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ORIGINAL PAPER

Comparing the Effectiveness of two Innovative Slow-Release Fertilizers on Chemical Release Dynamics and Lettuce Plant Growth

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Abstract

Purpose: The use of conventional fertilizers raises concerns about nutrient losses, soil degradation, and non-renewable resource consumption. Waste-derived slow-release fertilizers (SRFs) may reduce these impacts by combining gradual nutrient release with residue valorization. However, complete Nitrogen, Phosphorous, Potassium (NPK) glass-based SRFs remain poorly explored, especially when nitrogen is supplied through an organic phase. We hypothesized that cold- and hot-consolidated SRFs, despite having the same raw materials and NPK content, would differ in nutrient release and plant response. Therefore, this study compared their NPK release, compliance with European SRF criteria, and effects on *Lactuca sativa* L. growth and physiology. Methods: The SRFs, characterized by crystalline or amorphous phases depending on processing temperature, were evaluated through solubility tests in citric and acetic acid to assess macro elements (N,P,K) release dynamics. Two repeated lettuce cultivation growth tests were conducted to measure agronomic parameters (fresh biomass, plant height, leaf area) and substrate chemical parameters and quality. Plants physiological status was assessed using the Nitrogen Balance Index (NBI). Results: Cold-SRF exhibited notably higher nitrogen release (75.9% at 90 days) than hot-SRF (<5%). Phosphorus release in citric acid was comparable for both formulations (~80%), whereas in acetic acid it reached ~70% for cold-SRF but remained below 30% for hot-SRF. Potassium showed the opposite pattern, with hot-SRF releasing nearly completely (~100% in citric acid, ~90% in acetic acid) versus ~60% for cold-SRF in both media. Cold-SRF significantly improved plant biomass, height and leaf area ($p < 0.05$) compared with both the control and hot-SRF, and this agronomic performance was supported by enhanced NBI values. Conclusions: Cold-SRF provided a higher and more sustained overall Total Nutrient Release (TNR: 67.0% at 90 days) compared to hot-SRF (59.4%), driven primarily by the trends of N and P. This more sustained nutrient availability translated, in the 28-day lettuce trial, into superior plant performance: larger leaf area, greater plant height, higher above-ground biomass, and enhanced NBI values, indicating better nitrogen status and photosynthetic efficiency. Since the release remained active for up to three months while the agronomic trial already showed positive results within 28 days, cold-SRF also represents a promising option for low-maintenance fertilization strategies where fewer applications are desirable, reducing operational costs while maintaining adequate nutrition.

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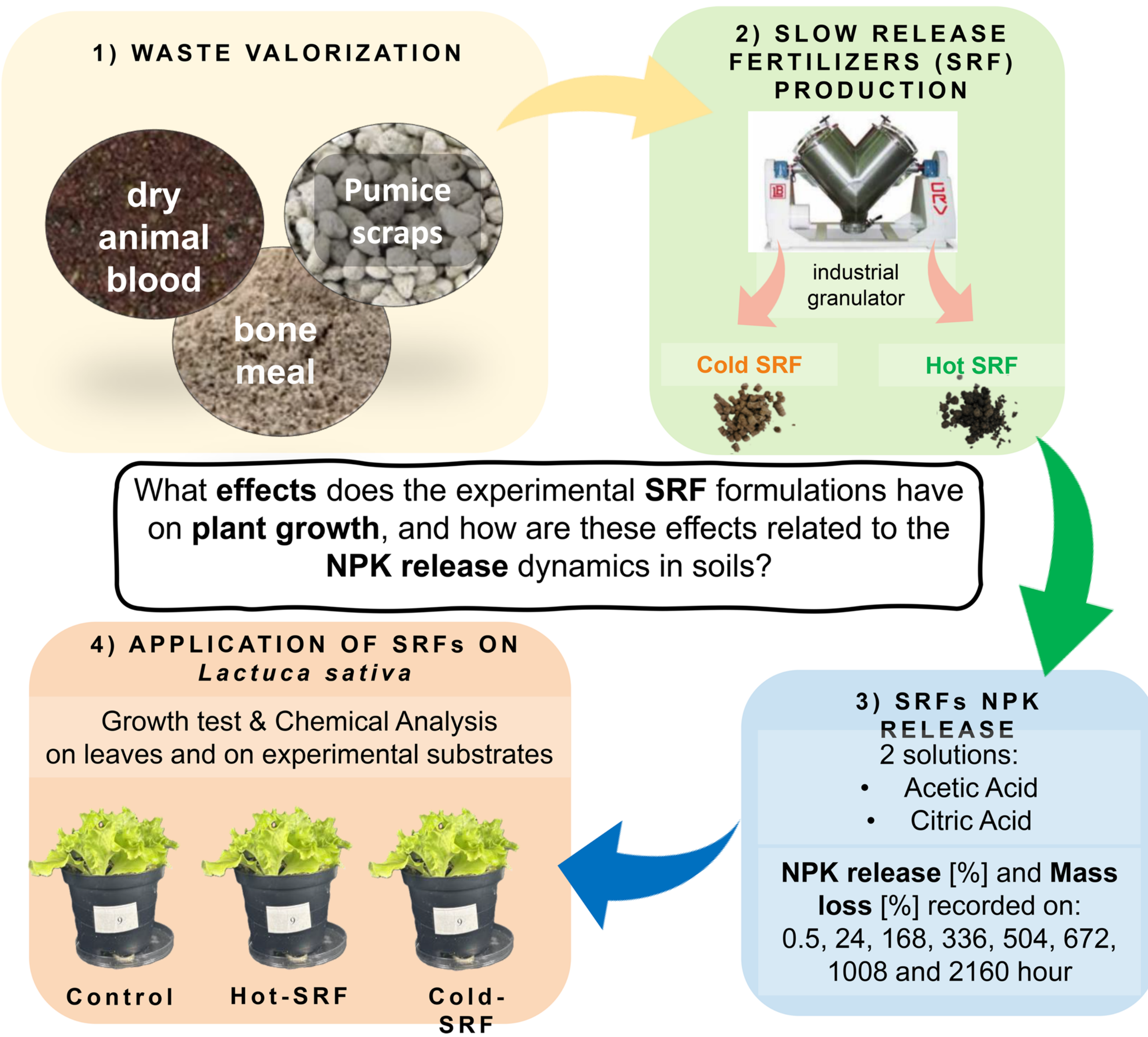
Graphical Abstract



Abbreviations

AGB	Above-Ground Biomass
BGB	Below-Ground Biomass
CNTR	Control
DW	Dry Weight
FW	Fresh Weight
HA	hydroxyapatite
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
NBI	Nitrogen Balance Index
NPK	Nitrogen, Phosphorous, Potassium
PCA	Principal Components Analysis
SRF	Slow-Release Fertilizer
TNR	Total Nutrient Release
TNC	Total Nutrient Content

1 Introduction

The world population is projected to reach approximately 9.7 billion by 2050, further increasing pressure on agricultural systems (van Dijk et al. 2021). Synthetic fertilizers have played a central role in sustaining crop productivity under this increasing demand; however, their inefficient use remains a major environmental concern (FAO 2025; Olad et al. 2018). FAOSTAT data show that cropland nutrient budgets are essential to quantify nutrient surpluses and deficits, since excessive nutrient inputs are associated with losses through leaching and runoff, soil and water pollution, and greenhouse gas emissions. Nitrogen management represents a critical challenge, as approximately 150 Tg of reactive nitrogen are added annually to the Earth's land surface through agriculture and industry, more than twice the pre-industrial rate (FAO 2025). Therefore, improving fertilizer-use efficiency while maintaining crop productivity is a key requirement for sustainable agriculture. In this context, the use of slow-release fertilizer systems (SRFs) obtained from waste-derived resources may represent a promising strategy to reduce nutrient losses, valorize residual materials, and support more circular fertilization practices. By enabling controlled nutrient release, these formulations enhance nutrient use efficiency while minimizing the risk of environmental pollution (Tarashkar et al. 2024). Among SRFs, glass or glass-ceramic form are particularly interesting because they provide a durable matrix for sustained nutrient delivery, enabling the incorporation of both micronutrients (Fe, Mo, Cu, Zn, Mn, etc.) and macronutrients (P, K, Ca, Mg, etc.) although nitrogen generally requires an additional non-glassy source. Their composition can therefore be tailored to crop requirements. Moreover, the different crystalline phases formed in glass-ceramic systems may exhibit different solubilities, further modulating nutrient release over extended periods (Martin Mariska 2000). These systems provide a series of advantages: their gradual nutrient release helps maintain a more stable nutrient supply in the soil, reducing the need for frequent applications and supporting plant growth (Soares et al. 2025; Vancea et al. 2021). Moreover, their slow and controlled nutrient release can reduce environmental impacts by limiting nutrient losses through leaching and runoff and by lowering the risk of pollution associated with conventional fertilization, including eutrophication and greenhouse gas emissions such as nitrous oxide (Sofyane et al. 2020). An additional strength is due to the possibility to tailor the nutrient composition to specific crop and soil requirements, supporting precision fertilization and improving farming efficiency (van Heerwaarden 2022). Furthermore, these systems can enrich the soil over very long periods, contributing to maintaining soil fertility over time (Li et al., 2021). In addition, SRFs derived from waste materials may combine controlled nutrient release with waste valorization, making them promising tools for sustainable nutrient management (Badagliacca et al. 2024; Chojnacka et al. 2020). Finally, studies have demonstrated the ecotoxicological safety of fertilizers based on a glassy matrix, showing no significant negative effects on seed germination or plant cell health (Albert and Bloem 2023; Soares et al. 2025).

