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Article

Fertilization and Pre-Sowing Seed Soaking Affect Yield and Mineral Nutrients of Ten Microgreen Species

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Abstract: Microgreens, vegetable or herb seedlings consumed at a young growth stage, are considered to be a functional food with high concentrations of mineral nutrients and healthy beneficial bioactive compounds. The production of microgreens has been increasing in recent years. Vegetable growers are interested in growing microgreens as a new specialty crop due to their high market value, popularity, and short production cycles. However, there is a lack of research-based crop-specific recommendations for cultural practices including fertilization, pre-sowing seed treatments, and their effects on nutritional facts of microgreens. Ten microgreen species were evaluated for their shoot growth and mineral nutrient concentrations as affected by one-time post-emergence fertilization and pre-sowing seed soaking in two repeated experiments, from November 2018 to January 2019, in a greenhouse. The microgreen species varied in fresh and dry shoot weights, shoot height, visual rating, as well as macro- and micro-nutrient concentrations. Fertilization with a general-purpose soluble fertilizer (20-20-20 with micronutrients) at a rate of 100 mg·L^{-1} nitrogen (N) increased fresh shoot weight, and macro- and micro-nutrient concentrations in one or both experiments, with the exception of decreasing concentrations of calcium (Ca), magnesium (Mg), and manganese (Mn). Seed soaking consistently decreased fresh or dry shoot weight and nutrient concentrations when there was a significant effect.

Keywords: leafy green; *Brassica*; nutrient management; seed treatment; fresh production

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1. Introduction

Microgreen is a collective term used for vegetable or herb seedlings consumed at a young growth stage, with expanding cotyledons or the first pair of true leaves, harvested 7 to 21 days after germination [1–4]. As a new specialty crop, the market demand for microgreens has been rapidly increasing in recent years [5]. Microgreens are popular items at local markets and are used by chefs and consumers to enhance flavor, color, and texture in various foods [2,6,7]. The market value of microgreens is 30 to 50 USD per pound [7], drawing interest among vegetable growers for the high value and short production cycles [8]. A number of plant species in the families of Amaranthacea, Apiaceae, Asteraceae, Brassicaceae, Fabaceae, and Lamiaceae have been produced as microgreens [5,6,9,10].

Microgreens are considered to be a functional food and have high concentrations of mineral nutrients and phytochemicals that contribute to health benefits [11,12]. For example, microgreens in the Brassicaceae family are rich sources of mineral nutrients including potassium (K), Ca, iron (Fe), and zinc (Zn) [5,13]. Basil (*Ocimum basilicum*) and Swiss chard (*Beta vulgaris* subsp. *vulgaris*) microgreens have been reported to be excellent sources of K and Mg [14]. *Brassica* microgreens also have high contents of healthy phytochemicals including ascorbic acid, phylloquinone, carotenoids, tocopherols, glucosinolates, and polyphenols [15]. Species vary in their nutrient profiles. Cultural practices including pre-sowing seed treatments, seeding rate, and fertilization, along with the microenvironment including temperature, light, and growth medium may all affect yield and nutrient compositions of microgreens [3,9,16,17].

Fast and uniform germination is a key aspect in successful production of microgreens. Pre-sowing seed treatments including seed priming and soaking were examined for their effects in advancing seedling emergence [16,18–20]. Lee et al. [19] found priming seeds in grade five vermiculite was the most effective pre-sowing treatment in advancing germination of microgreen beet (*Beta vulgaris*) and chard compared to seed soaking in a variety of solutions including water, hydrogen peroxide, or hydrochloric acids. Seed soaking in cold water was recommended for large seed crops including pea (*Pisum sativum*) and radish (*Raphanus sativus* var. *Longipinnatus*), but not for small seed species including a number of *Brassica* crops, or mucilaginous seeds such as basil that develop a jelly-like coating when wet [18,21]. Imbibing seeds can leak several compounds including amino acids, inorganic ions, sugars, phenolics, and proteins due to the inability of cellular membranes to function normally until seeds are fully hydrated [22]. There is lack of research-based information regarding the effects of seed soaking on crop yield and nutritional content of microgreens.

Fertilization management is another important aspect in promoting fast growth and high yield of microgreens [23]. Fertilizer premixed in growing substrate or applied post-emergence through soluble fertilizer were both investigated for their effect in the production of microgreens [3,23,24]. Daily post-emergence fertigation with 150 $mg \cdot L^{-1}$ N was found to be of the most economical fertilization treatment for improving fresh shoot weight of arugula (*Eruca vesicaria* subsp. *sativa*) microgreens [16]. Kou et al. [24] found 10 mM of calcium chloride solution applied daily for ten days increased biomass of broccoli (*Brassica oleracea* var. *italica*) microgreens as compared with water and improved visual quality during storage. Plant species, slow versus fast growing, may differ in their requirements for fertilization [7]. There is concern that some crops such as arugula tend to accumulate excessive nitrate [23]. Crop-specific fertilizer requirements with respect to shoot yield and mineral nutrients in microgreen production remain unclear.

The objective of this study was to investigate the effects of pre-sowing seed soaking and post-emergence fertilization on shoot growth and mineral nutrients of ten microgreen species.

2. Materials and Methods

2.1. Plant Materials and Microgreen Culture

Ten species were grown as microgreens (Table 1) and evaluated for shoot growth and mineral nutrient concentrations, in a greenhouse on the campus of Mississippi State University in Starkville, MS, USA (33.4552° N, 88.7944° W). Microgreen seeds of all selected species were purchased from True Leaf Market (Salt Lake City, UT, USA). Seed sowing rate for each species was as determined by the supplier's recommendation and summarized in Table 1. Hundred-seed weight of each species was measured in triplication. This study included two experiments with the first experiment conducted on 15 November 2018, and then repeated on 7 January 2019.

Seeds of appropriate weight were measured and manually sown into black plastic trays with drainage holes (width 25.72 cm, length 25.72 cm, depth 6.03 cm, T.O. Plastics, Clearwater, MN, USA) and filled with a peat-based soilless substrate (PRO-MIX BX general purpose, Premier Tech Horticulture, QC, Canada). After sowing, seeds were gently pressed into the medium with the bottom of another tray for maximum substrate contact and covered with an additional thin layer of substrate. The temperature in the greenhouse was set at 25 °C with natural light.

Prior to seed sowing, half of the seeds for each species were soaked with tap water (250 mL of water for pea seeds, and 100 mL for all other species) at room temperature (approximately 20 °C) for 6 h. Soaked seeds were drained and mixed with a handful of growing substrate and evenly distributed into the growing tray. All microgreens were hand watered as needed, approximately, once per day until harvest. After seed germination, half of the trays from each species 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%

molybdenum (Mo), and 0.025% Zn, ICL Specialty Fertilizers, Tel-Aviv, Israel) at a rate of 100 mg·L^{-1} N four days after planting. The fertilizer solution had a pH of 6.56 and electrical conductivity of 0.41 mS·cm^{-1}. As a control to the fertilization treatment, the other half of the trays were irrigated with the same volume of water (pH 7.54, EC 0.15 mS·cm^{-1}).

Table 1. Common name, scientific name, seeding rate, 100 seed weight, and harvest date of ten species grown as microgreens.

