



## Article

# Shoot Production and Mineral Nutrients of Five Microgreens as Affected by Hydroponic Substrate Type and Post-Emergent Fertilization

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**Abstract:** As a new specialty crop with high market value, microgreens are vegetable or herb seedlings consumed at a young age, 7–21 days after germination. They are known as functional food with high concentrations of mineral nutrients and health beneficial phytochemicals. Microgreen industry lacks standardized recommendations on cultural practices including species/variety selection, substrate choice, and fertilization management. This study evaluated shoot growth and mineral nutrient concentrations in five microgreens including four *Brassica* and one *Raphanus* microgreens as affected by four hydroponic pad types and post-emergent fertilization in two experiments in January and February 2020. The five microgreens varied in their shoot height, fresh, dry shoot weights, and mineral nutrient concentrations with radish producing the highest fresh and dry shoot weights. Radish had the highest nitrogen (N) concentration and mustard had the highest phosphorus (P) concentrations when grown with three hydroponic pads except for hemp mat. Hydroponic pad type altered fresh, dry shoot weights, and mineral nutrients in tested microgreens. Microgreens in hemp mat showed the highest shoot height, fresh, dry shoot weights, and potassium (K) concentration, but the lowest N concentration in one or two experiments. One time post-emergent fertilization generally increased shoot height, fresh, dry shoot weights, and macronutrient concentrations in microgreens.

**Keywords:** shoot growth; yield; mineral nutrients; hydroponic pad; fertilization



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## 1. Introduction

Microgreen is an emerging new specialty crop with high market value of USD 30–50 per pound (454 g) [1]. It is a collective term for vegetable, herb, grain, or flower seedlings consumed at a young stage [2–5]. Microgreens are harvested 7–21 days after germination with expanding cotyledons or first pair of true leaves [6]. A number of plant species in the families including Amaranthaceae, Apiaceae, Asteraceae, Brassicaceae, Fabaceae, and Lamiaceae, have been grown as microgreens, among which *Brassica* crops are of the most grown species [7–11]. Microgreens are used by chef and consumers to enhance flavor, color, and texture in various foods and have become increasingly popular in recent years as consumer awareness of microgreen dietary value increases [1,3,8]. The high market value, increasing customer demand, and short production cycle had drawn interest among vegetable growers to produce microgreens [7].

Microgreens are reported to be four to six times nutrient denser than their mature counterparts [12–15]. They are considered functional food with high mineral nutrient concentrations and health-beneficial phytochemicals [15]. Xiao et al. [16] reported microgreen greens in the Brassicaceae family are most abundant in macronutrients of K and calcium (Ca) and micronutrients of iron (Fe) and zinc (Zn) after evaluating 30 varieties. They also contain high levels of antioxidant phytochemicals including ascorbic acid, carotenoids, glucosinolates, and polyphenols with substantial variations within and between species [6].

Microgreen lettuce (*Lactuca sativa*) have higher mineral concentrations including Ca, Magnesium (Mg), Fe, manganese (Mn), Zn, selenium (Se), and molybdenum (Mo) and lower nitrate concentration than mature lettuce [10]. Basil (*Ocimum basilicum*) microgreens can be biofortified with Se to satisfy the human dietary need for this micronutrient [17]. There lacks standardized recommendations for cultural practices including species/variety and substrate choice, fertilization, and control of microenvironment in relation to shoot yield and nutrient compositions of microgreens [4,9,18,19].

Fertilization was considered optional in microgreen production since seeds have stored nutrient for initial seedling growth. However, fertilizer application, premixed in growing substrate, or applied post-emergent was reported to result in fast growth and high yield of microgreen shoots [20]. The most economic fertilization treatments for arugula (*Eruca vesicaria* subsp. *sativa*) microgreens were daily post-emergent fertigation with 150 mg·L<sup>-1</sup> N or daily application of 75 mg·L<sup>-1</sup> N plus preincorporation of 1000 mg·L<sup>-1</sup> N [18]. Preharvest daily spray of 10 mM calcium chloride solution on broccoli (*Brassica oleracea* var. *italica*) microgreens increased shoot biomass, Ca concentration, and improved visual quality during storage. Plant species/variety, slow versus fast growing, may differ in their requirements for fertilization [1]. With an increasing number of species and varieties grown as microgreens, crop-specific fertilizer requirements with respect to microshoot yield and mineral nutrients largely remain unclear.

Microgreens can be produced with peat-based potting mix or hydroponic pads that are made from synthetic or recycled fibrous materials [7]. A variety of hydroponic pads including biostrate (made from felt fiber), jute mat, and hemp mat are commercially available, some of which are compostable and can serve as a sustainable alternative to a peat-based substrate [21,22]. Physical and chemical properties including container capacity, air filled porosity, bulk density, pH, and electrical conductivity (EC) vary among substrate types and affect growth of microgreen crops [21]. Efficacy of using hydroponic mats to grow microgreens is species or variety dependent. Hydroponic pads made from textile fibers and jute-kenaf fibers were reported to produce similar fresh shoot yield of rapini (*Brassica rapa* L.) microgreens [21]. Hydroponic substrate type also affects nutritional facts of microgreens and food safety in microgreen production due to the humid conditions and warm temperatures. The latter has become one of the most important concerns in microgreen production [7], where hydroponic mat type was found to affect microbial populations on microgreens [21].

The objective of this study was to investigate shoot production and mineral nutrients of five microgreens grown with four types of hydroponic pads as affected by post-emergent fertilization.

## 2. Materials and Methods

### 2.1. Plant Materials and Cultivation

Shoot growth and mineral nutrient concentrations of five microgreens were evaluated (Table 1). Microgreen seeds of selected species were purchased from the True Leaf Market (Salt Lake City, UT, USA). Seed sowing rate for each microgreen was determined by supplier recommendation and summarized in Table 1. Hundred-seed weight of each microgreen was measured with three replications. This study was conducted in a greenhouse at Mississippi State University, USA. (33.4552° N, 88.7944° W) and included two experiments with the first conducted on 29 January 2020 and then repeated on 25 February 2020. The temperature in the greenhouse was set at 25 °C with natural light.

Each microgreen was grown with four types of hydroponic pad including biostrate, hemp mat, micro-mats, and jute mat (Table 2). Each hydroponic pad was pre-cut or manually cut into the size of approximately 25 cm by 25 cm to fit the bottom of the growing tray. Black plastic trays without drainage holes (width 25.72 cm, length 25.72 cm, depth 6.03 cm; T.O. Plastics, Clearwater, MN, USA) were used to grow microgreens in this study. Hydroponic pads were then hydrated by soaking them into tap water, drained with excessive water, and placed into each growing tray. Seeds of appropriate weight were measured and manually

sown onto the hydrated hydroponic pads, and covered with another black tray to provide a dark environment and maintain moisture during germination. Microgreen seeds were misted with a spray bottle every 12 h to moisturize the seeds and substrate, when the cover tray was removed shortly and put back on after misting. Four days after sowing, the cover tray was turned upside down with the bottom being placed on top of microgreen shoots for another 24–48 h. This practice was to provide some resistance and encourage microshoot elongation as recommended by the seed supplier. The cover tray was then removed at approximately 7 days after planting (DAP).