Despite these advantages, glass-based fertilizers are generally investigated as sources of specific nutrients, especially P, K, or micronutrients, whereas their use in complete Nitrogen-Phosphorous-Potassium (NPK) systems remains less explored, particularly when combined with organic nitrogen sources. This aspect is relevant because nitrogen is usually not incorporated into the glass network and must therefore be supplied through an additional phase, whose interaction with the glass-ceramic matrix may strongly influence N availability.

The present study focuses on the development and comparison of two experimental slow-release fertilizers formulated with the same waste-derived raw materials (animal blood, bone meal, and pumice scraps) and NPK content but processed through different ways. In cold-SRF, all precursors are granulated at room temperature: phosphorus remains in its native crystalline form as hydroxyapatite from bone meal, and potassium is added as soluble KNO_3. The cold-granulated formulation was selected based on a previous comparative study (Napolitano et al. 2025) that evaluated a series of formulations consolidated at room temperature. In those preliminary trials (lasted 21 days), agronomic assessments (including only fresh and dry biomass weight) showed that the cold-granulated formulation outperformed a commercial NPK fertilizer (8-17-8) applied at standard rates (3 g kg^{-1} of substrate). In hot-SRF, a vitrification step transforms bone meal and pumice into a glass-ceramic matrix, where phosphorus becomes part of the amorphous silicate network and potassium acts as a network modifier; this glass is then supplemented with animal blood as the organic nitrogen source to obtain a complete NPK fertilizer. In this study, both formulations are designed as complete NPK systems, allowing a direct comparison of how nutrient speciation and physical structure influence release behavior and plant response. The study focuses on the three primary macronutrients (N,P,K) as they are the main determinants of plant growth and yield. Micronutrients, while important, were not within the scope of this investigation.

Within this framework, the present study aims to compare the release dynamics of NPK between the two SRF formulations under simulated soil and environmental conditions (citric and acetic acid extraction), and their potential to meet the European criteria for slow-release fertilizer classification, where Total Nutrient Release (TNR) should remain $<15\%$ at 24 h and $<75\%$ at 28 days. The comparison is also aimed to go beyond laboratory testing by evaluating the practical consequences of the observed nutrient release patterns on plant growth, physiological status, and substrate chemistry during a 28-day controlled lettuce growth trial. This integrated approach linking in vitro release data with in vivo plant response aims to identify the best performing formulation and the most suitable application context for each formulation.

2 Materials and Methods

2.1 Experimental Design

The experimental design was structured to compare two waste-based slow-release fertilizer systems, produced through different consolidation strategies, in terms of nutrient-release behavior and agronomic performance. The first formulation, named cold-SRF, was obtained through an industrial cold granulation process, while the second, named hot-SRF, was produced by incorporating phosphorus and potassium into a glass-ceramic matrix through high-temperature melting followed by water quenching. Both formulations were adjusted to have comparable total nitrogen contents by adding dry animal blood as an organic nitrogen source. The release kinetics of N, P, and K from both SRFs were evaluated in simulated extraction environments using 2% citric acid and 0.5 M acetic acid solutions over different contact times, to assess their slow-release behavior and potential nutrient leaching. In parallel, the agronomic performance of the two formulations was tested on baby leaf lettuce (*Lactuca sativa* L.) grown under controlled conditions for 28 days. Three substrate treatments were compared: an unfertilized control substrate (CNTR), the same substrate amended with cold-SRF (cold-SRF) and the substrate amended with hot-SRF (hot-SRF), with both fertilizers applied at 5 g kg^{-1}. Each treatment was replicated in four pots per experimental trial, and the whole growth experiment was repeated twice. At the end of the cultivation period, plant growth, biomass allocation, leaf area, pigment-related physiological indices, and substrate and plant chemical composition were assessed to evaluate both fertilizer efficiency and potential accumulation of undesirable elements.

2.2 Treatments

The experimental slow-release solid fertilizer systems were based on two recovery local raw materials consisting of animal bone meal (main P-source) and pumice scraps (K-source) from mining extraction operations. Two SRFs systems with similar N content were developed using a different procedure: the first formulation, named cold-SRF, was obtained through a cold granulation process commonly used in industrial production. The second formulation, named hot-SRF, was produced by consolidating the main mineral components through high-temperature treatment followed by rapid quenching in water, according to a fritting technique. The complete workflows for both production processes are reported on Fig. 1.

For the cold-SRF formulation, animal bone meal, pumice scraps, dry animal blood, and KNO_3 were used as raw materials. Dry animal blood and KNO_3 were added to obtain a complete NPK fertilizer, providing slowly releasable organic nitrogen and rapidly available nitrogen, respectively. The animal blood was of industrial and local origin and had been heat-treated in accordance with Regulation European Economic Community Health Regulation 1069/2009, to ensure health and phytosanitary safety. Before granulation, all raw materials were ground and sieved to obtain a powder fraction below 0.25 mm, selected to promote efficient nucleation, granule growth, cohesion, and structural integrity while reducing binder and water requirements. The powders were mixed with an 8 w/w (%) calcium-lignosulfonate binder solution and granulated using an industrial granulator (GRV7 Model, provided by LB Officine Meccaniche S.p.A.) (Schwaeble et al. 2021). The resulting granules were sieved to obtain a 0.5–5 mm size fraction and dried at 110 °C until residual humidity was below 1.0%.

The final NPK composition, obtained from both theoretical calculations and experimental analyses, is reported in Table 1 and was based on a formulation previously optimized (Napolitano et al. 2025).

The hot-SRF fertilizer system was mainly based on a glass-ceramic powder obtained by mixing bone meal previously calcined at 950 °C for 1 h, pumice scraps and K_2CO_3. This mix was melted at 1400 °C for 1 h and then quenched in water at room temperature. The resulting glassy material was then mixed with dry animal blood as N-source and granulated following the same procedure used for cold-SRF, obtaining granules in the 0.5–5 mm size range with residual humidity below 1.0%.

The rationale underlying these formulation choices lies in the potential mechanisms governing nutrient release from the selected materials; in both SRFs, nitrogen is supplied in organic form, derived from animal blood and, in the case of cold-SRF, a little fraction also from uncalcined bone meal (Jilling et al. 2018). These organic nitrogen sources are not directly plant-available and require enzymatic or microbial degradation before conversion into ammonium or nitrate. In contrast, phosphorus and potassium are expected to follow distinct release dynamics depending on their chemical form and matrix incorporation. In cold-SRF, phosphorus is present as hydroxyapatite (HA), a calcium phosphate mineral with low solubility at neutral pH but higher dissolution under acidic conditions, whereas potassium is supplied as highly soluble KNO_3, whose release may be partially limited by diffusion through the granule matrix. In hot-SRF, the vitrification process leads to the incorporation of both P and K into a silicate glass matrix. Phosphorus acts as a network former and it is therefore expected to be strongly retained within the glass structure, with limited leaching except under conditions promoting matrix dissolution, such as the presence of complexing agents. Conversely, potassium

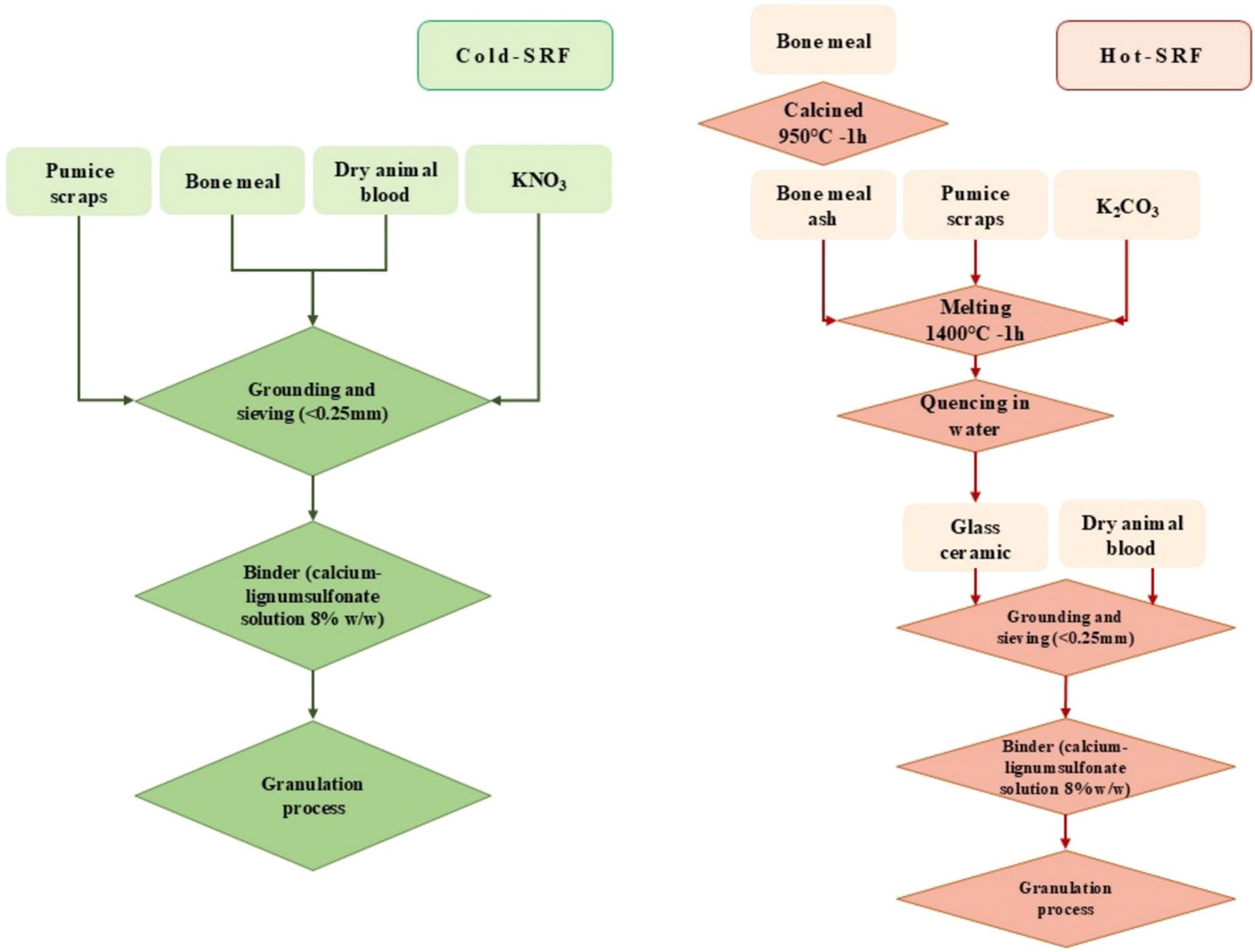


Fig. 1 Flow-chart of production process applied for slow-release fertilizer systems (SRFs): cold-SRF and hot-SRF, produced through cold and hot processes