Common Name	Scientific Name	Seeding Rate (g·m^{-2})	100 Seed wt. (g)	Harvest Date (DAP) [z]
Red Garnet amaranth	*Amaranthus tricolour*	60.5	0.087 ± 0.01	11–13
Slow bolt arugula	*Eruca sativa*	75.6	0.21 ± 0.05	10–15
Genovese basil	*Ocimum basilicum*	52.9	0.20 ± 0.005	16–18
Waltham broccoli	*Brassica oleracea* var. *italica*	98.3	0.44 ± 0.015	8–13
Red Acre cabbage	*Brassica oleracea* var. *capitata*	83.1	0.44 ± 0.016	8–14
Daikon radish	*Raphanus sativus* var. *longipinnatus*	173.8	1.63 ± 0.03	6–7
Red Russian kale	*Brassica napus* var. *pabularia*	75.6	0.23 ± 0.008	10–13
Early White Vienna kohlrabi	*Brassica oleracea* var. *gongylodes*	75.6	0.39 ± 0.02	9–13
Red Garnet mustard	*Brassica juncea*	60.5	0.18 ± 0.003	10–12
Green pea	*Pisum sativum*	1285	15.33 ± 0.08	6–8

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

2.2. Shoot Harvest and Data Collection

Microgreens grown 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. [4]. Fresh shoot weight of microgreens harvested from each tray was measured. Then, fresh microgreen shoots from each tray were oven dried at 60 °C until constant weight and measured for dry shoot weight (DW). Dry weight percentage (%) was also determined. Plant height was measured in each tray before being harvested from the substrate surface to the highest point of shoot growth. Each tray of microgreen species was given a visual quality rating from 1–5, where 1 = seedlings cover 20% of the growing surface area or less, 2 = seedlings cover 20% to 40% of the growing surface area, 3 = seedlings cover 40% to 60% of the growing surface area, 4 = seedlings cover 60% to 80% of the growing surface area, and 5 = seedlings cover over 80% of the tray surface area with healthy plant growth.

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 phosphorus (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 (mg·g^{-1} DW) and micronutrients (µg·g^{-1} DW) 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. Microgreen species (10 species), pre-sowing seed treatment (seed soaking or not), and fertilization (fertilizer or not) served as the three main factors. Each treatment had four 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 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 at $\alpha \leq 0.05$. Data from the two experiments were compared as repeated measures, where experimental date was used as a factor to analyze its effect. All statistical analyses were performed using SAS.

3. Results

The experimental date affected all measured dependent variables in this study, with varying trends between the November 2018 and January 2019 experiments (data not shown). Therefore, all data from the two experiments were presented separately. There were no significant three-way interactions among species, fertilization, or pre-sowing seed treatment on any measured dependent variable.

3.1. Fresh and Dry Shoot Weights

Microgreens were harvested at microgreen stage 1 or 2 with various harvest dates (Table 1). Fresh shoot weight sof microgreens were affected by the main effects of species and fertilization in November 2018, and by species, fertilization, and soaking but without interaction in January 2019. Pea produced the highest fresh shoot weight of 2484 g·m^{-2} in November 2018, higher than any other species. Daikon radish produced the highest fresh shoot weight of 1701 g·m^{-2} in January 2019 and pea producing ranked second with 1178 g·m^{-2} fresh shoot weight (Figure 1A). Amaranth produced the lowest fresh shoot weight of 630.6 and 497.8 g·m^{-2} in November 2018 and January 2019, respectively. Fertilization increased fresh shoot weight by 3.2% in November 2018 and by 14.3% in January 2019 (Tables 2 and 3). Soaking did not affect fresh shoot weight in November 2018 but decreased fresh shoot weight by 14.3% in January 2019 (Table 4).

Table 2. Microgreen shoot growth and nutrient concentrations on a dry weight basis affected by fertilization in November 2018.

	Shoot Growth and Nutrient Concentrations in November 2018									
	Fresh Shoot wt. (g·m^{-2})	Dry wt. Percentage (%)	Nitrogen (mg·g^{-1})	Phosphorus (mg·g^{-1})	Potassium (mg·g^{-1})	Calcium (mg·g^{-1})	Magnesium (mg·g^{-1})	Iron (µg·g^{-1})	Zinc (µg·g^{-1})	Boron (µg·g^{-1})
Fertilized ^{z}	1304	6.8	50.3	12.3	39.8	15.9	4.59	187.9	118	34.5
Not fertilized	1264	7.1	45.5	11.3	37.6	16.4	4.74	170.6	110.8	33.4
p-Value ^{y}	0.025	<0.0001	<0.0001	<0.0001	0.037	0.006	0.014	0.0008	0.0008	0.013

^{z} Means of the fertilized or not fertilized treatment were obtained by averaging data over ten tested microgreen species in both pre-sowing seed treatments. ^{y} $p \leq 0.05$ suggests significant difference between means within a column indicated by ANOVA.

Table 3. Microgreen shoot growth and nutrient concentrations on a dry weight basis affected by fertilization in January 2019.

	Shoot Growth and Nutrient Concentrations in January 2019									
	Fresh Shoot wt. (g·m^{-2})	Dry Shoot wt. (g·m^{-2})	Dry wt. Percentage (%)	Shoot Height (cm)	Visual Rating (1–5)	Phosphorus (mg·g^{-1})	Magnesium (mg·g^{-1})	Iron (µg·g^{-1})	Manganese (µg·g^{-1})	Boron (µg·g^{-1})
Fertilized ^{z}	1001	66.5	6.6	5.7	4.4	12.0	4.83	136.1	68.6	30.2
Not fertilized	875.4	61.7	7.0	5.6	4.3	11.2	5.02	129.6	72.2	28.2
p-Value ^{y}	<0.0001	<0.0001	<0.0001	0.043	0.021	<0.0001	0.004	0.0017	0.0014	<0.0001

^{z} Means of the fertilized or not fertilized treatment were obtained by averaging data over ten tested microgreen species in both pre-sowing seed treatments. ^{y} $p \leq 0.05$ suggests significant difference between means within a column indicated by ANOVA.

Table 4. Microgreen shoot growth and nutrient concentrations on a dry weight basis affected by pre-sowing seed soaking treatment in November 2018 and January 2019.

	Nutrient Concentrations in November 2018			Shoot Growth and Nutrient Concentrations January 2019						
	Nitrogen (mg·g^{-1})	Magnesium (mg·g^{-1})	Boron (µg·g^{-1})	Fresh Shoot wt. (g·m^{-2})	Dry Shoot wt. (g·m^{-2})	Shoot Height (cm)	Phosphorus (mg·g^{-1})	Magnesium (mg·g^{-1})	Iron (µg·g^{-1})	Zinc (µg·g^{-1})
Soaked ^{z}	47.5	4.6	33.3	875	61.0	5.5	11.4	4.82	129.6	77.9
Unsoaked	48.3	4.8	34.7	1001	67.2	5.8	11.8	5.03	136.1	80.4
p-Value ^{y}	0.036	0.004	0.0015	<0.0001	<0.0001	<0.0001	0.011	<0.0001	0.0016	0.015

^{z} Means of the soaked or unsoaked treatment were obtained by averaging data over ten tested microgreen species in both fertilization treatments. ^{y} $p \leq 0.05$ suggests significant difference between means within a column indicated by ANOVA.