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

Common Name	Scientific Name	Seeding Rate (g·m <sup>-2</sup> )	100 Seed Wt. (g)	Harvest Date (DAP)	Harvest Stage <sup>1</sup>
Broccoli	<i>Brassica oleracea</i> var. <i>italica</i> cv. 'Waltham'	98.3	0.44 ± 0.015	12–14	Stage 1
Cabbage	<i>Brassica oleracea</i> var. <i>capitata</i> cv. 'Red Acre'	83.1	0.44 ± 0.016	14–15	Stage 1
Kale	<i>Brassica napus</i> var. <i>pabularia</i> cv. 'Red Russian'	75.6	0.23 ± 0.008	14–16	Stage 1
Mustard	<i>Brassica juncea</i> cv. 'Red Garnet'	60.5	0.18 ± 0.003	15–16	Mixed with 70–80% stage 1 shoots
Radish	<i>Raphanus sativus</i> cv. 'Rambo'	189.0	1.10 ± 0.03	12–14	Stage 1

<sup>1</sup> Microgreens were harvested with the expanding cotyledons (microgreen stage 1) or with the first pair of true leaves (microgreen stage 2).

**Table 2.** Product name, constructed material, supplier, and manufacturer information of the four types of hydroponic pads used in this study.

Hydroponic Pad Product	Materials	Supplier	Manufacturer
Biostrate®	Felt fiber	True Leaf Market	Quick Plug, South Portland, ME, USA
Hemp mat	Hemp fiber	Amazon	Terrafibre, Drayton Valley, Canada
Jute mat	Jute fiber	True Leaf Market	Handy Pantry, Salt Lake City, UT, USA
Micro-Mats	Wood fiber	True Leaf Market	Handy Pantry, Salt Lake City, UT, USA

After covers were removed, half of the trays from each microgreen were fertigated once with 120 mL of water-soluble fertilizer 20N-8.7P-16.6K (Peters® Professional 20-20-20 General Purpose, also containing (wt/wt) 0.05% Mg, 0.05% Fe, 0.025% Mn, 0.013% boron (B), 0.013% copper (Cu), 0.005% Mo, and 0.025% Zn; ICL Specialty Fertilizers, Tel-Aviv, Israel) at a rate of 100 mg·L<sup>-1</sup> N. The fertilizer solution had a pH of 6.56 and EC of 0.41 mS·cm<sup>-1</sup>. As a control to the fertilization treatment, the other half of trays were irrigated with the same volume of water (pH 7.54; EC 0.15 mS·cm<sup>-1</sup>).

## 2.2. Data Collection and Shoot Harvest

Plant height was measured in each tray before shoot harvest from the substrate surface to the highest point of shoot growth. Microgreen shoot in each tray were carefully harvested above the substrate surface, with the expanding cotyledons (microgreen stage 1) or with the first pair of true leaves (microgreen stage 2) as described by Waterland et al. [5]. Fresh shoot weight of microgreens harvested from each tray was measured. Freshly harvested microgreen shoots were then oven dried at 60 °C until constant weight and measured for dry shoot weight (DW). Dry weight percentage (%) was also determined for each tray.

## 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 was used for the determination of total N concentration with 0.25 g of dry tissue

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 P, K, Ca, Mg, 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 ( $\text{mg}\cdot\text{g}^{-1}$ ) and micronutrients ( $\mu\text{g}\cdot\text{g}^{-1}$ ) in microgreens were presented on a dry weight basis.

#### 2.4. Experimental Design and Statistical Analyses

This experiment was conducted in a randomized complete block design with a factorial arrangement of treatments. Microgreens (5 species), hydroponic pad (4 types), and fertilization (fertilizer or not) were the three main factors contributing to 40 treatment combinations. Each treatment combination had five replications with an individual growing tray as the experimental unit. Significance of any main effect or the interaction among main factors were determined by analysis of variance (ANOVA) using PROC GLMMIX procedure of SAS (version 9.4; SAS Institute, Cary, NC, USA). Where indicated by ANOVA, means were separated by Tukey's honest significant difference (HSD) at  $\alpha \leq 0.05$ . Data from the two experiments were compared as repeated measures. All statistical analyses were performed using SAS.

### 3. Results

The experiment date affected all measured dependent variables in this study, showing varying trends between the January and February experiments (data not shown). Therefore, data from the two experiments were presented separately. There was no three-way interaction among microgreen species, hydroponic substrate type, and fertilization treatment for any measured variable.

#### 3.1. Shoot Height

Shoot height was affected by the interaction between microgreen species and hydroponic pad type in both experiments (Tables 3 and 4). Hemp resulted in the largest shoot height within each microgreen, with biostrate and jute mat resulting in similar shoot height in four microgreens next to hemp in January and February 2020. Micro-mats resulted in the lowest height in broccoli, kale, and radish in both experiments. Shoot height of radish and broccoli were higher than cabbage, kale, or mustard grown with any substrate type in both experiments. One time post-emergent fertilization increased shoot height by 3.2% and 2.5% in January and February 2020, respectively compared to the no fertilizer treatment (Table 5).

#### 3.2. Fresh and Dry Shoot Weights

Microgreen shoots were harvested with expanding cotyledons or the first pair of true leaves with varying days ranging from 12 to 16 DAP (Table 1, Figure S1). Fresh, dry shoot weights, and dry weight percentage were affected by the interaction between microgreen species and hydroponic pad type in January 2020 (Table 3). Hemp mat resulted in higher fresh and dry shoot weights in each microgreen than other substrates in January (Table 3). Micro-Mats resulted in the lowest fresh and dry shoot weights in broccoli, kale, and radish. Among the five microgreens, radish produced the highest fresh and dry shoot weights when grown with each substrate type, with broccoli being the second highest, higher than kale, cabbage, or mustard. Dry weight percentage ranged from 5.48% in kale grown with hemp to 9.21% in mustard grown with Micro-Mats. The comparable highest dry weight percentages were found in cabbage, kale, and mustard grown with Micro-Mats and cabbage grown with biostrate. Hemp resulted in the lowest dry weight percentages in cabbage (5.81%), kale (5.48%), and mustard (6.78%) among the four substrate types.

Fresh shoot weight and dry weight percentage were also affected by the interaction between species and fertilization treatment in January 2020, when one time post-emergent fertilization increased fresh shoot weight and decreased dry weight percentage in kale and radish, but resulted in similar values of both variables in broccoli, cabbage, and radish (Table 6). One time fertilization also increased overall dry shoot weight by 2.7% in the February experiment (Table 5).

**Table 3.** Shoot height, fresh shoot weight, dry shoot weight, and dry weight percentage of five microgreens affected by the interaction between species and hydroponic pad type in January 2020.