Table 1 Experimental chemical composition of hot and cold slow-release fertilizers systems (SRFs); in brackets calculated (theorical) composition is reported

Treatments	N_{tot} % [w/w]	$N\text{-}NH_4^+$ % [w/w]	$N\text{-}NO_3^-$ % [w/w]	$N_{organic}$ % [w/w]	P_{tot}% [w/w]	K_{tot}% [w/w]
cold-SRF	6.1 (5.9)	0.0	0.6	5.5	6.1 (6.4)	5.4 (5.4)
hot-SRF	5.8 (5.8)	0.0	0.0	5.8	6.6 (6.9)	9.1 (9.4)

behaves as a network modifier and exchangeable ion, so its release is expected to occur more gradually, mainly through cation-exchange reactions and diffusion across the hydrated silica layer forming on the glass surface (Ahmed A. and Youssof I.M., 1997; Zanini et al. 2023).

2.3 NPK-Release Kinetics

The release kinetics of NPK nutrients from cold- and hot-SRF granules were assessed using 2% citric acid and 0.5 M acetic acid solutions as extractants, following the specifications outlined in Italian legislations and standards (Decreto Legislativo 29 Aprile 2010, N° 75, 2010). The citric acid extractants solution was chosen to mimic the activity of soil humic substances due to its chelating properties. Conversely, the test performed in acetic acid solution provides an indication of the nutrients prone to leaching under low pH conditions (i.e. acid rain) (Decreto Legislativo 29 Aprile 2010, N° 75, 2010). In this context, a slower and more gradual release of nitrogen and phosphorus is desirable to minimize the potential environmental impact associated with excessive nutrient leaching.

The method to assess the kinetic of release included the following steps: for each extractant and formulation tested, 1 g of fertilizer reduced to dust below 100 microns was deposited in a 100 mL screw-capped polypropylene laboratory bottle. The bottle was then filled with the chosen solution up to 100 mL. An independent sample, serving as blank, was prepared for each of the selected contact times (0.5, 24, 168, 336, 504, 672, 1008 and 2160 h). Contact times were chosen to evaluate the samples' slow-release behavior. More specifically, European standards (International Standards 2016) require that the total NPK release (in % by weight) should not exceed 15% before 24 h and 75% before 672 h (28 days). The sealed bottles were shaken at room temperature (20 °C) at an agitation of 40 rpm for the specified contact time. The sample was then quantitatively filtered through paper filters. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) measurements were conducted within three days of filtration. The filtered solutions were stored at 10 °C until ICP-MS (triple quadrupole detector) analysis to determine the released P and K, using a reaction/collision cell employing O_2 as the reaction gas. For phosphorus, Q1 was set to m/z 31 (isotope ^{31}P) and Q2 to m/z 47 (adduct $^{31}P^{16}O$). For potassium, both Q1 and Q2 were set to m/z 39 (isotope ^{39}K). This operating mode is effectively able to eliminate interferences, particularly those due to adducts. Filters containing solid residue were dried at 105 °C for 24 h. The dried residue was weighed with an analytical balance to evaluate mass loss over time, then stored at room temperature for elemental analysis to estimate residual nitrogen content. The same procedures were applied for both experimental formulations and the blank samples, using 100 mL of 2% citric acid or 0.5 M acetic acid solution, which were processed in identical containers and under identical conditions for each contact time. Each kinetic release test has been replicated three times to collect statistically significant data.

The release kinetics were completed by processing the NPK release data to obtain two other parameters: the Total Nutrient Release (TNR) and the Total Nutrient Content (TNC). $TNR_{(t)}$ (w/w %) was calculated at 24, 672 and 2160 h in the citric acid environment simulating soil extraction conditions and according to Eq. 1:

$$TNR_{(t)} = \left\{\left[rN_{(t)} \cdot \frac{\%N}{TNC}\right] + \left[rK_{(t)} \cdot \frac{\%K}{TNC}\right] + \left[rP_{(t)} \cdot \frac{\%P}{TNC}\right]\right\} \cdot 100 \quad (1)$$

where %(N), % (P), % (K) were defined as the initial w/w % of N, P, and K in the fertilizer, $rN_{(t)}$, $rK_{(t)}$, $rP_{(t)}$ were defined as the w/w % fractions of N, P, and K that have been released at time t respectively.

Moreover, TNC (w/w %) at the initial time in the formulations was calculated according to Eq. 2.

$$TNC = \%(N) + \%(K) + \%(P) \quad (2)$$

where %(N), % (P), % (K) correspond to the initial w/w % of N, P, and K in the fertilizer.

2.4 Plant Growth Tests

2.4.1 Plant Material

The effects of cold-SRF and hot-SRF were evaluated on baby leaf lettuce plants (*Lactuca sativa* L., cultivar Chiara, ISI Sementi S.p.A., Fidenza, Italy). This cultivar was selected because of its short growth cycle, generally ranging from 20 to 28 days, and its compact size, which make it suitable for controlled-environment cultivation and horticultural experiments.

2.4.2 Substrates

The growth substrate was prepared by mixing agriperlite 120 Agrilit 3, Perlite Italiana S.r.l., Italy; particle size 2–5.6 mm; density 90 kg m^{-3}) with a universal peat substrate (UNICO, AL.FE S.r.l., Pomponesco, MN, Italy). The peat substrate consisted of a mixture of blond and brown peat with different particle-size fractions and was characterized by 23% organic C, 0.4% organic N, and 46% organic matter. Perlite and peat were mixed at 3:1 ratio. The resulting substrate was used as the unfertilized control treatment (CNTR). Two additional treatments were prepared by adding either cold-SRF or hot-SRF to the same control substrate. Both fertilizers were applied at a concentration of 5 g kg^{-1} of substrate, based on previous experimental findings that identified this dosage as optimal for these formulations (Napolitano et al. 2025). Overall, three substrate treatments were tested: CNTR, cold-SRF, and hot-SRF. Although the main aim of the present work was to study the comparison between the two experimental SRF systems, the comparison between cold-SRF and a commercial NPK fertilizer has already been reported in a previous study (Napolitano et al. 2025).

2.4.3 Growth test Conditions

The three substrates were used to fill plastic pots with a volume of approximately 500 cm^3. Four replicated pots were prepared for each treatment (CNTR, cold-SRF and hot-SRF) in each experimental trial. After filling, each pot was moistened with 50 mL of tap water, and five seeds were sown per pot. Irrigation was carried out every three days using the same water volume. The pots were placed in trays into a growth chamber (KW, WRS 85) set at 25 ± 1.5 °C, under a LED light panel, positioned 55 cm over the pots, maintaining this distance constant for all the duration of the study. The light regime was selected according to (Sgarbi et al. 2023), where an optimized light recipe for lettuce growth had been identified. During the first 7 days plants were exposed to hyper-red light 66.6% and far-red light 33.3% for 16 h day^{-1}, followed by 8 h of darkness. From day 8 to day 28, the light spectrum consisted of green 14%, hyper-red 59.3%, far-red 7.3% and white light 19.3%, with the same 16 h light / 8 h dark photoperiod. The growth test lasted 28 days and was repeated twice.

2.4.4 Agronomic and Physiological Parameters

At the end of the cultivation period, several agronomic parameters were measured. Fresh weight (FW) was determined for total above-ground biomass (AGB), total below-ground biomass (BGB), and mean fresh weight per shoot (shoot FW) using an analytical scale (Kern, d = 0.1 mg). Dry weight (DW) was determined after drying the samples in an oven (Argo Lab, TCN 50) at 55 °C for at least 48 h. Shoot and root height were measured using a digital caliper. Leaf area was determined on three fully expanded leaves per plant, excluding the smallest leaves and those showing visible defects. Image analysis was performed using ImageJ software, applying the function "Analyze particles", to identify the Region of Interest (ROI) on 8-bit images. Leaf physiological indices were measured using a Dualex® sensor (Metos, Pessl Instruments). Chlorophylls, anthocyanins, flavanols and Nitrogen Balance Index (NBI) were measured on one fully expanded leaf per plant (among the 3rd and the 5th position) and without no visible defeats. Chlorophylls are directly involved in photosynthesis, whereas anthocyanins and flavonols contribute to antioxidant activity and stress protection. The NBI, calculated as the ratio between chlorophylls and flavonols, was used as an indicator of plant nitrogen status and metabolic balance (Davies et al. 2022).