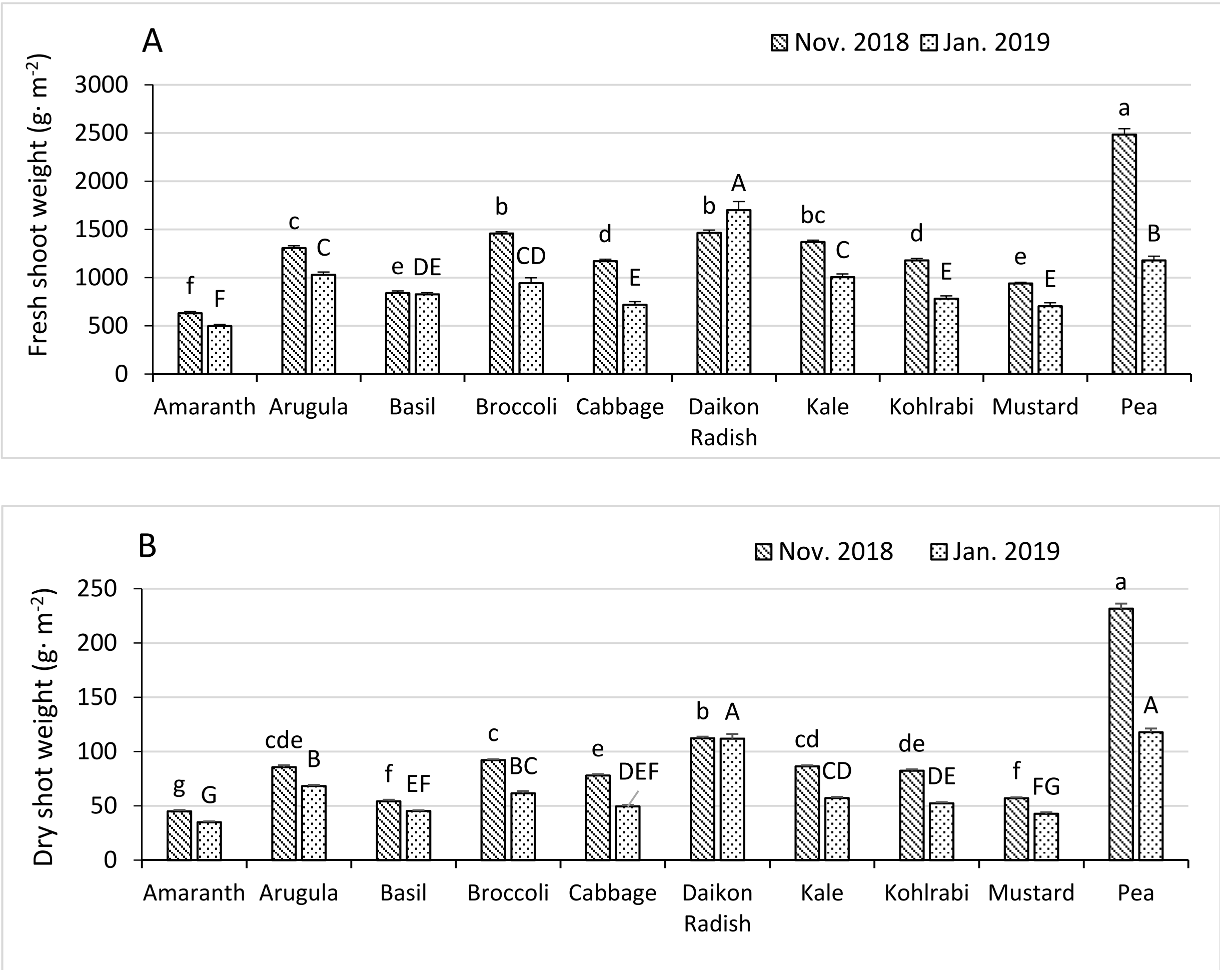


Figure 1. Fresh (**A**) and dry (**B**) shoot weights of ten species grown as microgreens. Means with standard error bars of each species were obtained by averaging data from both fertilization and pre-sowing seed treatments. Different lower case or capitalized letters suggest significant difference among species indicated by Tukey's HSD test at $\alpha \leq 0.05$ in November 2018 or January 2019, respectively.

Trends of dry shoot weight among species was similar to that of fresh shoot weight in each experiment. Pea and daikon radish ranked first and second in dry shoot weight in both experiments, i.e., 115.8 and 56.1 $g \cdot m^{-2}$ in November 2018, and 117.7 and 111.8 $g \cdot m^{-2}$ in January 2019, respectively, higher than the other eight species (Figure 1B). Lower than pea or daikon radish, broccoli and kale produced higher dry shoot weights than those of basil, amaranth, or mustard with the lowest dry shoot weights in November 2018 and January 2019. Fertilization did not affect dry shoot weight in November 2018 but increased dry shoot weight by 3.3% in January 2019 (Table 3). Seed soaking did not affect dry shoot weight in November 2018 but decreased dry shoot weight by 10.3% in January 2019 (Table 4).

Dry weight percentage out of fresh weight varied among species ranging from 6.1% in mustard to 9.3% in pea, in November 2018 (Figure S1A). Next to pea, daikon radish ranked second with a dry weight percentage of 7.7%, higher than any other species. Dry weight percentage in January 2019 was affected by the interaction between species and seed soaking (Figure S1B). Pea had the highest dry weight percentage of 10.3% in the soaked or 9.8% in the unsoaked treatment, higher than any other species. Basil generally had the lowest dry weight percentage of 5.6% in the soaked and 5.4% in the unsoaked treatment, respectively. Seed soaking increased dry weight percentage in broccoli, cabbage, daikon radish, and kohlrabi as compared with the unsoaked treatment but resulted in similar dry weight percentage in the other six species. Fertilization decreased dry weight percentage as compared with the no-fertilizer treatment in November 2018 and January 2019 (Tables 2 and 3).

3.2. Shoot Height and Visual Rating

Shoot height varied among species in both experiments ranging from 3.8 cm in basil to 9.9 cm in pea, in November 2018, and from 4.0 cm in basil to 9.2 cm in daikon radish, in January 2019 (Figure 2A). Broccoli ranked second in both experiments, with a height of 8.8 cm in November 2018 and 6.5 cm in January 2019, higher than other eight species. Kohlrabi, kale, cabbage, and arugula had intermediate heights, ranging from 6.4 to 8.0 cm in November 2018, and from 5.3 to 5.5 cm in January 2019. Higher than basil, amaranth and mustard had similar and second lowest height in both experiments. Fertilization increased shoot height in January 2019 as compared with the no-fertilizer treatment (Table 3). The soaking treatment decreased shoot height in January 2019 (Table 4).

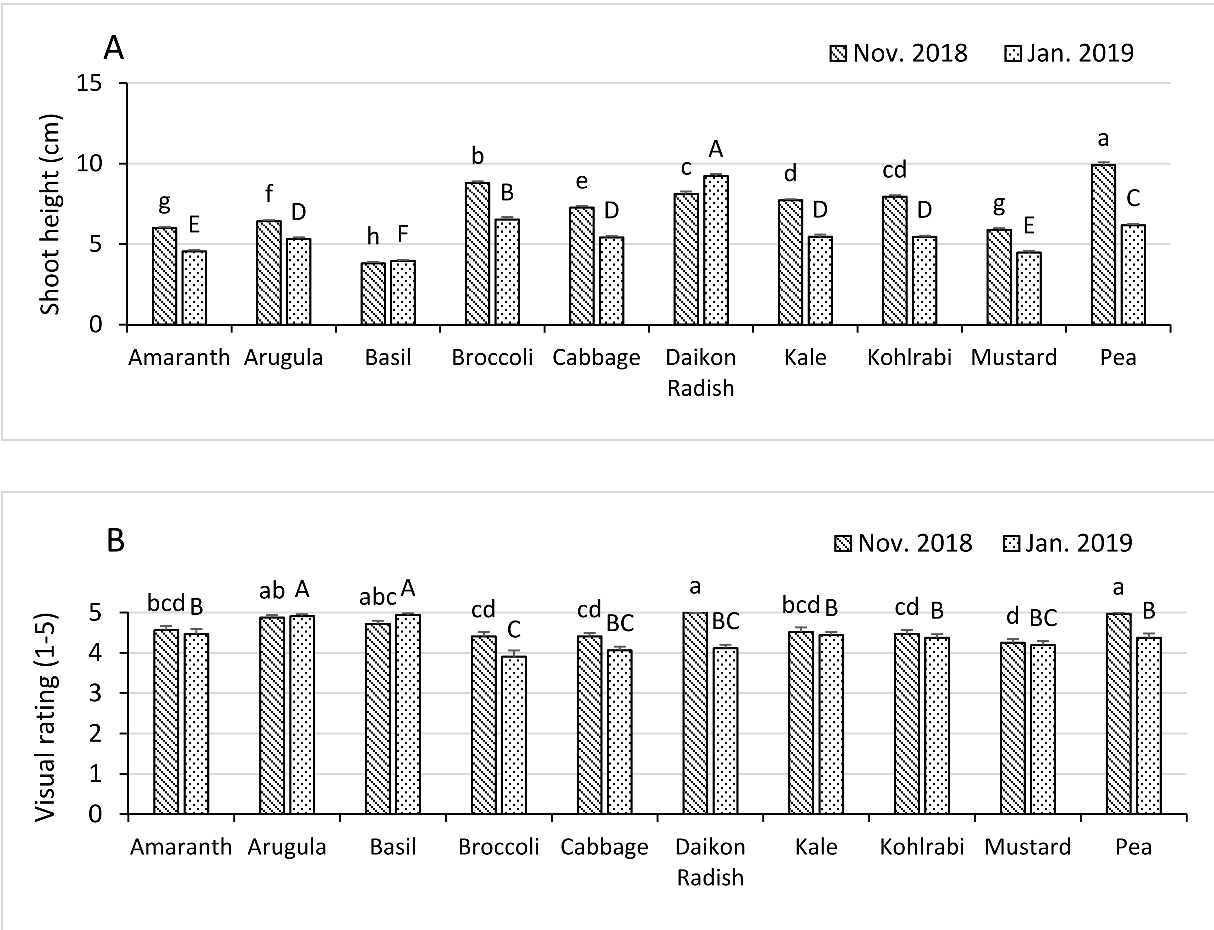


Figure 2. Shoot height (**A**) and visual rating (**B**) of ten species grown as microgreens. Means with standard error bars of each species were obtained by averaging data from both fertilization and pre-sowing seed treatments. Different lower case or capitalized letters suggest significant difference among species indicated by Tukey's HSD test at $\alpha \leq 0.05$ in November 2018 or January 2019, respectively.

