

Cultivation System Modulates Raspberry Bioactive Compounds and Fruit Quality

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Abstract

This study evaluated the effects of three cultivation systems—open-field, tunnel-soil, and tunnel-pot—on fruit quality, bioactive compounds, and mineral composition in raspberries (*Rubus idaeus* L. 'Vajole') over six harvest dates. Open-field cultivation promoted the highest quality, with total soluble solids up to 12% higher at early harvest and resulted in berries with significantly elevated anthocyanin (+ 35%) and ascorbic acid (+ 22%) contents, reflecting enhanced antioxidant potential under higher light exposure. In contrast, tunnel-pot cultivation increased average fruit weight by approximately 28% compared to open-field, while also elevating magnesium and manganese concentrations, favoring fruit firmness and storability. Tunnel-soil berries exhibited highest titratable acidity mid-season, suggesting suitability for processing. Principal component analysis revealed distinct multivariate quality profiles for each system, confirming that cultivation environment simultaneously alters sugar accumulation, acidity, pigment biosynthesis, and mineral nutrient uptake. These findings demonstrate that protected cultivation systems allow targeted modulation of raspberry composition to match specific market and processing demands, optimizing both product quality and production efficiency.

Introduction

Raspberries (*Rubus idaeus* L., Rosaceae) are among the most economically and nutritionally important small fruits in Europe, including Germany (Sawicka et al., 2023). Their high consumer acceptance is driven by both flavor and recognized health benefits linked to their content of vitamins, minerals, and polyphenolic compounds (Cosme et al., 2022). Anthocyanins represent the dominant class of phenolic compounds in raspberries, contributing both to the characteristic red pigmentation and to antioxidant activity, accounting for up to 25% of total antioxidant capacity (Burton-Freeman et al., 2016). Cyanidin- and pelargonidin-based anthocyanins, particularly cyanidin-3-glucoside, have been associated with protective effects against metabolic disorders, cardiovascular disease, and neurodegeneration due to their antioxidative and anti-inflammatory properties (Burton-Freeman et al., 2016). In addition, raspberries provide high concentrations of vitamin C, which supports immune function and contributes synergistically to overall antioxidant defense (Ponder & Hallmann, 2020; Akimov et al., 2021).

Minerals such as potassium, magnesium, calcium, and iron further enhance the nutritional profile of raspberries by contributing to electrolyte balance, neuromuscular function, and cellular metabolism (Akimov et al., 2021; Ispiryan et al., 2024). Due to this diverse composition of bioactive compounds, raspberries exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, and anticarcinogenic effects (Ispiryan et al., 2024; Anttonen and Karjalainen, 2005).

Modern raspberry production increasingly employs protected cultivation systems such as high tunnels to stabilize production, extend the harvest window, and improve fruit quality under variable climatic conditions (Demchak, 2009; N.C. Cooperative Extension, 2023). Tunnel-based cultivation alters the microclimate by modifying temperature, humidity, and light availability, which directly affect fruit development and compositional traits (Robson et al., 2022). High tunnels can improve fruit size,

firmness, shelf life, and marketability (Hanson et al., 2011; Pritts et al., 2017), while precise fertigation regimes optimize mineral uptake (Bushway et al., 2008). Cultivation approaches can differ both in substrate and management: tunnel-soil systems utilize the native ground soil under protected structures, whereas tunnel-pot systems combine controlled environments with soilless substrates and fertigation. In contrast, open-field cultivation exposes plants directly to natural environmental conditions, which may promote higher light exposure but also increases climatic stressors.

The composition and quality of raspberry fruit are strongly modulated by the environmental conditions created within each cultivation system. High tunnel production provides a partially controlled microclimate, buffering temperature fluctuations, limiting exposure to excessive rainfall or wind, and allowing regulated water and nutrient supply via fertigation systems (Bushway et al., 2008; Heidenreich et al., 2012). Under tunnel cultivation, reduced abiotic stress enhances physiological processes such as photosynthesis, which may contribute to larger and firmer berries. In some cases, it also results in higher sugar accumulation and elevated sugar-to-acid ratios (Kadir et al., 2006; Hanson et al., 2011). However, reduced light intensity under tunnel coverings limits the synthesis of anthocyanins, which are highly light-dependent, thereby lowering pigment accumulation and antioxidant capacity compared to open-field systems (Anttonen and Karjalainen, 2005; Olsson et al., 2006). In contrast, open-field cultivation exposes raspberry plants fully to natural light and temperature variations, which enhances anthocyanin biosynthesis and volatile aroma development (Anttonen and Karjalainen, 2005; Olsson et al., 2006; Hanson et al., 2011). The high irradiance conditions during ripening promote sugar accumulation and flavor development (Hanson et al., 2011), although fruit size and firmness are typically reduced compared to tunnel-grown berries, increasing susceptibility to mechanical damage and shortening postharvest shelf life (Kadir et al., 2006). Furthermore, open-field systems may enable a broader uptake of mineral nutrients, as roots have unrestricted access to native soil profiles with naturally occurring macro- and micronutrients (Bushway et al., 2008; Hanson et al., 2011). However, open-field production is also more vulnerable to yield variability due to biotic and abiotic stresses, including diseases and adverse weather events (Fuentelba et al., 2024).

In light of the growing adoption of protected cultivation systems in raspberry production, this study systematically compares the effects of open-field, tunnel-soil, and tunnel-pot cultivation on fruit quality, bioactive metabolite accumulation, and mineral nutrient profiles in raspberries. We specifically tested the following hypotheses: (i) light intensity and microclimatic buffering under protected cultivation significantly alter the accumulation of sugars, organic acids, and antioxidant compounds, particularly anthocyanins and ascorbic acid; (ii) cultivation system modifies mineral nutrient composition; and (iii) multivariate interactions between bioactive metabolites, minerals, and fruit size generate distinct quality profiles associated with each cultivation method. The overarching objective was to define how protected cultivation systems simultaneously influence raspberry fruit composition, enabling the optimization of production strategies for different market and processing needs.

