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LCA FOR AGRICULTURE

# Environmental impact of rocoto chili pepper production: integrated assessment of yield, bioactive composition, and environmental footprint

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## Abstract

**Purpose** Conventional Life Cycle Assessment (LCA) studies of agricultural systems frequently utilize mass-based functional units, neglecting the consideration of product quality integration. This study aims to address this gap by integrating agronomic productivity, bioactive quality (pungency), and environmental footprint into a unified LCA framework for Rocoto chili cultivation. The study will employ primary high-fidelity experimental data for the LCA to ensure attributable comparisons between eight different cultivation scenarios.

**Methods** An attributional LCA was conducted between scenarios in accordance with ISO 14040-44 standards. A $2^3$ factorial design was employed to evaluate eight scenarios, combining low-tech greenhouse (LGT) /open-field cultivation, normal/stress irrigation, and biochar/conventional fertilization. The system boundaries followed a cradle-to-gate approach, encompassing seedling production through harvest. The primary inventory data were derived from a one-year controlled field experiment. The environmental impacts were assessed in SimaPro, with a functional unit based on a pungency of 70,000 SHU. The yield was measured at the time of harvest. The quantification of capsaicinoid content was performed via high-performance liquid chromatography (HPLC).

**Results and discussion** The findings of this study demonstrate that conventional LTG cultivation with standard irrigation techniques achieved the highest yield, measuring 22.19 tons per hectare, but a weaker pungency of 69,457 SHU. In contrast, the organic LTG system with biochar and conventional irrigation methods yielded fruits with the highest pungency (125,601 SHU) and a substantial yield of 16.80 tons per hectare. This system also exhibited the lowest environmental impact in five of six assessed categories, including climate change (0.092 kg $CO_2$ eq/FU). The utilization of biochar in these systems has been demonstrated to result in a consistent reduction of environmental loads. Conversely, open-field conditions have demonstrated a tendency to yield suboptimal outcomes, exhibiting reduced yields and pungency results when compared to LTG systems.

**Conclusions** This study establishes that the environmental sustainability of Rocoto cultivation is governed by system choice and nutrient management. The organic LTG system with biochar emerged as the best strategy, balancing high quality with reduced environmental footprint. Crucially, employing a pungency-based functional unit redefined environmental efficiency, demonstrating that prioritizing product quality transforms Life Cycle Assessments. This integrated approach provides a transformative framework for high-value crop production.

**Keywords** Capsicum pubescens · Biochar · Bioactive compounds · Life cycle assessment · Sustainable agriculture · Water stress · Low-tech greenhouse cultivation

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## 1 Introduction

The genus Capsicum spp. is native to the tropics and after salt is the second most consumed spice in the world, whether fresh or as an ingredient in dehydrated and processed foods (Duranova et al. 2022). The global pepper harvest in 2023 from 3.9 million hectares was about 44 million tons. China is the world's leading producer followed by Mexico. In Colombia, the area under cultivation in 2024 was approximately 7,860 hectares, with a total production of 101,937 tons (FAO 2025). There are five of domesticated capsicum species (*C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum* and *C. pubescens*) cultivated primarily for human consumption (Tripodi and Kumar 2019). Rocoto (*C. pubescens)*, is one of the most consumed varieties due to its high nutritional content and wide use in gastronomy (Hernández-Amasifuen et al. 2021). Moreover, demonstrates optimal growth at temperate climates, rendering multiple regions of Colombia with considerable market potential for cultivation of this specie.

Colombia is a tropical country with strong climatic variability and characterized by medium and small-holders, so low- and medium-technology greenhouses predominate. The design and technological level of greenhouses exhibit significant variation on a global scale, influenced by regional climate conditions, investment capacity, and production objectives. These systems are generally distinguished by their simplicity, characterized by structures that are uncomplicated, plastic covers, passive ventilation, and limited or no automated environmental control (Marín-Garcia et al. 2023). The primary function of these structures is to provide physical protection against excessive radiation, precipitation, and wind, while enabling passive heat retention during cooler periods. (Villagrán et al. 2022). In this study low-tech greenhouse (LTG) systems were used.