2.4.5 Substrates and Plants Chemical Analysis

Chemical analyses were performed on substrates and lettuce leaves to assess nutrient release from the fertilizers, nutrient uptake by plants, and the possible accumulation of potentially undesirable elements. In the substrates, the following parameters were evaluated before and after the growth tests: total organic carbon (C_{tot}), total nitrogen (N), assimilable phosphorus (P), exchangeable potassium (K), Ca, Mg, Na and Fe. Additionally, concentrations of nitrates (NO_3^-), Al, Cr, Pb, Cu, and Si were analyzed to ensure compliance with regulatory limits (Commission Regulation EU 1258/2011 Amending Regulation (EC) No 1881/2006 as Regards Maximum Levels for Nitrates in Foodstuffs, 2011; Commission Regulation EU 2015/1005, 2015). Lettuce leaves were also analyzed to evaluate nutrient absorption from the substrate and to verify the absence of heavy metal accumulation or potentially toxic levels of nitrates or silicon. These analysis were conducted by the same certified external laboratory, using accredited methodologies. Nitrates, total carbon content, Al, Cr, Pb, Cu were extracted using acid digestion and quantified in accordance with UNI EN ISO 11885:2009. Silicon content was measured following alkaline digestion, in compliance with the same standard and based on the ISTISAN 1996/34 protocol (Istituto Superiore di sanità 1996). The organic carbon concentration was determined using the procedure outlined by APAT(APAT 2004).

2.4.6 Statistical Analysis

All the statistical analyses were carried out with R software, version 4.3.3. Data were first checked for normality and homoscedasticity. Differences among treatments and parameters were assessed using a non-parametric two-way ANOVA followed by post-hoc analyses. Pairwise comparisons were performed using Dunn's test with Bonferroni correction. Moreover, a multivariate analysis was performed to evaluate any possible correlation among chemical and agronomic parameters. Principal Component Analysis (PCA) was performed to reduce dataset dimensionality and visualize the multivariate structure of treatment responses. Trait loadings on the principal components were examined to identify the main variables contributing to group separation. For all statistical tests, differences were considered significant at $\alpha = 0.05$.

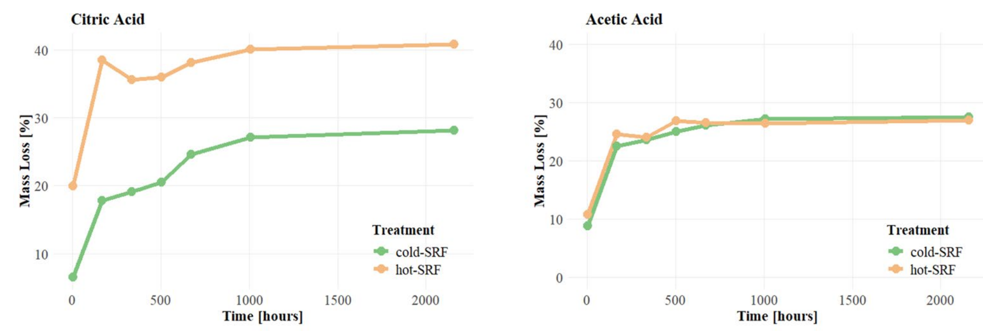
3 Results

3.1 SRFs Release Dynamics

The solubility in acetic and citric acids of the two SRFs formulations was compared, with slower solubility over time being desirable as an indicator of potential performance as a slow-release fertilizer (Fig. 2).

A marked difference in solubility between the two samples is shown in the citric acid solution (Fig. 2a), although the curve profiles are similar: a sharper mass loss takes place in the first 168 h, followed by a more gradual profile, reaching eventually approximately 40% mass loss for hot-SRF and <30% for cold-SRF. This is consistent with the composition of cold-SRF containing a more durable pumice fraction, and with the presence in the hot-SRF sample of a glass phase formed by silico-aluminate network that rapidly dissolves in organic acid such as citric acid (Lin et al. 2020). Cold-SRF mass loss remains largely unchanged in acetic acid (Fig. 2b), whereas the solubility of hot-SRF decreases, aligning with that of cold-SRF.

In this study, the analysis of NPK release was evaluated at defined intervals, both at short times (0.5 h and 24 h) and



(a) (b)

Fig. 2 Mass loss [%] of the two slow-release fertilizer (SRFs) types (cold and hot) in citric (**a**) and acetic (**b**) acid. Data were collected at time intervals of 0.5, 168, 336, 504, 672, 1008, and 2160 h

longer times until 90 days (specifically 168, 336, 504, 672, 1008 and 2160 h). Results shown in Fig. 3 were expressed in % in weight of nutrients (expressed as %P, %K and %N) released over time. The standard deviation, calculated on three independent replicates, was approximately 5% of the release values over time for P and K, and about 10% of the release values over time for N.

At 24 h, TNR was respectively 7.3% for both cold- and hot-SRF. While after 672 h (28 days), TNR reached 59.0% for cold-SRF and 55.9% for hot-SRF. At 2160 h (90 days) TNR was respectively 67.0% for cold-SRF, 59.4% for hot-SRF. Since European legislation (International Standards 2016) defines that a fertilizer can be considered slow release if TNR does not exceed 15% before 24 h and 75% before 672 h (28 days), we can consider our fertilizer systems as SRF. Furthermore, TNC parameter resulted in 17.7% and 22.1% for cold-SRF and hot-SRF, respectively.

Regarding the behavior of cold-SRF system, the release of N in citric acid (Fig. 3a) and acetic acid (Fig. 3b) differ not only in the total amount released but also in the release dynamics. For the cold-SRF system in acetic acid, most of the nitrogen is extracted within the first 0.5–168 h, with 12% of the total N released. By the end of the experiment, only 14% of N had been from cold-SRF sample, a fraction that coincides with its nitrate content. In citric acid (Fig. 3a), cold-SRF releases 75.9% of its total N at 90 days, well beyond the nitrate fraction, indicating that approximately 70% of the organic nitrogen pool is mobilized. Hot-SRF, however, releases less than 5% of its N under the same chelating conditions. Since the protein source is identical to that of cold-SRF, this behavior is likely attributable to the vitrified matrix.

The release of P in citric acid (Fig. 3c) exhibits similar trends for both SRFs, characterized by a major release in the first after 168 h (consistent with mass loss data) and reaching values of approximately 80% by the end of the experiment. The trend is maintained by cold-SRF in acetic acid (Fig. 3d) exhibiting a slightly smaller release (approximately 70% by the end of the experiment), while hot-SRF release does not exceed 30% at the end of the 90 days.

K release appeared to be mostly independent of the extractant solution, exhibiting consistently higher values for hot-SRF, 100% in citric acid (Fig. 3e) and 90% in acetic acid (Fig. 3f) approximately respect to cold-SRF (approximately 60% in both extraction media). This trend correlates better with mass loss in citric acid (Fig. 2a), since part of the potassium in cold-SRF is part of the durable pumice structure and is not mobilized in the experiment conditions. In both samples the trend seems to be characterized by a sharper release profile in the first 168 h, in accordance with the major mass loss from the samples registered at that time, followed by a more gradual release up to 90 days. pH trends (Fig. 4) document a higher increase in time for the sample hot-SRF in both citric (Fig. 4a) and acetic acid (Fig. 4b) respect to cold-SRF: the higher increment of pH is due to fast partial solubilization of glass matrix, which occurs thanks to the ion exchange between H_3O^+ ion present in the solution with mono-positive alkaline ions (K^+ and Na^+) present in the glass matrix (McGrail et al. 2001).

3.2 Plant Growth test

The plant growth results are reported in Fig. 5 and show clear treatment-dependent differences in lettuce growth and biomass allocation after the 28-day controlled growth trial. The cold-SRF treatment generally promoted the highest aboveground growth, showing significantly greater aboveground fresh weight (AGB FW) and shoot fresh weight than the control and hot-SRF treatments. Cold-SRF also resulted in the greatest plant height and leaf area, indicating a stronger stimulation of shoot development and canopy expansion. For these parameters, cold-SRF was often assigned to a different statistical group, confirming a significant improvement compared with the other treatments. In terms of above-ground growth, cold-SRF significantly improved lettuce shoot development compared with both CNTR and hot-SRF. Plants treated with cold-SRF showed the highest above-ground fresh biomass, with AGB FW reaching 7.6 ± 1.6 g, compared with 3.7 ± 1.3 g in CNTR plants and lower values in hot-SRF plants. Similarly, single