Materials and Methods

Plant material and experimental set-up

The raspberry cultivar 'Vajoret' (*Rubus idaeus* L.), cultivated in the Ortenaukreis district of Baden-Württemberg, Germany, was used in this study to evaluate and compare three distinct cultivation systems, each established with 0.5 m intra-row spacing. The open-field raspberry cultivation was located adjacent to the tunnel-soil system on the eastern side of the field, without any irrigation or fertigation. The tunnel-soil cultivation was conducted in native soil under polytunnels using a commercial drip fertigation system. In the tunnel-pot system, raspberry plants were grown in containers placed on black ribbon fabric under the same polytunnels; each pot contained two plants and was supplied with the same fertigation regime. The polytunnel structures were Meta Pat professional tunnels (Metasa, Gladbeck, Germany).

Raspberries were sampled from all three cultivation systems, with each sample consisting of 20 randomly selected class I fruits. Six replicate samples were collected per system across six harvest dates. To account for differences in fruit ripening dynamics between systems, harvest dates were normalized as harvest 1 to 6 based on practical maturity stages typical for commercial harvest. Specifically, the first harvest date was 30 May for the tunnel-pot system, 2 June for the tunnel-soil system, and 24 June 2024 for the open-field system. After each harvest, about 100 g of raspberries were harvested for each cultivation system, temporarily stored in a cool box at 4°C, and subsequently frozen at -22°C until analysis.

Light radiation

Photosynthetically active radiation (PAR; $\mu\text{mol m}^{-2} \text{s}^{-1}$) was measured inside and outside the tunnels using an LP-80 AccuPAR meter (Meter Group Inc., Pullman, USA) to assess differences in light availability. Measurements were taken around midday on six selected dates under varying weather conditions. Within the tunnel, the sensor was positioned along the central plant row to minimize edge effects and placed at canopy level to capture representative light incidence.

Fruit Quality

For weight measurements, 10 berries were randomly selected from each sample on all six harvest dates, and individual fruit weight was recorded in grams. For the analysis of total soluble solids (TSS) and titratable acidity (TA), frozen raspberries were thawed and centrifuged at 5,500 rpm for 15 minutes. TSS was measured in °Brix using a digital refractometer (ATAGO®, Atago Co. Ltd., Tokyo, Japan). For TA determination, 5 ml of the supernatant were diluted with 15 ml of distilled water and titrated with 0.25 M NaOH to an endpoint of pH 8.1 using an automatic titrator (TitroLine Easy, Schott Instruments AG, Mainz, Germany). The pH meter was calibrated with buffer solutions of pH 7.00 and 9.18. Acidity was expressed in g L^{-1} . The sugar-to-acid ratio (SAR), indicating the balance between sweetness and acidity, was calculated as $\text{SAR} = \text{TSS} / \text{TA}$. The Thiault index (TI), a maturity indicator based on the ratio of

sugars to acids, was calculated as $TI = ((TSS \times 10.6) - 20.6) / (TA \times 10)$, where TSS is expressed in °Brix and TA in g L⁻¹. Berry color was determined non-invasively using a spectrophotometer (X-Rite CA22, X-Rite, Planegg-Martinsried, Germany) and expressed as L*, a*, and b* values. The hue angle (H°) was calculated using the formula $H^\circ = \text{atan2}(b^*, a^*)$, with values around 20.5° indicating ripe fruit and values around 24.5° representing semi-ripe stages.

Bioactive compounds

Anthocyanins were analyzed by high-performance liquid chromatography with diode-array detection (HPLC-DAD) using a Prominence LC-2030C 3D Plus system (Shimadzu Deutschland GmbH, Duisburg, Germany) equipped with a C18 column (Nucleodur 100-5-C18, 5 µm, 125 × 2 mm; Macherey & Nagel, Düren, Germany). Frozen raspberry samples were ground in liquid nitrogen, and 5 g of the powder was extracted with 3 ml of methanol/HCl (98:2, v/v). After centrifugation (5500 rpm, 15 min; Heraeus Christ, Gemini, Apeldoorn, Netherlands), the supernatant was filtered through a 0.45 µm nylon syringe filter (13 mm) into a sealed HPLC vial. Separation was performed at 30°C with a flow rate of 1.0 ml min⁻¹ using gradient elution. Mobile phase A was water/formic acid (91.5:8.5, v/v); mobile phase B was formic acid/acetonitrile/methanol/water (8.5:25:25:41.5, v/v). The total run time was 60 minutes. Detection was carried out at 520 nm, and quantification was based on a calibration curve using cyanidin-3-O-glucoside chloride as external standard. The UV-Vis spectra from 200 to 900 nm were also recorded to confirm anthocyanin identity.

Ascorbic acid was quantified by HPLC-DAD using the same instrument as for anthocyanin analysis. Frozen raspberries were ground in liquid nitrogen, and 5 g of the powder was extracted with 15 ml of 3% metaphosphoric acid. After centrifugation (5000 rpm, 10 min), the supernatant was filtered through a 0.45 µm nylon syringe filter (13 mm) into a sealed HPLC vial. Chromatographic separation was performed at 30°C with a flow rate of 0.8 ml min⁻¹ under isocratic conditions using a mobile phase composed of tetrabutylammonium hydrogen sulphate solution (2.5 g in 55 ml methanol, diluted to 1 L with water). Detection was carried out at 254 nm, and quantification was based on a calibration curve generated with L-ascorbic acid as an external standard.

Mineral Analysis

For mineral analysis, 5 g of frozen raspberry pulp per sample were homogenized and approximately 200 mg of the homogenized material was weighed into quartz digestion vessels and treated with 2.5 mL of 69% nitric acid (HNO₃, supra quality; Carl Roth GmbH + Co. KG, Karlsruhe, Germany; product code HN50.3). Samples were digested using a microwave-assisted high-pressure digestion system (UltraClave V; MLS GmbH, Leutkirch, Germany) according to VDLUFA Method Book, Volume VII, Section 2.1.3 (Microwave-assisted pressure digestion). Digested samples were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES; Agilent 5110, Agilent Technologies, Santa Clara, CA, USA) following VDLUFA Method Book, Volume VII, Section 2.2.2.6 (Element determination in plant

material and feedstuffs). Concentrations of boron (B), calcium (Ca), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), and zinc (Zn) were determined and are expressed as mg/kg fresh weight.

Statistics

All quality parameters were analysed using univariate and multifactorial analysis of variance (ANOVA) in RStudio (version 4.0). The model included treatment and relative harvest date as fixed effects and replication as a random effect. Significant differences between means were assessed by Tukey's post hoc test at a significance level of $p \leq 0.05$. Data are presented as means with standard errors, and statistically significant differences are indicated in the boxplots.

