quality of two cress microgreens when affected by mineral nutrient supplementation, Keutgen et al. [17] reported that the response of bioactive compounds to mineral nutrient supplementation varied among species, compounds of interest (including carotenoids, total phenols, nitrate content, anthocyanin, etc.), and plant parts (cotyledons vs. stems). They concluded that the sensory quality was generally rated higher in the two tested cress microgreens when the highest mineral nutrients were supplied in the nutrient solution. However, the sensory quality of microgreens should not equal nutritional quality.

5. Conclusions

Microgreens in the Brassicaceae family including broccoli, cabbage, Daikon radish, kale, pea, and Rambo radish varied in shoot yields, height, visual quality, and mineral nutrient concentrations. Broccoli, Daikon radish, and kale similarly produced the highest fresh shoot weight of 916.5 to 984 $g{\cdot}m^{-2}$ in December 2020, while pea produced the highest fresh shoot weight of 2471 $g{\cdot}m^{-2}$ in January 2021. Such a fluctuation in shoot yield was likely attributed to the germination quality and fluctuating microenvironment between experiments. Pea microgreens also produced the largest shoot heights in both experiments, which is a desirable feature in microgreens, making it easier for shoot harvest. The fertigation rate of 140 $mg{\cdot}L^{-1}$ N was considered sufficient and economical for optimal fresh shoot production. While the supplementation of fertilizer solution improved the shoot yield in the tested microgreens, increasing N fertigation rate did not necessarily increase most macro- and micronutrients except for N. Variations in mineral nutrient compositions were more subject to microgreen species/cultivars than changing fertigation rate. Future research should focus on microshoot yield, bioactive phytochemicals, and nitrate concentrations in response to fertigation practices including fertilization frequency and delivery method.

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