January 2020					
Microgreens	Hydroponic Pad	Shoot Height <sup>1,2</sup> (cm)	Fresh Shoot Weight (g·m <sup>-2</sup> )	Dry Shoot Weight (g·m <sup>-2</sup> )	Dry Weight Percentage (%)
Broccoli	Biostrate	6.69 cd	1322 ef	89.1 ef	6.76 de
	Hemp	8.32 b	1843 c	109.8 d	6.04 efg
	Jute	6.87 c	1512 de	100.1 de	6.63 def
	Micro-Mats	5.78 ef	1136 fg	76.7 gh	6.77 de
Cabbage	Biostrate	4.85 ghi	533 j	46.0 l	8.78 ab
	Hemp	6.71 cd	1462 de	84.9 fg	5.81 fg
	Jute	5.3 fgh	817 hi	64.7 ij	7.92 bc
	Micro-Mats	4.61 hij	539 j	48.5 kl	9.10 a
Kale	Biostrate	5.24 fgh	1003 gh	70.7 hi	7.23 cd
	Hemp	6.9 c	1487 de	81.5 fgh	5.48 g
	Jute	5.35 efg	1000 gh	63.6 ij	6.55 def
	Micro-Mats	3.92 jk	656 ij	58.2 jk	9.06 a
Mustard	Biostrate	4.28 ijk	533 j	42.4 l	7.99 bc
	Hemp	6.05 de	941 gh	63.8 ij	6.78 de
	Jute	4.34 ij	612 ij	48.1 kl	7.87 bc
	Micro-Mats	3.58 k	468 j	43.0 l	9.21 a
Radish	Biostrate	7.25 b	2278 b	152.2 b	6.77 de
	Hemp	9.71 a	3158 a	186.4 a	5.92 efg
	Jute	8 b	2414 b	160.8 b	6.69 def
	Micro-Mats	6.01 de	1676 cd	122.9 c	7.46 cd
<i>p</i> -value	Species	<0.0001	<0.0001	<0.0001	<0.0001
	Substrate	<0.0001	<0.0001	<0.0001	<0.0001
	Species * Substrate <sup>3</sup>	<0.0001	<0.0001	<0.0001	<0.0001

<sup>1</sup> Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at  $p \leq 0.05$ .

<sup>2</sup> Each mean was obtained by averaging data from both the fertilized and no-fertilizer treatments. <sup>3</sup> Species \* Substrate suggests interaction between species and substrate type.

**Table 4.** Shoot height, fresh shoot weight, dry shoot weight, and dry weight percentage of five microgreens affected by the interaction between species and hydroponic pad type or the main effect of specie in February 2020.

February 2020					
Microgreens	Hydroponic Pad	Shoot Height <sup>1,2</sup> (cm)	Fresh Shoot Weight (g·m <sup>-2</sup> )	Dry Shoot Weight (g·m <sup>-2</sup> )	Dry Weight Percentage (%)
Broccoli	Biostrate	7.08 c			
	Hemp	8.31 a			
	Jute	7.45 b	1289 b	84.9 b	6.68 a
	Micro-Mats	6.55 cd			
Cabbage	Biostrate	4.59 f-i			
	Hemp	5.79 e			
	Jute	5.01 f	968 c	67.8 c	7.20 a
	Micro-Mats	4.18 hi			

Table 4. Cont.

February 2020					
Microgreens	Hydroponic Pad	Shoot Height <sup>1,2</sup> (cm)	Fresh Shoot Weight (g·m <sup>-2</sup> )	Dry Shoot Weight (g·m <sup>-2</sup> )	Dry Weight Percentage (%)
Kale	Biostrate	4.75 fgh	929 c	71.2 c	7.90 a
	Hemp	6.53 cd			
	Jute	4.93 f			
	Micro-Mats	3.96 i			
Mustard	Biostrate	4.26 fhi	626 d	46.9 d	7.71 a
	Hemp	6.25 de			
	Jute	4.83 f			
	Micro-Mats	4.25 ghi			
Radish	Biostrate	7.24 b	2325 a	156.3 a	7.97 a
	Hemp	8.92 a			
	Jute	7.24 b			
	Micro-Mats	6.3 de			
<i>p</i> -value	Species	<0.0001	<0.0001	<0.0001	0.58
	Substrate	<0.0001	<0.0001	<0.0001	0.24
	Species *	0.0002	0.81	0.18	0.39
	Substrate <sup>3</sup>				

<sup>1</sup> Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at  $p \leq 0.05$ .

<sup>2</sup> Means for shoot height were obtained by averaging data from both the fertilized and no-fertilizer treatments; means of each microgreen species for fresh, dry shoot weights, and dry weight percentage were obtained by averaging data from both fertilization treatments and all four substrate types. <sup>3</sup> Species \* Substrate suggests interaction between species and substrate type.

**Table 5.** Microgreen shoot growth and mineral nutrient concentrations affected by the main effect of post-emergent fertilization.

Fertilization	January 2020				February 2020		
	Shoot Height <sup>1</sup> (cm)	Dry Shoot Weight (g·m <sup>-2</sup> )	Mg <sup>2</sup> (mg·g <sup>-1</sup> )	S (mg·g <sup>-1</sup> )	Shoot Height (cm)	N (mg·g <sup>-1</sup> )	P (mg·g <sup>-1</sup> )
Fertilized	6.08	86.8	5.24	7.60	5.99	49.6	9.48
Not fertilized	5.90	84.5	5.38	7.87	5.85	48.6	8.74
<i>p</i> -value <sup>3</sup>	0.004	0.035	0.034	0.015	0.017	0.0003	<0.0001

<sup>1</sup> Means of the fertilized or not fertilized treatment were obtained by averaging data over five tested microgreens grown with four types of hydroponic substrates. <sup>2</sup> Mg = magnesium; S = sulfur; N = nitrogen; P = phosphorus. <sup>3</sup>  $p \leq 0.05$  suggests significant difference between means within a column indicated by Tukey's HSD test.

In February 2020, fresh and dry shoot weights were affected by the main effects of species and hydroponic pad type without interaction (Table 4). Ranking for both fresh and dry weights among microgreens are radish > broccoli > kale or cabbage > mustard. Hemp resulted in the highest fresh and dry weights of 1551 g·m<sup>-2</sup> and 101.9 g·m<sup>-2</sup> in microgreens, with jute and biostrate producing similar fresh and dry shoot weights. Micro-mats resulted in the lowest fresh shoot weight among all substrate types, but similar dry weight to biostrate or jute (Data not shown). Dry weight percentage was not affected by any experimental factor in February.

### 3.3. Macronutrient Concentrations

Macronutrient concentrations including N, P, K, Ca, Mg, and S in microgreens were affected by the interaction between species and hydroponic pad in January and February 2020 (Tables 7 and 8). Concentrations of N, P, and K in January and K concentration in February were also affected by the interaction between species and fertilization (Table 6).

**Table 6.** Fresh shoot weight, dry weight percentage, and macronutrient concentrations in five microgreens affected by the interaction between species and post-emergent fertilization treatment in January or February 2020.

Microgreens	Fertilization	January 2020					February 2020
		Fresh Shoot Weight <sup>1,2</sup> (g·m <sup>-2</sup> )	Dry Weight Percentage (%)	N <sup>3</sup> (mg·g <sup>-1</sup> )	P (mg·g <sup>-1</sup> )	K (mg·g <sup>-1</sup> )	K (mg·g <sup>-1</sup> )
Broccoli	Fertilized	1498 c	6.4 d	55.4 b	10.1 cd	18.8 b	14.8 c
	Not fertilized	1408 c	6.7 cd	54.8 b	9.9 d	16.9 cd	13.2 d
Cabbage	Fertilized	851 ef	7.8 a	51.1 c	9.5 de	18.9 b	13.3 d
	Not fertilized	824 f	8.1 a	49.2 de	8.5 f	17.9 bcd	11.0 e
Kale	Fertilized	1112 d	6.6 cd	50.7 cd	9.6 de	16.2 de	11.6 e
	Not fertilized	961 e	7.6 ab	47.9 e	8.8 ef	14.7 ef	9.5 f
Mustard	Fertilized	651 g	7.9 a	42.8 f	12.0 a	21.6 a	20.0 a
	Not fertilized	626 g	8.0 a	41.3 g	10.8 bc	18.3 bc	17.0 b
Radish	Fertilized	2235 a	6.3 d	61.0 a	11.0 b	14.5 ef	10.8 ef
	Not fertilized	2227 b	7.1 bc	60.8 a	11.2 ab	14.0 f	10.3 ef
<i>p</i> -value	Species	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Fertilization	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Species * fertilization <sup>3,4</sup>	0.0002	0.0012	0.0059	0.0016	0.014	0.013

<sup>1</sup> Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at  $p \leq 0.05$ .