A principal component analysis (PCA) was performed to reduce dimensionality and explore multivariate patterns among the 17 measured quality and mineral parameters. Prior to PCA, all variables were z-transformed (mean-centered and scaled to unit variance) to ensure equal weighting across variables with different measurement units. The PCA was conducted using the `prcomp` function in R (version 4.0), with default settings (`center = TRUE`, `scale. = TRUE`). The PCA results were visualized using `ggplot2` and `ggrepel` packages, with treatment groups indicated by color-coded ellipses.

Results

Light radiation

To quantify differences in light availability across cultivation systems, PAR was measured inside and outside the tunnel at plant canopy level under six different weather conditions. Table 1 summarizes the PAR values recorded on each date. PAR outside the tunnel ranged from 169 to 1978 $\mu\text{mol m}^{-2} \text{s}^{-1}$, reflecting varying solar irradiance. Relative reduction in tunnel PAR ranged from 24.3% (rainy conditions) to 56.6% (sunny conditions), with an overall mean of $35.9 \pm 13.4\%$.

Table 1

Weather conditions and photosynthetically active radiation (PAR) outside and inside the tunnel on selected dates in May and June 2024. Measurements were recorded at plant canopy level around midday. Relative reductions were calculated as percentage (%). Values below the table represent mean $\pm$ standard deviation across all dates (n = 6).

Date	Weather	PAR outside ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	PAR inside ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Relative reduction (%)
05.05.2024	Sunny	1978	1398	29.3
02.06.2024	Rain	169	128	24.3
09.06.2024	Sunny	1814	834	54.0
15.06.2024	Overcast	438	321	26.7
24.06.2024	Sunny	1743	756	56.6
30.06.2024	Rain	189	143	24.3
Mean		1222 $\pm$ 818	630 $\pm$ 487	35.9 $\pm$ 13.4

Fruit Quality

A significant effect of both harvest date and cultivation system on berry weight was observed (Fig. 1A). Across all six harvest dates, raspberries grown in the tunnel-pot system consistently exhibited the highest fruit weight, followed by tunnel-soil, while berries from the open-field had the lowest weight overall. Statistically significant differences between treatments were detected for harvest dates 1 to 3. From harvest date 4 onward, a general decline in fruit weight was observed, particularly in tunnel-grown berries, indicating possible seasonal effects on fruit development.

During early harvests (1–3), TSS values were significantly higher in open-field raspberries compared to both tunnel systems (Fig. 1B). However, at later harvest dates (4–6), no significant differences were observed across treatments. This pattern suggests that early-season conditions in the open-field favor sugar accumulation, while later in the season, sugar levels stabilize across systems.

TA shows that open-field berries consistently exhibited the lowest TA values across all harvest dates (Fig. 1C). Tunnel-soil berries showed the highest acidity from harvest dates 3 to 5, while tunnel-pot berries maintained relatively stable TA values throughout. These differences may reflect the influence of environmental buffering and soil composition on organic acid metabolism.

The SAR is illustrated in Fig. 1D, with open-field berries showing the highest SAR values across most harvest dates, indicating a more favorable sweetness-acidity balance for fresh consumption. Tunnel-pot and tunnel-soil berries displayed comparable SAR values throughout the season. Within individual harvest dates, significant differences between treatments were primarily observed in open-field fruit, particularly at harvest date 5.

The Thiault index, a combined maturity indicator derived from TSS and TA, is depicted in Fig. 1E. While Thiault values were similar across treatments during harvest dates 1, 2, and 6, tunnel-soil berries exhibited significantly higher values at harvest dates 3, 4, and 5. Open-field berries showed the lowest Thiault index at harvest date 5. These findings highlight the interaction between environmental conditions and physiological maturity, particularly in mid-season fruit.

Hue°, representing berry color, is shown in Fig. 1F. While values were similar across treatments at harvest date 1, berries from the tunnel-pot system displayed significantly lower hue values from harvest dates 2 to 6, indicating more intense coloration. Open-field and tunnel-soil berries showed comparable hue values across the season, suggesting that both systems allow sufficient light for stable anthocyanin development.

Bioactive metabolites

Figure 2A–C displays the concentrations of three major anthocyanins across six harvest dates and cultivation systems. In general, cyanidin-3-glucoside and cyanidin-3-sophoroside were the most abundant pigments, typically ranging between 30 and 60 mg 100 g⁻¹ fresh weight (FW), while pelargonidin-3-glucoside occurred at lower concentrations, between 2 and 5 mg 100 g⁻¹ FW. Raspberries grown in the open-field consistently exhibited the highest levels of all three anthocyanins. In contrast, tunnel-grown fruit—particularly from the pot-based system—showed significantly lower anthocyanin concentrations at most harvest dates. These differences were most pronounced during early and mid-season harvests.

As shown in Fig. 2D, ascorbic acid levels followed a similar pattern. Open-field berries consistently contained the highest concentrations of ascorbic acid across all harvest dates, whereas tunnel-grown berries, especially those from the pot system, exhibited markedly reduced values. The overall trend indicates that both light availability and cultivation system significantly influenced the accumulation of antioxidant compounds in raspberry fruit.

Mineral Analysis

Figure 3A–G presents the concentrations of seven mineral nutrients in raspberry fruit across three cultivation systems. B, Ca, P, and K concentrations were highest in berries from the open-field system. Notably, calcium and boron levels declined significantly under protected cultivation, with the lowest concentrations observed in tunnel-pot-grown fruit (Fig. 3A–B). Phosphorus levels also decreased significantly under tunnel conditions, although no significant difference was detected between tunnel-soil and tunnel-pot systems (Fig. 3E).

In contrast, magnesium (Mg), manganese (Mn), and zinc (Zn) concentrations were highest in fruit from the tunnel-pot system (Fig. 3C–D, G). Magnesium levels were significantly elevated in tunnel-pot samples compared to open-field berries, while the tunnel-soil treatment exhibited intermediate values. Manganese accumulation was markedly increased in tunnel-pot fruit, potentially due to reduced competition with other divalent cations under containerized root conditions. Zinc followed a similar

pattern, with the highest concentrations in tunnel-pot, intermediate levels in open-field, and the lowest in the tunnel-soil system.

