Communication

Inter-Day Instability in Plant Sap Composition Undermines Single-Day Diagnostics

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Inter-Day Instability in Plant Sap Composition Undermines Single-Day Diagnostics

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Abstract

Plant sap analysis has emerged as a practical, real-time tool for nutrient monitoring, complementing conventional tissue tests. As adoption expands, standardization has become a priority; yet current research emphasizes tissue selection and analytical procedures, whereas short-term variation in sap composition has been largely overlooked. Consequently, a single-day diagnostic paradigm has prevailed, resting on the unverified premise that inter-day shifts lack practical relevance. To test this, we conducted a five-day field trial in broccoli, quantifying sap pH, EC, K^+, NO_3^-, and °Bx daily under five extraction methods. Extraction method had a negligible effect on inter-day shifts (%Δ), whereas chemical parameters differed markedly, ranking pH (0.8%) < °Bx (3.7%) < K^+ (5.9%) < EC (6.2%) < NO_3^- (8.2%). The largest observed changes across successive days were 0.13 pH units, 0.49 °Bx, 6.94 mmol/L K^+ (271 mg/L), 1.17 dS/m EC, and 11.98 mmol/L NO_3^- (743 mg/L). These results show that inter-day instability can bias single-day diagnostics, motivating adoption of new sampling strategies. We urge researchers and advisors to account for inter-day variation and incorporate multi-day sampling into experimental design and decision-making until predictive frameworks are available. Recognizing and addressing this dimension will improve sap diagnostic reliability and support more sustainable, science-based nutrient management.

Keywords: crop nutritional assessment; fertilizer management; ion-selective electrodes; nutrient monitoring; on-farm quick tests; plant nutrition; plant sap

1. Introduction

Plant sap analysis has emerged as a promising technique for monitoring the nutritional status of crops in a practical and timely manner [1–5]. In contrast to conventional dry matter tissue analysis, which reflects total nutrient accumulation over extended periods, sap analysis measures the soluble and metabolically accessible fraction of key parameters [6–10]. By reflecting the plant's real-time nutritional status, this method supports more accurate and responsive fertilization strategies, particularly in short-cycle crops and intensive production systems [11–16]. However, as adoption of the technique expands across scientific and commercial contexts, concerns over protocol standardization intensify, reflecting unresolved issues in ensuring reproducible and comparable sap measurements [17–21].

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Efforts to enhance methodological consistency have focused on several critical aspects, including the selection of plant tissue for sap extraction [22–26], the time of day at which samples are collected [27–31], the sample size needed to ensure representativeness [32–35], and the analytical tools used for nutrient quantification [36–40]. Yet one dimension has received comparatively little attention: the role of temporal variation—specifically, how sap composition may fluctuate across consecutive days.

In practice, scientific studies and field monitoring programs often rely on single-day sampling at specific phenological stages, without accounting for potential shifts in baseline sap composition across nearby days [41–45]. However, routine practices—including irrigation events and the application of plant growth regulators—as well as environmental fluctuations (e.g., light intensity, temperature, vapor pressure deficit), can alter photosynthetic activity, plant water potential, and transpiration rates [46–50]. Such physiological processes shape the plant's metabolic profile and, consequently, are expected to influence the composition of the sap expressed from its tissues [51–53].

This calls into question the temporal stability of sap composition under field conditions. Most existing research has focused on contrasting sampling scales, either capturing intra-day fluctuations [54–58] or relying on weekly intervals [59–63], thereby overlooking the intermediate dynamics that unfold on a day-to-day basis. Based on our extensive literature review, Matthäus & Gysi [64] stands as the only published work that has explored sap composition over multiple successive days (>3), focusing exclusively on NO_3^- in broccoli. This underscores the need for empirical data to clarify the extent and implications of short-term variability. To address this gap, the present study provides a novel exploratory assessment in the same species, incorporating four additional sap chemical parameters and evaluating their daily variation over five consecutive days in an open-field production system.