<sup>2</sup> Each mean was obtained by averaging data over four hydroponic pad types. <sup>3</sup> N = nitrogen; P = phosphorus; K = potassium. <sup>4</sup> Species \* fertilization suggests interaction between species and fertilization treatment.

**Table 7.** Macronutrient concentrations in five microgreens affected by the interaction between species and hydroponic pad type in January 2020.

Microgreens	Hydroponic Pad	January 2020					
		N (mg·g <sup>-1</sup> ) <sup>1,2,3</sup>	P (mg·g <sup>-1</sup> )	K (mg·g <sup>-1</sup> )	Ca (mg·g <sup>-1</sup> )	Mg (mg·g <sup>-1</sup> )	S (mg·g <sup>-1</sup> )
Broccoli	Biostrate	58.1 b	10.1 d–g	13.9 de	7.2 hi	5.00 g–j	7.07 ef
	Hemp	50.2 fg	9.7 efg	30.1 b	12.7 cd	5.75 c–f	9.00 ab
	Jute	55.9 bc	10.2 d–g	14.0 d	11.0 de	5.53 d–h	8.97 abc
	Micro-Mats	56.2 bc	9.9 d–g	13.3 d–g	5.4 ij	4.30 jk	8.78 a–d
Cabbage	Biostrate	57.8 b	9.9 d–g	14.4 d	9.1 fg	5.23 f–i	8.77 a–d
	Hemp	41.5 j	7.8 h	35.2 a	19.6 a	6.38 bc	8.11 b–e
	Jute	50.2 fg	8.8 gh	12.2 d–h	14.4 c	5.68 c–g	8.68 a–d
	Micro-Mats	51.1 efg	9.4 g	11.7 d–h	5.1 j	3.67 k	9.93 a
Kale	Biostrate	52.5 def	9.2 g	10.5 gh	4.9 k	4.99 g–j	6.02 fg
	Hemp	42.6 ij	9.3 g	31.7 b	11.5 de	6.05 bcd	8.72 a–d
	Jute	53.2 de	9.4 fg	9.9 h	8.9 gh	5.52 d–h	7.70 cde
	Micro-Mats	48.8 g	9.0 gh	9.8 h	3.1 k	3.78 k	8.05 b–e
Mustard	Biostrate	45.2 h	12.2 ab	14.4 d	11.8 de	6.55 b	7.56 de
	Hemp	35.3 k	9.3 g	37.3 a	14.4 c	5.95 b–e	5.89 fg
	Jute	44.7 hi	12.5 a	13.7 def	17.2 b	7.59 a	8.57 bcd
	Micro-Mats	42.8 hij	11.6 abc	14.3 d	6.4 ij	4.72 ij	8.96 abc
Radish	Biostrate	61.7 a	11.9 abc	13.3 d–g	7.2 hi	5.03 ghi	4.31 h
	Hemp	54.7 cd	10.8 cde	21.8 c	10.8 ef	5.31 e–i	8.66 a–d
	Jute	63.1 a	10.7 c–f	10.9 fgh	9.1 fg	4.84 hij	5.64 g
	Micro-Mats	64.1 a	11.1 bcd	11.1 e–h	5.4 ij	4.30 jk	5.34 gh
<i>p</i> -value	Species	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Substrate	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Species * Substrate <sup>4</sup>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

<sup>1</sup> Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at  $p \leq 0.05$ .

<sup>2</sup> Each mean was obtained by averaging data over both the fertilized and no-fertilizer treatments. <sup>3</sup> N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; S = sulfur. <sup>4</sup> Species \* Substrate suggests interaction between species and substrate type.

**Table 8.** Macronutrient concentrations in five microgreens affected by the interaction between species and hydroponic pad in February 2020.

Microgreens	Hydroponic Pad	February 2020					
		N (mg·g <sup>-1</sup> ) <sup>1,2,3</sup>	P (mg·g <sup>-1</sup> )	K (mg·g <sup>-1</sup> )	Ca (mg·g <sup>-1</sup> )	Mg (mg·g <sup>-1</sup> )	S (mg·g <sup>-1</sup> )
Broccoli	Biostrate	59.9 ab	8.7 efg	13.5 cd	8.23 efg	4.75 e–h	6.14 bcd
	Hemp	49.6 fg	7.6 hi	19.4 b	14.3 ab	5.20 b–e	7.58 ab
	Jute	57.8 b	9.0 d–g	11.6 def	7.24 f–i	4.93 def	7.0 ab
	Micro-Mats	53.7 de	8.8 d–g	11.6 def	3.58 jk	3.98 ij	6.78 abc
Cabbage	Biostrate	50.8 ef	7.9 gh	11.8 de	10.2 de	4.59 fgh	2.93 ef
	Hemp	39.3 k	6.1 j	19.8 b	13.4 bc	5.07 c–f	3.08 ef
	Jute	45.9 hi	6.7 ij	8.8 g	7.57 e–h	4.22 hij	4.54 def
	Micro-Mats	49.1 fg	7.5 hi	8.2 g	2.35 k	2.93 k	2.72 f
Kale	Biostrate	47.4 gh	8.8 def	9.1 fg	3.87 jk	4.68 e–h	4.38 def
	Hemp	43.0 ij	8.5 e–h	13.9 cd	4.75 ijk	5.38 bcd	3.75 ef
	Jute	48.2 fgh	9.9 cd	10.1 efg	9.49 def	5.75 b	7.93 ab
	Micro-Mats	48.6 fgh	9.3 c–f	9.1 fg	3.32 jk	3.8 ij	6.94 ab
Mustard	Biostrate	43.9 ij	11.9 a	14.0 cd	11.5 cd	6.40 a	6.92 ab
	Hemp	30.3 l	8.3 fgh	34.6 a	13.3 bc	5.52 bc	6.13 bcd
	Jute	39.7 k	11.4 ab	12.4 cde	16.4 a	6.75 a	7.09 ab
	Micro-Mats	42.1 jk	12.2 a	13.1 cd	5.75 g–j	4.33 ghi	8.55 a
Radish	Biostrate	61.3 a	9.9 cd	10.2 efg	5.52 hij	4.58 fgh	4.48 def
	Hemp	54.7 cd	9.9 cd	14.6 c	10.1 de	4.90 d–g	6.87 ab
	Jute	58.4 ab	10.3 bc	8.5 g	8.53 ef	4.76 e–h	4.86 cde
	Micro-Mats	57.4 bc	9.5 cde	9.0 g	4.49 jk	3.74 j	4.53 def
<i>p</i> -value	Species	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Substrate	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Species * Substrate <sup>4</sup>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

<sup>1</sup> Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at  $p \leq 0.05$ .

<sup>2</sup> Each mean was obtained by averaging data over both the fertilized and no-fertilizer treatments. <sup>3</sup> N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; S = sulfur. <sup>4</sup> Species \* Substrate suggests interaction between species and substrate type.