Potassium concentrations (Fig. 3F) remained relatively consistent across treatments. No statistically significant differences were found between the open-field and tunnel-soil systems, although a slight but significant reduction was observed in tunnel-pot berries. These findings suggest that cultivation system and substrate type influence mineral nutrient accumulation in raspberry fruit, likely through differences in nutrient availability, irrigation dynamics, and root zone confinement.

Principal Component Analysis

The first three principal components accounted for 69.54% of the total variance (PC1: 34.62%, PC2: 18.94%, PC3: 15.98%). PC1 was mainly influenced by parameters associated with color and sugar accumulation, such as total anthocyanins, sugar-to-acid ratio, and red hue intensity. PC2 reflected gradients in mineral content (e.g., Mg, Mn, Zn) and pH, while PC3 captured further variation in acidity and individual anthocyanin components. The PCA biplots (Fig. 4a–c) revealed distinct separation between open-field and any of the tunnel-grown fruits, particularly along PC1, indicating substantial differences in metabolic profiles across cultivation systems. Tunnel-pot and tunnel-soil samples overlapped partially but separated further along PC3, suggesting additional differences in acidity-related traits. These results demonstrate that PCA effectively summarizes major quality differences induced by cultivation system, with certain traits co-varying strongly and forming interpretable gradients in principal component space.

Discussion

Light radiation

Photosynthetically Active Radiation (PAR) measurements confirmed the expected light attenuation inside the tunnels (Table 1). PAR levels outside and inside the tunnels varied depending on weather conditions, where tunnel-grown plants experienced a mean reduction of $35.9 \pm 13.4\%$ due to film-induced light scattering. As previously reported, tunnels modify light penetration by increasing diffuse radiation while reducing total incident PAR, particularly on sunny days (Maynard and O'Donnell, 2020; Robson et al., 2022). This buffering effect stabilizes light availability under variable weather conditions, contributing to a more uniform growing environment inside the protected cultivation systems.

Fruit Quality

Berry weight showed significant effects of both harvest date and cultivation system (Fig. 1A). Across all harvest dates, tunnel-pot-grown berries consistently exhibited the highest weight, followed by tunnel-soil, while open-field fruit displayed the lowest values. The progressive decline in weight observed in all treatments over the season is consistent with seasonal physiological changes in raspberry fruit development (Riesselman et al., 2013). Larger fruit size under tunnel cultivation is in agreement with previous studies reporting that moderate microclimatic buffering inside protected systems supports fruit enlargement (Clark and Finn, 2011; Thompson et al., 2009).

Total soluble solids (TSS) were significantly higher in open-field-grown fruit during the early harvests (Fig. 1B), similar to the case in apples likely reflecting enhanced sugar accumulation under higher light and temperature conditions (Remberg et al., 2010; Juillion et al., 2024). In contrast, TSS remained more stable across harvest dates under both tunnel systems, which suggests that the tunnel microclimate contributed to more uniform sugar levels throughout the season (Palonen et al., 2017).

Titrateable acidity (TA) followed an opposite pattern, with consistently lower values in open-field raspberries across all harvest dates (Fig. 1C), while tunnel-soil fruit exhibited the highest acidity during mid-season (harvest dates 3–5). These differences likely reflect the interaction between temperature, light exposure, and organic acid metabolism under varying cultivation conditions (Wang et al., 2009; Fuentealba et al., 2024; Palonen et al., 2017).

Building the sugar-to-acid ratio (SAR) from these data shows, that SAR was consistently highest in open-field berries across most harvest dates (Fig. 1D), reflecting the combined effects of higher sugar accumulation and lower titrateable acidity observed in this system (Remberg et al., 2010). In contrast, SAR values in tunnel-soil and tunnel-pot berries remained lower and relatively stable throughout the harvest season, with no significant differences between the two tunnel-based systems as supported by earlier findings in apples (Palonen et al., 2017; Juillion et al., 2023). From a sensory perspective, this suggests that open-field berries offered superior flavor attributes, particularly during early harvests, although their quality parameters exhibited greater variability across the season.

The Thiault index, which integrates both sugar and acid dynamics, displayed no significant differences between treatments at the beginning and end of the harvest period. However, distinct differences emerged during mid-season, where tunnel-soil-grown berries exhibited significantly higher Thiault values at harvest dates 3 to 5 (Fig. 1E). This pattern indicates that tunnel-soil cultivation may provide especially favorable conditions for achieving a balanced ripening phase during peak harvest (Remberg et al., 2010; Palonen et al., 2017).

Hue angle values, reflecting berry coloration, began to diverge between treatments from harvest date 2 onward (Fig. 1F). Tunnel-pot-grown berries on average exhibited higher hue angles than open-field and tunnel-soil berries, indicating a shift towards more orange-red coloration of $\sim 24^\circ$ hue angle, while open-field berries maintained lower hue values around 21° , corresponding to deeper red pigmentation. This confirms that cultivation system influences not only compositional but also visual aspects of fruit quality, which are critical for consumer acceptance (Voća et al. 2006).

Bioactive Compounds

Direct quantification of anthocyanins confirmed that total anthocyanin concentrations were highest in open-field fruit across nearly all harvest dates (Fig. 2A–C), reflecting the pronounced influence of cultivation system on pigment biosynthesis (Anbesse Girma et al., 2022; García-Viguera et al., 1998; Crespo et al., 2010). Anthocyanins, as major flavonoid pigments, are primarily responsible for the characteristic red color of raspberries, with cyanidin-based compounds dominating the anthocyanin

profile. In line with earlier studies, the most abundant anthocyanins detected were cyanidin-3-glucoside, cyanidin-3-sophoroside, and to a lesser extent, pelargonidin-3-glucoside (Crespo et al., 2010; Kassim et al., 2009; Bradish et al., 2015).

Light availability is a key environmental driver of anthocyanin accumulation. The open-field system, characterized by higher light intensity and greater temperature fluctuations, promoted significantly higher anthocyanin concentrations compared to both tunnel systems (Table 1). In contrast, tunnel-grown fruit, particularly under the pot system, exhibited markedly reduced levels of all major anthocyanins. These findings are consistent with previous reports showing that low light conditions in protected cultivation suppress anthocyanin biosynthesis, while increased irradiance enhances its accumulation (Tako et al., 2006; Wang et al., 2020; Xie et al., 2012). Among the individual compounds, cyanidin-3-glucoside showed the strongest environmental response, with substantially higher levels in open-field fruit across the season (Kassim et al., 2009; Tarara et al., 2008).