*C. pubescens* is distinguished by its notable content of capsaicinoids and phenolic compounds, which bestow upon it bioactive properties and therapeutic activities that are of considerable interest to the cosmetic and pharmaceutical industries (Wang et al. 2025). Agroclimatic conditions and cultivation factors, including water availability, light exposure, and the different stressors, can regulate bioactives metabolic pathways and accumulation (Kopecká et al. 2023). Numerous studies on bell pepper (*Capsicum annuum*) have documented the effects of various agricultural practices—including greenhouse cultivation (Ahmad et al. 2025), water stress irrigation (Zamljen et al. 2020; Mahmood et al. 2021), organic fertilization (Ribes-Moya et al. 2018), and disease exposure (Ahmad et al. 2024) —on phytochemical responses (Yang et al. 2024), particularly the accumulation of secondary metabolites, as well as on agronomic yield. Conversely, research conducted on the rocoto variety has been restricted to its bioactive composition quantification (Meckelmann et al. 2015), with no evaluation of how diverse agricultural practices affect its phytochemical profile or yield performance.

Life cycle assessment (LCA) is a robust tool widely used to assess the environmental sustainability of agricultural systems (Yi et al. 2023). However, comparative studies published often present a lack of conclusive results due to the absence of homogeneous conditions between evaluated systems (Meier et al. 2015). Regarding published LCA studies on chili peppers, no such assessment has yet been conducted for the rocoto variety. However, several studies have evaluated the environmental impacts of bell pepper systems under diverse cultivation conditions. These studies indicate that efficient nutrient management in organic fertilization systems leads to lower environmental impacts (Wang et al. 2018, 2020), whereas greenhouse-based systems exhibit high environmental burdens associated with infrastructure construction (Chatzisymeon et al. 2017; García García and García García 2022).

All these analyses employ mass-based functional units (FUs) (e.g., 1 kg or 1 tonne). The FU quantifies the function of the system and must be appropriately defined to provide a common basis for comparing results (Kopton et al. 2026). Mass-based FUs are the most widely used in the LCA literature for foods with different functional properties—appearing in nine out of ten studies (Fernández-Ríos et al. 2022). However, these units can lead to unfair comparisons, particularly in systems where both productivity and functional quality traits vary simultaneously due to external factors, which is precisely the case in the present study. In some cases, studies have adopted FUs based on the amount of food required to deliver a specific quantity of protein (McAuliffe et al. 2023), vitamin C (Svanes and Johnsen 2019), or Nutritional Density Units (Pérez et al. 2024) to address an specific daily dietary intake. These approaches offer a clearer perspective on environmental impact relative to the efficiency with which a system performs a defined function. Nevertheless, significant limitations persist regarding the ease of application of such units, particularly when comparing systems with pronounced compositional differences or when attempting to ensure cross-regional comparability of results.

Controlled, homogeneous comparisons of organic and conventional agricultural systems are necessary to assess their relative impacts in agronomic and environmental performance. Further research is also required on sustainable practices, such as cultivation under LTG structures and water stress management. Moreover, comparison framework that is both fair and accurate must consider not only productivity but also product bioactive composition (e.g. capsaicinoids), which enhance the nutritional and commercial value of products. This approach highlights the potential of agricultural strategies to modulate fruit quality attributes, including pungency levels, while simultaneously integrating environmental impact considerations.

Therefore, the objective of this study was to evaluate the influence of diverse agronomic practices on the yield, bioactive composition, and environmental footprint of *Capsicum pubescens* cultivation. To move beyond conventional mass-based assessments, this work integrates agronomic and biochemical quality parameters with a life cycle perspective. A key innovative aspect is the use of a pungency-based functional unit, which reframes environmental efficiency in terms of product quality. This integrated approach aims to provide a transformative framework for assessing and improving the sustainability of high-value crop production systems.