### 3.3.1. Nitrogen

Nitrogen concentration ranged from 35.3 mg·g<sup>-1</sup> in mustard grown with hemp to 64.1 mg·g<sup>-1</sup> in radish grown with micro-mats in January 2020 (Table 7) and from 30.3 mg·g<sup>-1</sup> in mustard grown with hemp to 61.3 mg·g<sup>-1</sup> in radish grown with biostrate in February 2020 (Table 8).

Radish microgreens had higher N concentration than other microgreen species when grown with any substrate type in January 2020 ranging from 54.7 mg·g<sup>-1</sup> in hemp to 64.1 mg·g<sup>-1</sup> in micro-mats. Mustard had the lowest N concentration among all microgreens grown with each substrate in both experiments ranging from 35.3 mg·g<sup>-1</sup> in hemp to 45.2 mg·g<sup>-1</sup> in biostrate in January, and from 30.3 mg·g<sup>-1</sup> in hemp mat to 43.9 mg·g<sup>-1</sup> in biostrate in February. In February, higher N concentrations were found in radish and broccoli than kale, cabbage, or mustard when grown with any substrate type. Hemp resulted in lower N concentrations in all five microgreens than the other three substrates in both experiments. One time post-emergent fertilization increased overall N concentration by 2.0% in February 2020 (Table 5).

### 3.3.2. Phosphorus

Phosphorus concentration ranged from 7.8 mg·g<sup>-1</sup> in cabbage grown with hemp to 12.5 mg·g<sup>-1</sup> in mustard grown with a jute mat in January 2020 (Table 7) and from



6.1 mg·g<sup>-1</sup> in cabbage grown with hemp to 12.2 mg·g<sup>-1</sup> in mustard grown with micro-mats in February 2020 (Table 8).

In January, four substrate types generally resulted in similar P concentrations in each microgreen, except that P concentration in mustard was lower when grown with hemp than other substrate types. Ranking of P concentration among microgreens varied in different substrate types, where mustard had the highest P concentrations of 12.2 mg·g<sup>-1</sup>, 12.5 mg·g<sup>-1</sup>, and 11.6 mg·g<sup>-1</sup> among the five species in three substrates including biostrate, jute mat, and micro-mats, respectively (Table 7). Phosphorus concentration in January was also affected by the interaction between microgreen species and fertilization treatment, where fertilization increased P concentration in cabbage and mustard, but resulted in similar P concentrations in broccoli, kale, and radish (Table 6).

In February, mustard grown with biostrate, jute, and micro-mats had comparable highest P concentrations ranging from 11.4 to 12.2 mg·g<sup>-1</sup> (Table 8). Hemp resulted in the lowest P concentrations of 7.6 mg·g<sup>-1</sup> and 8.3 mg·g<sup>-1</sup> compared to other substrate in broccoli and mustard, respectively. Fertilization increased overall P concentration by 8.4% compared to the no-fertilization treatment (Table 5).

### 3.3.3. Potassium

Potassium concentration ranged from 9.8 mg·g<sup>-1</sup> in kale grown with micro-mats to 37.3 mg·g<sup>-1</sup> in mustard grown with a hemp mat in January 2020 (Table 7) and from 8.2 mg·g<sup>-1</sup> in cabbage grown with micro-mats to 34.6 mg·g<sup>-1</sup> in mustard grown with a hemp mat in February 2020 (Table 8).

Hemp resulted in higher K concentrations than other substrate types in each microgreen in both experiments. Among all treatment combinations, the highest K concentrations were found in mustard grown with hemp in both experiments, 37.3 mg·g<sup>-1</sup> in January and 34.6 mg·g<sup>-1</sup> in February respectively. Cabbage had comparable K concentration with mustard in each substrate type in January. In February, broccoli and cabbage grown with hemp had the second highest K concentrations next to mustard grown with hemp.

Potassium concentration was also affected by the interaction between species and fertilization in January, where fertilization increased K concentrations in broccoli and mustard by 11.2% and 18.0%, but not in the other three microgreens (Table 6).

### 3.3.4. Calcium

Calcium concentration ranged from 3.12 mg·g<sup>-1</sup> in kale grown with micro-mats to 19.6 mg·g<sup>-1</sup> in cabbage grown with a hemp mat in January 2020 (Table 7) and from 2.35 mg·g<sup>-1</sup> in cabbage grown with micro-mats to 16.4 mg·g<sup>-1</sup> in mustard grown with a jute mat in February 2020 (Table 8).

The five microgreens varied in their Ca concentrations, and substrate type altered concentrations for each species. In January, cabbage and mustard had higher Ca concentrations than broccoli, kale, or radish when grown with biostrate, hemp, or jute mat. When grown with micro-mats, broccoli had higher Ca concentrations than the other four microgreens. The hemp mat resulted in the highest Ca concentrations of 19.6 mg·g<sup>-1</sup> in cabbage and 11.5 mg·g<sup>-1</sup> in kale, whereas the jute mat resulted in the highest Ca concentration of 17.2 mg·g<sup>-1</sup> in mustard.

There was not as much separation of means in February, where broccoli, cabbage, kale, and radish had similar Ca concentrations when grown with a jute mat or micro-mats. The hemp mat resulted in higher Ca concentrations in broccoli (14.3 mg·g<sup>-1</sup>) and cabbage (13.4 mg·g<sup>-1</sup>) than other substrates, whereas the jute mat resulted in higher Ca concentrations in kale (9.49 mg·g<sup>-1</sup>) and mustard (16.4 mg·g<sup>-1</sup>) than other substrate types.

### 3.3.5. Magnesium

Magnesium concentration ranged from 3.67 mg·g<sup>-1</sup> in cabbage grown with micro-mats to 7.59 mg·g<sup>-1</sup> in mustard grown with a jute mat in January 2020 (Table 7) and from

2.93 mg·g<sup>-1</sup> in cabbage grown in micro-mats to 6.75 mg·g<sup>-1</sup> in mustard grown with a jute mat in February 2020 (Table 8).

Broccoli, cabbage, and kale had similar Mg concentrations when grown with any of the four substrate types in January, and grown with biostrate or a hemp mat in February. Mustard had higher Mg concentrations than other microgreens when grown with biostrate or a jute mat in both experiments. Micro-mats resulted in lower Mg concentrations than the other three substrate types in each microgreen in January, and in broccoli, kale, cabbage, and mustard in February. One time fertilization decreased Mg concentration by 2.6% compared with the no-fertilization treatment (Table 5).

### 3.3.6. Sulfur

Sulfur concentration ranged from 4.31 mg·g<sup>-1</sup> in radish grown with biostrate to 9.93 mg·g<sup>-1</sup> in cabbage grown with micro-mats in January 2020 (Table 7) and from 2.72 mg·g<sup>-1</sup> in cabbage grown with micro-mats to 8.55 mg·g<sup>-1</sup> in mustard grown with micro-mats in February 2020 (Table 8), affected by the interaction between species and substrate interaction in both experiments.

In general, separation among microgreens within a substrate type, or among substrates within one microgreen were not as much in January or February. In January, radish had lower S concentrations of 4.31 mg·g<sup>-1</sup> to 5.64 mg·g<sup>-1</sup> than other microgreens when grown with biostrate, a jute mat, or micro-mats. The hemp mat, jute mat, and micro-mats resulted in similar S concentrations in broccoli (8.78–9.00 mg·g<sup>-1</sup>) and kale (7.70–8.72 mg·g<sup>-1</sup>). One time fertilization decreased S concentration by 3.6% compared with the no-fertilization treatment (Table 5). In February, four substrate types resulted in similar S concentrations in broccoli (6.14–7.58 mg·g<sup>-1</sup>) and cabbage (2.72–4.54 mg·g<sup>-1</sup>). Kale and cabbage had lower S concentrations than other microgreens when grown in a hemp mat. Broccoli, kale, and mustard had similar S concentrations when grown with a jute mat or micro-mats (Table 8).