These compositional differences are reflected in the observed hue angle values (Fig. 1F), which serve as a phenotypic proxy for color intensity. Tunnel-pot-grown berries exhibited higher hue values ($\sim 24^\circ$), indicating a shift towards more orange-red coloration, while open-field berries consistently showed lower hue values ($\sim 21^\circ$), corresponding to deeper red pigmentation. This shift directly mirrors the higher concentrations of cyanidin-based anthocyanins in open-field berries, particularly the almost double proportion of cyanidin-3-glucoside relative to cyanidin-3-sophoroside (Fig. 2A, B). This might be because cyanidin-3-glucoside exhibits higher molar absorptivity and slightly different color saturation than cyanidin-3-sophoroside, which results in more intense and darker red coloration under comparable pH conditions (Zhang et al., 2008). The balance between cyanidin and pelargonidin derivatives contributes to these visual differences, reinforcing the impact of cultivation system on both biochemical composition and consumer-perceived fruit quality.

Similarly, ascorbic acid (vitamin C) levels were significantly influenced by cultivation system (Fig. 2D), with highest concentrations consistently observed in open-field raspberries. This is likely due to enhanced light exposure, which not only stimulates anthocyanin biosynthesis but also promotes ascorbic acid synthesis via sugar availability as a metabolic precursor (Ponder et al., 2020; Wang et al., 2009). Across harvest dates, ascorbic acid concentrations declined slightly in later harvests, likely reflecting progressive metabolic turnover during extended fruit maturation (Wang et al., 2009). The parallel trends between anthocyanin and ascorbic acid accumulation further underline the shared regulatory role of light and microclimate in determining the antioxidant profile of raspberry fruit.

However, it should be noted that fruit weight was significantly higher under tunnel cultivation (Fig. 1A), especially in the tunnel-pot system. Since anthocyanin and ascorbic acid concentrations were expressed on a fresh weight basis, the larger berry size in tunnel-grown fruit likely contributed to a dilution effect. On a per-fruit basis, absolute amounts of antioxidants may therefore be more comparable across systems than the fresh weight concentrations suggest. This highlights the importance of considering

fruit water content and berry size when interpreting nutritional differences between cultivation systems (Fuentealba et al., 2024).

Mineral Analysis

The cultivation system strongly influenced mineral nutrient profiles in raspberry fruit, reflecting the combined effects of microclimate, substrate, and transpiration processes established in the previous sections. Open-field conditions consistently promoted higher boron, calcium, potassium, and phosphorus levels, while tunnel-pot cultivation favored magnesium, manganese, and zinc accumulation (Fig. 3).

Boron concentrations were highest under open-field conditions, consistent with the role of light and transpiration in driving boron uptake and transport via xylem flow (Noppakoonwong et al., 1993). Reduced boron levels in tunnel-grown fruit suggest that shading diminishes boron demand and mobility, as light-dependent transpiration is suppressed. Similarly, calcium accumulation was enhanced in open-field berries, likely reflecting higher evaporative demand and transpiration-driven calcium transport under lower relative humidity (Blanco et al., 2021).

In contrast, magnesium, manganese, and zinc concentrations were markedly elevated under tunnel-pot conditions. The stable moisture regime, specialized soilless substrate, and reduced competitive ion interactions likely facilitated improved uptake of these micronutrients (Hirzel et al., 2020). Zinc and manganese levels exhibited parallel increases, reflecting their known positive interaction in plant nutrient dynamics (Adamczuk et al., 2023). As previously shown in other horticultural species, magnesium enrichment may also reflect enhanced chlorophyll synthesis under these buffered conditions (Zhang et al., 2019).

Potassium and phosphorus levels were generally highest in open-field berries and declined under tunnel cultivation. The reduced availability of phosphorus and potassium under tunnel conditions aligns with previous observations in protected systems, where both nutrient concentration and uptake efficiency may decrease with lower transpiration and altered root-zone conditions (Kızılkaya et al., 2010; Hirzel et al., 2020). Overall, these findings emphasize that protected cultivation modifies not only fruit composition and bioactives but also mineral nutrient profiles in complex, element-specific ways depending on both macronutrient and micronutrient group.

Principal Component Analysis

The PCA clearly visualized the multidimensional relationships between fruit quality parameters and cultivation systems, with the first three principal components explaining 70% of the total variance (Fig. 4). PC1 (35% explained variance), primarily driven by bioactive compounds and sugar-related traits, effectively separated open-field samples from tunnel-grown berries. Total anthocyanins, individual anthocyanins, ascorbic acid, and TSS clustered strongly with open-field samples (Fig. 4A, B), confirming that increased light exposure in open-field conditions simultaneously enhanced both sugar accumulation and the biosynthesis of antioxidant pigments. This co-localization supports earlier findings that

anthocyanin synthesis is highly light-dependent, with strongly reduced levels observed under shaded tunnel environments (Takos et al., 2006; Wang et al., 2020; Fukuoka et al., 2014).

In addition, Fig. 4A reveals that fruit weight and anthocyanins are positioned almost directly opposite along PC1, indicating a negative correlation between berry size and anthocyanin concentration per gram fresh weight. Thus, larger berries produced under tunnel conditions tend to have lower pigment concentrations due to a dilution effect.

On PC2 (19%), acidity-related traits (TA, Thiault) and hue angle separated tunnel-soil-grown berries from other systems (Fig. 4A, C). The tunnel-soil environment maintained stable acidity and slightly elevated hue angles, reflecting its intermediate light exposure and buffered temperature regime, consistent with enhanced organic acid stability under controlled microclimatic conditions (Fuentealba et al., 2024; Both et al., 2019).

PC3 (16%) primarily captured mineral nutrient variation (Fig. 4B, C). The tunnel-pot system distinctly clustered with Mg, Mn, Zn and fruit weight, reflecting the influence of soilless substrates and controlled irrigation on micronutrient availability and fruit expansion (Hirzel et al., 2020; Adamczuk et al., 2023). The known positive correlation between Mn and Zn accumulation was also evident here, further emphasizing that nutrient availability in soilless systems may favor certain mineral uptakes while simultaneously promoting larger berry size due to higher water content and cell expansion.