2. Materials and Methods

2.1. Study Site

The study was conducted during the 2024-2025 summer season in a broccoli crop (*Brassica oleracea* var. *italica*, cv. Zafiro) cultivated in Quillota, Valparaíso Region, Chile, at coordinates 32°54′32.63″S and 71°15′24.93″W. Plants were evaluated at the preharvest phase, corresponding to stage 49 on the BBCH scale, as described by Meier [65].

The crop was established at a planting density of 0.40 × 0.65 m, with inter-row paths inserted after every eight planting lines. This spatial arrangement resulted in a plant population of ~35,000 individuals per hectare. At the time of sampling, plants exhibited an average of 23 [20–25] leaves, a height of 23 [21–27] cm, and a basal inflorescence diameter—measured below the first bract—of 54 [48–63] mm.

The soil at the site was classified as clay loam, with a pH of 7.68 determined in a soil-to-water suspension at a 1:2.5 ratio, electrical conductivity (EC) of 1.02 dS/m measured in a saturated paste extract, and organic matter content of 4.0%. Available nitrogen (sum of $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$) and potassium levels in the soil were 22.3 mg/kg and 250 mg/kg (0.64 cmol/kg), respectively. Irrigation was provided through a drip system using water with a pH of 7.15, EC of 0.73 dS/m, nitrate (NO_3^-) concentration of 177 mg/L (2.85 mmol/L), and potassium levels below the analytical detection limit (<1 mg/L; <0.03 mmol/L).

2.2. Experimental Design

In a 0.1-hectare section of the field, 525 broccoli plants were randomly selected over five consecutive days (13-17 January 2025). To minimize potential bias in sap nutrient composition, only healthy, undamaged, and unshaded plants were included in the sampling [66,67].

The most recently mature leaves (MRML) were selected as the sampling organ, following the recommendations of several authors [68–71]. These leaves were consistently located at the third or fourth node below the inflorescence and were identified in the field by their insertion angle, ranging from 65° to 75° [72]. The adaxial surface exhibited vivid green hues (5 G 3/2, 2.5 G 3/4, and 2.5 G 4/2),

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while the abaxial side displayed lighter, moderately saturated tones such as 5 G 6/2 and 7.5 G 6/2. Lamina thickness ranged from 300 to 500 μm, with surface texture varying from smooth to slightly rough.

The central third of each MRML petiole was excised and cleared of any remaining leaflets. Each day, 7 biological replicates were formed by grouping 15 petiole segments, each taken from a different individual plant. To avoid confounding effects from diurnal variation, all samples were collected daily between 9:00 and 9:30 a.m.

Immediately after collection, samples were transported to the Agronomy campus of the Pontificia Universidad Católica de Valparaíso, situated about 10 km away from the field site (32°53′44.29″S, 71°12′31.22″W). To preserve tissue integrity and minimise post-harvest handling effects, we followed previous recommendations for short-term storage [54,56,68,73,74]. Insulated containers with ice packs maintained the samples at a mean 4.1 °C during the 18 min field-to-laboratory transfer. Upon arrival, they were transferred to zip-lock plastic bags and stored at 4.0 °C in complete darkness until analysis (<8h).

Petiole samples were removed from refrigeration just prior to sap extraction, then immediately cut into ~5 mm segments and thoroughly homogenized to produce a uniform composite sample. Sap was extracted using five distinct methods, each defined by a manually operated device and its specific mode of operation, applying an average hand grip force of 33 kg: (1) A citrus squeezer (Zulay Kitchen, "2-in-1 Lemon Squeezer"); (2) a potato press (Metaltex, "Mr Mash"); and (3) a garlic press (MI Store, "Kitchen Series n°9191"), each operated according to its typical culinary use; (4) a domestic juicer (Sindelen, "BM-490IN 450W"), where samples were processed twice at power level 1 (5 s each) and sap subsequently separated from tissue residues using the garlic press; and (5) a garlic grinder (Ilko, "New Line"), operated through 40 rotations of 45° under constant hand pressure. For each method, a portion of the composite sample was used, with mean fresh weights of 30 g (26 segments), 18 g (15 segments), 8.4 g (7 segments), 13 g (10 segments), and 18 g (16 segments), respectively.