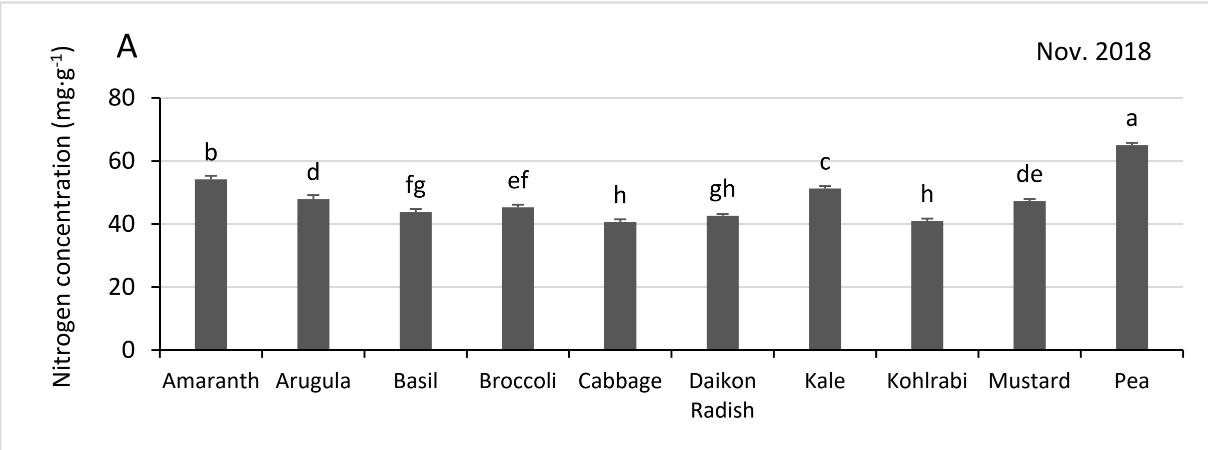
Visual rating varied among species in both experiments. In November 2018, daikon radish, pea, arugula, and basil had comparable highest ratings ranging from 4.7 in basil to 5 in daikon radish, higher than mustard with a rating of 4.3 (Figure 2B). In January 2019, basil and arugula had the highest ratings of 4.9, higher than other species with ratings ranging from 3.9 in broccoli to 4.5 in amaranth. Visual rating of all ten species were above 4 in November 2018, with nine species, excluding broccoli, above 4 in January 2019.

Fertilization increased overall rating as compared with the no-fertilizer treatment in January 2019 (Table 3) but did not affect visual rating of microgreen species in November 2018.

3.3. Macronutrient Concentrations

3.3.1. Nitrogen

Nitrogen concentration in microgreens was affected by the main effects of species, fertilization, and soaking treatment without interaction in November 2018 (Figure 3A) but affected by the species and fertilization interaction in January 2019 (Figure 3B). In November 2018, pea, amaranth, and kale ranked first (65.1 mg·g^{-1}), second (54.1 mg·g^{-1}), and third (51.2 mg·g^{-1}), respectively, in N concentration, higher than any other microgreen species. Cabbage, daikon radish, and kohlrabi microgreens had the lowest N concentration from 40.6 to 42.6 mg·g^{-1}. Fertilization increased N concentration by 10.6% in November 2018 (Table 2). Soaking decreased overall N concentration by 1.62% across microgreen species (Table 4).



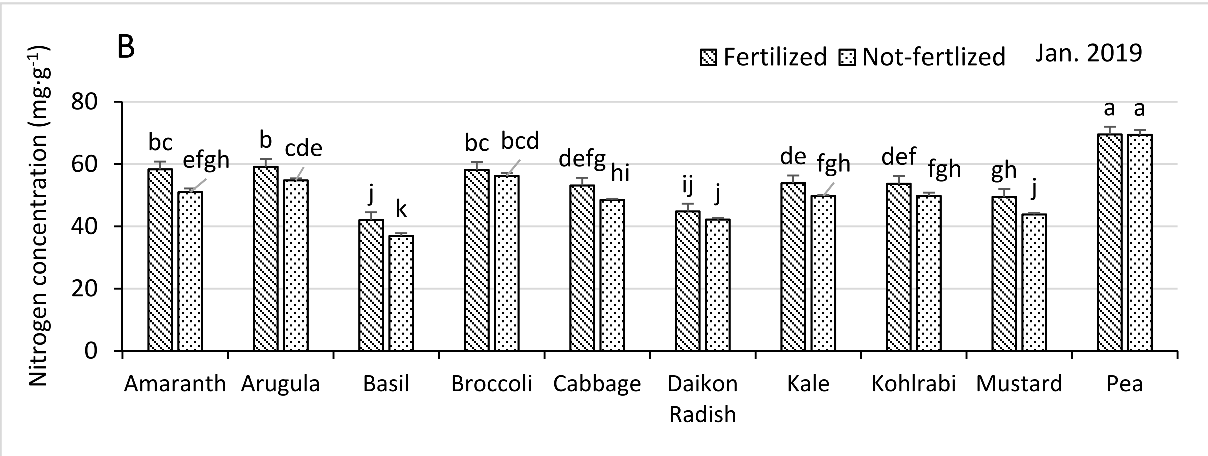


Figure 3. Nitrogen concentrations on a dry weight basis in ten microgreens affected by the main effect of species in November 2018 (**A**) or by the interaction between species and fertilization in January 2019 (**B**). Means with stannard error bars of each species were obtained by averaging data from both fertilization and pre-sowing seed treatments (**A**), or by averaging data from both pre-sowing seed treatments (**B**). Different lowercase letters within a chart suggest significant difference among treatments indicated by Tukey's HSD test at $\alpha \leq 0.05$.

In January 2019, pea microgreen also had the highest N concentration among all species, fertilized or not. Amaranth and arugula fertilized, and broccoli fertilized or not had the second highest N concentrations from 56.2 to 59.1 mg·g^{-1}. Basil, daikon radish, and mustard had the lowest N concentrations among all species fertilized or not. Fertilization increased N concentrations in amaranth, arugula, basil, cabbage, kale, and mustard, but not in daikon radish, broccoli, kohlrabi, or pea.

3.3.2. Phosphorus

Phosphorus concentration in microgreens were affected by the main effects of species and fertilization in November 2018 and affected by species, fertilization, and seed soaking in January 2019, but without interactions. Amaranth ranked first and second in P concentrations in November 2018 and January 2019, i.e., 16.6 and 15.5 $mg \cdot g^{-1}$, respectively (Figure S2). Mustard ranked third and first in November 2018 and January 2019, with P concentrations of 13.7 and 15.7 $mg \cdot g^{-1}$, respectively. Pea and cabbage ranked last and second last in P concentration in both experiments, lower than other species.

Fertilization increased P concentration by 8.8% and 7.8% in November 2018 and January 2019, respectively (Tables 2 and 3). Soaking decreased P concentration by 3.3% in January 2019 (Table 4).