Conclusion

Although limited to a single season, this study provides evidence consistent with earlier findings that protected cultivation systems exert distinct effects on raspberry fruit quality by modifying light exposure, microclimate, and nutrient dynamics. In support of hypothesis (i), open-field cultivation with higher light intensity and natural temperature variation promoted significantly greater accumulation of sugars, anthocyanins, and ascorbic acid, resulting in superior sweetness, deeper red coloration, and enhanced antioxidant capacity. Tunnel-based systems, characterized by partial shading and environmental buffering, reduced anthocyanin and vitamin C concentrations but stabilized sugar levels and acidity over the harvest season. Regarding hypothesis (ii), cultivation system significantly affected mineral nutrient profiles: open-field berries exhibited higher concentrations of calcium, potassium, phosphorus, and boron, whereas tunnel-pot cultivation increased magnesium, manganese, and zinc levels at least in this experiment. Finally, hypothesis (iii) was supported by principal component analysis, which revealed distinct multivariate quality profiles for each cultivation system, reflecting the integrated effects of bioactive compounds, mineral nutrients, and fruit size. Collectively, these results demonstrate that cultivation system selection can be strategically used to tailor raspberry fruit composition according to specific market objectives, whether targeting fresh consumption, processing, or nutritional enhancement.

Declarations

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Author Contributions

Pooja Anand: Investigation, data curation, chemical analysis, manuscript writing.

Tobias Müller: Sample collection and quality analysis, Preliminary data analysis

Elke Sprich: Resources, methodology and editing.

Michael Helmut Hagemann: Project administration, conceptualization, supervision, manuscript editing.

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Data Availability Statement

Data will be made available upon reasonable request.

Conflict of Interest

The authors declare that they have no competing interests.

Ethical Standards

This study did not involve human participants or animals. All research procedures were conducted following institutional and scientific guidelines for plant-based research.

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Figures

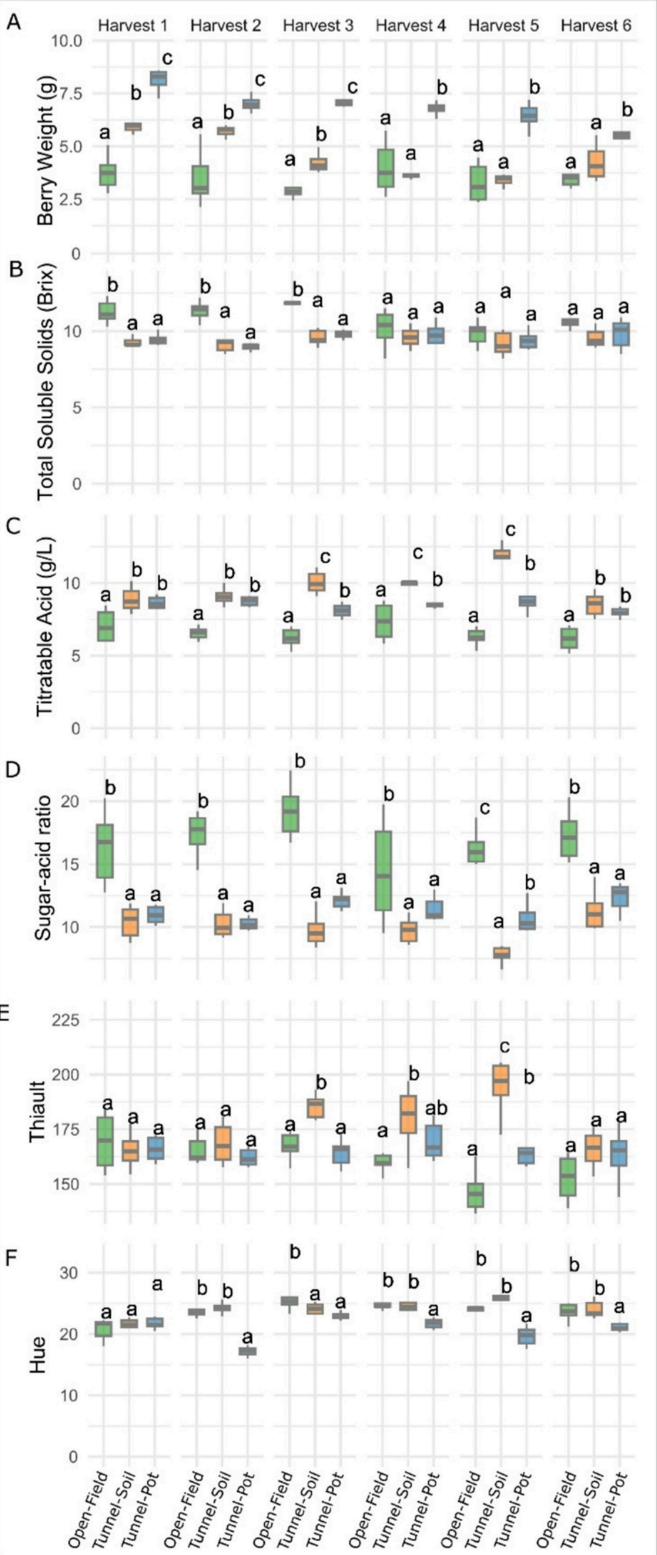


Figure 1

Boxplots showing six fruit quality parameters of raspberries across six harvest dates (1–6) under three cultivation systems: open-field (green), tunnel-soil (orange), and tunnel-pot (blue). (A) Berry weight (g), (B) total soluble solids (°Brix), (C) titratable acidity (g L⁻¹), (D) sugar-to-acid ratio, (E) Thiault index, and (F) hue angle (°). Different lowercase letters above the boxplots indicate statistically significant differences between treatments at each harvest date (Tukey’s HSD test, $p \leq 0.05$). To enhance visual comparison of treatment effects for the Thiault and sugar-acid ratio, y-axes do not start at zero.

