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Four herbal microgreen species varied in optimal seeding densities and growing substrate for maximized shoot yield and mineral nutrients

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Abstract

The microgreen industry in North America has experienced continuous growth, with an increasing number of species, including vegetables, herbs, and grains, being produced as microgreens. Cultural practices significantly impact the productivity and nutrient composition of various microgreen species. The hypothesis of this study was that optimal cultural practices, including seeding density and substrate type in microgreen production, should be species dependent. This study investigated shoot growth, visual quality, and mineral nutrient compositions of four herbal microgreen species, including basil, chives, scallion, and shiso, in two experiments as affected by five seeding densities, including 75, 150, 225, 300, and 375 g·m^{-2}, when grown with a peat-based soilless substrate, and a hydroponic mat made from jute fibers. Microgreen fresh shoot weights generally increased with increasing seeding density, with scallion producing the highest fresh shoot weight at 375 g·m^{-2}, whereas the 375 g·m^{-2} seeding density increased fresh shoot weight compared with 75 to 225 g·m^{-2}, but resulted in similar fresh shoot yield to 300 g·m^{-2} in basil, chives, and shiso. Scallion and shiso microgreens produced higher fresh shoot weight when grown on peat-based substrate, while basil and chives produced higher fresh shoot weight on jute mat compared with peat in one or both experiments. Substrate type also altered mineral nutrient concentrations in tested microgreens with peat substrate increasing phosphorus (P), potassium (K), calcium (Ca), and sulfur (S), and jute mat increasing nitrogen (N) and magnesium (Mg) in one or more species.

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Introduction

Microgreens can be defined as young seedlings grown to be harvested at an early stage of development, usually 7 to 21 d after sprouting occurred[1,2]. A large number of species and varieties from families including Alliaceae, Amaranthaceae, Apiaceae, Asteraceae, Brassicaceae, Fabaceae, and Lamiaceae have been reported to be grown as microgreens, including vegetables, herbs, and grains[3−5]. The global microgreens market was valued at 1.3 billion USD in 2019 and is projected to grow to 2.2 billion USD by 2028, with a compound annual growth rate of 11.1% from 2021 to 2028[6]. North America accounted for 50% of global sales in 2019 and is expected to maintain this dominance, led by the United States, Mexico, and Canada. By 2030, the North American and European microgreen markets are estimated to grow to 960 million USD and 715 million USD, respectively[6].

Microgreens are considered a functional food due to high levels of mineral nutrients, and health-beneficial phytochemicals, which include glucosinolates, ascorbic acid, tocopherols, carotenoids, and phenolic compounds[7,8]. Interest in microgreens has been on the rise in recent years as consumer preference for a healthy diet has increased[9]. Species-specific nutrient profiles highlight the diversity of microgreen species. For example, red cabbage (*Brassica oleracea* var. capitata) is characterized by high levels of Ca and S[10−12], while amaranth (*Amaranthus tricolor*) offers significant levels of Mg and nitrate[2]. Scallion (*Allium fistulosum*) accumulates elevated nitrate, Ca, and S concentrations, consistent with the known sulfur-rich profile of *Allium* species[13,14]. Lemon balm (*Melissa officinalis*) and shiso (*Perilla frutescens*), both members of the Lamiaceae family, are rich in phenolic compounds such as rosmarinic acid, and contain high P and nitrate levels[14,15]. These findings support the growing consensus that microgreens offer a concentrated source of essential minerals and phytochemicals, contributing meaningfully to dietary quality even in small serving sizes.

Microgreens are commonly grown on peat-based substrates due to their high porosity, and water holding capacity, which are conducive to fresh shoot production[10,16,17]. Peat-based substrates are considered a non-renewable resource and are becoming increasingly expensive, which has sparked interest in exploring alternative substrates in microgreen production[17]. Alternative mat products made from fibers, including felt, bamboo, coconut coir, hemp, jute etc., have been used to cultivate microgreens[16−19]. Compared to peat substrate, jute mats resulted in similar flavor, fresh shoot yield, phenolic, and chlorophyll content in mustard (*Brassica nigra*) microgreens, but required more frequent irrigation[18]. Several substrates including mats made from felt, hemp, and jute fibers resulted in similar fresh and dry shoot weights in leek (*Allium ampeloprasum*) and parsley (*Petroselinum crispum*) microgreens compared with peat substrate[19]. Alternative substrate types also altered mineral nutrients and phytochemical compositions of varying microgreens[16,18−20]. The effects of substrate type on microgreen growth, nutrient profile, and bioactive compound concentrations varied among reports, microgreens species, and merit further investigation[21].

Seeds are the largest recurring expense and a significant part of production cost in microgreen cultivation. Factors including average seed weight, germination percentage, the desired shoot density, and fresh shoot yield should all be considered when deciding the optimal seeding rate for a given species[22]. However, seeding rates recommended by microgreen seed suppliers varied significantly from each other and are not necessarily reliable. Microgreen producers commonly rely on in-house experimentation when determining the seeding rate, which may not be optimal and can lead to inconsistencies in production[18]. Fresh shoot yield of several microgreens species was reported to increase as seeding density increased[23,24]. Murphy & Pill reported that increasing seed sowing rate increased shoot number and shoot fresh weight per unit area, but decreased mean fresh weight per shoot for arugula microgreens (*Eruca vesicaria* subsp. sativa)[25]. Increasing seeding rates also increases seed cost, affects shoot height, and growth rate, and may affect visual and eating quality as well as profitability of microgreen production, according to multiple reports[23,25,26]. Scientific research needs to determine the optimum seeding densities for various microgreen species based on shoot yield, quality, and nutrient compositions.

The lack of species-specific recommendations for cultural practices, as well as their effects on productivity and microgreen nutrient compositions has hindered the adoption of various species to be used in microgreen production. It is hypothesized that optimal cultural practices, including seeding density and substrate type, in microgreen production should be species dependent. Therefore, the objective of this study was to evaluate the effect of five seeding densities and two growing substrates on the shoot growth and mineral nutrient compositions of four herbal microgreen species.

Materials and methods

Plant cultivation and experiment setup

Four herbal microgreen species, including chives (*Allium schoenoprasum*), scallion (*Allium fistulosum* cv. 'Evergreen Hardy White'), shiso (*Perilla frutescens var. crispa*), and basil (*Ocimum basilicum* cv. 'Dark Opal') were evaluated for shoot growth, visual quality, and mineral nutrient concentrations (Table 1). Chives and shiso seeds were purchased from True Leaf Market (Salt Lake City, UT, USA). Scallion and basil seeds were purchased from Johnny's Selected Seeds (Winslow, ME, USA). The study was carried out in a greenhouse located at Mississippi State University (33.4552° N, 88.7944° W) with temperature set at 25 °C day/night for the duration of the experiment, and with no supplemental lighting. This study contained two experiments, the first was initiated on January 3, 2023 (referred to as the January experiment), and the second initiated on January 31, 2023 (referred to as the February experiment).