## 3.4. Micronutrient Concentrations

Micronutrient concentrations including Cu, Fe, Mn, Zn, and B were all affected by the interaction between species and hydroponic pad type, but not affected by the fertilization treatment.

### 3.4.1. Copper

Copper concentration ranged from 0.51 to 1.92 µg·g<sup>-1</sup> in mustard grown with a hemp mat in January 2020 (Table 9) and from 0.39 to 5.09 µg·g<sup>-1</sup> in cabbage grown in a hemp mat in February 2020 (Table 10).

**Table 9.** Micronutrient concentrations in five microgreens affected by the interaction between species and hydroponic pad type in January 2020.

		January 2020				
Microgreens	Hydroponic Pad	Cu (µg·g <sup>-1</sup> ) <sup>1,2,3</sup>	Fe (µg·g <sup>-1</sup> )	Mn (µg·g <sup>-1</sup> )	Zn (µg·g <sup>-1</sup> )	B (µg·g <sup>-1</sup> )
Broccoli	Biostrate	0.51 d	94.8 d–i	35.1 i–l	78.8 de	16.5 h–k
	Hemp	0.51 d	86.2 f–i	45.7 ghi	74.4 def	34.6 bc
	Jute	0.71 cd	119.5 bc	62.7 de	110.5 b	23.9 e–h
	Micro-Mats	0.51 d	116.9 bcd	33.4 jkl	87.3 cd	17.1 g–j
Cabbage	Biostrate	0.51 d	113.3 b–e	58.4 def	76.1 de	19.9 f–i
	Hemp	0.51 d	134.7 ab	118.6 a	84.8 d	45.0 a
	Jute	0.51 d	156.3 a	116.5 a	133.3 a	28.0 cde
	Micro-Mats	0.51 d	134 ab	51.5 efg	78.5 de	23.9 d–g
Kale	Biostrate	1.29 bc	84.8 c–h	45.3 ghi	53.3 hij	9.65 k
	Hemp	0.67 d	97.4 c–h	65.9 cd	68.6 efg	37.3 b
	Jute	1.29 bc	90.3 e–i	74.3 c	75.3 def	14.5 ijk
	Micro-Mats	1.29 bc	90.4 e–i	39.0 h–k	50.8 ij	11.9 jk

Table 9. Cont.

January 2020						
Microgreens	Hydroponic Pad	Cu ( $\mu\text{g}\cdot\text{g}^{-1}$ ) <sup>1,2,3</sup>	Fe ( $\mu\text{g}\cdot\text{g}^{-1}$ )	Mn ( $\mu\text{g}\cdot\text{g}^{-1}$ )	Zn ( $\mu\text{g}\cdot\text{g}^{-1}$ )	B ( $\mu\text{g}\cdot\text{g}^{-1}$ )
Mustard	Biostrate	1.29 bc	107.3 c–f	49.4 fgh	67.6 e–h	26.3 def
	Hemp	1.92 a	101 c–g	42.7 g–k	61.1 f–i	52.0 a
	Jute	1.29 bc	112.8 b–e	88.4 b	100.5 bc	30.4 bcd
	Micro-Mats	1.29 bc	107 c–f	33.4 jkl	59.6 g–j	24.4 def
Radish	Biostrate	1.72 ab	83.4 ghi	31.5 kl	53.6 hij	11.5 jk
	Hemp	1.29 bc	79.8 ghi	44.6 g–j	57.4 g–j	22.8 f–h
	Jute	1.29 bc	76.9 hi	44.3 g–j	65.3 e–i	10.6 jk
	Micro-Mats	1.29 bc	74.0 i	25.3 l	47.3 j	11.5 jk
<i>p</i> -value	Species	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Substrate	0.5972	<0.0001	<0.0001	<0.0001	<0.0001
	Species * Substrate <sup>4</sup>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

<sup>1</sup> Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at  $p \leq 0.05$ .

<sup>2</sup> Each mean was obtained by averaging data over both the fertilized and no-fertilizer treatments. <sup>3</sup> Cu = copper; Fe = iron; Mn = manganese; Zn = zinc; B = boron. <sup>4</sup> Species \* Substrate suggests interaction between species and substrate type.

**Table 10.** Micronutrient concentrations in five microgreens affected by the interaction between species and hydroponic pad type in February 2020.

February 2020						
Microgreens	Hydroponic Pad	Cu ( $\mu\text{g}\cdot\text{g}^{-1}$ ) <sup>1,2,3</sup>	Fe ( $\mu\text{g}\cdot\text{g}^{-1}$ )	Mn ( $\mu\text{g}\cdot\text{g}^{-1}$ )	Zn ( $\mu\text{g}\cdot\text{g}^{-1}$ )	B ( $\mu\text{g}\cdot\text{g}^{-1}$ )
Broccoli	Biostrate	2.82 abc	80.4 b–e	29.2 f–i	63.7 bc	12.9 f–k
	Hemp	2.82 abc	71.1 def	40.8 c–f	60.0 bcd	26.4 bc
	Jute	1.20 bcd	81.2 b–e	47.5 bc	68.0 ab	19.5 c–g
	Micro-Mats	0.82 cd	75.9 c–f	25.8 hi	55.6 bcd	16.5 e–j
Cabbage	Biostrate	3.50 ab	62.7 fg	34.9 e–h	24.8 hg	12.0 g–k
	Hemp	5.09 a	70.6 def	56.6 b	30.9 fgh	21.3 b–f
	Jute	4.92 a	99.9 a	71.8 a	61.3 bcd	18.3 c–h
	Micro-Mats	0.88 cd	41.4 h	27.5 ghi	17.0 h	7.7 k
Kale	Biostrate	0.50 cd	42.6 h	37.5 c–h	26.3 hg	9.7 ijk
	Hemp	4.26 a	42.8 h	48.2 bc	21.0 hg	15.2 e–j
	Jute	0.39 d	83.3 bcd	76.3 a	70.2 ab	17.8 d–i
	Micro-Mats	0.39 d	65.7 efg	37.8 c–g	45.4 def	11.0 h–k
Mustard	Biostrate	0.39 d	94.8 ab	43.5 cde	66.4 ab	25.7 bcd
	Hemp	0.39 d	69.0 d–g	32.9 e–h	48.7 cde	37.9 a
	Jute	0.71 cd	90.1 abc	81.9 a	83.3 a	28.2 b
	Micro-Mats	0.39 d	94.5 ab	29.1 f–i	55.4 bcd	23.4 b–e
Radish	Biostrate	0.39 d	54.3 gh	20.2 i	48.6 cde	15.4 e–k
	Hemp	0.39 d	44.5 h	45.5 bcd	47.1 c–f	15.0 e–k
	Jute	0.39 d	53.7 gh	42.4 cde	61.9 bcd	10.2 h–k
	Micro-Mats	0.39 d	40.6 h	19.4 i	37.1 efg	8.7 jk
<i>p</i> -value	Species	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Substrate	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Species * Substrate <sup>4</sup>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

<sup>1</sup> Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at  $p \leq 0.05$ .

