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Avocado (*Persea americana* cv. 'Hass') Fruit Mineral Composition at Canopy Level towards Sustainable Quality

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Abstract: Sustaining avocado fruit quality is crucial to maintain customer satisfaction and confidence. Among fruit qualities, mineral nutrient composition is an important contributor to postharvest robustness. Towards better understanding and addressing variability within the plant canopy, 'Hass' fruit from across seven orchard blocks were individually characterised. From five representative trees in each block, five fruit were harvested (one from each of five positions: top (sun-exposed), bottom (shaded), middle (shaded), East (sun-exposed), and West (sun-exposed)). Fruit dry matter was significantly higher ($p \leq 0.001$) in fruit from the top, East, and West sun-exposed positions. No significant ($p > 0.05$) effect of position was discerned for fruit weight at harvest or for either stem end rot (SER) or body rot (BR) incidence at eating soft. Shaded fruit had significantly higher ($p \leq 0.05$) [N], [K], [Mg], N:Ca, K:Ca, and K + Mg:Ca in their flesh. Significant negative linear correlations ($p \leq 0.001$) were obtained between fruit DM and flesh [N] (r = −0.75), [K] (r = −0.67), and N:Ca (r = −0.57). SER and BR incidence were significantly positively correlated ($p \leq 0.01$) with flesh and skin mineral ratios of N:Ca, K:Ca, Mg:Ca, and K + Mg:Ca. Skin and flesh [Ca] were significantly negatively correlated with SER (r = −0.51, $p \leq 0.01$) and BR (r = −0.74, $p \leq 0.001$) incidences. Soil cation (Ca, Mg, K) availability (%base saturation of cation exchange capacity (CEC)) was not ($p > 0.05$) correlated with skin or flesh mineral concentrations or ratios. Considered collectively, results suggest that selective harvest of sun-exposed fruit with inherently lower mineral nutrient ratios yields relatively robust fruit. Such fruit lots should better tolerate the rigours of harvest and postharvest treatment and handling. In this context, they should better maintain quality upon passage through long, in terms of accumulated time-temperature increments, export supply chains. In contrast, shaded fruit could be directed into shorter domestic supply chains. As a harvest strategy, segregating fruit lots from harvest could underpin the quality offered to consumers at the end of 'short' and 'long' supply chains.

Keywords: Calcium; disease; flesh; N:Ca; nutrients; quality; skin

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1. Introduction

Avocado (*Persea americana*) is an important subtropical fruit in high demand worldwide. Global production increased by over 170% from 2009 to 2019 [1]. In Australia, production rose exponentially from 40,000 tonnes in 2009/10 to over 120,000 tonnes in 2021/22 [2]. Per capita consumption simultaneously increased from 2.2 to 4.8 kg [2]. The variety 'Hass' accounts for 83% of total Australian avocado production [2]. The simultaneous increase in production and demand are incentivising avocado industry interests around the world to establish, grow, and protect both domestic and export markets. Demand can be seriously curtailed by quality, quantity, and/or consistency falling short of market expectations and consumer preferences [3].

A sustainable supply of quality fruit free from internal defects, including stem end rot (SER), body rot (BR), skin damage, flesh bruising, flesh browning, and vascular discolouration [4] is important globally. Despite a relatively mature industry, a relatively recent quality monitoring report for domestic retail sales in Australia suggested that one in every

10 domestic 'Hass' fruit had ≥10% internal flesh defects, barely meeting acceptability [5]. For export markets, circumstances are more challenging due to 'longer' supply chains in terms of time temperature unit (TTU) integrals and more treatment (e.g., phytosanitary), handling (e.g., consolidation, cross docking), and procedural (e.g., exporter, importer) activities [3,6–8].

If fruit is not inherently robust at harvest, then quality is likely to decline relatively quickly, leading to increased wastage and even whole consignment losses, market dissatisfaction, reduced financial returns, and repeat orders [6]. Selection of inherently robust fruit for 'long' (export), but also for 'short' (domestic) supply chains, is vital to establishing and maintaining a sustained supply of quality fruit to retailers and consumers, and to mitigating product losses from harvest to consumption [9].

However, realising, measuring, and monitoring robustness for optimum outturn quality is challenging, including in terms of establishing reliable indices. This is primarily because fruit robustness is not well characterised nor appreciated in the context of 'long' (e.g., export) versus 'short' (e.g., domestic) supply chains. Fruit dry matter (DM) and size at harvest are often used as indices of robustness [6,8]. Mineral nutrition also interacts with postharvest qualities, including product size, DM, shelf life, and susceptibility to rots and disorders [10]. Calcium (Ca) and nitrogen (N) are implicated in fruit susceptibility to diseases (e.g., body and stem end rots) and disorders (e.g., diffuse discoloration and vascular and flesh browning) [10–20].

While individual mineral nutrients influence fruit development [10–20], their balance, such as N/Ca and K/Ca ratios in fruit skin and flesh tissues, are considered useful indices of robustness and quality [16,21,22]. However, specific target ratios as predictors of fruit qualities are somewhat elusive, perhaps because of inherent variability across genotype, environment, and management practices. Marques et al. [15] reported marked tree-to-tree variability in 'Hass' avocado fruit quality and flesh mineral concentrations within the orchard. Fruit position in the canopy also contributes to variability. Perring and Jackson [23] and Kalcsits et al. [24] reported that mineral concentrations in individual apple fruit on a tree may vary by two to threefold, and differentially impact postharvest quality.

Relatively intensive single fruit sampling is required to quantify and qualify quality variation in mineral composition towards decision support tools for sustainable production and marketing of robust fruit fit for long and/or arduous horticulture supply chains, including in terms of steps, treatments, time, and distance. More certainty and/or reliability around critical fruit nutrient ratios should facilitate decisions in relation to 'short' (e.g., domestic) versus long (e.g., 'export') supply chains towards more predictable quality for consumers and better returns to producers and other supply chain stakeholders.

The present work explores fruit-to-fruit variability in mineral nutrients and shelf life for 'Hass' avocado fruit sampled at different spatial levels within the canopies of individual trees across orchard blocks. It aims to inform fruit harvesting protocols towards a more sustainable supply of robust fruit, particularly for 'long' (e.g., export) supply chains.

2. Materials and Methods

2.1. Experimental Sites and Fruit Sampling

'Hass' avocado fruit sampling was conducted in the fruiting season (2022) on three commercial avocado farms in south-central Queensland, Australia (Table 1). Farms 1 and 2 were ca. 15 km apart and Farms 1 and 3 were 122 km apart. Orchard blocks denoted B1–7 were selected across farms based on differences in tree age, management practices, and past fruit quality. All blocks were self-pollinated. Five representative trees within each block were selected and tagged for sampling in consultation with farm staff and considering uniformity in tree size, vigour, and crop load. All trees in each block were in 'on' year cropping season, with good crop loads. The blocks were irrigated with under-tree sprinklers and rows were orientated North-South with a planting density of 10 m interrow and 5 m within a row. Fruit were sampled from each tree from five positions in the canopy:

viz., top (sun-exposed), bottom (shaded), middle (shaded), East (sun-exposed), and West (sun-exposed).

Table 1. Site information on 'Hass' avocado tree blocks used for fruit sampling. 'F' and 'B' represent sampled farms and blocks within each farm, respectively. '-' no information available.