Seeding density recommendations for selected species varied drastically among suppliers, ranging approximately from 80 to 350 g·m^{-2}, up to six times different for a given species. Therefore, five seeding densities, including 75, 150, 225, 300, and 375 g·m^{-2} were designed and tested in the current study, to determine the optimum seeding density for the four selected species. Each microgreen was grown with two types of substrates, including a peat-based substrate (PRO-MIX BX General Purpose, Premier Tech Horticulture, Quebec, Canada), and a hydroponic mat made from jute fibers (True Leaf Market, Salt Lake City, UT, USA). The jute mats were precut and measured 25 cm, by 25 cm which allowed the mats to fit in the selected growing trays. The growing trays used for both substrate types were black plastic with dimensions measuring 25.72 cm × 25.72 cm, with a depth of 6.03 cm (T.O. Plastics, Inc., Clearwater, MN, USA). Trays used for the peat-based substrate had drainage holes, while those used for the jute mat did not have drainage holes. Jute mats were hydrated by being soaked in tap water for approximately five minutes before use. Excess water was allowed to drain, and the saturated mats were then placed in trays to be used for planting. Microgreen seeds were manually sown by spreading the seeds at various seeding rates evenly over the substrate surface. After seed sowing, a thin layer of peat was added on top of each tray filled with peat substrate to provide the dark environment that is beneficial for germination. The trays with jute mats were covered with another black plastic tray during germination, and then removed 6 to 7 d after planting (DAP). Trays with jute mats were irrigated once or twice daily as needed, by misting with a hand-held half-gallon sprayer until saturation. Trays with peat substrate were overhead irrigated with a water hose, every day, or every other day as needed. No fertilizer was applied to the microgreens in either experiment.

Data collection

Microgreen shoot height was measured from the substrate surface to the tallest point of shoot growth before harvest, with one height measured approximately at the center of each tray. A visual quality rating was given to each tray by evaluating the percentage of microgreens shoot coverage out of the entire growing area using a scale of 1 to 5 with 20% increments: 1 suggesting 20% or less surface coverage, 2 suggesting 20% to 40% coverage, 3 suggesting 40% to 60% coverage, 4 suggesting 60 to 80% coverage, and 5 suggesting over 80% growth coverage with healthy growth. Once the plant height and visual rating data were collected, microgreens were harvested at the stage with expanding cotyledon(s), or the first true leaf just above the substrate surface. Fresh shoot weight for each tray was immediately measured after harvesting. Individual shoot weight from each tray was calculated by measuring the fresh weight of 100 shoots sampled from a representative area and divided by 100. Harvested microgreens were then dried in an oven at 60 °C, to the point where a constant weight was achieved. Dry shoot weight of microgreens harvested from each tray was measured.

Mineral nutrient analyses

Oven-dried microgreen samples were processed using a grinder (Wiley mini mill, Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1 mm sieve and then used for mineral nutrient analyses. Combustion analysis with 0.25 g of the dry microgreen samples was conducted to measure the total N concentration using an elemental analyzer (vario MAX cube; Elementar Americas Inc., Long Island, NY, USA). Dried samples were also tested for P, K, Ca, Mg, and S, using inductively coupled plasma optical emission spectrometry (SPECTROBLUE; SPECTRO Analytical Instruments, Kleve, Germany). Concentrations of macronutrient (mg·g^{-1}) in microgreen samples

Table 1. Microgreen species, seed sowing rate, germination percentage, hundred-seed weight, and harvest date of four herbal microgreens species.

Common name^z	Scientific name	Seeding rates^y (g·m^{-2})	Germination percentage	100 Seed weight (g)	Harvest date (DAP)
Basil	*Ocimum basilicum* cv. 'Dark Opal'	75–375	90%	0.12 ± 0.0048	16
Chives	*Allium schoenoprasum*	75–375	90%	0.13 ± 0.0035	19
Scallion	*Allium fistulosum* cv. 'Evergreen Hardy White'	75–375	94%	0.24 ± 0.0046	16–18
Shiso	*Perilla frutescens var. crispa*	75–375	91%	0.44 ± 0.0096	16

^{z} Seed source of each species is as follows: basil (www.johnnyseeds.com/vegetables/microgreens/microgreen-herbs/basil-dark-opal-microgreen-seed-902M.html); chives (https://trueleafmarket.com/products/chives-microgreens-seeds); scallion (https://www.johnnyseeds.com/vegetables/microgreens/microgreen-vegetables/scallion-evergreen-hardy-white-microgreen-seed-502M.html?); shiso (https://trueleafmarket.com/products/shiso-seeds-perilla-green?jml_clid=123e531c8f6aff4c2a482a5831e44b94c97f3346&aidaptive_cohort=test). ^{y} Each microgreen species was grown with five seeding densities including 75, 150, 225, 300, and 375 g·m^{-2}.

were presented on a dry weight basis. The mineral nutrient analyses were conducted by the Mississippi State University Extension Service Soil Testing Laboratory (MS, USA).

Experimental design and data analyses

This study was conducted in a randomized complete block design with a factorial arrangement of treatments for both experiments. The three experimental factors included microgreen species (4), seeding density (5), and the type of substrate (2), resulting in 40 treatment combinations. Each experiment consisted of five replications, with one tray in each replication and each tray serving as an experiment unit. The significance of any main effect and interactions among the main factors were determined by the analysis of variance (ANOVA) using the PROC GLIMMIX procedure in SAS (version 9.4, SAS Institute, Cary, NC, USA). Means were separated using Tukey's honest significance difference (HSD) test at $\alpha \leq 0.05$. All statistical analysis was conducted using SAS.

Results

Shoot height

In the January experiment, shoot height varied among microgreen species and was not affected by seeding density or substrate type (Table 2). Shoot height in the February experiment was affected by the two-way interactions between microgreen species and seeding density and between substrate type and seeding density (Tables 2 and 3).

In the January experiment, chives, and scallion microgreens produced significantly larger shoot heights of 8.1 and 8.8 cm, respectively, than basil with a shoot height of 4.6 cm (Table 2). As affected by the interaction between species and seeding density in the February experiment, scallion microgreens grown with the seeding densities of 225 to 375 g·m^{-2} produced the highest shoot height, ranging from 8.8 to 9.1 cm, among all treatment combinations. Shoot height varied among the five seeding densities for chives, scallion, and basil during the February experiment. Higher seeding densities of 300 and 375 g·m^{-2} resulted in larger shoot height than 75 or 150 g·m^{-2} in chives and scallion microgreens. The highest seeding density of 375 g·m^{-2} also increased shoot height compared with 75 g·m^{-2} in shiso microgreens.