## 2 Materials and methods

### 2.1 Experimental plot and crop yield

Consecutive Rocoto pepper (*Capsicum pubescens*) crops were produced in an experimental field between October 2023 and December 2024. The experimental plot for was established at the facilities of the Institute of Biotechnology and Agroindustry (IBA) at the National University of Colombia in Manizales, Caldas, Colombia. The site is located at an altitude of 2150 m above sea level, with average temperatures ranging from 14 °C to 22 °C, a latitude of 5.028°N, and a longitude of -75.47°W. Temperature and rainfall conditions were monitored throughout the evaluation period using a local meteorological station (Meteostat 2024), and these data were used to calculate the crop's water requirements.

An experimental design was set up with eight treatments following a factorial design with three variables on two levels ($2^3$) in a field plot arrangement with three replicates. The factors evaluated included cultivation method (LGT – open field), irrigation regime (normal – stress), and fertilization type (conventional – biochar). The cultivation conditions scenarios were coded as shown in Table 1.

Table 1 Experimental design and coding of Rocoto pepper cultivation conditions

| Scenario code | Crop | Irrigation | Fertilization |
|---|---|---|---|
| 1 | LTG | Normal Irrigation | Biochar |
| 2 | LTG | Normal Irrigation | Conventional |
| 3 | LTG | Stress Irrigation | Biochar |
| 4 | LTG | Stress Irrigation | Conventional |
| 5 | Open Field | Normal Irrigation | Biochar |
| 6 | Open Field | Normal Irrigation | Conventional |
| 7 | Open Field | Stress Irrigation | Biochar |
| 8 | Open Field | Stress Irrigation | Conventional |

Each experimental unit consisted of four rows, with each row measuring 4.0 m in length and 2.5 m in width. Seedlings with a height of 25 centimeters were transplanted into the field, with a spacing of 50 centimeters between plants, resulting in a plant density of (2 plants/m$^2$). Three experimental units were subsequently grouped, each sharing the same cultivation conditions.

A fertilization plan was proposed for each cultivation scenario based on a soil analysis. For the scenarios with conventional fertilization, Solufos ©, MAP, Ammonia Sulfate, Potassium nitrate and Agrimins © fertilizers were applied monthly depending on the phenological stage of the crop according with the fertilization plan (Supplementary information Table A2). For the scenarios with organic fertilization, biochar was utilized. The biochar was prepared by mixing crushed vegetable charcoal (12.6 kg) with pig manure leachate (43.7 kg). This mixture was allowed to rest for at least 24 h before application in the field. Biochar improves soil structure, increases water retention capacity, and enriches the soil with essential micro and macronutrients, promoting healthier and more sustainable plant growth. The macronutrient composition of the biochar (70.31% carbon, 2.95% nitrogen, 21.79% phosphorus, and 4.93% potassium) was assumed based on values reported by (Adebayo 2025).

Irrigation scheduling was guided by soil moisture monitoring using a portable resistive soil moisture meter, which provides a relative-dimensionless moisture index ranging from 1 (dry) to 10 (wet). The device operates based on changes in electrical resistance between electrodes and therefore does not measure soil water potential. For conventional irrigation treatments, the irrigation process was initiated when the soil moisture index decreased to values of 8 or below. For the water-stress treatments, irrigation was delayed until the index reached values between 3 and 4, inducing controlled water deficit conditions. A drip irrigation system was utilized to ensure the provision of water at consistent intervals. Moreover, this system functioned as a nutrient delivery mechanism. Conventional irrigation was administered three times daily for 15 min, while stress irrigation followed the same duration and frequency but was limited to three days per week. This approach ensured consistent differentiation between irrigation regimes while maintaining comparable application protocols across plots.