Sampling Areas	Farms	Location	Block	Tree Age	Rootstock
Childers	F1	25°13′30.0″ S 152°17′49.2″ E	B1	15	Birdwood (BW19)
			B2	15	Birdwood (BW19)
			B3	12	Velvick
			B4	12	Zutano
	F2	25°08′22.1″ S 152°16′00.2″ E	B5	8	Birdwood (BW19)
			B6	8	Birdwood (BW19)
Gunalda	F3	26°00′51.3″ S 152°31′11.2″ E	B7	-	Velvick + Zutano

At commercial harvest, one representative fruit free from damage or defect from each position in each tree in each block was harvested. A 0–30 cm depth soil sample, where most feeder roots are present, was taken for each tree using a handheld tube corer. Harvested fruit and soil samples were labelled and transported in an air-conditioned van to a postharvest laboratory at The University of Queensland, Gatton.

2.2. Fruit Quality Parameters

Upon arrival at the postharvest laboratory, each fruit was weighed and subsequently held at 20 °C and > 90% relative humidity in the shelf life room for ripening. Hand firmness was the criteria for fruit ripening and shelf life. Firmness was assessed on a 0–7 scale after the International Avocado Quality Manual [25]. Fruits were assessed every second day until each reached a firmness rating of 5 (eating soft), signifying the end of shelf life.

Incidence and severity of disease and/or disorder were assessed by eating soft upon longitudinally cutting through the fruit flesh. The fruits were rated according to the International Avocado Quality Manual [25]. The internal quality of each fruit was visually assessed as flesh proportion (%) affected by disease and/or disorder. Consumer acceptability was judged as < 10% of affected flesh and % incidence was: (number of fruit having ≥ 10% affected flesh/total fruit) × 100. Fruit having both SER and BR symptoms were counted in both disease categories for % incidence calculation.

Individual fruits were assessed at the end of shelf life for DM and duly prepared for mineral analysis. For DM, flesh samples were taken using a fruit corer from the equatorial region of each fruit, weighed for fresh weight, and dried at 65 °C in a fan-forced oven to constant weight. DM was calculated as: (dry weight/fresh weight) × 100.

2.3. Mineral Analyses

For soil mineral analysis, samples were oven-dried at 40 °C for 3–4 days and sieved to pass a <2 mm mesh. Nitrate-nitrogen and ammonium-nitrogen were measured upon extracting samples with 2 M potassium chloride solution. Ammonium-nitrogen was measured colourimetrically after dilution and nitrate-nitrogen was reduced to nitrite through a copperised cadmium column and measured colourimetrically [26]. Exchangeable cation concentrations of Ca, Mg, Na, K, and Al, soil samples were extracted using 0.1 M ammonium chloride and barium chloride 1:10 mixture and measured using inductively coupled plasma spectroscopy (ICPS) [26]. Results were expressed as meq/100 g. Soil boron (B) was measured by extraction with 0.01 M calcium chloride (1:4) heated to 90 °C and analysed by ICPS [26]. Plant-available sulphur (S) in soil was determined by extraction with 0.25 M potassium chloride solution and analysed with ICPS [26]. Plant-available phosphorus (P) was measured upon extraction with 0.5 M sodium bicarbonate solution (pH 8.5) for 16 h at a 1:100 ratio and measured colourimetrically [26].

Fruit flesh from the 'equatorial region' and whole fruit skin samples were used for mineral analyses. Skin and flesh samples were dried in a fan-forced oven at 65 °C for 3 days, ground to a fine powder in a laboratory-scale mini-ball mill (Retsch Mixer Mill MM 400), and analysed for mineral concentrations. The dried samples were digested with hydrogen peroxide and nitric acid and then analysed using ICPS [27]. Results were expressed as mg/kg dry weight for B, Mn, Zn, Fe, and Cu and as % dry weight for Ca, K, S, P, and Na. Total N was measured using a LECO (Laboratory Equipment Corporation) combustion analyser with a combustion temperature of 950 °C [28], and results were expressed as % N on a dry weight basis. All mineral analyses were carried out by the NATA-accredited CSBP Soil and Plant Analysis Laboratory, Western Australia (https://www.csbplab.com.au/ (accessed on 13 November 2023)).

2.4. Statiscial Analyses

Each fruit position was considered a treatment unit, and each tree was considered a replicate within each orchard block. Data on fruit quality and mineral nutrient concentrations were analysed by two-way analysis of variance (ANOVA) for fruit position within the canopy and orchard block as the factors. Treatment means were compared at the 5% confidence level by Tukey's HSD test [29]. Significance levels are indicated as: * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$, with ns for not significant ($p > 0.05$). Pearson correlation coefficients (r) were used to relate mineral nutrient concentrations in flesh, skin, and soil samples to fruit quality [30].

Furthermore, the whole data set was divided into two categories based on ANOVA results as sun-exposed (top + east + west) and shaded (middle + bottom) fruit mineral nutrient concentrations (viz., N, K, N/Ca, K/Ca, and K + Mg/Ca) were significantly ($p \leq 0.05$) different between these two aggregate canopy position categories. These data were used to calculate sample size for mineral ratios to inform future sampling strategies. The sample size was calculated at the farm level using Cochran's formula of minimum sample size [31] as based on the standard deviation of population; i.e., $n = (z/d)^2 \times SD^2$, where, n is the minimum sample size required, z is critical value for desired level of confidence (e.g., 1.96 for 95% confidence), d is desired level of precision (i.e., maximum allowable difference between sample mean and true population mean), and SD is standard deviation of population. All statistical analyses were performed using SPSS (IBM® SPSS® Statistics version 27) and R (4.0.2).

3. Results

3.1. Soil Characteristics

From soil properties and nutrient status from the top 0–30 cm soil profile for the different orchard blocks as evaluated at fruit harvest time, B1–3 were clay and B4–7 were loam or loam/clay in texture with a neutral pH range (6.0–7.5) (Table 2). Soil organic carbon % was significantly ($p \leq 0.05$) higher (>2%) in B1–3 compared to other orchard blocks. Influences of organic C and clay were evident on the nutrient holding capacity of soil with higher ($p \leq 0.05$) cation exchange capacity (CEC) (>20 meq/100 g) in B1–3 compared to B4–7 (Table 2). Exchangeable [Ca] and [Mg] were higher ($p \leq 0.05$) also in B1–3. In contrast, exchangeable [Na] and [K] generally did not differ ($p > 0.05$) among blocks, except in B5 and B6 having low exchangeable [K] (Table 2). As % base saturation of CEC, cation concentrations were not significantly ($p > 0.05$) different among blocks (Table 2). The % base saturation represents the ready availability of cations in soil solution. Ammonium-N and nitrate-N concentrations were highest in B3 and B1, respectively, while B6 showed significantly ($p \leq 0.05$) lower concentrations of both N forms. Overall, B6 had lower concentrations of almost all mineral elements compared to other blocks (Table 2).

Table 2. Soil characteristics and mineral concentrations from the 0–30 cm depth across seven orchard blocks at 'Hass' avocado fruit harvest. Each data point represents an average of five analyses. Means followed by same letter in each row do not differ statistically at $p \leq 0.05$ according to Tukey's HSD test.