When affected by the species and substrate interaction in February 2023, peat resulted in higher shoot height measuring 7.1, 9.0, and 6.2 cm in chives, scallion, and shiso compared with jute mat, which produced shoot heights of 6.4, 8.1, and 5.0 cm, respectively. No significant difference in shoot height was found in basil microgreens between the two substrate types (Table 3).

Visual rating

Visual rating in both experiments was affected by the two-way interactions between microgreen species and seeding density, and between substrate type and seeding density (Tables 2 and 3).

When affected by the species and seeding density interaction, visual ratings were similar with all five seeding densities in scallion in January, and in shiso in both experiments (Table 3). The seeding densities of 300 and 375 g·m^{-2} resulted in higher visual rating scores of 4.5 in January and 5 in February, compared with 75 g·m^{-2} in chives in both experiments. The seeding densities of 150 to 375 g·m^{-2} all resulted in a visual rating of 5 in scallion microgreens in the February experiment, higher than that from 75 g·m^{-2}. In basil microgreens, the lowest seeding density of 75 g·m^{-2} resulted in a higher visual rating of 4.6 compared with 300 or 375 g·m^{-2}, with ratings of 3.0 to 3.2 in January due to shoot rotting in basil

Table 2. Shoot height, visual rating, fresh and dry shoot weights, and individual shoot weight that varied among species or affected by the interaction between microgreen species and seeding density in two experiments in January and February 2023.

Species	Seeding density (g·m^{-2})	January 2023					February 2023				
		Shoot height^{z,y} (cm)	Visual rating (1–5)	Fresh shoot weight (g·m^{-2})	Dry shoot weight (g·m^{-2})	Individual shoot weight (mg)	Shoot height (cm)	Visual rating (1–5)	Fresh shoot weight (g·m^{-2})	Dry shoot weight (g·m^{-2})	Individual shoot weight (mg)
Basil	75	4.6 b	4.6 a–c	480 l	34.1 l	15.9 e–g	4.4 j	4.3 a–d	455 jk	33.8 j	15.1 c
	150		4.2 a–e	797 i–l	53.9 j–l	14.9 f–h	4.8 ij	4.8 ab	880 g–i	69.0 h–j	
	225		3.6 c–f	1,037 g–j	74.3 h–k	15.1 f–h	5.0 h–j	3.9 cd	859 g–k	65.9 h–j	
	300		3.0 f	1,505 d–g	120.3 ef	14.6 f–h	4.9 ij	3.7 d	1,291 d–g	91.5 f–h	
	375		3.2 ef	1,605 d–f	121.8 ef	15.6 e–h	5.1 h–j	4 b–d	1,459 d–f	112.4 e–g	
Chives	75	8.1 a	3.4 d–f	339 l	34.9 l	11.6 h	5.4 g–i	4 b–d	327 k	36.5 j	10.8 d
	150		4.3 a–d	645 j–l	66.4 i–k	11.6 h	6.5 de	4.8 ab	734 h–k	77.5 g–i	
	225		4.2 a–e	1,000 h–k	100.3 f–h	12.2 gh	7.0 cd	4.9 a	1,106 e–h	114.1 e–g	
	300		4.7 ab	1,400 e–h	131.5 de	12.8 gh	7.4 c	5 a	1,593 b–e	162.6 b–d	
	375		4.5 ab	1,782 de	164.3 bc	12.3 gh	7.5 c	5 a	2,016 bc	193.2 ab	
Scallion	75	8.8 a	3.8 b–f	532 kl	44.9 kl	19.4 de	7.5 c	4 b–d	498 jk	51.3 ij	18.5 b
	150		4.4 a–d	1,066 g–j	86.3 g–i	19.7 de	8.3 b	5 a	1,055 f–i	96.2 f–h	
	225		4.6 a–c	1,806 de	143.0 c–e	17.4 d–f	8.8 ab	5 a	1,498 c–f	140.0 de	
	300		4.5 a–c	2,386 bc	183.6 b	19.6 de	9.1 a	5 a	2,059 b	179.3 bc	
	375		4.7 ab	2,980 a	230.7 a	21.2 d	9.1 a	5 a	2,629 a	227.0 a	
Shiso	75	6.9 ab	4.8 ab	766 j–l	48.0 kl	53.6 a	4.7 j	4.7 a–c	568 i–k	46.4 ij	36.4 a
	150		5.0 a	1,266 f–i	80.6 h–j	45 b	5.5 f–h	5 a	1,109 e–h	83.1 f–i	
	225		4.7 ab	1,759 de	114.9 e–g	43 b	5.7 fg	4.8 ab	1,319 d–g	118.2 ef	
	300		4.8 ab	1,983 cd	127.7 ef	37.5 c	6.0 e–g	4.8 ab	1,612 b–e	143.4 c–e	
	375		4.7 ab	2,595 ab	160.7 b–d	38.4 c	6.1 ef	4.5 a–d	1,706 b–d	163.0 b–d	
p-value	Species	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Density	0.15	0.24	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.40
	Interaction	0.36	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.24

[z] Different lower-case letters within a column suggest there is a significant difference among means as indicated by Tukey's HSD at $p < 0.05$. [y] Means for a species for shoot height in January, and individual shoot weight in February 2023 were averaged across all five seeding densities and both substrates from a total of 50 data points; and when the interaction between species and seeding density was significant, each mean was sourced from a total of 10 data points from both the jute and peat substrates.

Table 3. Shoot height, visual rating, fresh and dry shoot weights, and individual shoot weight that varied among species or affected by the interaction between microgreen species and substrate type in January and February 2023.