2.3. Measurements

Five chemical parameters were assessed: pH, electrical conductivity (EC), nitrate (NO_3^-), potassium (K^+), and soluble solids (°Bx). pH and EC measurements were obtained using Horiba® LAQUATwin compact meters (models pH-33 and EC-33), each fitted with flat sensors (S010 and S070, respectively). NO_3^- and K^+ concentrations were determined using ion-selective electrodes (ISEs) from the same equipment line: NO3-11 with sensor S040 and K-11 with sensor S030. New electrodes were used to ensure optimal performance and minimize the risk of measurement drift caused by the progressive degradation of their PVC gel-filled membranes [40,75,76]. °Bx was measured using a digital refractometer (Refratec®, model DR32).

Instruments were rinsed with distilled water and dried with tissue paper between samples. Calibration was performed every five measurements using reference standards [77]. To reduce thermal interference in ISE responses, both sap samples and calibration solutions were equilibrated to 18.5 °C in a temperature-controlled room [78–80].

2.4. Statistical Analyses

All analyses were conducted in R (v4.4.2). For each chemical parameter and extraction method, raw observations were aggregated to day-level means. Relative inter-day shifts were then derived as the classical relative change (%Δ), using successive day pairs 1-2, 2-3, 3-4, and 4-5. We first tested the null hypothesis that extraction method had no effect on relative inter-day shifts using a linear mixed-effects model, with extraction method as a fixed effect and day pair as a random intercept; no significant method effects were detected. Consequently, data were pooled across methods, and inter-day variability within each parameter was assessed by one-way ANOVA with Tukey's HSD (α = 0.05). A separate one-way ANOVA evaluated differences in inter-day shifts among parameters. Model assumptions were checked using the Shapiro-Wilk test and the Levene test.

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3. Results

There was no evidence that the extraction method affected relative inter-day shifts (p = 0.071-0.718 across parameters), implicating plant and environmental drivers rather than the sap extraction procedure. The magnitude of these shifts was parameter-dependent ($p < 0.05$), with means ranked as follows: pH (0.8 ± 0.4% [0.2-1.2]) < °Bx (3.7 ± 1.9% [2.1-6.2]) < K^+ (5.9 ± 2.1% [2.8-7.5]) < EC (6.2 ± 2.1% [3.1-8.7]) < NO_3^- (8.2 ± 5.9% [2.7-15.9]) (Figure 1). In practical terms, the observed changes across successive days reached 0.13 pH units, 0.49 °Bx, 6.94 mmol/L (i.e., 271 mg/L) for K^+, EC 1.17 dS/m, and 11.98 mmol/L (i.e., 743 mg/L) for NO_3^- (Table 1).

Beyond differences in shift magnitude, parameters exhibited distinct inter-day variability. K^+ shifts did not differ significantly across days (p = 0.058), whereas pH, EC, and °Bx varied ($p < 0.05$, η^2 = 0.39-0.53), with Tukey contrasts resolving at most two groups (a and b, with occasional ab overlaps). For NO_3^-, heterogeneity was greater, with three groups (a-c; $p < 0.001$, $\eta^2 = 0.74$) and inter-day shifts ranging from 2.7% (days 1-2) to 16% (days 3-4), suggesting a more complex, externally driven dynamic rather than a uniform oscillation.

Table 1. Sap composition over five consecutive days by extraction method (mean ± SD).