Parameters	Unit	Orchard Blocks							
		B1	B2	B3	B4	B5	B6	B7	Significance
Texture	-	Clay	Clay	Clay	Loam/clay	Loam	Loam	Loam/clay	-
pH (H_2O)	-	6.90 ^{ab}	7.06 ^{a}	6.96 ^{a}	6.56 ^{abc}	6.24 ^{c}	6.32 ^{bc}	6.12 ^{c}	***
EC	dS/m	0.46 ^{a}	0.37 ^{abc}	0.44 ^{a}	0.09 ^{c}	0.40 ^{ab}	0.16 ^{bc}	0.16 ^{bc}	**
Organic carbon	%	2.41 ^{ab}	2.13 ^{ab}	2.82 ^{a}	1.30 ^{cd}	1.74 ^{cd}	1.08 ^{d}	1.16 ^{cd}	*
CEC	meq/100 g	25.0 ^{ab}	24.5 ^{ab}	28.8 ^{a}	11.1 ^{c}	12.1 ^{bc}	7.95 ^{c}	8.26 ^{c}	**
Exc. Ca	meq/100 g	21.2 ^{ab}	20.1 ^{ab}	24.4 ^{a}	8.20 ^{cd}	10.6 ^{cd}	6.55 ^{d}	6.22 ^{d}	**
Exc. Mg	meq/100 g	3.00 ^{ab}	3.53 ^{a}	3.59 ^{a}	2.32 ^{b}	1.19 ^{c}	1.13 ^{c}	1.30 ^{c}	*
Exc. K	meq/100 g	0.57 ^{a}	0.63 ^{a}	0.54 ^{a}	0.40 ^{ab}	0.21 ^{bc}	0.11 ^{c}	0.41 ^{ab}	*
Exc. Na	meq/100 g	0.24	0.21	0.22	0.13	0.13	0.14	0.26	n.s.
Ca base saturation	% of CEC	83.2	80.4	83.6	73.7	86.9	82.2	74.8	n.s.
Mg base saturation	% of CEC	13.2	15.7	13.2	21.3	9.95	14.4	15.9	n.s.
K base saturation	% of CEC	2.40	2.74	2.11	3.64	1.84	1.46	5.00	n.s.
Ammonium—N	mg/kg	3.40 ^{ab}	3.20 ^{ab}	6.60 ^{a}	3.40 ^{ab}	3.75 ^{ab}	2.60 ^{b}	2.80 ^{b}	**
Nitrate—N	mg/kg	12.2 ^{a}	11.2 ^{a}	6.60 ^{ab}	1.50 ^{b}	3.40 ^{ab}	1.33 ^{b}	11.2 ^{a}	*
B	mg/kg	3.40 ^{abc}	3.57 ^{ab}	4.03 ^{a}	2.41 ^{cd}	2.26 ^{cd}	1.39 ^{d}	2.43 ^{bcd}	***
P	mg/kg	241 ^{a}	232 ^{a}	188 ^{ab}	255 ^{a}	151 ^{ab}	95.6 ^{b}	124 ^{b}	*
S	mg/kg	312 ^{ab}	217 ^{bc}	354 ^{a}	28.8 ^{c}	291 ^{ab}	75.6 ^{bc}	73.4 ^{bc}	**

* $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$, with n.s. for not significant ($p > 0.05$).

3.2. Fruit Quality Variations

Incidence of SER and BR diseases was evident in fruit from all canopy positions across orchard blocks (Figure 1, Table 3). In contrast, no incidence of any physiological disorders was discerned. Fruit position in the canopy had no evident effect ($p > 0.05$) on fruit weight or incidence of SER and BR (Table 3). In contrast, sun-exposed fruit from the top, East, and West canopy positions had higher ($p \leq 0.001$) DM content compared to shaded fruit from the middle and bottom canopy (Table 3, Figure S1). Fruit from the West canopy had longer ($p \leq 0.001$) shelf life compared to all other canopy positions (Table 3).

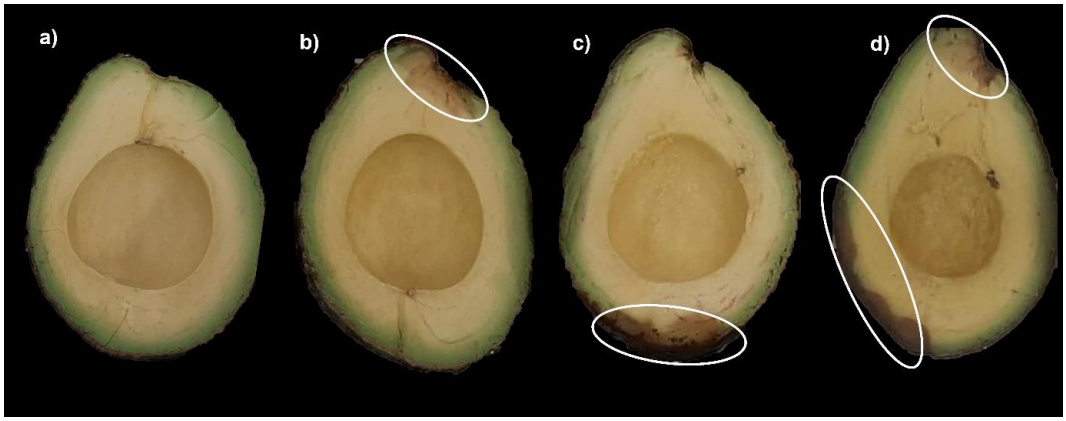

Figure 1. Disease expression highlighted by white ellipses as observed in 'Hass' avocado fruit at eating ripe stage: (**a**) Healthy, (**b**) Stem end rot (SER) affected, (**c**) Body rot (BR) affected, and (**d**) SER + BR affected.

Table 3. Avocado fruit quality attributes and % incidence of SER and BR in 'Hass' avocado fruit with ≥ 10% affected flesh as sourced from top, middle, bottom, East, and West positions within tree canopies across seven different orchard blocks. Means followed by same letter in each column are not significantly different at $p \leq 0.05$ according to Tukey's HSD test.

	DM (%)	Fruit Weight (g)	Shelf Life (d)	% Incidence SER	% Incidence BR
Fruit position (FP)					
Top	30.9 ^{a}	322.4	11.2 ^{b}	23	40
Middle	27.3 ^{b}	325.5	11.7 ^{b}	57	29
Bottom	26.0 ^{b}	331.1	11.8 ^{b}	43	46
East	29.6 ^{a}	341.7	12.1 ^{ab}	40	40
West	29.5 ^{a}	325.3	13.2 ^{a}	40	34
Significance	***	n.s.	***	n.s.	n.s.
Block (B)					
B1	29.6 ^{b}	335.3 ^{a}	11.7 ^{ab}	36 ^{ab}	48 ^{a}
B2	26.3 ^{c}	330.0 ^{a}	12.2 ^{ab}	56 ^{ab}	32 ^{ab}
B3	26.9 ^{c}	344.6 ^{a}	11.9 ^{ab}	40 ^{ab}	60 ^{a}
B4	27.4 ^{c}	354.5 ^{a}	10.9 ^{b}	76 ^{a}	64 ^{a}
B5	31.9 ^{a}	324.9 ^{a}	12.1 ^{ab}	20 ^{b}	4 ^{c}
B6	30.8 ^{ab}	327.9 ^{a}	11.9 ^{ab}	32 ^{ab}	12 ^{b}
B7	27.7 ^{c}	286.9 ^{b}	13.2 ^{a}	24 ^{b}	44 ^{ab}
Significance	***	***	***	***	***
Interaction (FP × B)					
Significance	n.s.	n.s.	n.s.	n.s.	n.s.

*** $p \leq 0.001$, with n.s. for not significant ($p > 0.05$).

Fruit from different orchard blocks differed significantly ($p \leq 0.05$) in all quality attributes. DM was highest in fruit from B5 and B6, followed by B1 (Figure S1, Table 3). Fruit weight generally did not differ ($p > 0.05$) across all orchard blocks, except for B7, with the lowest average value of 286 g. SER incidence was lowest in B5 (20%) and B7 (24%) fruit and was highest in B4 (76%) fruit compared to all other orchard blocks. A similar pattern was observed for BR, with the lowest incidence in B5 (4%), followed by B6 (12%), and with the highest incidence in B4 (64%) fruit (Table 3). Overall, fruit from B5 and B6 were of sound quality with relatively high DM content and low BR and SER incidence at eating soft. By comparison, fruit from B1–4 were relatively less sound with high disease incidence.