Species	Substrate	January 2023					February 2023				
		Shoot height[z,y] (cm)	Rating (1–5)	Fresh shoot weight (g·m^{-2})	Dry shoot weight (g·m^{-2})	Individual shoot weight (mg)	Shoot height (cm)	Rating (1–5)	Fresh shoot weight (g·m^{-2})	Dry shoot weight (g·m^{-2})	Individual shoot weight (mg)
Basil	Peat	4.6 b	2.8 c	1131 c	98.8 c	15.9 de	4.9 e	3.6 b	749 d	74.5 c	16.3 d
	Jute		4.6 a	1,039 cd	62.9 e	14.6 ef	4.8 e	4.6 a	1,229 bc		13.8 e
Chives	Peat	8.1 a	3.7 b	831 d	82.4 d	13.0 fg	7.1 c	4.7 a	1,083 c	116.8 b	11.1 f
	Jute		4.8 a	1,236 c	116.6 b	11.2 g	6.4 d	4.8 a	1,227 c		10.5 f
Scallion	Peat	8.8 a	4.0 b	1,953 a	144.3 a	22.0 c	9.0 a	4.8 a	1,631 a	138.7 a	20.2 c
	Jute		4.8 a	1,555 b	131.1 ab	16.9 d	8.1 b	4.8 a	1,464 ab		16.8 d
Shiso	Peat	6.9 ab	4.6 b	2,124 a	118.3 b	56.1 a	6.2 d	4.6 a	1,525 a	110.8 b	43.2 a
	Jute		4.9 a	1,223 c	94.5 cd	30.9 b	5.0 e	4.9 a	1,000 cd		29.6 b
p-value	Species	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Substrate	0.29	< 0.0001	< 0.0001	0.0006	< 0.0001	< 0.0001	< 0.0001	0.72	0.086	< 0.0001
	Interaction	0.58	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.51	< 0.0001

[z] Different lower-case letters within a column suggest significant difference among means as indicated by Tukey's HSD at $p < 0.05$. [y] Means for shoot height in January, and dry shoot weight in February 2023 were averaged across all five seeding densities and both substrate types from a total of 50 data points; and when the interaction between species and substrate type was significant, each mean was sourced from a total of 25 data points averaged across all five seeding densities.

microgreens when grown with higher seeding densities of 300 and 375 g·m^{-2}. The seeding density of 150 g·m^{-2} also resulted in a higher visual rating in basil than 225 or 300 g·m^{-2} in February.

When affected by the species and substrate interaction, visual rating was higher in each tested species when grown on the jute mat compared to the peat substrate in January (Table 3). In the February experiment, visual ratings were similar, ranging from 4.6 to 4.9 in chives, scallion, and shiso microgreens grown with either substrate. Whereas the jute mat increased the visual rating in basil compared with peat.

Fresh shoot weight

Fresh shoot weight was affected by the interaction between microgreen species and seeding density, as well as the interaction between species and substrate type in both experiments (Tables 2 and 3).

When affected by the species and seeding density interaction, fresh shoot weight had a generally increasing trend as seeding density increased within a species in both experiments (Table 2). In January, the highest seeding density of 375 g·m^{-2} resulted in higher fresh shoot weight than 75 to 225 g·m^{-2} in basil and chives microgreens, and higher than seeding densities of 75 to 300 g·m^{-2} in scallion and shiso microgreens. Scallion and shiso grown at 375 g·m^{-2} produced the highest fresh shoot weights of 2,980 and 2,595 g·m^{-2}, respectively, among all treatment combinations. In the February experiment, the highest seeding density of 375 g·m^{-2} resulted in higher fresh shoot weight than all other seeding densities in scallion, than 75 to 300 g·m^{-2} in basil and chives, and higher than 75 or 150 g·m^{-2} in shiso microgreens.

When affected by the interaction between microgreen species and substrate, scallion and shiso produced higher fresh shoot weights of 1,953 and 2,124 g·m^{-2} when grown on the peat substrate compared to the jute substrate, which produced fresh shoot weights of 1,555 and 1,223 g·m^{-2}, respectively, in the January experiment. Chives microgreens produced higher fresh shoot weight of 1,236 g·m^{-2} on the jute mat compared to 831 g·m^{-2} when grown with peat. Peat and jute mat resulted in similar fresh shoot weight in basil microgreens in January 2023 (Table 3). In the February experiment, basil microgreens grown on jute produced a higher fresh shoot weight of 1,229 g·m^{-2} compared to 749 g·m^{-2} when grown on peat. Chives and scallion produced similar fresh shoot weights between the two types of substrates within a species. Shiso microgreens produced a higher fresh shoot weight of 1,525 g·m^{-2} when grown on the peat substrate compared with jute resulting in a fresh shoot weight of 1,000 g·m^{-2}.

Dry shoot weight

Dry shoot weight was affected by the interaction between species and seeding density in both experiments (Table 2). Dry shoot weight in the January experiment was also affected by the interaction between species and substrate type and varied among species in the February experiment (Table 3).

When affected by the species and seeding density interaction, high seeding densities of 300 and 375 g·m^{-2} resulted in higher dry shoot weights in each species than seeding densities of 75 to 225 g·m^{-2} in the January experiment (Table 2). While in the February experiment, the seeding density of 375 g·m^{-2} resulted in higher dry shoot weight in each species than 75 to 225 g·m^{-2}. Dry shoot weight increased significantly with increasing seeding density from 75 to 375 g·m^{-2} in chives in January, and in scallion in both experiments. Overall, scallion microgreens grown with the highest seeding density of 375 g·m^{-2} produced the highest dry shoot weights of 230.7 g·m^{-2} in January, and 227.0 g·m^{-2} in February among all treatment combinations, respectively.

When affected by the species and substrate type interaction in the January experiment, basil and shiso produced higher dry shoot weights of 98.8 and 118.3 g·m^{-2}, respectively, on the peat-based substrate compared to 62.9 and 94.5 g·m^{-2} on the jute mats (Table 3). Whereas the jute mat resulted in a higher dry shoot weight of 116.6 g·m^{-2} in chives compared with peat with dry shoot weight of 82.4 g·m^{-2}. Scallion had similar dry shoot weights when grown on the peat and jute substrates. In the February experiment, dry shoot weight varied among species and was not affected by substrate type. The ranking of dry shoot weight among microgreen species was: scallion (138.7 g·m^{-2}) > chives (116.8 g·m^{-2}), or shiso (110.8 g·m^{-2}) > basil (74.5 g·m^{-2}).

Individual shoot weight

Species and seeding density significantly interacted to affect the individual shoot weight in the January experiment (Table 2). Basil, chives, and scallion had similar individual shoot weights within a species when grown with five different seeding densities. Shiso produced a significantly higher individual shoot weight of 53.6 mg when seeded at 75 g·m^{-2} compared to the other seeding densities, ranging from 37.5 to 45.0 mg. In the February experiment, individual shoot weight varied among species and was not affected by seeding density. The ranking of individual shoot weight among species was: shiso (36.4 mg) > scallion (18.5 mg) > basil (15.1 mg) > chives (10.8 mg).

Individual shoot weight was also affected by the interaction between species and substrate type in both January and February 2023 (Table 3). The two substrates resulted in similar individual shoot weight in basil in January and in chives in both experiments. Peat substrate increased individual shoot weight in scallion and shiso microgreens in both experiments and in basil microgreens in the February experiment.