Parameter	Extraction method	Day 1	Day 2	Day 3	Day 4	Day 5
pH	Citrus squeezer	6.33 ± 0.02	6.27 ± 0.03	6.35 ± 0.03	6.28 ± 0.04	6.26 ± 0.03
	Potato press	6.31 ± 0.03	6.26 ± 0.03	6.28 ± 0.02	6.20 ± 0.02	6.19 ± 0.02
	Garlic press	6.25 ± 0.01	6.22 ± 0.03	6.19 ± 0.02	6.21 ± 0.03	6.24 ± 0.03
	Juicer	6.49 ± 0.01	6.45 ± 0.02	6.47 ± 0.03	6.34 ± 0.02	6.35 ± 0.01
	Garlic grinder	6.31 ± 0.01	6.18 ± 0.02	6.26 ± 0.02	6.19 ± 0.03	6.20 ± 0.02
EC (dS/m)	Citrus squeezer	9.20 ± 0.10	9.07 ± 0.20	9.47 ± 0.13	10.29 ± 0.16	9.67 ± 0.12
	Potato press	9.48 ± 0.13	8.95 ± 0.18	9.53 ± 0.13	10.70 ± 0.18	9.72 ± 0.10
	Garlic press	8.96 ± 0.14	8.97 ± 0.09	9.71 ± 0.14	10.19 ± 0.18	9.63 ± 0.11
	Juicer	9.69 ± 0.17	9.22 ± 0.17	9.79 ± 0.17	10.57 ± 0.24	10.08 ± 0.15
	Garlic grinder	9.52 ± 0.19	9.16 ± 0.09	9.79 ± 0.13	10.72 ± 0.12	9.96 ± 0.11
NO_3^- (mmol/L)	Citrus squeezer	52.07 ± 1.71	50.23 ± 4.35	52.30 ± 1.96	61.29 ± 5.29	58.53 ± 4.16
	Potato press	56.91 ± 2.19	56.91 ± 5.63	51.61 ± 1.99	61.98 ± 4.22	57.37 ± 2.75
	Garlic press	56.68 ± 3.56	60.37 ± 5.66	55.07 ± 3.77	61.52 ± 4.75	64.98 ± 2.63
	Juicer	65.44 ± 2.79	66.82 ± 6.54	60.83 ± 3.06	68.66 ± 5.98	67.05 ± 5.76
	Garlic grinder	67.74 ± 3.30	68.66 ± 5.56	56.68 ± 3.18	66.59 ± 7.36	68.43 ± 2.08
K^+ (mmol/L)	Citrus squeezer	63.21 ± 2.28	68.69 ± 1.97	62.11 ± 1.74	64.30 ± 1.03	68.32 ± 2.07
	Potato press	65.40 ± 2.29	67.96 ± 3.10	62.48 ± 2.00	69.05 ± 1.12	68.32 ± 2.07
	Garlic press	61.38 ± 1.37	68.32 ± 4.01	62.48 ± 1.10	67.96 ± 1.46	66.13 ± 2.33
	Juicer	66.13 ± 2.26	69.42 ± 2.92	64.30 ± 2.26	67.23 ± 1.65	68.32 ± 2.72
	Garlic grinder	65.40 ± 1.35	67.96 ± 2.89	65.40 ± 1.66	69.78 ± 1.55	67.96 ± 2.00
Bx (°)	Citrus squeezer	4.67 ± 0.04	4.79 ± 0.06	4.84 ± 0.13	5.09 ± 0.13	4.99 ± 0.10
	Potato press	4.81 ± 0.04	4.89 ± 0.09	5.07 ± 0.20	5.21 ± 0.07	5.04 ± 0.06
	Garlic press	4.73 ± 0.09	5.03 ± 0.09	5.01 ± 0.09	5.29 ± 0.09	4.97 ± 0.11
	Juicer	5.04 ± 0.08	5.10 ± 0.16	4.87 ± 0.14	5.26 ± 0.07	5.04 ± 0.06
	Garlic grinder	5.13 ± 0.09	5.10 ± 0.06	5.06 ± 0.10	5.54 ± 0.21	5.24 ± 0.10