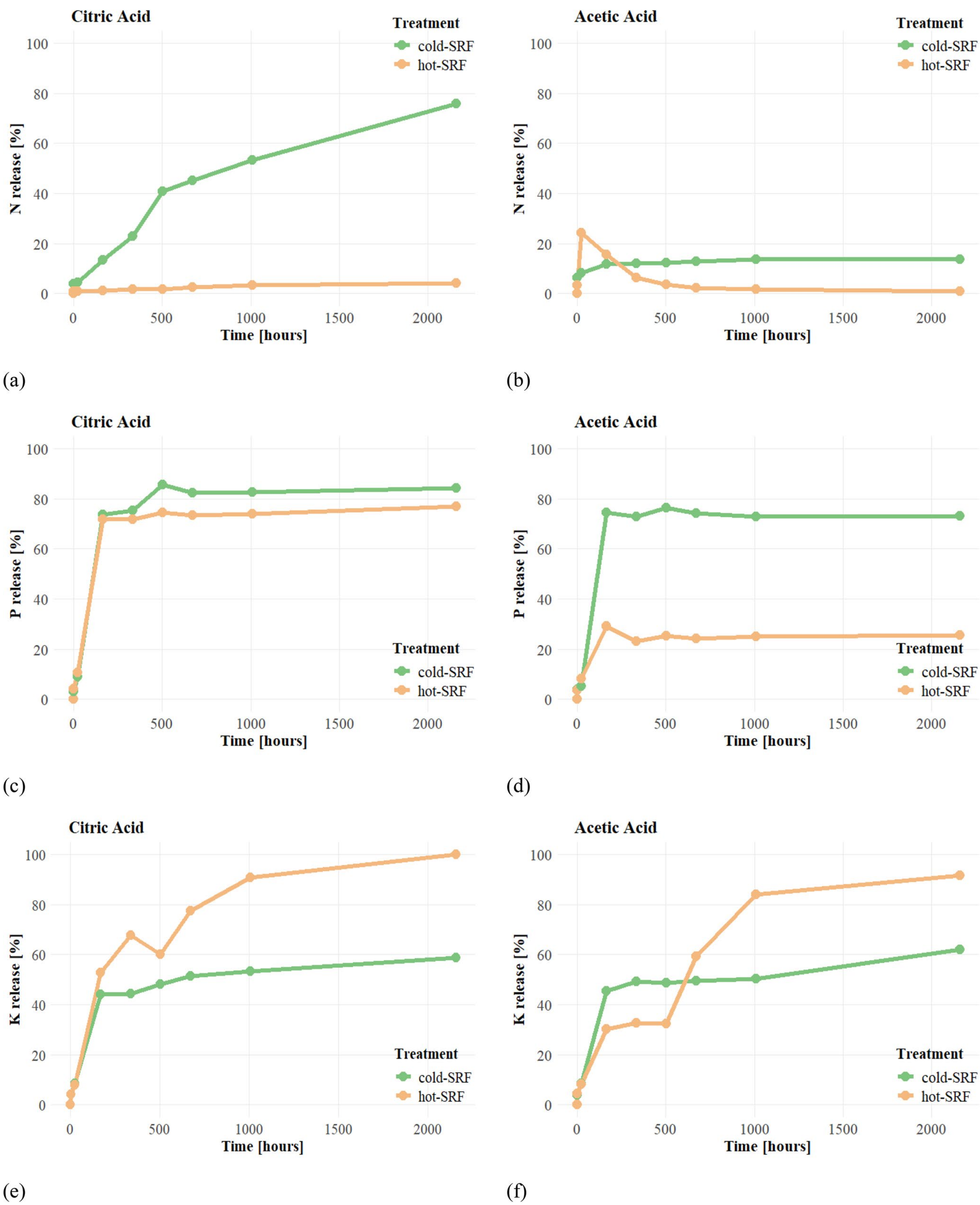


Fig. 3 NPK releases [%] of the two slow-release fertilizer types (cold-SRF and hot-SRF) in citric acid (a, c, e, respectively) and acetic acid (b, d, f, respectively). Data were collected at time intervals of 0.5, 24, 168, 336, 504, 672, 1008, and 2160 h

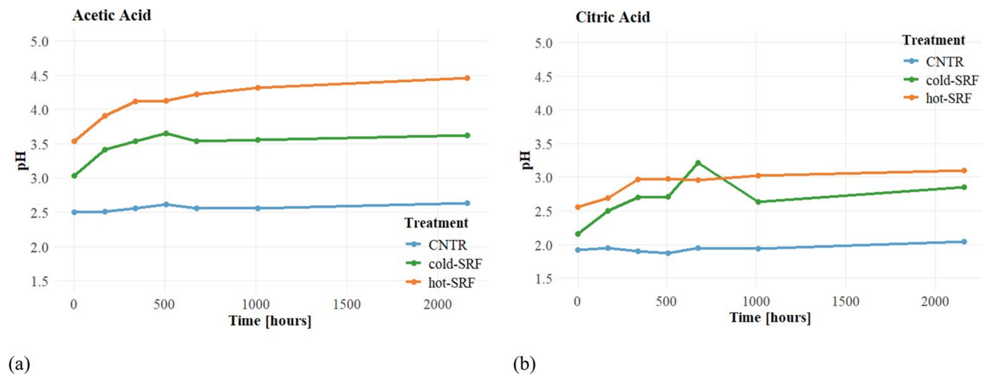


Fig. 4 pH values of extractant solutions using citric (**a**) and acetic (**b**) acids on the two slow-release fertilizers (cold-SRF, hot-SRF) and CNTR (here referred as "Control", represented by blank solution) samples at different soaking times

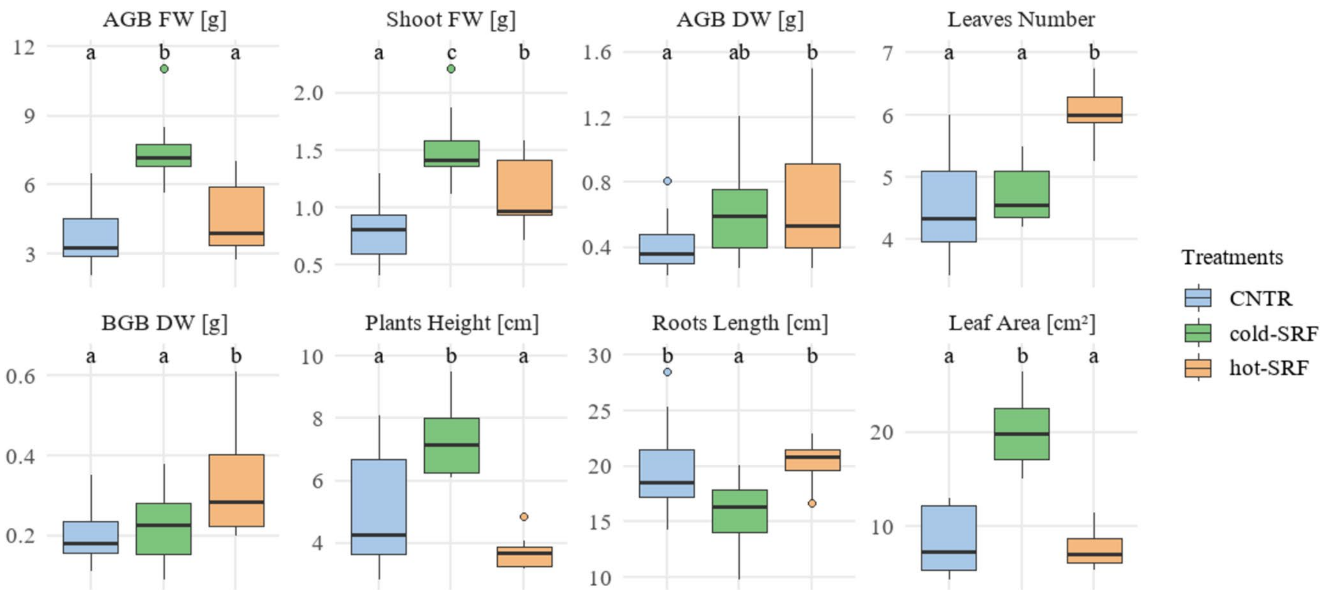


Fig. 5 Boxplot of plant growth parameters on the three experimental treatments: slow-release fertilizer types (cold-SRF, hot-SRF) and CNTR (unfertilized control substrate): Total Above-Ground Biomass Fresh Weight (ABG FW), Above-Ground Biomass Dry Weight (AGB DW), mean single shoot fresh weight (Shoot FW), total Below Ground Biomass Dry Weight (BGB DW), Plants Height, Roots Length, Leaf Area and Leaves Number per plant Data were collected on each plant grown on 4 pots per treatment, for 2 repeated trials ($n=8$ per treatment). Different letters indicate significant differences among treatments: ($p<0.05$)

shoot fresh weight increased from 0.9 ± 0.3 g in CNTR to 1.5 ± 0.3 g under cold-SRF treatment. Shoot FW was also significantly higher in hot-SRF plants than in the control, reaching 1.1 ± 0.3 g. These differences were statistically significant ($p<0.05$), indicating a clear positive effect of SRF application, particularly cold-SRF, on shoot biomass production. Plant height followed a similar pattern. Cold-SRF plants reached the greatest height (7.3 ± 1.8 cm), significantly exceeding both CNTR (5.1 ± 1.8 cm) and hot-SRF plants (3.7 ± 0.7 cm; $p<0.05$). Leaf area further confirmed the strong stimulation of shoot growth under cold-SRF, with a mean leaf surface of 20.1 ± 4.0 cm², compared with 8.5 ± 3.7 cm² in control plants. This indicates that cold-SRF primarily promoted leaf expansion and above-ground biomass accumulation. By contrast, hot-SRF induced a different growth response, mainly associated with leaf number and below-ground development. Plants grown with hot-SRF produced, on average, a higher number of leaves than both CNTR and cold-SRF plants, although their individual leaf area remained smaller ($p<0.05$). Moreover, below-ground biomass dry weight was higher in hot-SRF plants, reaching 0.3 ± 0.1 g, compared with 0.2 ± 0.1 g in both CNTR and cold-SRF plants. Root length was also greater in hot-SRF (20.2 ± 2.3 cm) and CNTR plants (19.5 ± 3.7 cm) than in cold-SRF plants (15.8 ± 3.2 cm), suggesting a stronger investment in root development under hot-SRF and control conditions.