Nitrogen concentration

Nitrogen concentration was affected by the interactions between microgreen species and seeding density (Tables 4 and 5), and between species and substrate type in both experiments (Tables 6 and 7).

When affected by the species and seeding density interaction, the five seeding densities resulted in similar N concentrations in basil (47.95 to 52.33 mg·g^{-1}) and scallion (42.97 to 46.61 mg·g^{-1}) in the January experiment, and in basil (41.57 to 43.07 mg·g^{-1}) in the February experiment (Tables 4 and 5). Chives had significantly higher N concentrations when grown at 375 g·m^{-2} compared to 75 or 150 g·m^{-2} in both experiments. High seeding densities of 300 and 375 g·m^{-2} resulted in higher N concentration in shiso microgreens than seeding densities of 75 to 225 g·m^{-2} in the February experiment. Overall, shiso microgreens grown at 225 to 375 g·m^{-2} had the highest N concentrations of 54.31 to 58.27 mg·g^{-1} among all treatment combinations in January. Similarly, in February 2023, shiso microgreens grown at 225 to 375 g·m^{-2} and chives grown at 225 and 375 g·m^{-2} had the highest N concentrations, ranging from 49.4 to 52.45 mg·g^{-1}, among all treatment combinations.

When affected by the species and substrate interaction, all four microgreen species produced higher N concentrations when grown with jute mat compared to the peat substrate in both experiments (Tables 6 and 7). In January, basil, chives, and shiso grown with jute had similarly the highest N concentrations of 59.07, 57.14, and 59.09 mg·g^{-1}, respectively, among all treatment combinations. In February 2023, chives and shiso grown on jute had the highest N concentrations of 52.06 and 52.03 mg·g^{-1} compared to the other treatment combinations. Basil grown with peat substrate produced the lowest N concentration in both experiments.

Phosphorus concentration

Phosphorus concentration was affected by the interactions between microgreen species and seeding density (Tables 4 and 5) and between species and substrate type in both experiments (Tables 6 and 7).

When affected by the species and seeding density interaction, P concentrations were generally similar within a given species except for minor separations within basil and shiso in both experiments (Tables 4 and 5). In January 2023, shiso microgreens had the highest N concentrations, ranging from 12.11 to 13.71 mg·g^{-1}, compared with the other species at each seeding density (Table 4). Chives had the lowest P concentrations, ranging from 5.32 to 6.05 mg·g^{-1}, among species regardless of seeding density. In February 2023, basil and shiso had higher P concentrations than chives or scallion regardless of seeding density (Table 5).

When affected by the species and substrate interaction, peat increased P concentrations in basil and scallion microgreens in January and in shiso in both experiments, and otherwise resulted in similar P concentrations within a species (Tables 6 and 7). Shiso microgreens grown with peat had the highest P concentrations of 14.50 mg·g^{-1} in January and of 13.59 mg·g^{-1} in February among all treatment combinations.

Potassium concentration

Potassium concentration was affected by the interactions between species and seeding density (Tables 4 and 5) and between

Table 4. Nitrogen, phosphorous, potassium, calcium, magnesium, and sulfur concentrations as affected by the species and seeding density interaction in January 2023.

Species	Seeding density (g·m^{-2})	Nitrogen^{z,y} (mg·g^{-1})	Phosphorous (mg·g^{-1})	Potassium (mg·g^{-1})	Calcium (mg·g^{-1})	Magnesium (mg·g^{-1})	Sulfur (mg·g^{-1})
Basil	75	47.95 c–f	9.49 cd	30.28 bc	10.36 b–e	4.37 de	3.67 b–d
	150	52.33 bc	10.01 c	26.66 c–e	10.01 de	4.66 de	3.72 b–d
	225	48.63 c–f	9.29 cd	22.85 d–g	10.42 b–e	4.57 de	3.59 cd
	300	48.71 c–f	8.52 de	18.82 g–j	10.62 b–e	4.21 e	3.50 cd
	375	51.45 b–d	9.81 c	20.35 f–i	10.67 b–e	4.82 d	3.48 cd
Chive	75	46.43 e–g	5.75 gh	22.29 e–h	10.37 b–e	3.19 f	4.71 a
	150	47.12 d–g	5.32 h	16.78 ij	10.17 de	3.11 f	3.57 cd
	225	51.29 b–e	5.99 gh	17.68 h–j	10.08 de	3.32 f	3.76 b–d
	300	51.46 bc–d	6.05 gh	16.47 ij	10.39 b–e	3.42 f	3.77 b–d
	375	52.37 bc	5.47 h	13.82 j	9.422 e	3.20 f	3.34 d
Scallion	75	42.97 g	7.48 ef	33.78 b	12.33 a	4.35 de	4.37 ab
	150	44.23 fg	7.22 f	26.80 c–e	11.35 a–d	4.37 de	4.16 a–c
	225	46.58 d–g	7.37 ef	25.82 c–e	10.51 b–e	4.30 de	3.68 b–d
	300	46.47 e–g	7.38 ef	22.20 e–h	10.36 b–e	4.49 de	3.17 d
	375	46.61 d–g	6.86 fg	18.63 g–j	10.29 c–e	4.70 de	3.50 cd
Shiso	75	49.46 b-–e	12.11 b	39.01 a	11.88 ab	4.62 de	2.22 e
	150	52.53 bc	11.97 b	30.16 bc	11.07 a–d	5.75 c	2.12 e
	225	54.31 ab	12.73 ab	27.69 cd	11.47 a–d	6.26 c	2.21 e
	300	57.55 a	13.12 ab	24.90 d–f	11.06 a–d	7.38 b	2.28 e
	375	58.27 a	13.71 a	24.63 d–f	11.83 a–c	8.00 a	2.28 e
p-value	Species	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Seeding density	< 0.0001	0.32	< 0.0001	0.0086	< 0.0001	< 0.0001
	Interaction	0.0001	0.0002	0.0004	0.0009	< 0.0001	< 0.0001

[z] Different lower-case letters within a column suggest there is a significant difference among means as indicated by Tukey's HSD at $p < 0.05$. [y] Each mean was sourced from a total of 10 data points from both the jute and peat substrates.

Table 5. Nitrogen, phosphorous, potassium, calcium, magnesium, and sulfur concentrations as affected by the species and seeding density interaction in February 2023.