3.3. Mineral Nutrient Variations within Canopy

Flesh minerals: Canopy position associations for all flesh mineral concentrations and their ratios were evident with the exceptions of [Ca] and [B] (Table 4). Fruit from canopy-shaded positions (viz., middle, and bottom) had higher ($p \leq 0.001$) flesh [N], [K], and [Mg] than did sun-exposed fruit (viz., canopy top, East, and West) (Table 4). A non-significant trend towards higher flesh [Ca] was noted for sun-exposed compared to canopy-shaded fruit. The mineral ratios of N/Ca, K/Ca, Mg/Ca, and K + Mg/Ca ratios were higher ($p \leq 0.001$) in the flesh of canopy-shaded position fruit compared to sun-exposed fruit (Table 4).

Irrespective of fruit position in the canopy, orchard blocks differed significantly ($p \leq 0.05$) for individual flesh minerals and their ratios (Table 4). Overall, fruit from B5 and B6 had higher ($p \leq 0.05$) flesh [Ca] and relatively low [N], [K], N/Ca, K/Ca, Mg/Ca, and K + Mg/Ca ratios compared to other orchard blocks. The interaction of orchard blocks versus canopy positions was not significant ($p > 0.05$) (Table 4).

Table 4. 'Hass' avocado fruit flesh mineral concentrations from five within canopy positions across seven different orchard blocks. Means followed by same letter in each column are not statistically significantly different at $p \leq 0.05$ according to Tukey's HSD test.

'Hass' Flesh Mineral Concentrations (D.W.)									
	Ca (%)	**N (%)**	**K (%)**	**Mg (%)**	**B (mg/kg)**	**N/Ca**	**K/Ca**	**Mg/Ca**	**K + Mg/Ca**
Fruit position (FP)									
Top	0.0220	0.91 ^{b}	1.89 ^{b}	0.087 ^{c}	94.59	46.11 ^{b}	94.80 ^{b}	4.24 ^{ab}	99.04 ^{b}
Middle	0.0208	1.16 ^{a}	2.35 ^{a}	0.096 ^{ab}	106.92	62.60 ^{a}	125.10 ^{a}	5.00 ^{a}	130.09 ^{a}
Bottom	0.0212	1.22 ^{a}	2.41 ^{a}	0.099 ^{a}	112.61	64.12 ^{a}	124.63 ^{a}	5.02 ^{a}	129.65 ^{a}
East	0.0239	1.01 ^{b}	1.97 ^{b}	0.092 ^{bc}	107.18	47.53 ^{b}	91.80 ^{b}	4.17 ^{b}	95.86 ^{b}
West	0.0248	0.98 ^{b}	2.04 ^{b}	0.092 ^{bc}	109.03	43.65 ^{b}	89.45 ^{b}	3.95 ^{b}	93.41 ^{b}
Significance	n.s	***	***	***	n.s.	***	***	***	***
Block (B)									
B1	0.0198 ^{b}	1.04 ^{b}	2.23 ^{ab}	0.085 ^{b}	93.93 ^{bc}	55.75 ^{a}	119.76 ^{a}	4.49 ^{bc}	124.25 ^{a}
B2	0.0241 ^{ab}	1.18 ^{ab}	2.22 ^{ab}	0.098 ^{a}	114.35 ^{ab}	54.09 ^{a}	101.89 ^{ab}	4.42 ^{bc}	106.31 ^{ab}
B3	0.0191 ^{b}	1.26 ^{a}	2.32 ^{a}	0.096 ^{a}	114.40 ^{ab}	69.76 ^{a}	128.78 ^{a}	5.21 ^{ab}	133.99 ^{a}
B4	0.0188 ^{b}	1.21 ^{a}	2.24 ^{ab}	0.101 ^{a}	80.61 ^{c}	70.21 ^{a}	128.48 ^{a}	5.65 ^{a}	134.13 ^{a}
B5	0.0269 ^{a}	0.79 ^{c}	1.87 ^{c}	0.097 ^{a}	115.67 ^{ab}	32.73 ^{b}	75.69 ^{b}	3.84 ^{c}	79.51 ^{b}
B6	0.0268 ^{a}	0.81 ^{c}	1.98 ^{bc}	0.096 ^{a}	121.58 ^{a}	32.93 ^{b}	81.11 ^{b}	3.86 ^{c}	84.97 ^{b}
B7	0.0222 ^{ab}	1.11 ^{ab}	2.07 ^{abc}	0.081 ^{b}	101.92 ^{abc}	54.11 ^{a}	100.25 ^{ab}	3.86 ^{c}	104.11 ^{ab}
Significance	***	***	***	***	***	***	***	***	***
Interaction (FP × B)									
Significance	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*** $p \leq 0.001$, with n.s. for not significant ($p > 0.05$).

Skin minerals: Canopy position had no apparent influence ($p > 0.05$) on skin [Ca], [B], and Mg/Ca ratio (Table 5). However, skin [N] was higher ($p \leq 0.001$) in fruit from the canopy middle compared to East and West canopy fruit, while top and bottom canopy position fruit skin [N] was no different from other canopy positions. Fruit from canopy East had the lowest skin [K] and [Mg] (Table 5). N/Ca ratio was significantly ($p \leq 0.05$) lower in sun-exposed (East and West) and higher in shaded fruit (middle and bottom). K/Ca and K + Mg/Ca ratios were also higher in fruit skin from the canopy bottom and lower in the canopy West (Table 5).

Orchard blocks were also significantly ($p \leq 0.05$) different, with lower skin [N] and [Mg] in B5 and B6 and lower skin N/Ca, K/Ca, Mg/Ca, and K + Mg/Ca ratios in B5–7 (Table 5).

Table 5. 'Hass' avocado fruit skin mineral concentrations from five within canopy positions across seven different orchard blocks. Means followed by same letter in each column do not differ statistically at $p \leq 0.05$ according to Tukey's HSD test.

'Hass' Skin Mineral Concentrations (D.W.)									
	Ca (%)	**N (%)**	**K (%)**	**Mg (%)**	**B (mg/kg)**	**N/Ca**	**K/Ca**	**Mg/Ca**	**K + Mg/Ca**
Fruit position (FP)									
Top	0.0467	1.05 ^{ab}	2.41 ^{ab}	0.13 ^{a}	175.77	25.39 ^{bc}	59.95 ^{ab}	3.29	63.24 ^{ab}
Middle	0.0447	1.19 ^{a}	2.57 ^{a}	0.13 ^{a}	174.64	30.82 ^{ab}	66.84 ^{ab}	3.34	70.18 ^{ab}
Bottom	0.0384	1.09 ^{ab}	2.37 ^{ab}	0.12 ^{ab}	163.24	32.79 ^{a}	70.55 ^{a}	3.58	74.13 ^{a}
East	0.0430	0.93 ^{b}	2.20 ^{b}	0.11 ^{b}	148.52	24.05 ^{c}	57.07 ^{ab}	2.90	59.96 ^{ab}
West	0.0488	0.99 ^{b}	2.38 ^{ab}	0.12 ^{ab}	173.41	22.60 ^{c}	54.58 ^{b}	2.85	57.43 ^{b}
Significance	n.s.	***	**	*	n.s.	***	*	n.s.	**