3.3.3. Potassium

Potassium concentrations varied among species in November 2018 and January 2019 (Figure S3). There was no interaction among factors in either experiment. Ranking of K concentration among species were generally similar in the two experiments. Amaranth had the highest K concentrations of 69.2 and 67.4 $mg \cdot g^{-1}$ in November 2018 and January 2019, respectively. Mustard, basil, and kale had lower K concentration than amaranth, but higher than those in arugula, kohlrabi, or cabbage, with similar K concentrations in both experiments. Broccoli, daikon radish, and pea had the lowest K concentrations of 25.0 to 28.6 $mg \cdot g^{-1}$ in November 2018 and 25.7 to 28.6 $mg \cdot g^{-1}$ in January 2019.

Fertilization resulted in increased overall K concentration by 3.4% in November 2018 (Table 2). Soaking did not affect K concentration of microgreen species in either experiment.

3.3.4. Calcium

Calcium concentration in November 2018 was affected by the main effects of species and fertilization without interaction. Cabbage ranked first with Ca concentration of 25.2 $mg \cdot g^{-1}$, higher than any other species (Figure 4A). Broccoli had the second highest Ca concentration of 20 $mg \cdot g^{-1}$. Pea and daikon radish had the lowest Ca concentrations of 8.6 $mg \cdot g^{-1}$ among all microgreen species. Fertilization decreased overall Ca concentration by 3.3% as compared with the no-fertilizer treatment in November 2018 (Table 2).

Calcium concentration was affected by the interaction between species and soaking in January 2019 (Figure 4B). Basil and cabbage in the soaking treatment had comparable highest Ca concentrations of 18.1 and 17.4 $mg \cdot g^{-1}$, respectively, among all treatment combinations. Similar to results in November 2018, pea had the lowest Ca concentrations of 7.3 $mg \cdot g^{-1}$ (soaked) and 5.8 $mg \cdot g^{-1}$ (unsoaked) among species. Soaking decreased Ca concentrations in basil, cabbage, and kale as compared with untreated dry seeds but resulted in similar Ca concentrations in the other seven species.

3.3.5. Magnesium

Magnesium concentration was affected by the main effects of species, fertilization, and soaking without interactions in November 2018 and January 2019. Amaranth and basil ranked first and second in both experiments, i.e., 7.6 and 5.4 $mg \cdot g^{-1}$ in November 2018 and 7.5 $mg \cdot g^{-1}$ and 5.7 $mg \cdot g^{-1}$ in January 2019, respectively (Figure S4). Pea had the lowest Mg concentrations of 3.0 $mg \cdot g^{-1}$ in November 2018 and 2.5 $mg \cdot g^{-1}$ in January 2019, lower than any other species. The other seven species including arugula, cabbage, daikon radish, broccoli, kale, mustard, and kohlrabi had intermediate Mg concentrations, varying between the two experiments.

One-time fertilization decreased Mg concentrations as compared with the no-fertilizer treatment by 3.2% in November 2018 and by 3.8% in January 2019 (Tables 2 and 3). Soaking decreased Mg concentrations as compared with untreated dry seed in both experiments as well (Table 4).

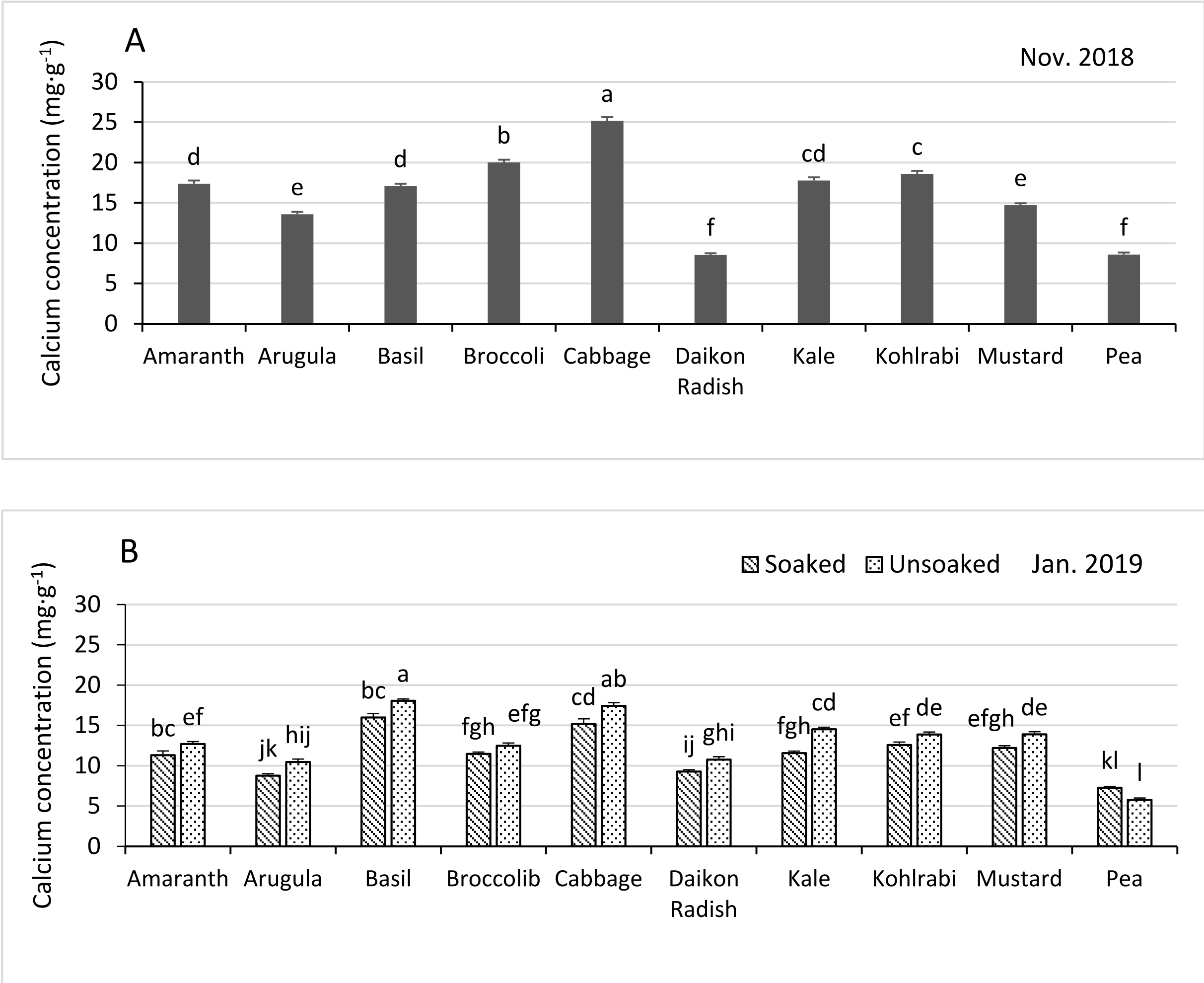


Figure 4. Calcium concentrations on a dry weight basis in ten microgreens affected by the main effect of species in November 2018 (**A**) or by the interaction between species and seed soaking in January 2019 (**B**). Means with standard error bars of each species were obtained by averaging data from both fertilization and pre-sowing seed treatments (**A**), or by averaging data from both fertilization treatments (**B**). Different lowercase letters within a chart suggest significant difference among treatments indicated by Tukey's HSD test at $\alpha \leq 0.05$.

3.4. Micronutrient Concentrations

3.4.1. Copper

Copper concentrations were affected by the interaction between species and soaking in both experiments (Figure 5). Fertilization did not affect copper concentration in November 2018 or January 2019. Pea, amaranth, and basil, soaked or not, ranked first, second, and third in Cu concentration among species in both experiments, ranging from 12.4 to 23.4 $\mu g \cdot g^{-1}$ in November 2018, and from 7.8 to 11.3 $\mu g \cdot g^{-1}$ in January 2019, respectively, higher than other species, except for kale in November 2018. Arugula, broccoli, kohlrabi, and mustard had the lowest Cu concentrations among all species in November 2018, ranging from 1.0 to 5.1 $\mu g \cdot g^{-1}$. In January 2019, arugula, broccoli, daikon radish, and kohlrabi had comparable lowest Cu concentrations among all species, soaked or not, ranging from 1.2 to 1.7 $\mu g \cdot g^{-1}$. Soaking or not generally resulted in similar Cu concentration within one species, except soaking increased Cu concentration in pea in November 2018 and January 2019.