Species	Seeding density ($g \cdot m^{-2}$)	Nitrogen[z,y] ($mg \cdot g^{-1}$)	Phosphorous ($mg \cdot g^{-1}$)	Potassium ($mg \cdot g^{-1}$)	Calcium ($mg \cdot g^{-1}$)	Magnesium ($mg \cdot g^{-1}$)	Sulfur ($mg \cdot g^{-1}$)
Basil	75	41.57 i–k	12.23 a	40.82 a	13.51 ab	5.59 a	2.86 a–e
	150	41.74 h–k	11.39 ab	31.91 ab	13.95 a	5.46 a	2.67 b–e
	225	43.07 g–j	11.13 ab	28.37 ab	13.22 ab	5.46 a	3.26 a–c
	300	42.29 h–k	11.13 ab	25.96 ab	14.05 a	5.50 a	3.29 a–c
	375	41.99 h–k	10.55 b	24.49 b	14.19 a	5.44 a	3.30 a–c
Chive	75	44.54 f–i	6.23 cd	23.66 cd	11.64 bc	3.50 e	2.69 b–e
	150	48.01 c–f	6.28 cd	20.74 cd	11.13 c	3.54 e	2.96 a–d
	225	50.47 a–c	6.57 c	20.10 c	10.49 c–e	3.51 e	3.30 a–c
	300	48.64 b–e	6.09 cd	16.32 cd	10.47 c–e	3.38 e	3.05 a–d
	375	52.45 a	6.69 c	17.65 c	9.96 c–f	3.47 e	3.15 a–c
Scallion	75	37.15 l	5.76 cd	27.93 cd	8.68 d–g	3.62 de	3.30 a–c
	150	38.70 kl	5.63 cd	25.35 cd	8.96 d–g	3.68 de	3.57 ab
	225	40.30 j–l	5.80 cd	18.42 cd	7.39 g	3.72 de	3.82 a
	300	46.54 d–g	4.99 cd	15.75 d	8.35 fg	3.50 e	3.73 a
	375	45.35 e–h	6.27 d	15.07 cd	8.52 e–g	4.17 cd	1.92 e
Shiso	75	43.71 g–j	11.96 a	38.74 ab	10.61 cd	4.67 bc	2.38 c–e
	150	47.81 c–f	11.67 ab	31.15 cd	10.31 c–f	5.13 ab	2.15 de
	225	50.27 a–c	11.52 ab	27.37 c–f	9.81 c–f	5.37 a	2.15 de
	300	49.40 a–d	11.44 ab	25.64 c–g	10.04 c–f	5.57 a	2.12 de
	375	52.07 ab	10.37 b	18.33 h–j	8.66 d–g	5.35 a	1.90 e
p-value	Species	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Seeding density	< 0.0001	0.0087	< 0.0001	0.0019	0.045	0.0004
	Interaction	< 0.0001	0.0002	0.0003	0.047	0.0001	< 0.0001

[z] Different lower-case letters within a column suggest there is a significant difference among means as indicated by Tukey's HSD at $p < 0.05$. [y] Each mean was sourced from a total of 10 data points from both the jute and peat substrates.

Table 6. Nitrogen, phosphorous, potassium, calcium, magnesium, and sulfur concentrations as affected by the species and substrate type interaction in January 2023.

Species	Substrate	Nitrogen[z,y] ($mg \cdot g^{-1}$)	Phosphorous ($mg \cdot g^{-1}$)	Potassium ($mg \cdot g^{-1}$)	Calcium ($mg \cdot g^{-1}$)	Magnesium ($mg \cdot g^{-1}$)	Sulfur ($mg \cdot g^{-1}$)
Basil	Peat	40.56 e	8.60 d	27.73 c	12.36 b	4.02 d	4.11 c
	Jute	59.07 a	10.24 c	19.85 e	8.47 e	5.03 b	3.08 d
Chive	Peat	42.32 de	5.48 f	22.49 d	11.29 c	2.97 f	4.99 b
	Jute	57.14 a	5.96 ef	12.32 f	8.88 de	3.53 e	2.67 e
Scallion	Peat	43.50 d	8.10 d	39.20 b	12.44 b	4.55 c	5.37 a
	Jute	47.24 c	6.43 e	11.96 f	9.50 d	4.33 c	2.19 f
Shiso	Peat	49.76 b	14.50 a	47.84 a	13.33 a	7.88 a	3.05 d
	Jute	59.09 a	10.96 b	10.71 f	9.60 d	4.92 b	1.39 g
p-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
	Interaction	< 0.0001	< 0.0001	< 0.0001	0.0003	< 0.0001	< 0.0001

[z] Different lower-case letters within a column suggest there is a significant difference among means as indicated by Tukey's HSD at $p < 0.05$. [y] Each mean was sourced from a total of 25 data points averaged across all five seeding densities.

species and substrate type in both January and February (Tables 6 and 7).

When affected by the species and seeding density interaction in January, low seeding density of 75 $g \cdot m^{-2}$ resulted in higher K concentrations than densities of 150 to 375 $g \cdot m^{-2}$ in scallion and shiso microgreens, higher than 225 to 375 $g \cdot m^{-2}$ in basil, and higher than 150, 300, or 375 $g \cdot m^{-2}$ in chives. Shiso grown at 75 $g \cdot m^{-2}$ also produced the highest K concentration of 39.01 $mg \cdot g^{-1}$ among all treatment combinations (Table 4). In February 2023, the five seeding densities resulted in similar K concentrations within a species in chives and scallion microgreens. At each seeding density, basil produced significantly higher K concentrations, ranging from 24.49 to 40.82 $mg \cdot g^{-1}$, than any other species. Shiso grown at 75 $g \cdot m^{-2}$ produced the highest K concentration of 38.74 $mg \cdot g^{-1}$ compared with the other seeding densities within this species (Table 5).

When affected by the species and substrate type interaction, peat substrate increased K concentrations in all microgreen species compared with jute mat in both experiments (Tables 6 and 7). Shiso grown with peat produced the highest K concentration, 47.84 $mg \cdot g^{-1}$ in January and 47.49 $mg \cdot g^{-1}$ in February, respectively, among all treatment combinations. Whereas chives, scallion, and shiso grown on jute produced the lowest K concentrations of 10.71 to 12.32 $mg \cdot g^{-1}$ in January, and of 8.7 to 13.65 $mg \cdot g^{-1}$ in February, respectively.

Calcium concentration

Calcium concentration was affected by the interactions between species and seeding density (Tables 4 and 5) and between species and substrate type in both January and February (Tables 6 and 7).

When affected by the species and seeding density interaction, the five seeding densities resulted in similar Ca concentrations in basil, chives, and shiso in January (Table 4). Scallion produced a significantly higher Ca concentration of 12.33 $mg \cdot g^{-1}$ at 75 $g \cdot m^{-2}$ compared to 225, 300, or 375 $g \cdot m^{-2}$ seeding densities, which was also higher than basil or chives, regardless of seeding density, similar to the Ca concentration in shiso grown with any density. In

Table 7. Nitrogen, phosphorous, potassium, calcium, magnesium, and sulfur concentrations as affected by the species and substrate type interaction in February 2023.