<sup>2</sup> Each mean was obtained by averaging data over both the fertilized and no-fertilizer treatments. <sup>3</sup> Cu = copper; Fe = iron; Mn = manganese; Zn = zinc; B = boron. <sup>4</sup> Species \* Substrate suggests interaction between species and substrate type.

Four hydroponic pad types resulted in similar Cu concentrations in radish and broccoli in both experiments, in cabbage in January, in mustard in February. Biostrate, jute mate,

and micro-mats resulted in similar Cu concentrations in Kale in both experiments and in mustard in January.

Radish, kale, and mustard had similar Cu concentrations when grown with biostrate or micro-mats, higher than broccoli or cabbage in January but lower than broccoli or cabbage in February when grown with biostrate. When grown with a hemp mat, broccoli, kale, and cabbage had similar Cu concentrations in both experiments. Micro-mats resulted in similar Cu concentrations among all five microgreens in February.

#### 3.4.2. Iron

Iron concentration ranged from  $74.0 \mu\text{g}\cdot\text{g}^{-1}$  in radish grown with micro-mats to  $156.3 \mu\text{g}\cdot\text{g}^{-1}$  in cabbage grown with a jute mat in January 2020 (Table 9) and from  $40.6 \mu\text{g}\cdot\text{g}^{-1}$  in radish grown with micro-mats to  $99.9 \mu\text{g}\cdot\text{g}^{-1}$  in cabbage grown with a jute mat in February 2020 (Table 10).

Four substrates resulted in similar Fe concentrations in radish, kale, and mustard in January and in radish and broccoli in February. Cabbage grown with a jute mat had the highest Fe concentrations of  $156.3 \mu\text{g}\cdot\text{g}^{-1}$  in January and  $99.9 \mu\text{g}\cdot\text{g}^{-1}$  in February among all treatment combinations, respectively. In addition, cabbage grown with a hemp mat and micro-mats also had higher Fe concentrations than other microgreens in January 2020. Mustard had higher Fe concentrations of  $94.8 \mu\text{g}\cdot\text{g}^{-1}$  and  $94.5 \mu\text{g}\cdot\text{g}^{-1}$  than other microgreens when grown with biostrate and micro-mats in February.

#### 3.4.3. Manganese

Manganese concentration ranged from  $25.3 \mu\text{g}\cdot\text{g}^{-1}$  in radish grown with micro-mats to  $118.6 \mu\text{g}\cdot\text{g}^{-1}$  in cabbage grown with a hemp mat in January 2020 (Table 9) and from  $19.4 \mu\text{g}\cdot\text{g}^{-1}$  in radish grown with micro-mats to  $81.9 \mu\text{g}\cdot\text{g}^{-1}$  in mustard grown with a jute mat in February 2020 (Table 10).

In January, cabbage had higher Mn concentrations of  $118.6 \mu\text{g}\cdot\text{g}^{-1}$ ,  $116.5 \mu\text{g}\cdot\text{g}^{-1}$ , and  $51.5 \mu\text{g}\cdot\text{g}^{-1}$  than other microgreens when grown with hemp, jute, or micro-mats, respectively. Hemp and jute mats resulted in similar Mn concentrations in kale, cabbage, and radish, higher than those in biostrate or micro-mats.

In February, hemp and jute mats resulted in higher Mn concentrations than biostrate or micro-mats in kale, cabbage, and radish, while a jute mat resulted in the highest Mn concentrations in broccoli and mustard.

#### 3.4.4. Zinc

Zinc concentration ranged from  $47.3 \mu\text{g}\cdot\text{g}^{-1}$  in radish grown with micro-mats to  $133.3 \mu\text{g}\cdot\text{g}^{-1}$  in cabbage grown with a jute mat in January 2020 (Table 9) and from  $17.0 \mu\text{g}\cdot\text{g}^{-1}$  in cabbage grown with micro-mats to  $83.3 \mu\text{g}\cdot\text{g}^{-1}$  in mustard grown with a jute mat in February 2020 (Table 10).

In January, broccoli and cabbage had higher Zn concentrations than kale, mustard, or radish when grown with a jute mat or micro-mats. The jute mat resulted in the highest Zn concentration of  $110.5 \mu\text{g}\cdot\text{g}^{-1}$ ,  $133.3 \mu\text{g}\cdot\text{g}^{-1}$ , and  $100.5 \mu\text{g}\cdot\text{g}^{-1}$  than the other three substrate types in broccoli, cabbage, and mustard, respectively.

In February, jute mat also resulted in higher Zn concentrations of  $61.3 \mu\text{g}\cdot\text{g}^{-1}$ ,  $70.2 \mu\text{g}\cdot\text{g}^{-1}$ , and  $83.3 \mu\text{g}\cdot\text{g}^{-1}$  than other substrate types in kale, cabbage, and mustard, respectively. Separation among microgreens was not as much when grown with a certain substrate.

#### 3.4.5. Boron

Boron concentration ranged from  $9.65 \mu\text{g}\cdot\text{g}^{-1}$  in kale grown with biostrate to  $45.0 \mu\text{g}\cdot\text{g}^{-1}$  in cabbage grown with a hemp mat in January 2020 (Table 9) and from  $7.7 \mu\text{g}\cdot\text{g}^{-1}$  in cabbage grown with micro-mats to  $37.9 \mu\text{g}\cdot\text{g}^{-1}$  in mustard grown with a hemp mat in February 2020 (Table 10).

In January, cabbage had higher B concentrations of  $45.0 \mu\text{g}\cdot\text{g}^{-1}$  and  $28.0 \mu\text{g}\cdot\text{g}^{-1}$  than other microgreens when grown with a hemp or jute mat, respectively. Kale, mustard, and

radish grown with biostrate, jute, and micro-mats had similar B concentrations, lower than most other treatment combinations. Hemp mat resulted in higher B concentrations of  $34.6 \mu\text{g}\cdot\text{g}^{-1}$ ,  $45.0 \mu\text{g}\cdot\text{g}^{-1}$ ,  $37.3 \mu\text{g}\cdot\text{g}^{-1}$ ,  $52.0 \mu\text{g}\cdot\text{g}^{-1}$ , and  $22.8 \mu\text{g}\cdot\text{g}^{-1}$  than other substrates in broccoli, cabbage, kale, mustard, and radish, respectively.

In February, mustard had higher B concentrations of  $23.4 \mu\text{g}\cdot\text{g}^{-1}$  in micro-mats to  $37.9 \mu\text{g}\cdot\text{g}^{-1}$  in jute mat than other microgreens when grown with each hydroponic pad type. Hemp resulted in higher B concentrations than other substrates in broccoli and mustard.

#### 4. Discussion

Fresh shoot yield is of the most limiting factor in microgreen production [4,20,23–25]. Microgreens varied in their fresh shoot yield, which was altered by hydroponic substrate type and fertilization treatment. Fresh, dry shoot weights, and dry weight percentage of tested microgreens in this current study are in similar ranges as our previous study when similar microgreen species/varieties were grown with a peat-based soilless substrate [26], suggesting selection of high-yielding microgreen species or varieties can potentially be applied to different growing systems including using peat-based substrate, or hydroponic pads of various types. Microgreen shoot yield (fresh or dry) results are also in agreement with previously reported ranges [9,20,21].