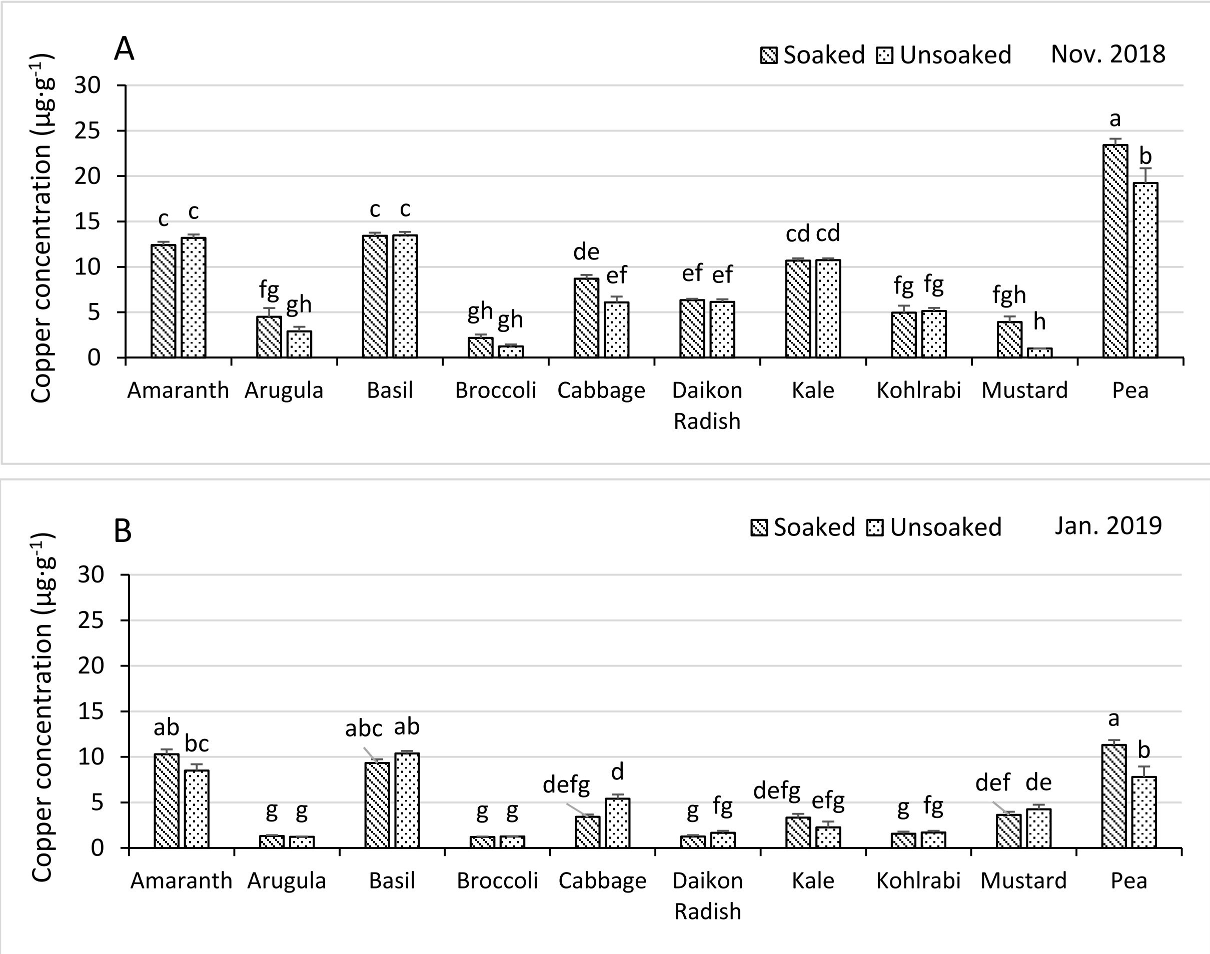


Figure 5. Copper concentrations on a dry weight basis in ten microgreens affected by the interaction between species and seed soaking in November 2018 (**A**) and in January 2019 (**B**). Means with standard error bars of each species were obtained by averaging data from both fertilization treatments. Different lowercase letters within a chart suggested significant difference among treatments combinations indicated by Tukey's HSD test at $\alpha \leq 0.05$.

3.4.2. Iron

Iron concentrations varied among species in November 2018 and January 2019 (Figure S5). Iron concentrations ranged from 73.5 $\mu g \cdot g^{-1}$ in daikon radish to 389.1 $\mu g \cdot g^{-1}$ in pea, in November 2018, and from 71.6 $\mu g \cdot g^{-1}$ in daikon radish to 187.9 $\mu g \cdot g^{-1}$ in basil, in January 2019. Mustard, broccoli, and kohlrabi had similar Fe concentrations in both experiments, ranging from 175 to 185.3 $\mu g \cdot g^{-1}$ in November 2018 and from 118.1 to 121.3 $\mu g \cdot g^{-1}$ in January 2019.

Fertilization increased Fe concentrations by 10.1% in November 2018 (Table 2) and by 5.0% in January 2019 (Table 3). Soaking decreased Fe concentration by 5.0% in January 2019 (Table 4).

3.4.3. Manganese

Manganese concentration varied among species ranging from 38.7 $\mu g \cdot g^{-1}$ in daikon radish to 207.5 $\mu g \cdot g^{-1}$ in cabbage in November 2018, with amaranth and cabbage producing comparable highest Mn concentrations (Figure 6A). Next to amaranth or cabbage, broccoli and basil had similar Mn concentrations of 110.8 and 97.5 $\mu g \cdot g^{-1}$, respectively, higher than kohlrabi, kale, arugula, mustard, or daikon radish. Mustard and daikon radish had comparable lowest Mn concentrations among all tested species.