Species	Substrate	Nitrogen^{z,y} (mg·g^{-1})	Phosphorous (mg·g^{-1})	Potassium (mg·g^{-1})	Calcium (mg·g^{-1})	Magnesium (mg·g^{-1})	Sulfur (mg·g^{-1})
Basil	Peat	35.75 e	11.08 b	38.28 b	15.57 a	5.07 c	3.60 b
	Jute	48.51 b	11.49 b	22.38 d	12.00 b	5.91 b	2.59 cd
Chive	Peat	45.04 c	6.37 d	26.01 c	11.06 c	3.18 f	3.84 ab
	Jute	52.06 a	6.38 d	13.38 e	10.41 c	3.78 e	2.22 d
Scallion	Peat	37.82 d	5.80 de	27.35 c	9.00 d	3.66 e	4.06 a
	Jute	45.40 c	5.58 e	13.65 e	7.76 e	3.82 e	2.47 d
Shiso	Peat	45.27 c	13.59 a	47.79 a	12.06 b	6.32 a	3.03 c
	Jute	52.03 a	9.20 c	8.70 f	7.72 e	4.12 d	1.25 e
p-value	Species	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Substrate	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0073	< 0.0001
	Interaction	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0069

^{z} Different lower-case letters within a column suggest there is a significant difference among means as indicated by Tukey's HSD at $p < 0.05$. ^{y} Each mean was sourced from a total of 25 data points averaged across all five seeding densities.

February 2023, the five seeding densities resulted in similar Ca concentrations within each species (Table 5). Among species, basil microgreens produced the highest Ca concentrations of 13.22 to 14.19 mg·g^{-1}, higher than any other species at a given seeding density. Scallion microgreens had the generally lowest Ca concentrations among species.

When affected by the interaction between microgreens species and substrate type, peat increased Ca concentrations in basil, scallion, and shiso in both experiments and in chives in January 2023 (Tables 6 and 7). In January, shiso grown on peat produced the highest Ca concentration of 13.33 mg·g^{-1}. In February, basil grown on peat produced the highest Ca concentration of 15.57 mg·g^{-1} among treatment combinations.

Magnesium concentration

Magnesium concentration was affected by the interactions between species and seeding density (Tables 4 and 5) and between species and substrate type in both January and February (Tables 6 and 7).

When affected by the species and seeding density interaction, the five seeding densities resulted in similar Mg concentrations in basil, chives, scallion in January, except for that the seeding density of 300 g·m^{-2} resulted in a lower Mg concentration in basil than 375 g·m^{-2}. Basil and scallion had similar Mg concentrations at each of the five seeding densities, and higher than that in chives, which produced the lowest Mg concentrations, ranging from 3.11 to 3.42 mg·g^{-1}, regardless of seeding density. Shiso had higher Mg concentration than any other species when grown at each seeding density from 150 to 375 g·m^{-2}, with 375 g·m^{-2} resulting in the highest Mg concentration of 8.00 mg·g^{-1} among all treatment combinations in January 2023 (Table 4). In February, basil and shiso generally produced similar Mg concentrations within a species except that shiso grown at 75 g·m^{-2} had a lower Mg concentration than other seeding densities. At each seeding density, basil and shiso had higher Mg concentration than chives or scallion microgreens, with Mg concentrations ranging from 3.38 to 4.17 mg·g^{-1} (Table 5).

When affected by the interaction between microgreen species and substrate type, microgreen species varied in their response to substrate type (Tables 6 and 7). Basil and chives had higher Mg concentrations when grown on jute compared to being grown on peat, whereas shiso grown on peat had higher Mg concentrations than jute mat in both experiments. Shiso microgreens grown on peat also produced the highest Mg concentrations, 7.88 mg·g^{-1} in January and 6.32 mg·g^{-1} in February, respectively, among all treatment combinations. Scallion had similar Mg concentrations when grown on peat and jute in both experiments.

Sulfur concentration

Sulfur concentration was affected by the interactions between species and seeding density (Tables 4 and 5) and between species and substrate type in both January and February 2023 (Tables 6 and 7).

When affected by the species and seeding density interaction, the five seeding densities resulted in similar S concentrations in basil, chives, and shiso in both experiments, except that chive grown at 75 g·m^{-2} seeding density produced a higher S concentration than any other seeding density in January 2023 (Tables 4 and 5). In January 2023, chives grown at 75 g·m^{-2} and scallion grown at 75 and 150 g·m^{-2} produced the highest S concentrations of 4.16 to 4.71 mg·g^{-1} among all treatment combinations. Shiso had lower S concentrations of 2.12 to 2.28 mg·g^{-1} than any other species at each seeding density (Table 4). In February 2023, basil grown at 75, 225, 300, and 375 g·m^{-2}, chives grown at 150 to 375 g·m^{-2}, and scallion grown at 75 to 300 g·m^{-2} all had similar S concentrations ranging from 2.86 to 3.82 mg·g^{-1}.

When affected by the interaction between microgreens species and substrate type, each microgreen species grown on peat had significantly higher S concentrations compared to those grown on jute mats in both experiments (Tables 6 and 7). Scallion grown on peat produced the highest S concentration, 5.37 mg·g^{-1} in January and 4.06 mg·g^{-1} in February, respectively, while shiso grown on jute had the lowest S concentration, 1.39 mg·g^{-1} in January and 1.25 mg·g^{-1} in February, among all the treatment combinations in both experiments.

Discussion

In both of our experiments, fresh shoot weight in tested microgreen species generally had an increasing trend as seeding density increased from 75 to 375 g·m^{-2}. The highest seeding density of 375 g·m^{-2} resulted in higher fresh shoot weight than seeding densities of 75 to 300 g·m^{-2} in scallion microgreens. In basil, chives, and shiso, the 375 g·m^{-2} seeding density increased fresh shoot weight compared with 75 to 225 g·m^{-2}, but resulted in similar fresh shoot yield to 300 g·m^{-2}, suggesting seeding density for these three species should not exceed 300 g·m^{-2} for maximized yield per unit area. Fresh shoot yields across species were comparable to those reported in other studies at lower seeding densities of 75 to 225 g·m^{-2}, whereas the use of higher densities, including 300 and 375 g·m^{-2} in the current work, resulted in substantially greater fresh yields[15,27]. Kyriacou et al. noted that seed costs can account for up to 50% of total production expenses in microgreens, and that optimizing seeding rates is essential for economic efficiency[22]. Besides, high seeding rates can elevate moisture being held among micro shoots, increase the risk of disease, and potentially reduce the marketability of microgreens[3]. Therefore, maximizing fresh shoot yield in microgreen production should be weighed against shoot quality, seed weight, and seed cost per unit area to better evaluate production profitability, which requires further investigation.