The results indicate that the two SRF formulations affected lettuce growth differently. Cold-SRF mainly enhanced above-ground growth, increasing fresh biomass, shoot weight, plant height, and leaf area, whereas hot-SRF promoted a more compact growth pattern, characterized by a higher number of leaves and greater below-ground biomass. This suggests that differences in SRF processing influenced

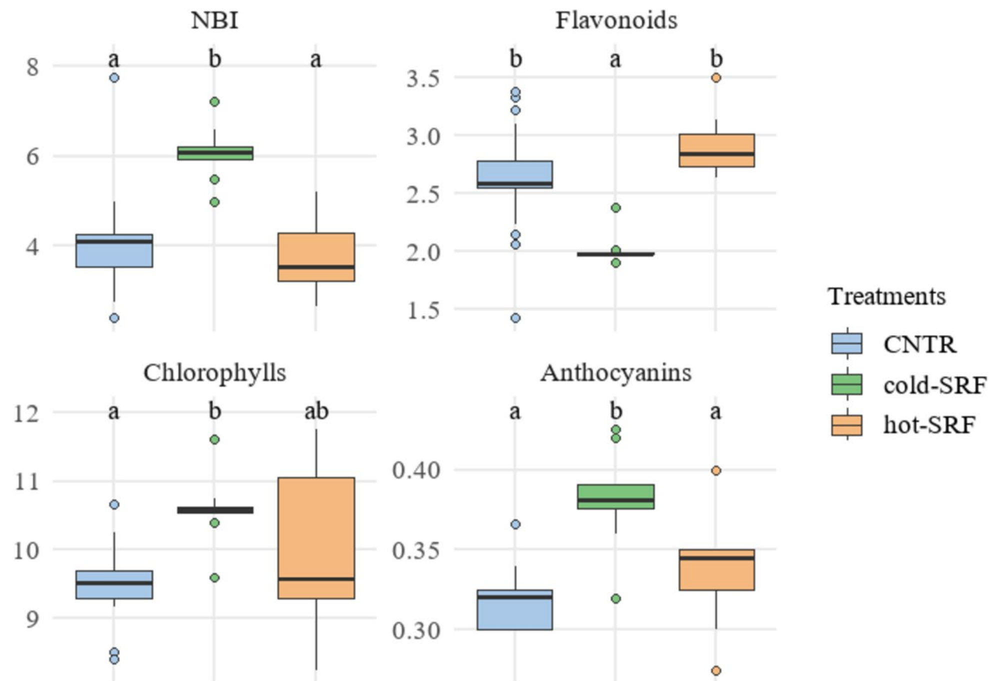


Fig. 6 Boxplot showing Dualex values (Nitrogen Balance Index -NBI-, Flavonoids, Chlorophylls and Anthocyanins) measured on leaves from two slow-release fertilizer types (cold-SRF, hot-SRF) and CNTR (unfertilized control substrate). Results are the means of data collected on 3 leaves per sample, on 4 samples per treatment, for 2 repeated tests ($n=12$ per treatment). Different letters indicate significant differences among treatments ($p<0.05$)

Table 2 Chemical content of *Lactuca sativa* leaves at harvest time. The values are the means (±SD) of the results obtained from 4 samples per treatment, for 2 repeated tests ± standard deviation ($n=8$ per treatment). Different letters indicate significant differences ($p<0.05$) among the treatments (slow-release fertilizer types: cold-SRF, hot-SRF and unfertilized control substrate referred as CNTR)

Treatments	C_{tot} [%]	NO_3^- [mg kg^{-1}]	Al [mg kg^{-1}]	Cu [mg kg^{-1}]	Pb [mg kg^{-1}]	Cr [mg kg^{-1}]
CNTR	4.28 ± 2.27 a	<2.00 a	1.80 ± 0.80 a	0.57 ± 0.46 a	<0.02 a	0.20 ± 0.30 a
cold-SRF	3.03 ± 1.38 a	<2.00 a	0.84 ± 0.41 ab	0.19 ± 0.23 a	<0.02 a	0.10 ± 0.00 a
hot-SRF	3.88 ± 0.58 a	<2.00 a	0.80 ± 0.30 b	0.30 ± 0.40 a	<0.02 a	0.10 ± 0.00 a

nutrient availability and plant allocation patterns during the 28-day controlled growth trial. Conversely, a significant difference was detected for the fertilization treatment.

Physiological indices measured on lettuce leaves using the Dualex sensor, including chlorophylls, anthocyanins, flavonols and Nitrogen Balance Index (NBI) are reported in Fig. 6. Significant differences among treatments were observed for alla parameters: NBI was significantly higher ($p<0.05$) in leaves from plants treated with cold-SRF (6.10 ± 1.00) compared to both control (4.14 ± 1.37) and hot-SRF (3.86 ± 0.76), which did not differ from each other ($p>0.05$). Since NBI is calculated as the ratio between chlorophyll and flavonoid contents, this increase suggests an improved nitrogen nutritional status in cold-SRF plants. Flavonoid levels were significantly lower in cold-SRF leaves (1.97 ± 0.05) compared to those treated with both control and hot-SRF (2.95 ± 0.28) ($p<0.05$), which showed similar values. Chlorophyll content was significantly higher in cold-SRF plants than in the control ($p<0.05$) while hot-SRF showed intermediate values and did not differ significantly from either treatment ($p>0.05$). Anthocyanin content was also significantly higher under cold-SRF compared with both control and hot-SRF ($p<0.05$), while no significant difference was observed between control and hot-SRF ($p<0.05$). These findings suggest that plants treated with cold-SRF enhanced nitrogen availability and/or uptake efficiency, as reflected by the higher NBI and chlorophyll values. These results suggest moreover changes in secondary metabolism for plants grown with cold-SRF, while the treatment hot-SRF showed a response generally comparable to the control for all physiological parameters.

Table 2 reports the concentrations of selected chemical elements in lettuce leaves under different treatments. No significant differences among treatments ($p>0.05$), were observed on the most parameters measured: total carbon content (C_{tot}), nitrates (NO_3^-), Cu, Pb, Cr. Nitrates amounts are always below to the legislation's threshold: <5000 mg kg^{-1} of fresh biomass, as defined by European Regulation n° 1258/2011 (Commission Regulation EU 1258/2011 Amending Regulation (EC) No 1881/2006 as Regards Maximum Levels for Nitrates in Foodstuffs, 2011).

Table 3 (sections *a*) reports the chemical properties of substrates analyzed before and after lettuce cultivation, following 28 days of growth under different treatments. The chemical analyses showed that the addition of the two SRF formulations affected substrate nutrient availability, with

Table 3 Chemical content of substrates before growth and at harvest time. Section (a) with the values of : total organic carbon (C_{tot}), total nitrogen (N), concentrations of nitrates (NO_3^-), Cu, Cr; Section (b) assimilable phosphorus (P), exchangeable potassium (K), Ca, Mg, Na. The values are the means (±SD) of the results obtained from 4 samples per treatment, for 2 repeated tests±standard deviation (n=8 per treatment). Different letters indicate significant differences (p<0.05) among the treatments (slow-release fertilizer types: cold-SRF, hot-SRF and unfertilized control substrate named as CNTR)

Treatments	C_{tot} [%]	N [%]	NO_3^- [mg kg^{-1}]	Cu [mg kg^{-1}]	Pb [mg kg^{-1}]	Cr [mg kg^{-1}]
Section a)						
Substrate before growth	10.32±0.62 ab	0.28±0.06 a	8.42±1.19 a	4.28±0.46 a	2.06±0.05 a	148±42 a
CNTR	16.22±2.84 a	0.29±0.05 a	3.42±2.31 a	6.20±2.28 a	2.48±0.78 a	137±97 a
cold-SRF	13.05±3.12 ab	0.32±0.07 a	9.63±3.82 b	6.56±3.48 a	2.49±0.80 a	136±10 a
Section b)						
Treatments	P [mg kg^{-1}]	K [mEq kg^{-1}]	Ca [mEq kg^{-1}]	Mg [mEq kg^{-1}]	Na [mEq kg^{-1}]	Si [mg kg^{-1}]
Substrate before growth	61.40±26.01 a	1.91±0.92 a	26.92±3.36 a	3.21±0.62 a	3.04±0.34 ab	553±154 a
CNTR	40.83±17.84 a	0.9 2±0.63 a	36.37±5.18 a	4.32±0.72 a	2.36±0.46 a	499±190 a
cold-SRF	42.75±18.70 a	0.73±0.32 a	38.52±16.15 a	4.23±0.92 a	2.74±0.67 ab	474±185 a
hot-SRF	97.43±11.82 a	1.43±0.74 a	32.00±16.11 a	3.91±0.67 a	3.16±0.59 b	296±83 a

more evident changes for nitrogen and phosphorus. After growth test (Table 3, section *b*), hot-SRF substrate exhibited a significantly higher total nitrogen (N%) content (p<0.05) compared to both cold-SRF and control substrates, as well as compared with the initial substrate before plant growth (p<0.05). Total carbon content significantly decreased in the hot-SRF treatment compared with the control substrate (p<0.05), while similar values were observed between the hot-SRF and cold-SRF treatments. Both SRFs treatments resulted in higher NO_3^- concentrations than controls, which in turn displayed lower NO_3^- levels compared to baseline substrate prior to cultivation. P availability also differed among treatments. The substrate amended with hot-SRF showed a significantly higher concentration of assimilable P than both the control and cold-SRF substrates (p<0.05), suggesting a greater release or retention of available P in this treatment after plant growth. By contrast, exchangeable K levels remained relatively low across all treatments, with values below 2.0 mg kg^{-1} and no significant differences among treatments (p>0.05). No significant differences were observed among treatments for Pb and Cr content (p>0.05). Similarly, Na, Ca, Mg, Si content did not show remarkable differences among treatments or compared with the initial substrate (p>0.05).

Data from two experiments were merged and analyzed together for PCA and descriptive statistics, following an additive approach (Arteaga et al. 2019; Fred Pyrczak, 2016). The outputs of PCA were reported in Fig. 7 (a, b) and illustrate the distribution of the three treatments according to multiple plant growth, physiological, and chemical parameters. The first two principal components, PC1 and PC2, explained 53.4% of the total variance, indicating that the PCA captured a relevant proportion of the variability within the dataset. The multivariate analysis revealed a clear separation among treatments (Fig. 7a). Samples treated with cold-SRF appear distinctly separated from hot-SRF mainly along PC2. This separation was primarily associated with shoot-related growth traits, including fresh above-ground biomass, single shoot biomass, and plants height, which show a strong contribution to PC2 (Fig. 7b). Hot-SRF samples were more widely distributed along PC1, suggesting that this treatment was associated with a different set of variables and showed greater variability in plant response. Hot-SRF was mainly related to substrate nitrate content, assimilable P content, and root dry biomass. This distribution may reflect the more complex nutrient-release dynamics of the hot-SRF formulation, which could have influenced below-ground growth and substrate nutrient availability differently among replicates. The control group clusters closely, indicating a more homogeneous and less dynamic nutrient environment, with limited variance in plant response. The dispersion may also reflect a higher variability in plant response, due to the formulation's complex release dynamics.