Table 5. *Cont.*

'Hass' Skin Mineral Concentrations (D.W.)									
	Ca (%)	**N (%)**	**K (%)**	**Mg (%)**	**B (mg/kg)**	**N/Ca**	**K/Ca**	**Mg/Ca**	**K + Mg/Ca**
Block (B)									
B1	0.0481 ^{a}	1.16 ^{a}	2.83 ^{a}	0.14 ^{ab}	192.32 ^{a}	26.77 ^{b}	65.06 ^{bc}	3.19 ^{bc}	68.25 ^{abc}
B2	0.0427 ^{ab}	1.10 ^{a}	2.27 ^{cd}	0.12 ^{bc}	178.28 ^{ab}	28.45 ^{b}	59.80 ^{cd}	3.08 ^{c}	62.88 ^{bcd}
B3	0.0326 ^{b}	1.18 ^{a}	2.41 ^{bc}	0.13 ^{bc}	207.44 ^{a}	37.64 ^{a}	77.77 ^{ab}	4.10 ^{ab}	81.87 ^{ab}
B4	0.0339 ^{b}	1.19 ^{a}	2.62 ^{ab}	0.16 ^{a}	162.77 ^{abc}	36.96 ^{a}	81.10 ^{a}	4.91 ^{a}	86.02 ^{a}
B5	0.0433 ^{ab}	0.77 ^{b}	2.20 ^{cd}	0.08 ^{d}	129.57 ^{c}	20.86 ^{bc}	59.45 ^{cd}	2.35 ^{c}	61.80 ^{cd}
B6	0.0550 ^{a}	0.80 ^{b}	2.31 ^{cd}	0.11 ^{c}	161.62 ^{abc}	16.20 ^{c}	46.55 ^{d}	2.27 ^{c}	48.82 ^{d}
B7	0.0547 ^{a}	1.14 ^{a}	2.08 ^{d}	0.12 ^{bc}	137.81 ^{bc}	23.01 ^{bc}	42.86 ^{d}	2.42 ^{c}	45.28 ^{d}
Significance	***	***	***	***	***	***	***	***	***
Interaction (FP × B) Significance	n.s.	n.s.	n.s.	n.s	n.s.	n.s.	n.s.	n.s.	n.s.

* $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$, with n.s. for not significant ($p > 0.05$).

3.4. Correlations between Fruit Quality and Mineral Composition

Fruit flesh minerals were significantly ($p \leq 0.05$) correlated linearly to skin minerals, except for [B] and [Mg] (Table 6). Fruit flesh [Ca] and [N] were highly ($p \leq 0.01$) correlated with skin [Ca] (r = 0.57) and [N] (r = 0.66), respectively (Figure S2, Table 6). A strong linear relationship was observed between skin and flesh minerals for N/Ca (r = 0.86), followed by K/Ca (r = 0.73), Mg/Ca (r = 0.74), and K + Mg/Ca (r = 0.74) at $p \leq 0.001$ (Figure S2, Table 6). By contrast, [P], [K], and [Si] showed only moderate correlations of r = 0.44, r = 0.44, and r = 0.31, respectively, between 'Hass' skin and flesh samples.

Table 6. Pearson correlation coefficients (r) between 'Hass' avocado fruit flesh and skin mineral concentrations and fruit quality attributes for data aggregated across five canopy positions and seven different orchard blocks. '*' denotes correlation significance at $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$, Tukey's HSD test.

Variables	**Skin Mineral**	**DM (%)**	**Shelf Life (d)**	**Fruit Weight (g)**	**SER Incidence (%)**	**BR Incidence (%)**
Flesh mineral						
B	0.18	−0.19	−0.10	0.07	−0.22	−0.36 *
Ca	0.57 **	0.31 *	0.38 **	−0.11	−0.34 *	−0.74 ***
Mg	−0.16	−0.36 **	−0.24	0.27	0.41 *	−0.14
P	0.44 **	−0.44 **	−0.25	0.11	0.46 **	0.43 *
K	0.44 **	−0.67 ***	−0.32 *	0.04	0.38 *	0.35 *
Si	0.31 *	−0.33 *	0.19	−0.21	0.01	0.37 *
N	0.66 ***	−0.75 ***	−0.32 *	0.11	0.48 **	0.50 **
N/Ca	0.86 ***	−0.57 **	−0.45 **	0.10	0.43 **	0.59 **
K/Ca	0.73 ***	−0.50 **	−0.46 **	0.06	0.36 *	0.55 **
Mg/Ca	0.74 ***	−0.42 **	−0.51 **	0.20	0.46 **	0.49 **
K + Mg/Ca	0.74 ***	−0.49 **	−0.46 **	0.07	0.37 *	0.55 **
Skin mineral						
B	-	−0.11	−0.01	0.03	0.12	0.52 **
Ca	-	0.25	0.37 *	−0.30	−0.51 **	−0.39 *
Mg	-	−0.20	−0.11	−0.10	0.32	0.60 **
P	-	−0.13	−0.29	0.08	0.32	0.57 **
K	-	−0.02	−0.33 *	0.07	0.14	0.35 *
Si	-	−0.30	0.13	−0.27	−0.06	−0.12
N	-	−0.40 **	−0.24	−0.12	0.25	0.53 **
N/Ca	-	−0.44 **	−0.43 **	0.14	0.47 **	0.54 **
K/Ca	-	−0.23	−0.47 **	0.25	0.45 **	0.45 **
Mg/Ca	-	−0.30 *	−0.38 **	0.17	0.55 **	0.62 **
K + Mg/Ca	-	−0.24	−0.47 **	0.24	0.45 **	0.47 **

Fruit flesh mineral concentrations and their ratios correlated negatively with DM content, except for [Ca] (r = 0.31, $p \leq 0.05$). DM showed strong negative correlations with fruit flesh [K] (r = −0.67, $p \leq 0.001$) and [N] (r = −0.75, $p \leq 0.001$) and intermediate correlations with flesh mineral ratios (N/Ca: r = −0.57, K/Ca: r = −0.50, Mg/Ca: r = −0.42, K + Mg/Ca: r = −0.49, $p \leq 0.01$) (Table 6). In contrast, this was not so for skin mineral concentrations, where only intermediate correlations were noted with fruit DM for [N] (r = −0.40, $p \leq 0.01$) and N/Ca (r = −0.44, $p \leq 0.01$). A similar case was observed for shelf life, with a significant ($p \leq 0.05$), but intermediate, positive correlation with fruit flesh and skin [Ca] and negative correlations with [K], [N], and their mineral ratios (Table 6). However, fruit weight at harvest was not correlated to fruit flesh or skin mineral concentrations.

SER incidence correlated negatively with fruit flesh [Ca] (r = −0.34, $p \leq 0.05$) and positively with [Mg] (r = 0.41, $p \leq 0.05$), [P] (r = 0.46, $p \leq 0.01$), [K] (r = 0.38, $p \leq 0.05$), [N] (r = 0.48, $p \leq 0.01$), and mineral ratios (Table 6). Overall, correlation coefficients were poor (r < 0.5). For skin analyses, SER incidence was not correlated ($p > 0.05$) with any individual mineral concentrations, except for [Ca] (r = −0.51, $p \leq 0.01$) and mineral ratios (Table 6).

BR incidence was correlated negatively with fruit flesh [Ca] (r = −0.74, $p \leq 0.001$), suggesting higher [Ca] in flesh renders fruitless susceptible to BR. Other fruit flesh minerals, except [B] and [Mg], were significantly ($p \leq 0.05$) positively correlated to BR incidence with moderate correlation strengths (Table 6). However, BR was highly correlated with skin [Mg] (r = 0.60, $p \leq 0.01$) and Mg/Ca ratio (r = 0.62, ≤ 0.01), followed by [P] (r—0.57, ≤ 0.01) and mineral ratios (Table 6).

The relationship of soil CEC and % base saturation of cations (i.e., Ca, Mg, and K) with fruit minerals was assessed to determine whether they reflect 'at-harvest' fruit mineral status. Soil CEC and %Ca-base saturation did not significantly ($p > 0.05$) correlate to any fruit flesh and skin minerals nor their ratios (Table 7). The %Mg-base saturation values showed significant ($p \leq 0.05$), albeit relatively poor correlation with the flesh [N] (r = 0.40) and N/Ca ratio (r = 0.35) and to the skin [Mg] (r = 0.37) and Mg/Ca ratio (r = 0.34). In contrast, %K-base saturation only showed a correlation with fruit flesh N (r = 0.38, $p \leq 0.05$), (Table 7).