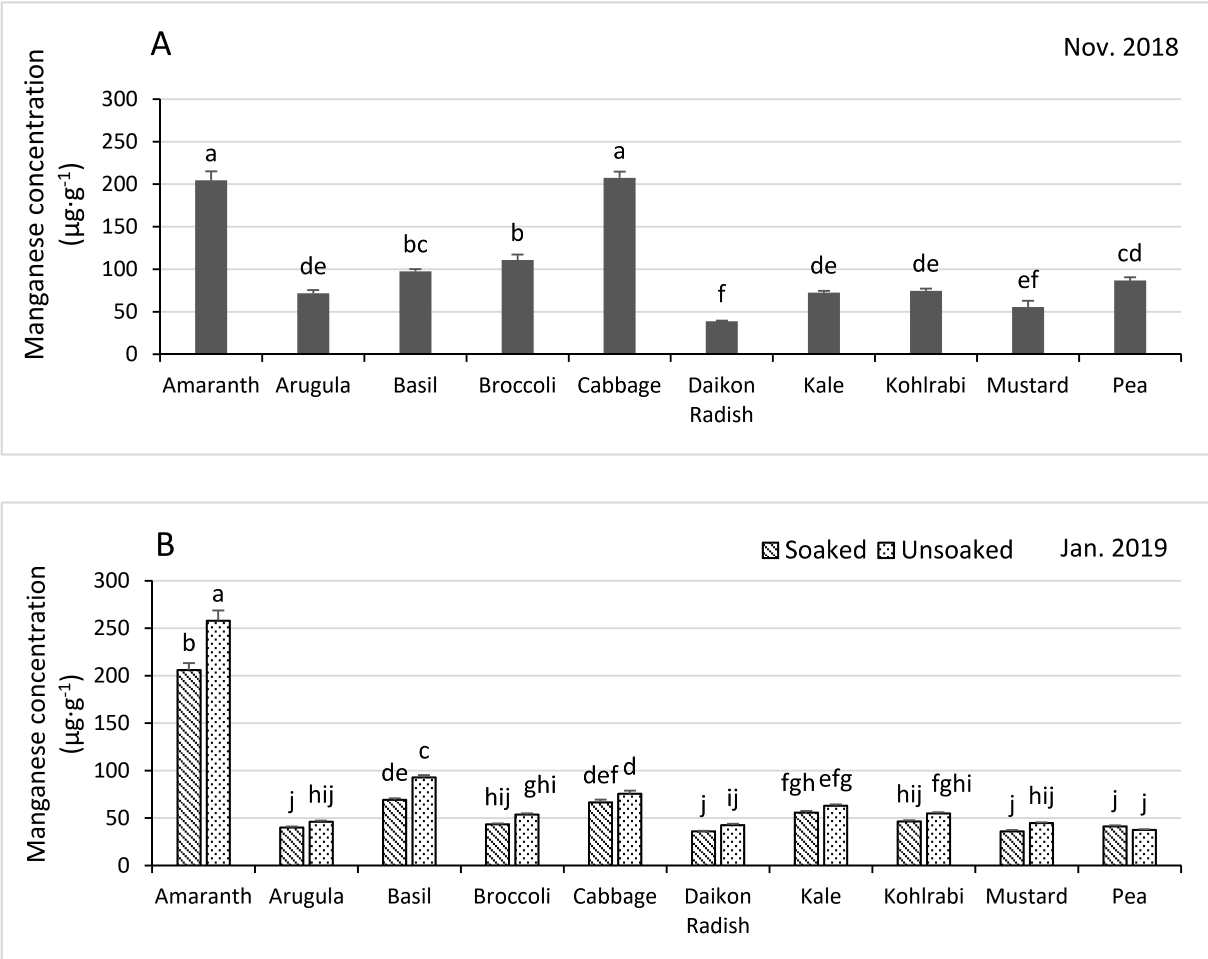


Figure 6. Manganese concentrations on a dry weight basis in ten microgreens affected by the main effect of species in November 2018 (**A**) or by the interaction between species and seed soaking in January 2019 (**B**). Means with standard error bars of each species were obtained by averaging data from both fertilization and pre-sowing seed treatments (**A**), or by averaging data from both fertilization treatments (**B**). Different lowercase letters suggested significant difference among treatments indicated by Tukey's HSD test at $\alpha \leq 0.05$.

Manganese concentration was affected by the interaction between species and soaking in January 2019 (Figure 6B). Amaranth had the highest Mn concentrations of 205.9 and 257.9 $\mu g \cdot g^{-1}$ in soaked and unsoaked treatment respectively, higher than any other species. Arugula, daikon radish, mustard, and pea had comparable lowest Mn concentrations from 35.9 to 46.1 $\mu g \cdot g^{-1}$ regardless of the soaking treatment. Soaking decreased Mn concentration in amaranth and basil but resulted in similar Mn concentrations in other eight species.

Fertilization did not affect Mn concentration in November 2018, but decreased Mn concentration by 5.0% in January 2019 (Table 3).

3.4.4. Zinc

Zinc concentrations varied among species in both experiments ranging from 65.6 $\mu g \cdot g^{-1}$ in kale to 199.7 $\mu g \cdot g^{-1}$ in arugula, in November 2018, and from 51.5 $\mu g \cdot g^{-1}$ in daikon radish to 114 $\mu g \cdot g^{-1}$ in arugula, in January 2019 (Figure S6). Arugula ranked first in Zn concentrations in both experiments, higher than any other species. In November 2018, pea, cabbage, and broccoli had similar Zn concentrations ranging from 141.5 to 152.4 $\mu g \cdot g^{-1}$, next to arugula, higher than basil, amaranth, or kale with comparable lowest Zn concentrations from 67.7 to 92.1 $\mu g \cdot g^{-1}$. Ranking of Zn concentrations in January 2019 was separated into the following five groups: Arugula > basil, or cabbage > broccoli, amaranth or pea > kohlrabi, kale, or mustard > daikon radish.

Fertilization increased Zn concentration by 6.5% in November 2018 (Table 2), while soaking decreased Zn concentration by 3.3% in January 2019 (Table 4).

3.4.5. Boron

Boron concentrations varied among species in November 2018, ranging from 14.1 $\mu g \cdot g^{-1}$ in daikon radish to 46.4 $\mu g \cdot g^{-1}$ in mustard (Figure 7A). Broccoli, kohlrabi, and cabbage had similar B concentrations ranging from 40.1 to 41.3 $\mu g \cdot g^{-1}$ next to mustard, higher than arugula, basil, pea, kale, or daikon radish.

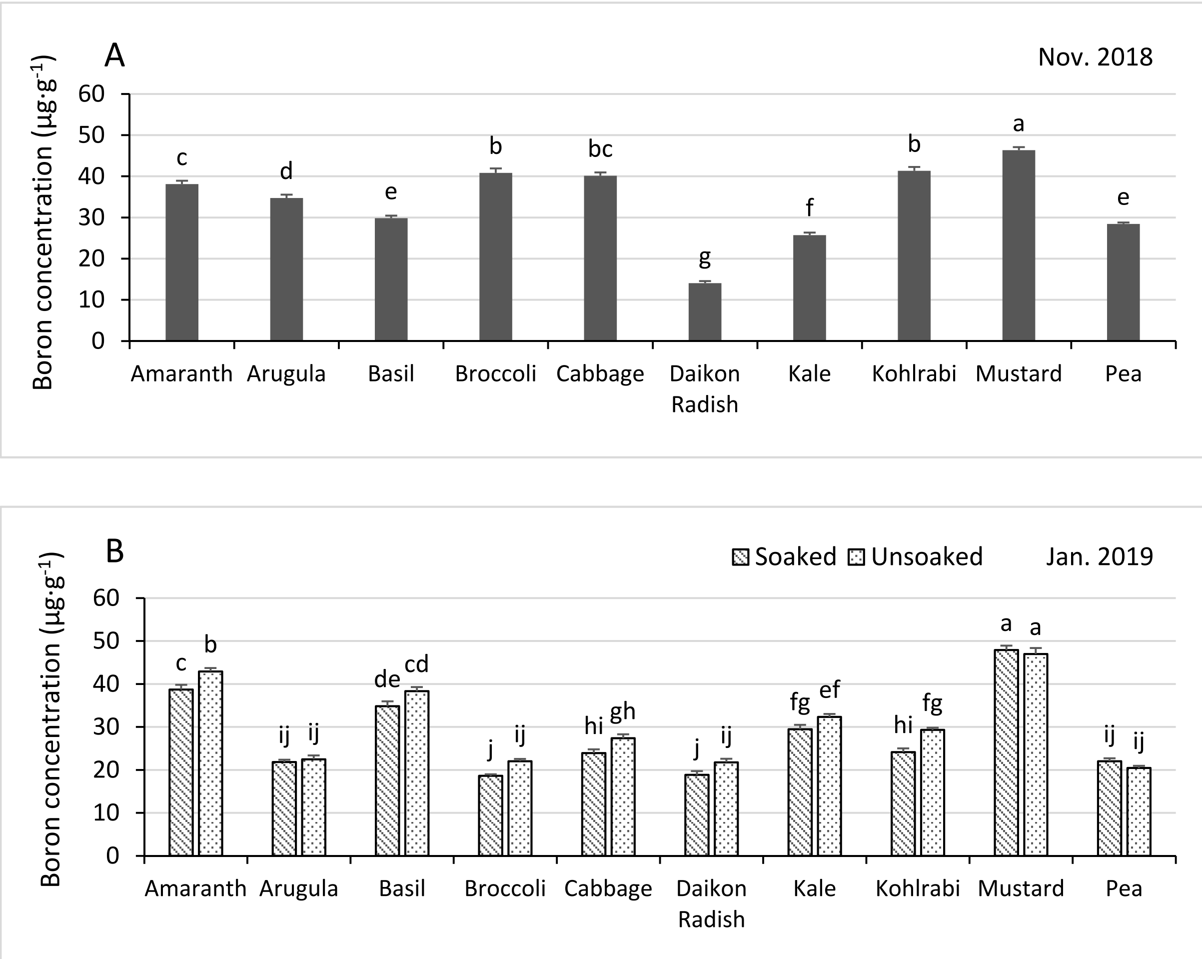


Figure 7. Boron concentrations on a dry weight basis in ten microgreens affected by the main effect of species in November 2018 (**A**) or by the interaction between species and seed soaking in January 2019 (**B**). Means with standard error bars of each species were obtained by averaging data from both fertilization and pre-sowing seed treatments (**A**), or by averaging data from both fertilization treatments (**B**). Different lowercase letters within a chart suggest significant difference among treatments indicated by Tukey's HSD test at $\alpha \leq 0.05$.