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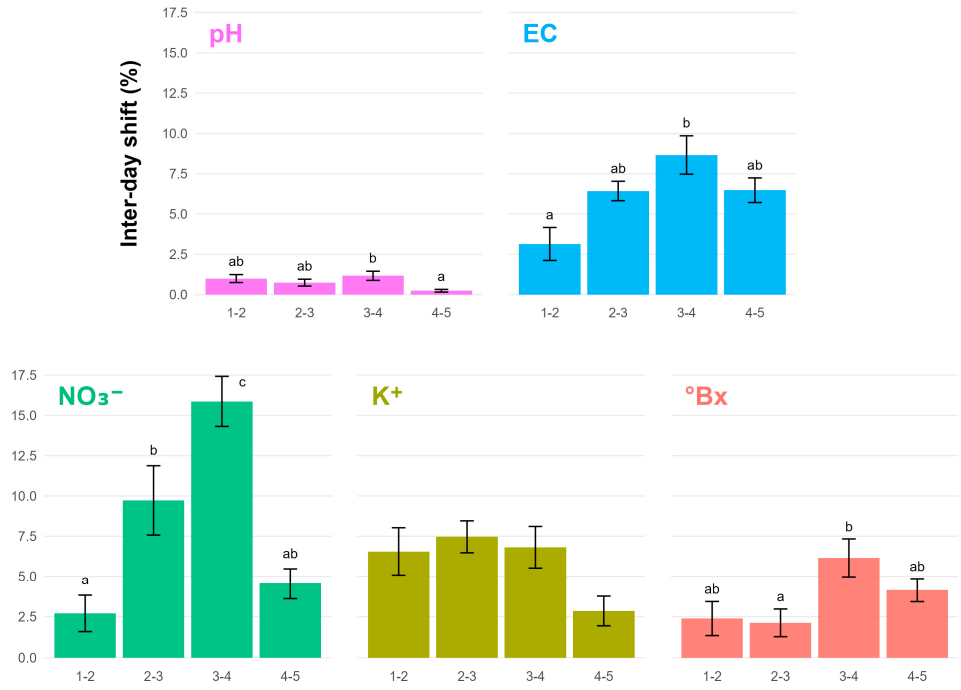

Figure 1. Inter-day shifts (%) in sap parameters, pooled across extraction methods. Lowercase letters denote Tukey HSD groups within each parameter ($p < 0.05$).

4. Discussion

Several studies have shown that the nutritional profile of plant sap changes over time, from intra-day periods (<24h) to broader stages monitored at fixed intervals of 7, 10, or ≥14 days [81–84]. However, short-term dynamics unfolding within a single week remain scarcely addressed in the literature. Our findings provide novel evidence that sap composition fluctuates across successive days, underscoring the need to account for this temporal dimension in sap-analysis protocols.

Among the measured parameters, pH exhibited the smallest relative shifts and NO_3^- the largest. To date, only the two parameters have previous evidence enabling direct magnitude comparisons; however, most of this assessed a single interval between two days, yielding isolated cases rather than time-series behaviours. Exceptions are the 3-day and 4-day assessments conducted by Loehwing [85] and Matthäus & Gysi [64], respectively.

For pH, we observed modest shifts of 0.8% [0.2-1.2] across successive days, in agreement with the 0.8% [0.0-2.5] reported by Loehwing [85] in wheat, but markedly lower than the 6.9% [2.8-12] documented by Ingalls & Shive [86] in *Bryophyllum* and *Tradescantia*. In contrast, sap NO_3^- exhibited shifts nearly tenfold higher (mean of 8.2%), reaching up to 16% in one of the intervals evaluated in this study. This exceeded the 3.8% [3.5-4.1] reported in potato by Vitosh & Silva [30] yet remained below other previously documented values. Among these, Matthäus & Gysi [64] reported shifts of 16% [1.9-36] in broccoli, Justes et al. [87] 26% [14–41] in wheat, and Papastylianou [88] 27% [0.0-158] in barley. Overall, this evidence supports day-to-day variation as a persistent, cross-species phenomenon, with sap nutrient concentrations rarely remaining constant on successive days. Multiple factors likely modulate these shifts and explain the wide ranges reported across studies.