Table 7. Pearson correlation coefficients (r) between 'Hass' avocado fruit flesh, skin, and soil mineral concentrations for data aggregated across five canopy positions and seven different orchard blocks. '*' denotes correlation significance at $p \leq 0.05$, Tukey's HSD test.

Variables	Soil CEC	% Ca-Base Saturation	% Mg-Base Saturation	% K-Base Saturation
Flesh mineral				
Ca	−0.23	0.18	−0.22	−0.18
Mg	−0.05	0.02	0.21	−0.33
K	0.31	−0.22	0.30	0.14
N	0.31	−0.32	0.40 *	0.38 *
N/Ca	0.31	−0.28	0.34 *	0.30
K/Ca	0.32	−0.20	0.26	0.16
Mg/Ca	0.23	−0.15	0.30	0.03
K + Mg/Ca	0.32	−0.19	0.27	0.15
Skin mineral				
Ca	−0.22	0.05	−0.16	−0.01
Mg	0.15	−0.30	0.37 *	0.22
K	0.29	0.09	0.04	−0.19
N	0.31	−0.25	0.26	0.31
N/Ca	0.33	−0.16	0.26	0.14
K/Ca	0.33	0.02	0.14	−0.08
Mg/Ca	0.22	−0.21	0.34 *	0.13
K + Mg/Ca	0.33	0.00	0.15	−0.07

3.5. Fruit Minerals versus Disease Severity

Individual 'Hass' avocado fruits were categorised based on overall disease severity (% disease-affected flesh). Fruits with <10% affected flesh (0 and 5%) were considered above the market acceptable threshold and ≥10% disease-affected flesh was considered unacceptable. Fruit flesh [Ca] was low ($p \leq 0.05$) and [K], [N], N/Ca, K/Ca, Mg/Ca, and K + Mg/Ca ratios were high ($p \leq 0.05$) in ≥10% BR-affected fruit compared with healthy fruit (0% disease expression) (Table 8). For SER, only flesh [K] was significantly ($p \leq 0.05$) higher in ≥10% of affected fruit (Table 8). For skin minerals, all individual minerals and their ratios were significantly higher in ≥10% BR-affected fruit, except for [Ca]. There was a trend for lower skin [Ca] with high BR severity (Table 8). For SER, skin [K], [N], and N/Ca ratio were low ($p \leq 0.05$) in healthy fruit (0% disease expression) compared to in ≥10% SER-affected fruit. Other skin mineral concentrations (Ca and Mg) and ratios (K/Ca, Mg/Ca, and K + Mg/Ca) had no significant ($p \leq 0.05$) association with SER severity (Table 8).

Table 8. Flesh and skin mineral concentrations in individual 'Hass' avocado fruit expressing different severity levels of body rots (BR) and stem end rots (SER). Fruit with < 10% affected flesh were considered acceptable and ≥10% disease-affected flesh was not considered acceptable by consumers. Means followed by the same letter in each column are not statistically significantly different at $p \leq 0.05$ according to Tukey's HSD test.

Fruit Part	Disease	% Affected Flesh	Sample Number	Ca (mg/kg)	Mg (mg/kg)	K (%)	N (%)	N/Ca	K/Ca	Mg/Ca	K + Mg/Ca
Flesh	BR	0	80	255.9 ^{a}	943.1	1.99 ^{b}	0.92 ^{b}	39.5 ^{b}	84.9 ^{b}	3.95 ^{b}	88.9 ^{b}
		5%	31	225.9 ^{b}	940.8	2.26 ^{a}	1.17 ^{a}	58.6 ^{a}	111.4 ^{a}	4.58 ^{a}	116.0 ^{a}
		≥10%	64	186.6 ^{c}	915.0	2.25 ^{a}	1.18 ^{a}	66.6 ^{a}	127.3 ^{a}	5.09 ^{a}	132.4 ^{a}
		Sig.		***	n.s.	***	***	***	***	***	***
	SER	0	62	242.6	947.8	2.01 ^{b}	0.97 ^{b}	46.5	93.7	4.29	98.0
		5%	41	218.2	899.3	2.20 ^{a}	1.12 ^{a}	57.3	111.1	4.43	115.6
		≥10%	72	214.4	937.9	2.20 ^{a}	1.09 ^{ab}	55.6	111.6	4.67	116.2
		Sig.		n.s.	n.s.	**	**	n.s.	n.s.	n.s.	n.s.
Skin	BR	0	80	461.0	1036.6 ^{b}	2.22 ^{b}	0.89 ^{b}	21.9 ^{b}	55.0 ^{b}	2.52 ^{c}	57.5 ^{b}
		5%	31	482.9	1337.7 ^{a}	2.50 ^{a}	1.12 ^{a}	26.9 ^{b}	60.6 ^{ab}	3.19 ^{b}	63.7 ^{ab}
		≥10%	64	401.9	1475.0 ^{a}	2.55 ^{a}	1.22 ^{a}	33.8 ^{a}	70.9 ^{a}	4.03 ^{a}	74.9 ^{a}
		Sig.		n.s.	***	**	***	**	**	***	***
	SER	0	62	471.5	1179.8	2.25 ^{b}	0.94 ^{b}	23.4 ^{b}	56.6	2.92	59.6
		5%	41	456.1	1254.8	2.47 ^{a}	1.12 ^{a}	27.7 ^{ab}	60.4	3.01	63.4
		≥10%	72	411.7	1308.4	2.46 ^{a}	1.10 ^{a}	30.0 ^{a}	67.0	3.53	70.5
		Sig.		n.s.	n.s.	**	***	*	n.s.	n.s.	n.s.

* $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$, with n.s. for not significant ($p > 0.05$).

3.6. Sample Size Estimation

Mineral nutrients play a role in improving fruit's innate ability to withstand diseases and disorder expression from pre- to post-harvest [10,11]. However, the question remains as to how many samples are enough to capture and reflect sample size and/or position variability. To address this, the present dataset was utilised for sample size estimation using the standard deviation of the sample population [31]. Based on ANOVA results of Tables 4 and 5, orchard blocks were assessed at the farm level (Table 1) and two sampling positions; viz., sun-exposed and shaded (Table S1).

An example sample size calculation for flesh and skin N/Ca ratios is presented using Cochran's formula for minimum sample number. This approach can be applied to other parameters, including DM, weight (mass), or various individual minerals of interest. Visual representations of sample size calculations for flesh (Figure 2) and for skin N/Ca ratio (Figure 3) represent differences in means (x-axis) that can be detected for a given standard

deviation. The *y*-axis presents sample numbers required to detect mean differences at each of the three significance levels $p = 0.1$, $p = 0.05$, and $p = 0.01$.