Increasing seeding rates can have diminishing returns on yield, shoot quality, and economic efficiency[28]. As shoot density increases, crowding and competition among microshoots can happen, and individual shoot weight may decrease as a result. Previous research has shown that individual shoot weight of table beet microgreens declined as sowing density increased from 50.3 to 201 g·m^{-2}[29]. Reductions in individual shoot weight were reported in *Brassica* and *Raphanus* microgreens such as mustard, kale, cabbage, broccoli, and radish grown as densities increased from 60.5 to 189.0 g·m^{-2}[30]. A similar trend was observed in shiso in the current study, where higher seeding densities of 300 and 375 g·m^{-2} resulted in a significant decline in individual shoot weight compared to 150 or 225 g·m^{-2}, and the lowest seeding density of 75 g·m^{-2} resulted in higher individual shoot weight than any other seeding densities. However, microgreen species varied in their response to increasing seeding density. In the current study, individual shoot weight within a species, i.e. basil, chives, and scallion, was similar regardless of seeding density in January, and was not affected by seeding density in February, suggesting overcrowding did not occur in these three species, likely due to their thin shoot size.

Substrate type, peat vs jute mat, affected shoot growth of selected microgreen species, including shoot height, visual rating, fresh and dry shoot weights, and individual shoot weight in this study (Table 3). Scallion and shiso microgreens produced higher fresh shoot weight when grown on peat-based substrate compared with jute mat in one or both experiments. This was in agreement with previous reports revealing that peat substrate was the most satisfactory in maximizing fresh shoot yield compared with other alternative substrates. For example, Hoang & Vu reported that peat mixed with vermiculite produced the highest fresh weight and dry biomass in five *Brassica* microgreens, including red cabbage, broccoli, mizuna, green mustard, and Pak choy, compared to alternatives such as soil-coco coir mixes, or coco coir with rice husk[31]. The superior performance of peat was attributed to its optimal water-holding capacity and aeration, which supported better germination and seedling development while reducing disease incidence. Contrary to these findings, chives and basil microgreens produced higher fresh shoot weight on jute mat compared to the peat substrate when there was a significant difference. The water-holding capacity of fiber mats can be significantly lower than peat[10], where the lower water retention could reduce the risk of waterlogging stress in chives and basil microgreens that are sensitive to excess moisture during their slow germination and long growth period. Severe rotting problems in basil microgreens were observed when grown with peat, especially at high seeding densities. Moreover, jute and other fibrous mats offer practical advantages such as cleaner harvests without substrate particle adherence, reduced postharvest labor, which can potentially compensate for their water management demands[19].

In agreement with numerous reports, and our previous findings, microgreen species varied in their mineral nutrient profiles, which are subject to influence by various cultural practices, including fertigation, use of different substrates, as well as seeding density[5,19,30,32]. For example, chives and scallion were generally shown to have high S contents, supporting previous findings that *Allium* species are naturally rich in sulfur-containing compounds[14,33]. Di Gioia et al.[14] noted that basil microgreens are a good source of Ca, and shiso microgreens are a good source of P and Mg. Substrate type altered mineral nutrient concentrations in the four microgreens species in the current study. For example, peat substrate increased P, K, Ca, and S concentrations in the tested microgreens, whereas jute mat generally increased N and Mg in one or more species (Tables 6 and 7). Such effects were likely attributed to the peat substrate being a source of K, P, Ca, and S as agreed by Di Gioia et al.[10], and jute being a source of N and Mg as revealed by testing results from the MSU Extension Service Soil Testing Laboratory (personal communication).

Compared with the clear trend of nutrient change as affected by substrate, the five seeding densities resulted in less separation of mineral nutrient concentrations, including P, Ca, Mg, and S, within a certain microgreen species. On the other hand, separation of N and K concentrations caused by different seeding densities were more frequently found, and was species dependent (Tables 4 and 5). There was an increasing trend in N concentrations in shiso and chives, and in Mg in shiso as seeding density increased, with values generally agreed by reported ranges[34]. Such results suggest that the generally good growth under high seeding densities might have driven the increased nutrient uptake of N and Mg in shiso and/or chives microgreens, and that the nutrient-rich substrates, including peat and jute, likely sustained high microgreen density without causing dilution in mineral nutrient concentrations. In comparison, a decreasing trend in K concentrations was found in basil, scallion, and shiso, in one or both experiments. The decreasing trend of mineral concentrations as seeding density increases was also found by Ntsoane et al. in *Brassica* microgreens, and was attributed to the competition for nutrient uptake by the high number of microshoots[35]. Environmental conditions, such as light quality, intensity, temperature, and humidity, may also have a substantial influence on nutrient accumulation, as reported by Kopsell et al.[36] and Weber[37], who documented changes in mineral nutrient profiles in response to lighting spectrum and controlled growth conditions in microgreens. Together, these results emphasize the interactive roles of species, substrate, and seeding density in shaping mineral nutrient concentrations in microgreens and that cultural practices and environmental manipulations can be strategically applied to improve nutrient profiles of microgreens.

Conclusions

For maximum fresh yield per unit area, optimal seeding density for basil, chives, and shiso microgreens should not exceed 300 g·m^{-2}, while scallion can be grown at 375 g·m^{-2} without a reduction in shoot visual quality. Increasing seeding density did not decrease individual shoot weight in basil, chives, and scallions, but caused a reduction in individual shoot weight in shiso microgreens that indicated shoot crowding. Moreover, recommendation for optimal seeding density should take into account seed cost, germination, and shoot quality, as increasing seed sowing rate not only increase production cost but may potentially cause a reduction in microgreen quality. Overall, scallion and shiso grown with peat produced the highest fresh shoot yield and are recommended herbal species for microgreen production. Mineral nutrient concentrations varied among microgreen species and were altered by both seeding density and growing substrate. Peat substrate increased P, K, Ca, and S concentrations in the tested microgreens, whereas jute mats are generally a source of N and Mg. Species-dependent cultural practices including seeding density and substrate selection, should be applied to maximize shoot yield and improve nutrient profiles in microgreen production.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Li TL; experiment operation and data collection: Arthur JA, Li TL, Pennington AP; draft manuscript preparation: Arthur JA; review and editing: Li TL, Bi GB, White SW, Bheemanahalli RB; funding acquisition, Li TL, Bi GB, White SW. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article.

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Conflict of interest

The authors declare that they have no conflict of interest.

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