4 Discussion

The present study was designed to compare two complete NPK slow-release fertilizer systems produced from the recovered waste-based raw materials, as animal blood, animal bone meal, and pumice scraps, but processed through two different consolidation strategies. The underlying hypothesis was that the cold and hot processing routes would modify nutrient speciation and physical organization within the fertilizer matrix, thereby influencing both nutrient-release dynamics and plant response. The results support this hypothesis, showing that cold-SRF and hot-SRF behaved differently in terms of nutrient solubility, substrate chemistry, and lettuce growth allocation. Both formulations fulfilled the criteria for slow-release fertilizer classification

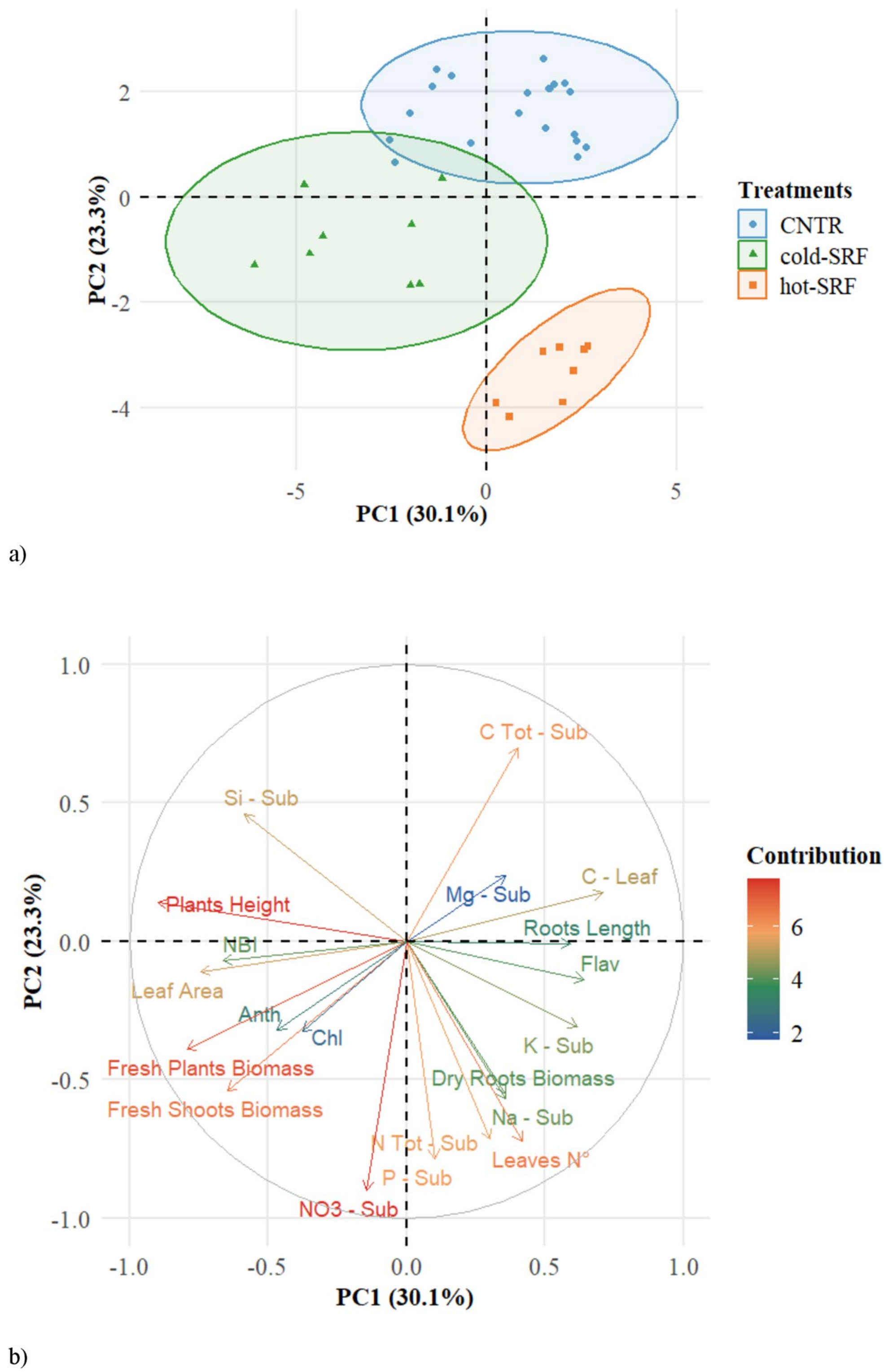


Fig. 7 Principal Component Analysis (PCA) plot of agronomic and chemical (on leaves and on substrates, these lasts indicated with "*Sub*") parameters of 2 repeated experimental tests ($n=8$ per treatment): (**a**) clusters of three different thesis: slow-release fertilizer: cold-SRF, hot-SRF, and unfertilized control substrate, named as CNTR. (**b**) Plot showing the relationships among variables on PC1 (30.10%) and PC2 (23.33%). Arrow colors represent the relative contribution of each variable to the principal components, with warmer colors indicating higher contributions and cooler colors lower contributions. Arrows direction indicate the sign and pattern of association with the principal components: variables pointing in the same direction are positively correlated, those pointing in opposite directions are negatively correlated, and approximately perpendicular arrows indicate weak or no correlation

(USDA Natural Resources Conservation Service, 2014) under the tested conditions, as their total nutrient release in citric acid remained below 15% after 24 h and below 75% after 28 days. This confirms that both systems can be considered slow-release fertilizers and demonstrates the potential of agro-industrial waste as valuable secondary raw materials for producing functional fertilizer systems (Neethu CB & Vardhanan YS, 2023). By aligning nutrient availability with plant uptake, particularly during early growth stages, SRFs minimize fertilizer waste runoff (Shanmugavel et al.

2023). When produced from agricultural or industrial waste, they also contribute to resource recovery (Fyi 2023; Priya et al. 2024). However, their nutrient-specific release behavior differed markedly, reflecting the different chemical forms in which N, P, and K were incorporated. In cold-SRF, P was mainly present as hydroxyapatite from bone meal, K was supplied partly as soluble KNO_3, and N was mainly provided by organic animal blood. In hot-SRF, P and K were incorporated into a glass-ceramic matrix, while N was supplied by animal blood added after vitrification. Therefore, the two fertilizers had a comparable overall NPK composition, but substantially different nutrient speciation and physical organization.

The solubility profiles obtained in citric and acetic acid solutions provided important insights into the expected behavior of the two formulations under simulated soil and potentially acidic environmental conditions. In cold-SRF, N and K showed a progressive release trend, suggesting that total nutrient release may continue beyond 90 days. In contrast, hot-SRF shows markedly different behavior: N is not released at all, and P reaches a plateau at around 75%, while K is the only nutrient to achieve a complete release, reaching 100% by day 90th. Consequently, the overall TNR for hot-SRF is unlikely to exceed 60%, even with extended incubation, mainly due to the limited N release and the partial immobilization of P within the glassy matrix. The near absence of N release from hot-SRF represents the most distinctive divergence between the two formulations. Previous studies on animal-derived organic fertilizers indicate that blood meal and related protein-rich materials are generally characterized by relatively rapid N mineralization, with a mean of organic nitrogen release of about 50% within 150 days (Sradnick and Feller 2020). Likewise, protein-fortified waste-derived fertilizers have been shown to exhibit delayed, but still measurable, nitrogen availability, with ammonium release occurring after 72 to 168 h (Olbrycht et al. 2020; Smith & Hadley, 1989). In the present formulation, however, the co-localization of the organic nitrogen source and the vitrified phase within the same granule may have limited N mobility. A possible explanation could be the interaction between the glass and blood meal; proteins are known to absorb onto silica surfaces depending on pH and net charge. In acetic acid, the pH of the sample containing hot-SRF is between 3.5 and 4.5, and silanol groups of silica should be partially deprotonated (the zero-point charge of silica is 2.5-3) (Cloarec et al. 2016), resulting in an overall negative surface charge. Since the system of pH is below the isoelectric points of many blood-derived (De et al. 2009), proteins are likely positively charged and therefore prone to electrostatic binding with the silica surface in hot-SRF, reducing their solubility. This effect should be weaker in citric acid (pH 2–3), where the silica surface should be closer to neutral. Dissolution and re-precipitation of silica under acidic conditions, which could create a silica-gel (Olgenblum et al. 2024), can also play a role in reducing proteins mobility, since proteins may become physically entrapped in this silica matrix (Gorrepati et al. 2010). Such encapsulation is intentionally exploited to protect proteins from degradation (Besanger and Brennan 2006; Olgenblum et al. 2024; Potnis et al. 2023), and this could also potentially explain the lack of N uptake from hot-SRF in substrate due to limited bioavailable nitrogen formation. This interpretation is further supported by “ensilication” experiments showing that proteins encapsulation in a silica is attributable to both physical encapsulation and electrostatic interactions (Chen et al. 2017). In addition, studies on bioactive glasses show that even when protein encapsulation is not the intended goal, proteins are nevertheless found embedded within the hydrated silica surface-reaction layer that forms upon glass–solution interaction (Barberi et al. 2023). While no prior fertilizer-specific literature has directly characterized N release in a system closely matching the present hot-SRF formulation, or directly demonstrated the mechanism proposed here, the observations reported in this study—together with the direct comparison with cold-SRF—support this interpretation. To our knowledge, the closest waste-derived NPK glass-containing systems was reported so far by (Barbi et al. 2020), which mainly describe retention effects for P and K whereas they do not show an equally strong suppression of N release. However, in that system, the nitrogen-rich biomass-derived fraction was applied as an external coating that protected the glass-containing core, and N was reported to be released rapidly from this coating. By contrast, in the present hot-SRF formulation the organic N source and the vitrified phase are co-localized within the same granule and exposed simultaneously to the extraction medium, making direct interactions between the organic fraction and the hydrated glass surface. A different behavior was observed for cold-SRF: in citric acid, N was released progressively over the 90-day extraction period; in acetic acid, instead, only a fraction of N—likely attributable to KNO_3—was released during the first 7 days. This difference is probably related to the strong chelating ability of citric acid, which can complex Al^{3+} released from pumice and Ca^{2+} released from hydroxyapatite. By binding these ions, citric acid may prevent them from cross-linking organic nitrogen compounds and inducing precipitation. Acetic acid has a much weaker chelating capacity. Under these conditions, Al^{3+} released from pumice may promote the coagulation of blood-derived proteins, thereby reducing their solubility (Chanphai et al. 2017a, b; Zhu et al. 1990). These findings are consistent with other studies where blood-meal fertilizers have demonstrated that in the presence of calcium, strong aggregation occurs, reducing solubility (Yunta et al. 2013), while citric acid can dissolve the metal bridges in soil organic matter increasing N availability (Jilling et al. 2018).