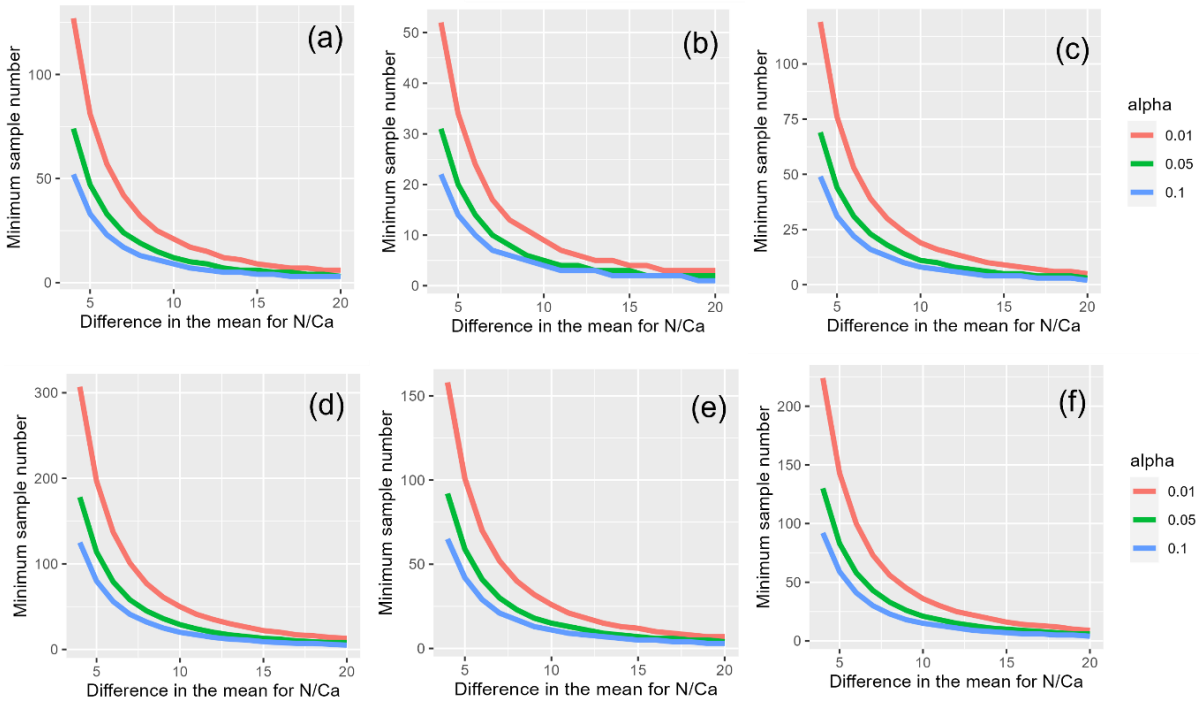

Figure 2. Sample size estimation for flesh N/Ca ratio in 'Hass' avocado fruit from three different farms by Cochran's method using standard deviations from the collected dataset at three significance levels; $p = 0.01$, $p = 0.05$, and $p = 0.1$. Here (**a**–**c**) represents sun-exposed fruit and (**d**–**f**) represents shaded fruit from Farms 1, 2, and 3, respectively.

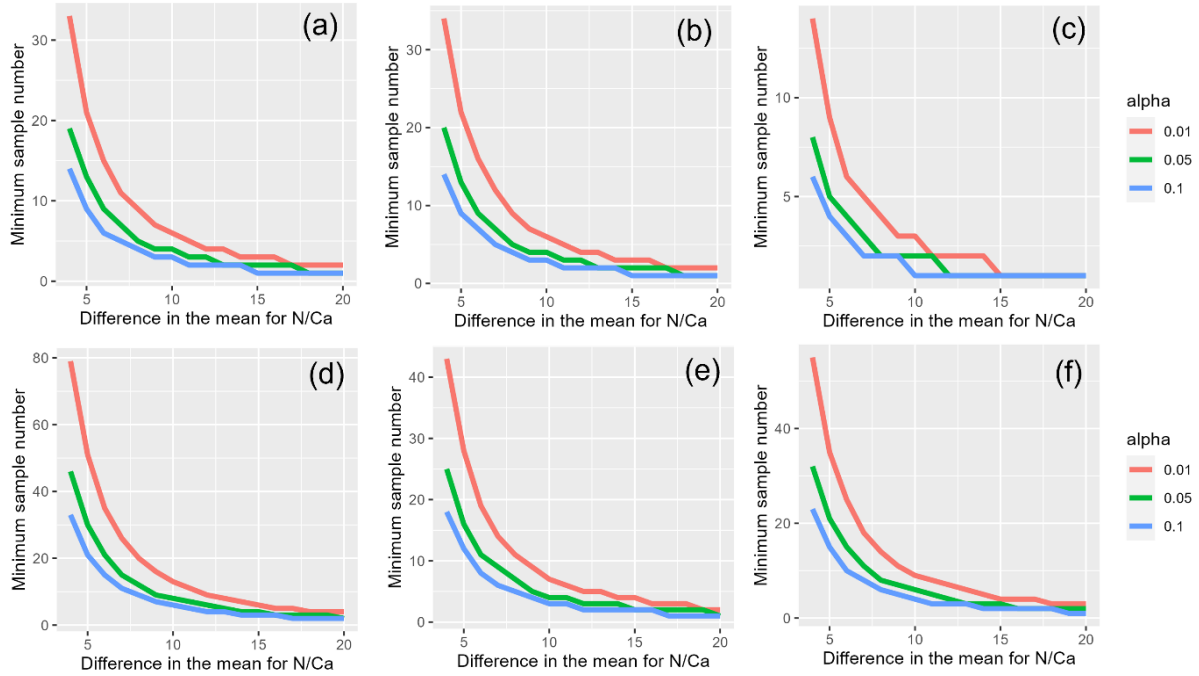

Figure 3. Sample size estimation for skin N/Ca ratio in 'Hass' avocado fruit from three different farms by Cochran's method using standard deviations from the dataset at three significance levels; $p = 0.01$, $p = 0.05$, and $p = 0.1$. Here (**a**–**c**) represent sun-exposed fruit, and (**d**–**f**) represent shaded fruit from Farms 1, 2, and 3, respectively.

Differences between farms and sun-exposed versus shaded fruit for flesh and skin N/Ca ratio sample size estimation were evident. For example, to detect a difference of 5 units between two means in sun-exposed fruit flesh at $p = 0.05$, 50 samples from Farm 1 are required (Figure 2a) versus 20 samples from Farm 2 (Figure 2b). Higher sample numbers for Farm 1 reflect higher standard deviation and variance in datasets (Table S1). Also, shaded fruit had higher standard deviation and variance compared to sun-exposed fruit, thus requiring a higher number of samples (Figure 2d–f, Table S1).

Fruit skin N/Ca ratios have low variability compared to flesh samples, with lower standard deviation and variance (Table S1). However, the trend of high variability in shaded fruit skin N/Ca ratio compared to sun-exposed was like for shaded and sun-exposed flesh N/Ca ratios, respectively (Table S1, Figure 3).

4. Discussion

A sustainable supply of robust relatively unblemished avocado fruit for both domestic and overseas markets is crucial to sustaining market demand and producer profitability. Avocado trees typically do not produce consistent quality due to variations in tree vigour [12,32,33], crop load [10], fruit position [32,34], and microclimate, including light, humidity, and sun-exposure [15,32,35] as well as various edaphic factors [36]. In-canopy differences are likely to influence fruit attributes, such as size, DM, and mineral composition, and hence ripened fruit quality, including expression of diseases and disorders. Thorp et al. [18] reported increased ($p < 0.001$) body rot and vascular browning in 'Hass' avocado fruit with relatively high maturity (DM) and low fruit [Ca]. In this context, differences in 'Hass' fruit harvest and postharvest quality characteristics, including flesh and skin mineral concentrations, were investigated across orchard blocks regarding 'Hass' avocado fruit position within the canopy.

Fruit weight at harvest was not differentially affected by fruit position within the canopy (Table 3). DM content was higher ($p \leq 0.001$) in sun-exposed positions (viz., canopy top, East, and West) compared to shaded fruit (viz., canopy middle and bottom), suggesting that exposed fruit matured earlier (Table 3).