For instance, daily fluctuations in soil nutrient availability [89,90] can affect its uptake and translocation by plants, ultimately leading to measurable shifts in sap concentrations. Direct evidence of this was early provided by Hoagland & Broyer [91], who traced the uptake of K^+ and Br^- in barley roots, reporting that sap composition changed markedly within 8h of exposure to modified external concentrations, reaching its maximum after 24h. Similarly, Justes et al. [87] showed that, following fertilization with ^{15}N-labeled nitrate, the tracer became detectable in wheat sap within 3h and attained its maximum isotopic excess at 48h. However, the extent of this uptake may be influenced by soil

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fertility, yet evidence remains scarce and inconsistent. Whereas one study reported nearly threefold greater inter-day shifts in sap NO_3^- under N fertilization relative to N deficiency [87], another found levels remained stable at high N but fluctuated in the absence of fertilization [88].

Environmental conditions can also play a crucial role in shaping the nutrient profile of plant sap. Hoagland & Broyer [91] and Zhang et al. [92] reported increases in sap NO_3^- and K^+ with rising temperature, likely reflecting enhanced root activity and increased xylem transport driven by higher transpiration rates. Conversely, testing three temperature levels, Palenski & Kemp [29] detected significant differences in sap NO_3^- concentration, yet no clear relationship between both—a result also reported by Coltman [93] and Papastylianou [88] suggesting that temperature effects are contingent on additional factors.

In the case of sap pH, prior work documents light-driven diurnal oscillations, characterized by daytime alkalinization followed by overnight reversion to baseline. However, the recovery rate appears temperature-dependent—an inference from graphical evidence rather than explicit authorial interpretation—with low temperatures precluding full restoration and thereby increasing inter-day shifts [85,94].

Soil moisture may also be a key driver of short-term variability in sap composition, as suggested by convergent evidence from three studies. First, Smith [95] found that irrigation pulses temporarily elevated sap phosphorus and other nutrients (not specified). Then, MacKerron et al. [96] noted rainfall-coincident peaks in sap NO_3^-, hypothesizing greater rhizosphere ion availability; and more recently, Janeiro-Cid et al. [97] documented a linear positive relationship between soil moisture and sap NO_3^- in strawberry ($R^2 = 0.78$).

Therefore, multiple sources of variability likely act concurrently, shaping the temporal stability of individual sap chemical parameters. Disentangling these influences will require multivariate designs that jointly evaluate candidate drivers to yield adjusted estimates, or else define standardized sampling conditions that reduce variability to a practical scale. A contemporary study by Llanderal et al. [98] begins to address this gap at the weekly scale in greenhouse tomato, offering a clear methodological precedent for future work targeting inter-day dynamics. By jointly evaluating soil-solution chemistry, climatic drivers, and canopy attributes, they found that ETc, VPD, and LAI were the most informative predictors of weekly changes in sap profile, with nutrient-specific response patterns.

However, pending predictive frameworks, standardizing sampling protocols remains the pragmatic priority for both scientific research and real-world fertilization decisions. In line with previous evidence and published guidelines, sampling should be avoided immediately after cloudy weather, irrigation or rainfall, or under atypical temperature conditions, which can perturb sap dynamics [99–101]. Furthermore, given inter-day variability, samples should be collected on successive days and averaged rather than relying on single-day measurements to better capture intra-week nutrient status and reduce the influence of anomalous readings, especially when monitoring NO_3^-.

5. Conclusions

Our results demonstrate that meaningful short-term dynamics in the sap nutritional profile occur and can bias single-day diagnostics. Pending a mechanistic understanding of their drivers and the development of predictive frameworks to adjust measurements, moving toward standardized protocols can enhance precision by minimizing measurement noise. Therefore, we encourage researchers to explicitly incorporate day-to-day variation into experimental design, and agricultural advisors to account for it when planning field measurements and making fertilization decisions. Addressing these gaps will improve diagnostic reliability and advance a more sustainable, science-based agriculture.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

°Bx	Degree Brix (soluble solids)
BBCH	Phenological growth stage scale
EC	Electrical conductivity
ETc	Crop evapotranspiration
ISE	Ion-selective electrode
K^+	Potassium
LAI	Leaf area index
MRML	Most recently mature leaves
NO_3^-	Nitrate
VPD	Vapor pressure deficit

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