Microgreen yield varied between the two experiments, with higher fresh shoot weight, macro-, and micronutrient concentrations except for Cu in the January experiment than February (data not shown). This is likely due to the fluctuating microenvironment conditions in the greenhouse including temperature, relative humidity, and light conditions [26]. Air temperature was set to be  $25\text{ }^{\circ}\text{C}$  in the greenhouse, and was observed to fluctuate within  $8\text{ }^{\circ}\text{C}$  of the setting. Relative humidity ranged from 20% to 80%, and daily light integral ranged from 3 to  $15 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  within the experiment duration. Producers could experience similar changes in microgreens between production cycles. Supplemental lighting may help offset such fluctuations.

Fresh microgreen yield was shown to increase with increasing seed sowing rate. However, as seeding rate increases, individual shoot weight decreases and production cost increases since seeds contribute to a significant portion of microgreen production cost [4,18]. The sowing rates used in this study was recommended by the seed supplier ranging from  $60.5 \text{ g}\cdot\text{m}^{-2}$  in mustard to  $189 \text{ g}\cdot\text{m}^{-2}$  in radish (Table 1), equivalent to 17,182 seeds per  $\text{m}^2$  in radish to 33,611 seeds per  $\text{m}^2$  in mustard. This sowing rate was generally in agreement with reported ranges of 10,000–40,000 seeds per  $\text{m}^2$  for microgreens, yet crop-specific optimum sowing rate needs to be determined based on germination percentage, seed weight, and desired shoot density [27]. Besides fresh shoot yield, choice of microgreen species is also affected by appearance, texture, flavor, nutritional values, and phytochemical compositions, with genetic variability between and within taxa for traits of interest [9,28].

Compared to a number of studies investigating the effect of light on minerals and phytochemical concentrations in microgreens [19,29,30], there lacks standardization in cultural practices including fertilization application, or guidelines for microgreen quality [4,20,24,25]. One time post-emergent fertigation increased overall shoot height in both experiments, increased overall dry shoot weight and fresh shoot weight in kale and radish in January 2020, and increased various macronutrient concentrations in different microgreens. This is in agreement with our previous results evaluating ten microgreens using a peat-based soilless substrate, when one time post-emergent fertigation with  $100 \text{ mg}\cdot\text{L}^{-1}$  N also increased overall fresh shoot yield, macro-, and micronutrient concentrations in selected microgreen species. Murphy et al. [4] recommended daily fertigation with  $150 \text{ mg}\cdot\text{L}^{-1}$  N for optimal yield in arugula microgreens [18]. In addition to increased yield, large shoot height is a desirable characteristic in assisting with easy harvest of microgreens manually or mechanically [11]. Hydroponic pads generally have lower water holding capacity than peat-based substrate [21], microgreens grown in a hydroponic system may benefit from more frequent fertigation. Slow growing species may require more frequent fertilization than fast growing ones [1].

Mineral nutrient profiles varied among microgreens. For example, radish and broccoli had the highest N concentrations and mustard had the highest P concentration when grown in three substrate types except for hemp. Mustard also had the lowest N concentration among tested microgreens when grown with each substrate type. Ranking of most other mineral nutrients between microgreens varied among hydroponic pad types, suggesting substrate type can affect nutrient profile of microgreens, which should be taken into consideration in microgreen production. Analyses of 30 varieties of *Brassica* microgreens revealed that they are a rich source of mineral nutrients, especially K, Mg, Fe, and Zn [16]. Concentrations of macro- and micronutrients in the current study were generally in consistent ranges with previous reports. The five tested microgreens had lower concentrations of K, Cu, Fe, and B in this study grown with hydroponic pads than when grown with a peat-based potting mix in our previous study [26]. This agrees with Di Gioia et al. [21] in that peat resulted in higher nitrate, potassium, and sodium concentrations in microgreen rapini than three hydroponic pads including Sure to Grow (made from polyethylene-terephthalate), textile fiber, and jute-kenaf fiber. This may result from the facts that water holding capacity of peat was tested to be higher than hydroponic pads, and that peat itself contains higher mineral nutrient concentrations than the hydroponic pads [21]. Mineral nutrients may also change among harvest stages from microgreen stage 1, microgreen stage 2, baby leaf, to adult stage, with microgreen stages having higher mineral nutrient concentrations than the adult stage [5].

An ideal hydroponic growing substrate should be readily available, relatively inexpensive, and derived from renewable materials [21]. They should also have an adequate ratio between micropores to macropores to have high container capacity and sufficient air filled porosity. The four tested hydroponic pads are all constructed with biodegradable fibers with different physical and chemical characteristics. Hemp mat was superior in producing the highest shoot height, fresh and dry shoot weights in each microgreen species, or overall, in one or two experiments compared with the other three pad types. Hemp also resulted in the lowest N concentrations among substrate types in each microgreen in both experiments, which could result from the dilution effect of nutrient due to the highest fresh and dry shoot weights in microgreens. Fast growth and high yield of microgreens may benefit from fertilization with a high N formula, with caution on the balance between nitrate and ammonium forms of N since some microgreen crops like arugula tends to accumulate more nitrate [20]. However, microgreens generally have lower nitrate concentrations than their mature counterparts [10,12]. Compared to the hemp mat, jute, and micro-mats have denser texture, likely contributing to a higher water holding capacity but less air filled porosity, while biostrate is thin and lose moisture easily as observed.

Hydroponic pads constructed with different fiber materials can be sources of certain mineral nutrients [21]. A hemp mat could be a source of K and B, resulting in the highest K (in both experiments) and B concentrations (in January) in each microgreen. Highest micronutrient concentrations were mostly found in the jute or hemp mat if there was significant difference among substrate types, where the higher nutrient concentrations may also result from better shoot growth in these two substrate types. Further analyses of mineral compositions of different hydroponic pads will be required to confirm their effects on microgreen mineral nutrient concentrations.

Food safety concerns including microbial contamination by human pathogens in microgreen production has emerged with the expanding industry [7]. Microbial contamination can be introduced through seeds, growing substrate, equipment, or a lack of hygienic practices by workers [31]. Microgreens are more vulnerable to internalization of bacteria than mature vegetable plants [32]. However, foodborne pathogen outbreaks have not been associated with microgreen consumption as much as in sprouts, where sprouts are generally grown in a dark and moist condition that are more conducive to microbial proliferation [9,33–35]. We did observe mold-like occurrence on hydroponic pads, with higher mold counts on micro-mats and hemp mats, less in our previous study using a peat-based substrate [26]. It was recently reported that microgreens grown in a hydroponic

system and soil-substitute are both subject to pathogen proliferation with contaminated seeds, with higher microbial population in the hydroponic system [33,36,37]. Effective treatments for seed surface sterilization and antimicrobial action need to be developed for sustainable microgreen production [9,38]. Disinfecting practices should also be identified for hydroponic pads since they could be potential source of microbial contamination [21]. Growers should also select reputable supplier for certified microgreen or sprout seeds with high germination quality and potentially lower health risks.

## 5. Conclusions

Microgreens varied in their shoot growth and mineral nutrient concentrations. Hydroponic pads with varying physical and chemical properties affected shoot yield and mineral nutrients of microgreens. Microgreens in the hemp mat showed the highest fresh and dry shoot weight, likely due to the appropriate balance between water holding capacity and air filled porosity. One time post-emergent fertilization increased overall shoot height of tested microgreens in both experiments. Recommendation for fertilization should be adjusted to species/varieties and production conditions.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/horticulturae7060129/s1>, Figure S1: Growth stage of five microgreens at harvest including 'Waltham' broccoli (A), 'Red Acre' cabbage (B), 'Red Russian' kale (C), 'Red Garnet' mustard (D), and Rambo radish (E).

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