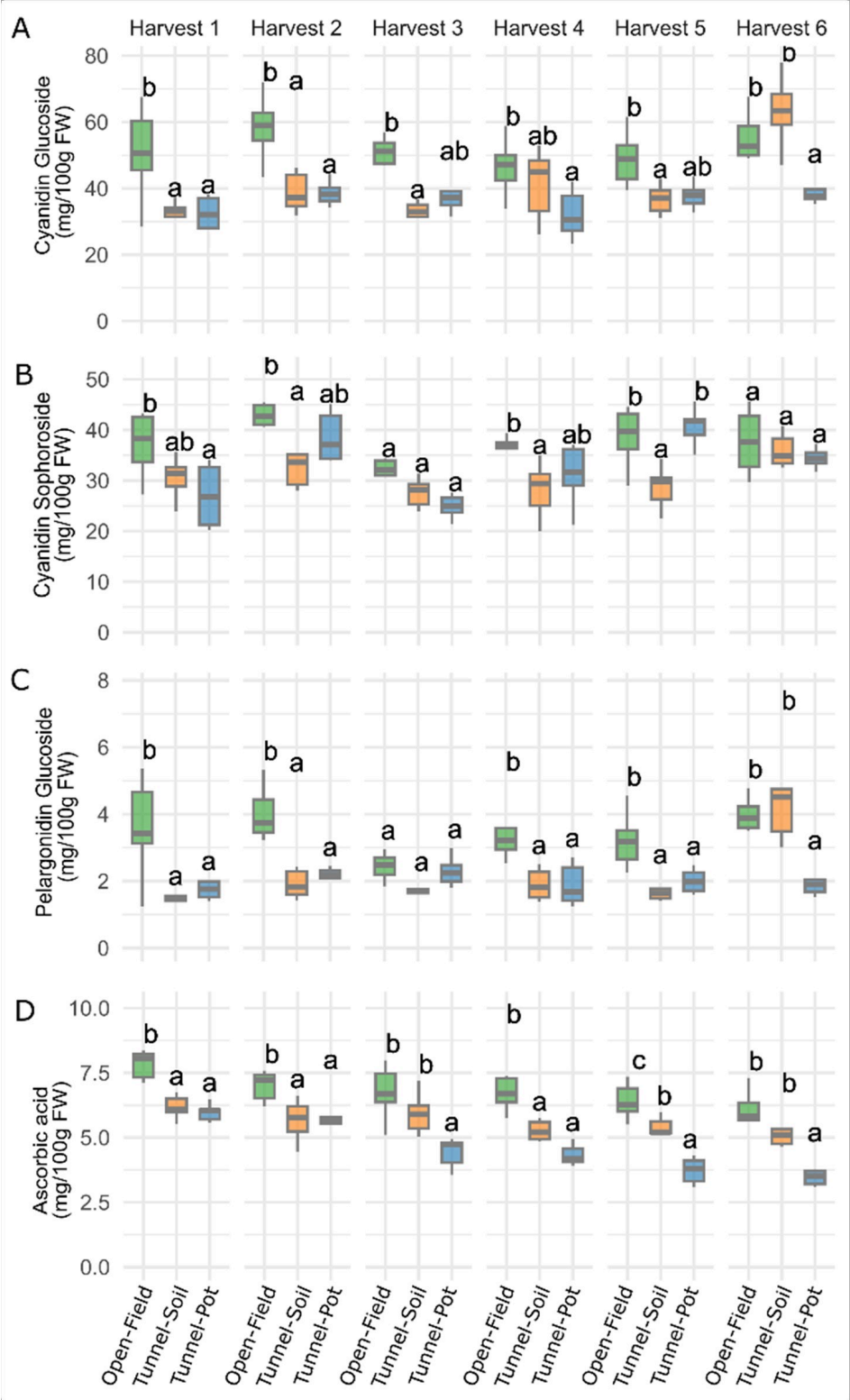


Figure 2

Boxplots showing raspberry fruit bioactive compound concentrations across six harvest dates (1–6) under three cultivation systems: open-field (green), tunnel-soil (orange), and tunnel-pot (blue). (A) Cyanidin-3-glucoside, (B) cyanidin-3-sophoroside, (C) pelargonidin-3-glucoside, and (D) ascorbic acid. All values are expressed in mg per 100 g fresh weight (FW). Different lowercase letters above the boxplots indicate statistically significant differences among treatments at each harvest date (Tukey's HSD test, $p \leq 0.05$).