In January 2019, boron concentration was affected by the interaction between species and soaking (Figure 7B). Mustard had the highest B concentration, with 47.9 $\mu g \cdot g^{-1}$ in soaked and 47.0 $\mu g \cdot g^{-1}$ in unsoaked treatment, respectively, followed by amaranth, with B concentrations of 38.7 $\mu g \cdot g^{-1}$ and 42.9 $\mu g \cdot g^{-1}$ in soaked and unsoaked treatments. Arugula, broccoli, daikon radish, and pea had comparable lowest B concentrations ranging from 18.6 to 22.5 $\mu g \cdot g^{-1}$, soaked or not. Soaking or not generally resulted in similar B concentrations within one species, except that soaking decreased B concentration in amaranth and kohlrabi.

Fertilization increased B concentration by 3.2% in November 2018 and by 7.3% in January 2019 (Tables 2 and 3). Soaking decreased B concentration by 4.2% in November 2018 (Table 4).

4. Discussion

The ten tested microgreen species varied in their yields in terms of fresh and dry shoot weights, consistent with reported ranges [3,16,23–25]. Several factors affect yield of microgreens, including seeding rate, fertilization, growing medium, pre-sowing seed treatment, harvest stage, and microenvironment including temperature and lighting conditions [3,5,9,16,23–26]. Low yield has been considered to be one of the limiting factors in microgreen production [24,27]. Microgreen yield of a given species varied between the two experiments (November 2018 and January 2019) in this study, likely due to changing microenvironment in the greenhouse. Air temperature in the greenhouse mostly fluctuated within 5 °C as compared with the setting of 25 °C, and relative humidity ranged from 30% to 70% within the experiment duration. Light conditions in the greenhouse were not recorded in this study and fluctuated drastically within a year. According to past records, daily light integral between November and February was generally the lowest of the year in the local area [28], which limits microgreen growth without supplemental lighting. Growers could experience fluctuations in microgreen yield between production cycles throughout a year.

A meaningful evaluation of microgreen yield should take seed cost into consideration, since seeds are used in large quantity and represent a major part of production costs [9,29]. Seeding rate in this study ranged from 52.9 $g \cdot m^{-2}$ in basil to 1285 $g \cdot m^{-2}$ in pea (Table 1), equivalent to 8378 seeds per m^2 for pea to 69,807 seeds per m^2 for amaranth, mostly consistent with ranges of 10,000 to 40,000 seeds per m^2, as reported by Di Gioia and Santamaria [29]. Increasing seedling rate increases microgreen fresh yield but decreases mean shoot weight [3,16]. Crop-specific information investigating the interaction between seeding rate and yield, and quality of microgreens merits further investigation. This is especially challenging with the expanding microgreen industry and the constantly increasing number of species and varieties being produced as microgreens [5,8,30].

Microgreens varied in their nutrient profiles including mineral nutrient and phytochemical concentrations [13,17,31]. The mineral nutrient concentrations of microgreens tested, in this study, were generally within the ranges as reported by Waterland et al. [4] and Xiao et al. [13]. There are a number of reports regarding how light quality and quantity affect nutritional content of microgreens [5,17,32–34], with fewer reports investigating the effects of cultural practices on such variables.

One-time fertilization increased fresh shoot weight and concentrations of N, P, K, Fe, Zn, and B in microgreens in one or both experiments in this study. On the one hand, the fertilization treatment decreased Ca concentration in November 2018, decreased Mg in both experiments, and decreased Mn concentration in January 2019. The water-soluble fertilizer, used in this study, provided macronutrients (20N:8.7P:16.6K), 0.05% Mg, and micronutrients including 0.013% Cu, 0.05% Fe, 0.025% Mn, 0.013% B, and 0.025% Zn, and therefore increased concentrations of macro- and micro-nutrients in the microgreens. The decreased Ca concentration could be due to the dilution effect in microgreens without a source of Ca from the fertilizer. Bulgari et al. [23] reported that low Ca concentration was commonly found in some microgreens. On the other hand, calcium chloride application was reported to increase Ca concentration, increase biomass, and improve postharvest quality in broccoli microgreens, making it a better Ca source to human nutrition [24].

Further investigation is needed regarding crop-specific requirements for fertilization rate and frequency with respect to microgreen yield and quality. There are concerns that some microgreens such as arugula accumulate high levels of nitrate, which is considered to be an unhealthy factor [23]. However, this was believed to be controlled by lowering fertilization rate or selectively controlling N fertilizer form [28]. A positive aspect is that microgreen Swiss chard and arugula were reported to have lower nitrate concentrations as compared with their baby leaf or adult stage counterparts [23]. In other cases, seeds were intentionally biofortified to increase certain beneficial micronutrients, for example, selenium (Se), to increase Se concentration in basil microgreens [35].

The seed soaking treatment consistently decreased fresh and dry shoot weights, shoot height, and concentrations of macro- and micro-nutrients in tested microgreen species when there was an effect, with the exception that soaking increased Cu concentration in pea in November 2018 and January 2019. This possibly resulted from the fact that dehydrated cellular membranes in imbibing seeds are dysfunctional, resulting in a leak of nutrients such as inorganic ions, amino acids, carbohydrate, and phenolics [22]. When evaluating pre-sowing seed treatments including seed soaking or priming, their effects on shoot production and nutritional content of microgreens should be examined in addition to the efficacy in advancing seedling emergence [19].

Most microgreen species, investigated in our study, were considered to be fast growing microgreens. Nine species germinated within 36–48 h regardless of seed soaking, with basil having the slowest germination. We observed advanced germination of approximately 12 to 24 h, but similar harvest dates within a given species. Seed soaking might be valued more in slow growing species like basil than fast-growing species for accelerating germination and shortening the production cycle. The use of seed soaking treatment should be weighed against the fact that it may decrease microgreen yields, since, in our study, fresh shoot weight and shoot height of microgreens were decreased by soaking in January 2019. Human pathogen contamination has become one of the major food safety concerns in microgreen and sprout production [36–38]. It is important to purchase high quality seeds from reliable suppliers that have a high germination percentage, 85% to 98% in this study according to the label, and the seeds should be certified for microgreen and sprout production for reduced pathogen risks [5,39].

5. Conclusions

The ten tested microgreen species in this study varied in fresh and dry shoot weights, shoot height, and mineral nutrient concentrations. Selection of microgreen species should consider shoot yield, seeding rate, and seed cost. One-time fertilization increased fresh shoot yield and macro- and micro-nutrient concentrations in microgreens but may have resulted in diluted nutrient concentrations of elements not included in the fertilizer. The use of pre-sowing seed soaking treatment to advance seed germination should be weighed against its possible effects in reducing microgreen yield and mineral nutrient concentrations. Changes in shoot yield and mineral nutrient concentrations could be expected between production cycles due to a fluctuating microenvironment.

Supplementary Materials: The following are available online at https://www.mdpi.com/2311-7524/7/2/14/s1, Figure S1: Dry weight percentage of ten microgreens affected by the main effect of species in November 2018 (A) or by the interaction between species and seed soaking in January 2019 (B), Figure S2: Phosphorus concentrations on a dry weight basis of ten species grown as microgreens, Figure S3: Potassium concentrations on a dry weight basis of ten species grown as microgreens, Figure S4: Magnesium concentrations on a dry weight basis of ten species grown as microgreens, Figure S5: Iron concentrations on a dry weight basis of ten species grown as microgreens, Figure S6: Zinc concentrations on a dry weight basis of ten species grown as microgreens.

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