Sun-exposed fruit experience relatively higher temperatures. Woolf et al. [37] reported a significant ($p < 0.05$) increase in 'Hass' avocado DM content associated with 35 °C flesh temperatures in sun-exposed fruit, while maximum surrounding air temperatures were ca. 20 °C. Extended exposure to direct sunlight-induced expression of mRNA and heat shock proteins in 'Hass' avocado flesh [38]. This mechanism conferred protection from postharvest heat and/or chilling injury [38]. Woolf et al. [38] also determined that sun-exposed fruit took longer to ripen. This, however, is contrary to present results, in that shelf life was not influenced ($p > 0.05$) by fruit position in the canopy, except for canopy West (Table 3). Hofman et al. [39] found no significant effect ($p > 0.05$) for DM between sun-exposed positions (viz., North, South, East, and West) in 'Hass' fruit. However, there was no direct comparison of sun-exposed versus shaded positions. Shezi et al. [40] found consistently higher ($p < 0.05$) DM from outside versus inside the canopy in 'Carmen' and 'Hass' fruit across a 16-week sampling period up to commercial maturity.

In the present study, disease incidence for SER and BR was not influenced by fruit position in the canopy (Table 3). Similarly, Willingham et al. [33] found no effect ($p > 0.05$) of fruit position (viz., North vs. South) on anthracnose incidence in 'Hass' avocado fruit. In their work, tree vigour was considered the major contributing factor to disease incidence. However, Kimeu et al. [41] reported significantly ($p = 0.05$) lower disease incidence of *Botrytis cinerea* in fruit outside the canopy (i.e., sun-exposed) compared to inside (i.e., shaded). Apart from inherent robustness, disease incidence has been linked to pathogen load in the orchard [42] and orchard conditions (e.g., humidity, rain, tree vigour) [12] along with postharvest handling practices [43,44] from farm to farm.

In the present work, fruit flesh and skin [Ca] and [B] were not influenced by fruit position in the canopy (Tables 4 and 5). Witney et al. [32] found no effect of fruit canopy position on flesh [Ca] in 'Fuerte' and 'Hass' avocado fruit sourced from vigorous and

non-vigorous trees. However, our results showed higher ($p < 0.001$) flesh [N], [K], [Mg], N/Ca, K/Ca, and K + Mg/Ca in shaded fruit towards the middle and bottom of the canopy (Table 4). In contrast, Woolf et al. [37] reported a marked increase in fruit flesh [Ca], [Mg], and [K] in sun-exposed fruit.

Fruit flesh and skin minerals were correlated with DM, shelf life, and disease incidence (Table 6). DM trended to be negatively correlated with flesh [K] (r = −0.67) and [N] (r = −0.75), suggesting that their higher concentrations are associated with lower DM content in individual 'Hass' fruit (Table 6). However, this apparent association was not evident between skin minerals and DM.

Relationships among individual minerals and their ratios with fruit quality are widely reported in the literature [10–12,14–18,20,22,45–47]. However, the strength and significance of correlation are variable, likely due to regional, seasonal, environmental, and/or management differences. For instance, body rot incidence in 'Hass' fruit was correlated ($p < 0.05$) with flesh Ca + Mg/K ratio in one season, but not the following season [12]. Similarly, site-specific differences in correlations between 'Hass' avocado flesh [Ca], [Mg], and [K] with % incidence of body rot were evident in only one of four sampling sites in two growing seasons [15].

More generally, Ca is critical in fruit development, cell wall strengthening, and signal transduction pathways [48]. In cell walls, Ca-pectin cross-links provide mechanical strength to fruit tissues, conferring protection against cell-wall degrading enzymes, and biotic and abiotic stresses [49]. In addition to cell walls, Ca also stabilizes cell membranes through interaction with phospholipids. Ca deficiency lessens cellular integrity and predisposes fruit to diseases and disorders [11,12]. Ca is accumulated primarily during the first 7–12 weeks after fruit set and tends not to change markedly thereafter [32,50]. Hence, enhancing Ca availability in early fruit development stages is likely to improve Ca concentrations at harvest.

'Hass' avocado flesh and skin minerals correlate with each other (Table 6). N/Ca ratio for flesh and for skin had a strong positive correlation (r = 0.86, $p < 0.001$) compared to their individual concentrations (N: r = 0.66, $p < 0.001$, and Ca: r = 0.57, $p < 0.01$) (Table 6). The regression correlation showed significant ($p < 0.001$) correlations between skin and flesh N (r2 = 0.44), Ca (r2 = 0.32), and N/Ca (r2 = 0.74) (Figure S2). Kämper et al. [51] also reported a significant positive correlation of [Ca] between 'Hass' skin and flesh samples (r = 0.75, $p < 0.001$).

However, determining how many individual fruit samples are sufficient to accurately reflect N:Ca variability at the site level is problematic in terms of representativeness and also, time and money. In the present study, sample size estimation based on standard deviation suggested different sample size requirements across farms and for respective fruit positions in the canopy; viz., sun-exposed versus shaded (Figures 2 and 3). A similar sample size estimation approach was explored by Schaffer and Baranowski [52] for 'Booth 8′ and 'Peterson' avocado cultivars. They used sample variances to estimate the number of trees and successive years required per experiment to capture yield variability at 5% and 10% significance levels. It is imperative to account for inherent differences in sampling across orchard blocks. That is, to recognize the management, edaphic, and environmental variables that affect fruit mineral concentrations in sample size to capture variability towards informed decisions.

In contrast, soil mineral levels reflected in % base saturation of CEC did not relate to flesh or skin minerals and/or their respective ratios (Table 7). Prior application of Ca as microfine gypsum slightly improved Ca availability in soil; however, it failed to raise [Ca] in fruit flesh [11].

Nutrient availability in soil alone does not guarantee uptake and accumulation in fruit. Other factors, including fruit yield [11,45] and tree vigour [32,33], rootstock-scion combination [14,16,17,45,53], growing temperatures [37,38,40], root health and distribution [33], and environmental (rainfall, humidity) fluctuations [54] are likely influences. Producing robust fruit is challenging due to the diversity of interacting deterministic factors prevailing

throughout fruit set, growth, and development, at harvest, and postharvest across genotype × environment × management [7,22,46,55].

5. Conclusions

The relationship between position in the tree canopy and 'Hass' fruit mineral status and postharvest quality was investigated. Sun-exposed fruit had lower flesh [N], [K], and [Mg]. They also had lower N/Ca, K/Ca, and K + Mg/Ca ratios in their flesh and skin. Positive linear correlations between mineral nutrient ratios and disease incidence levels were evident. Fruit with <40 flesh and <23 skin N/Ca ratios were relatively free of body rot and stem end rot diseases. Other potentially indicative mineral ratios of K/Ca, Mg/Ca, and K + Mg/Ca in fruit flesh and skin were also lower ($p < 0.001$) in fruit with no BR. If logistically feasible in a harvest protocol context, selective harvest and handling of sun-exposed fruit separately from shaded fruit should facilitate segregation of low mineral ratio fruit to enable assignment of more robust fruit for the more arduous (e.g., export vs. domestic) supply chains to consumers. Judicious harvesting and postharvest management could benefit all supply chain stakeholders, including by contributing toward UN SDGs like No. 12 to 'Ensure sustainable consumption and production patterns' (https://sdgs.un.org/goals/goal12 (accessed on 13 November 2023)).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16020750/s1, Figure S1: Data distribution indicated by box and whiskers plots for DM (%) and fruit weight (g) in 'Hass' avocado fruit at five different positions within the canopy across seven orchard blocks; Figure S2: Regression correlation plots between fruit flesh and skin mineral concentrations and their ratios at harvest (n = 175).; Table S1: Fruit flesh and skin N/Ca parameters for 'Hass' avocado sourced from three farms at different positions in the canopy. Data were rearranged as sun exposed (top + east +west) and shaded fruit (middle + bottom) based on ANOVA results and divided into three farms containing total of seven sampling blocks. F: farm, B: sampling block, and n: number of samples in each category.

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