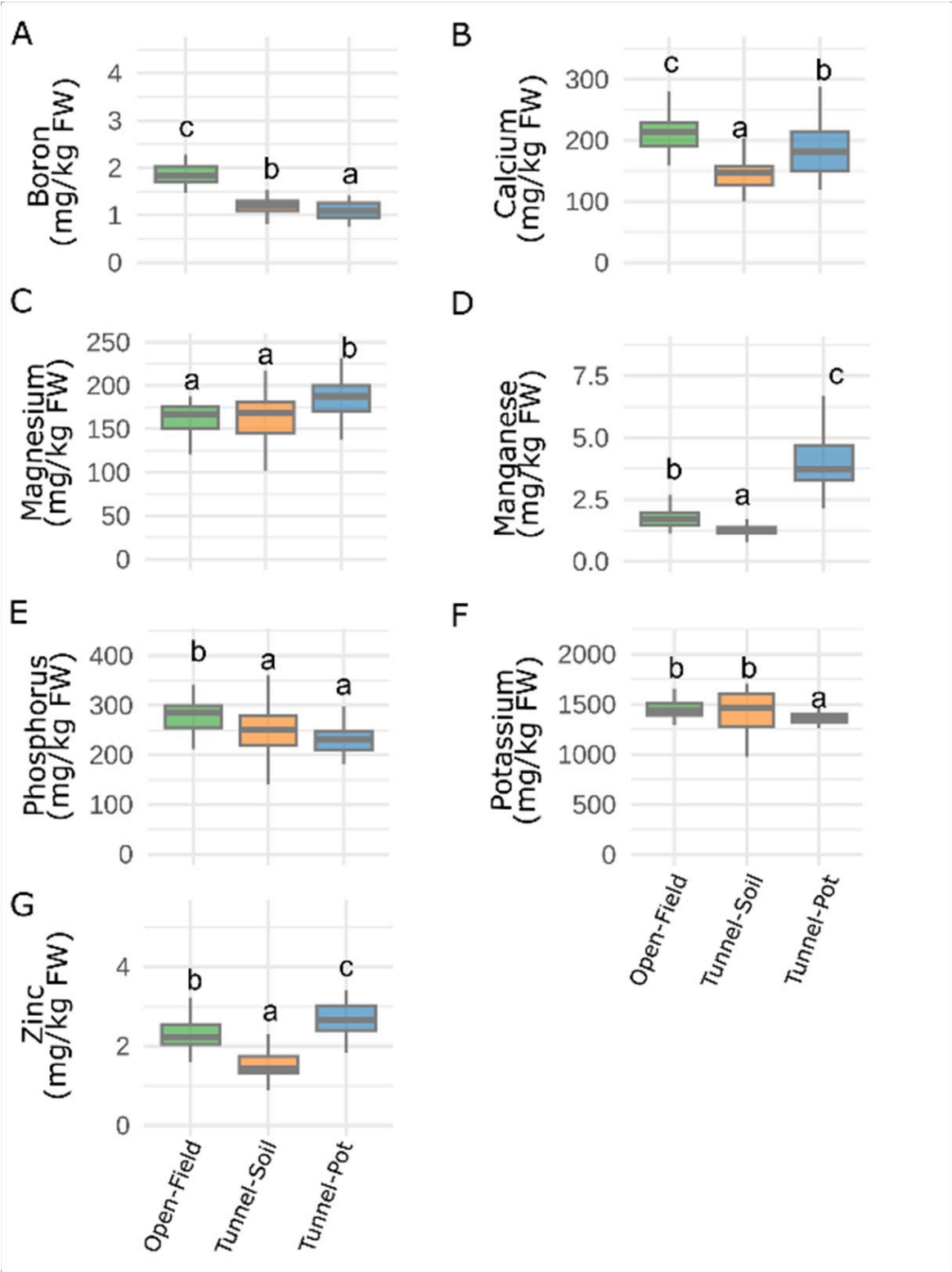


Figure 3

Boxplots showing concentrations of seven mineral elements in raspberry fruit across three cultivation systems: open-field (green), tunnel-soil (orange), and tunnel-pot (blue). (A) Boron, (B) calcium, (C) magnesium, (D) manganese, (E) phosphorus, (F) potassium, and (G) zinc. All values are expressed in mg/kg fresh weight (FW). Each dot represents an individual biological replicate. Different lowercase letters above the boxplots indicate statistically significant differences between treatments (Tukey's HSD test, $p \leq 0.05$).

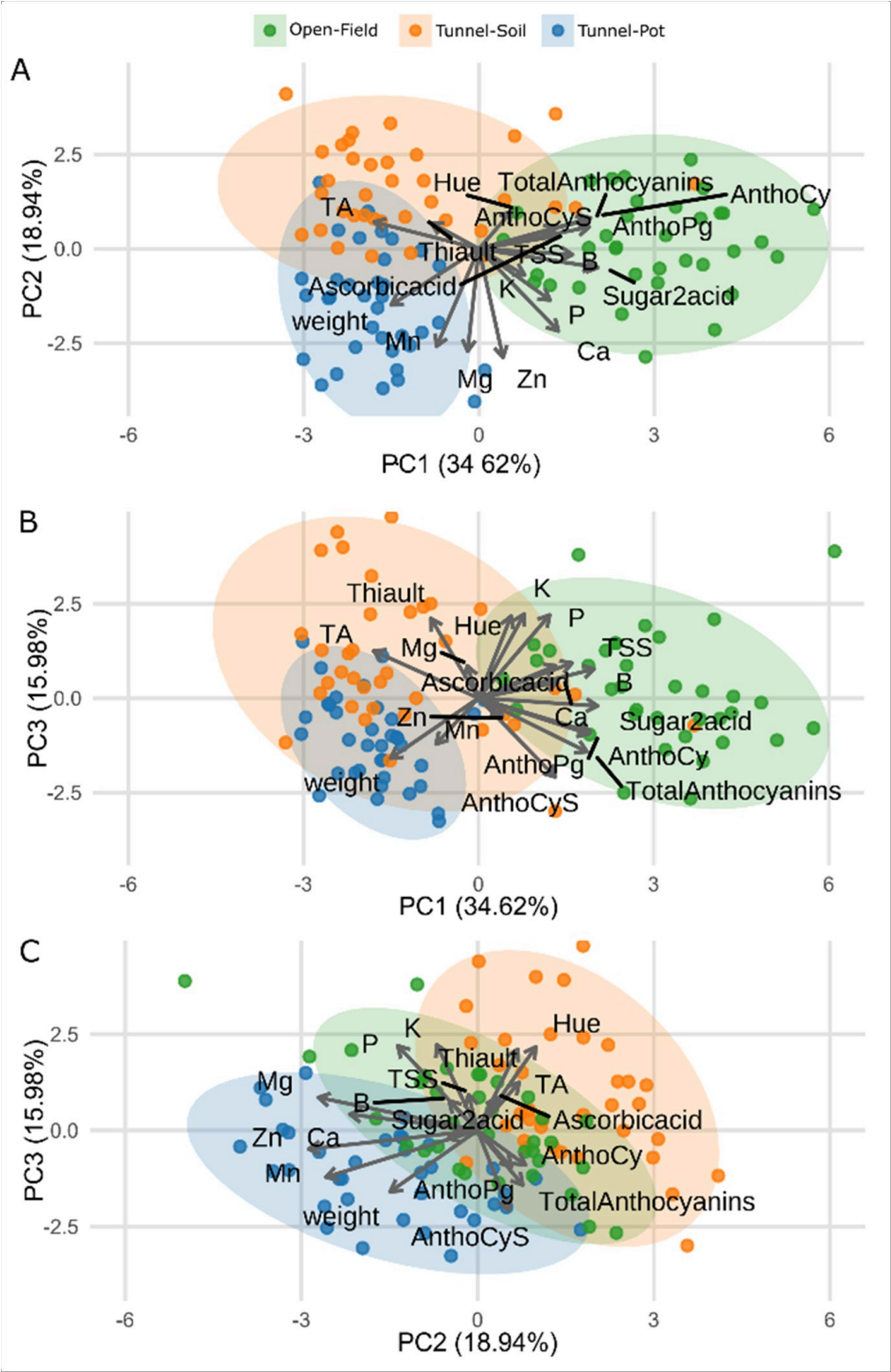


Figure 4

Principal component analysis (PCA) biplots showing the relationship between raspberry fruit quality parameters and cultivation systems. Each point represents one sample. Vectors indicate the direction and relative contribution of fruit quality variables to the principal components. Ellipses represent 95% confidence intervals for each cultivation system: green for open-field, orange for tunnel-soil, and blue for tunnel-plot. Subfigure (a) shows PC1 vs PC2 (explaining 34.62% and 18.94% of the total variance, respectively), (b) shows PC1 vs PC3 (34.62% and 15.98%), and (c) shows PC2 vs PC3 (18.94% and 15.98%).