Phosphorous release follows different patterns depending on whether the phosphate source is crystalline (hydroxyapatite, cold-SRF) or incorporated in a glass network (hot-SRF). In citric acid, both formulations release most of their phosphorous, with hydroxyapatite dissolution at pH ~ 2 mainly driven by proton attack. The chelating ability of citrate provides an additional, ligand-promoted contribution by complexing Ca^{2+} at the crystal surface and in solution, shifting the dissolution equilibrium. For hot-SRF, citric acid promotes glass dissolution through both proton exchange and chelation of network-modifying and forming cations, accelerating the breakdown of the silicate network. The high mass loss (~40% at 168 h) confirms extensive matrix dissolution. In acetic acid, a non-chelating medium, the two formulations diverge sharply: the absence of chelation only marginally reduces the final extent of P release from HA, while in the absence of chelation, the glass dissolution stalls. Accordingly, mass loss for hot-SRF drops from ~40% in citric acid to ~25% in acetic acid, a value comparable to cold-SRF, suggesting that without chelation only superficial glass alteration occurs. As P is incorporated in the glass as a network former, the dissolution of the structure is a necessary step for its release (Berezicka et al. 2025).

Potassium is the only macronutrient released in higher amounts from hot-SRF in both extraction media, with a multi-stage release profile characterized by distinct kinetic steps rather than a linear trend. The release proceeds through a rapid initial phase, likely driven by surface ion exchange, followed by progressively slower rates related to less accessible portion of the glass. In acetic acid, the released amount decreases only slightly, in contrast to the pronounced drop observed for phosphorus, despite both elements being supplied by the same glass phase. This difference stems from the distinct structural roles of the two elements within the glass: K is not a network former and is therefore mobilized easily through ion exchange mechanism, without requiring complete solubilization of the silicate network. In cold-SRF, the readily mobile K fraction is supplied primarily by KNO_3, while the portion originating from pumice is not mobile and determines the lower cumulative release observed.

The differences in nutrient-release behavior were reflected in substrate chemistry and plant performance. Cold-SRF promoted the highest above-ground biomass, single-shoot fresh weight, plant height, and leaf area, indicating that this formulation provided a nutrient availability pattern better synchronized with the short growth cycle and high nutrient demand of baby leaf lettuce. The higher Nitrogen Balance Index recorded in cold-SRF plants further supports this interpretation, suggesting improved N nutritional status and greater photosynthetic potential. In this treatment, the combined availability of N, P, and K likely supported rapid shoot development, with no need for plants to strongly invest in root expansion.

Hot-SRF induced a different plant response. Although this treatment showed higher residual total nitrogen, nitrate, and assimilable phosphorus in the substrate after cultivation, plants grown with hot-SRF did not develop comparable shoot biomass. This suggests that nutrient presence in the substrate did not necessarily correspond to effective nutrient uptake or use during the 28-day crop cycle. Recent research (Napolitano et al. 2025) has shown that hot-SRF requires more than 28 days—the duration of the growth test—for nitrification processes to occur. This delay likely explains the reduced leaf biomass production observed in plants treated with hot-SRF, whereas cold-SRF promotes earlier nitrification, making nitrogen available to plants sooner (Jones et al. 2005). Delayed N mobilization, together with partial P retention within the glass-derived matrix, may have limited shoot development during early growth. At the same time, plants grown on hot-SRF substrate showed higher root dry weight and root length, indicating a shift in biomass allocation toward the below-ground compartment. This response may represent a compensatory strategy under delayed or limited availability of readily usable nutrients, particularly nitrogen, allowing plants to increase the absorptive surface and explore the substrate more effectively (Hermans et al. 2006). The higher residual assimilable P detected in hot-SRF substrates may also be linked to the greater root development observed in this treatment. Indeed, previous research (Ohyama & Sueyoshi, 2010) indicated as P is essential for energy transfer, root growth; therefore, its gradual release from the glass-ceramic matrix may have contributed to root elongation and biomass accumulation. However, the persistence of higher P levels in the substrate after cultivation suggests that P release and plant uptake were not fully synchronized during the short lettuce cycle. Similarly, although K release from hot-SRF was high in-vitro, it did not translate into superior above-ground growth.

Moreover, physiological data of leaves are consistent with this interpretation. Cold-SRF plants showed higher NBI values, indicating better nitrogen status, whereas hot-SRF and control plants showed higher flavonoid accumulation. Since flavonoids are involved in antioxidant protection and stress responses, their significant decrease in cold-SRF plants may indicate an improved nutritional status during the early growth stages. This interpretation is further supported by the chlorophyll data, as cold-SRF plants showed significantly higher chlorophyll levels than control plants, suggesting a greater photosynthetic capacity and a more efficient nitrogen assimilation, given the close relationship between leaf nitrogen and chlorophyll biosynthesis. In contrast, hot-SRF plants showed intermediate chlorophyll values, with greater variability, indicating that nutrient availability may have been less synchronized with plant demand (Karami et al. 2022).

The multivariate analysis further confirmed the divergent behavior of the two formulations. Cold-SRF samples were mainly associated with shoot-related traits, such as above-ground biomass, shoot fresh weight, plant height, and leaf area. Hot-SRF samples were more closely related to substrate nitrate content, assimilable phosphorus, and root biomass. This separation indicates that the two SRFs did not simply differ in overall fertilizer efficiency, but induced distinct chemical, physiological, and agronomic responses.

From a food-safety perspective, both formulations showed promising results. Chemical analyses of lettuce leaves indicated that nitrate and heavy metal concentrations remained below the limits established by European regulations for safe consumption (Commission Regulation EU 1258/2011 Amending Regulation (EC) No 1881/2006 as Regards Maximum Levels for Nitrates in Foodstuffs, 2011). Pb values were always below 0.1 mg kg^{-1} fresh weight (Commission Regulation EU 2015/1005, 2015), and nitrate concentrations never exceeded 5000 mg kg^{-1} fresh weight (Commission Regulation EU 1258/2011 Amending Regulation (EC) No 1881/2006 as Regards Maximum Levels for Nitrates in Foodstuffs, 2011). These values were comparable with those reported in previous studies on *Lactuca sativa* grown with other SRF systems, suggesting that neither cold-SRF nor hot-SRF caused unsafe accumulation of undesirable elements in the edible tissues under the tested conditions (Sgarbi et al. 2023).

In summary, cold-SRF showed a more balanced and progressive nutrient release, better synchronized with the short growth cycle of baby leaf lettuce, resulting in greater shoot growth, higher leaf area, and improved N nutritional status. Hot-SRF, instead, acted as a more delayed-release system, with high K mobility but strong N immobilization and partial P retention within the glass-derived matrix. This reduced short-term shoot development and promoted greater root allocation, likely as a response to delayed nutrient availability. Cold-SRF appears more suitable for short-cycle leafy crops, whereas hot-SRF may be more appropriate for longer-term applications where prolonged nutrient retention and reduced early nutrient release are desirable. Although cold-SRF and hot-SRF were produced from the same recovered raw materials and designed as complete NPK fertilizers, their different nutrient speciation and physical structure resulted in distinct release mechanisms and plant responses. The study demonstrates that the processing strategy of SRFs could represent a key factor controlling the functional behavior of waste-derived SRFs.

5 Conclusions

This study demonstrates that waste-derived raw materials such as animal blood, bone meal, and mineral porous fillers can be successfully integrated into granular slow-release fertilizer systems (SRFs) through different processing strategies. The comparison between cold-SRF and hot-SRF highlights that the production procedure is not only a technological variable, but a key factor controlling the agronomic function of the final fertilizer.

Rather than identifying one formulation as universally superior, the results indicate that the two SRF systems may be suitable for different agronomic contexts. Cold-SRF formulation appears more appropriate for short-cycle crops or cultivation systems requiring earlier nutrient availability and rapid above-ground biomass production. In contrast, the hot-SRF formulation, characterized by slower nutrient mobilization and stronger interaction between the organic and vitrified phases, may be more suitable for longer crop cycles, situations where prolonged nutrient retention is desirable, or applications in which gradual nutrient release and root system development are relevant targets. From an application perspective, both formulations represent promising examples of circular-economy fertilizers, as they combine nutrient recovery from waste materials with controlled-release behaviour and potential soil-conditioning functions. Their use could therefore contribute to reducing dependence on conventional mineral fertilizers while valorizing residues from animal and mineral-based waste streams.

Future research should evaluate these systems under longer cultivation periods, real soil conditions, and different crop species to better define their most appropriate agronomic uses and to assess their long-term performance at field scale.

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Data Availability The data that supports the findings of this study is available from the corresponding author, upon reasonable request.

Declarations

Conflict of Interest The authors declare no conflicts of interest.

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