During productive stage of the crop, ten plants were selected for each experimental unit and the total number of flowers and fruits per plant were documented. The first harvest was determined when 90% of the fruits changed color from green to red. Subsequent harvesting occurred every 15 days for eight months. After the collection of these data, the yield for each scenario was calculated using Eq. (1). In this equation, $PY$ represents the chili yield (tons/hectare), $TWHP$ is the total harvested weight per plant (tons/plant), and 2000 corresponds to the plant density (plants/ha).

$$PY = TWHP * 2000 \tag{1}$$

## 2.2 Chili biochemical characterization

### 2.2.1 Sample preparation

Ripe chili peppers from each experimental unit by scenario were manually harvested, grouped and stored, ensuring triplicate samples for each treatment. Marketable defect-free fruits were subsequently selected and washed with running water at a rate of 0.75 L/kg, followed by a disinfection with a 100 ppm sodium hypochlorite solution, maintaining a solid-to-liquid ratio of 4:3 (w/w). Afterwards, fruits were blanched in a thermostatic bath at 80 °C for one minute and then immediately cooled in a water bath at 4 °C for five minutes (Castro et al. 2008). The seeds and stems were manually removed, and the fruit was pulped using a blender until a homogeneous paste was obtained (20,000 rpm) (Oster, Bogotá, Colombia).

The pulp was freeze-dried in a Virtis Genesis SQ XL-70 freeze-dryer (Virtis, Gardiner, NY, USA). The freezing phase was carried out at a controlled rate of 0.75 °C min$^{-1}$ until reaching −40 °C. During the primary drying phase, the temperature was gradually increased from −40 °C to 40 °C. In the secondary drying, the temperature was maintained at 40 °C with a pressure of 0.75 mbar. The end point of the drying process was determined by stabilizing the pressure readings from the Pirani and capacitive sensors. After the drying process, the dehydrated powder was vacuum-sealed in polyethylene/nylon metalized bags and stored at 4 °C for further analysis.

### 2.2.2 Ultrasonic extraction

The extractions were conducted according to the methodologies outlined by Castro-Concha et al. (2014) and Murillo-Franco et al. (2023), with slight modifications. Powdered samples (0.150 ± 0.001 g) were combined with 1 ml of methanol in a conical flask. The flask was immersed in an ultrasonic bath operating at 37 kHz with a power output of 240 W for two hours. The temperature was kept close to ambient conditions (22 ± 2 °C) by periodically replacing some of the bath water with fresh cold water. After extraction, the samples were centrifuged at 13,500 rpm for 20 min. The supernatant was then collected and stored in an amber glass jar at 4 °C in the dark for further analysis as extracts.

### 2.2.3 Total phenolic content (TPC), flavonoid content and antioxidant activity

Total phenolic content of the extract was measured by triplicate using the Folin–Ciocalteu colorimetric method. In this method, 15 µL of the extract was mixed with 240 µL of distilled water, 15 µL of Folin–Ciocalteu reagent (1 N), and 30 µL of sodium carbonate (20% w/v). After incubating in the dark for 2 h, the absorbance was measured at 765 nm using a Spectramax Abs Plus microplate reader. The results were expressed as milligrams of gallic acid equivalents per 100 g of dry sample (Singleton et al. 1999).

Flavonoid content was quantified using a colorimetric assay based on the method described by Meneses et al. (2013). In brief, 30 µL of the sample was combined with 90 µL of methanol, 5 µL of aluminum chloride (10% w/v), 5 µL of potassium acetate (1 mol/L), and 170 µL of distilled water in a 96-well microplate. The mixtures were incubated in the dark at room temperature for 30 min, and absorbance was measured at 415 nm. The results were expressed as quercetin equivalents in milligrams per 100 g of dry sample.

Antioxidant activity was assessed using the ABTS+· radical cation decolorization assay, as described by Ozgen et al. (2006) with minor modifications. The assays were conducted by mixing 10 µL of the sample with 231 µL of ABTS+· solution. The mixtures were incubated in the dark for 30 min, and absorbance was measured at 734 nm using a microplate reader. The results were expressed as Trolox equivalents in millimoles per 100 g of dry sample. Calibration curves were generated for each method and sample results adjusted with distilled water serving as the control for each method.

### 2.2.4 Capsaicin (CC), dihydrocapsaicin (DC) and pungency

Capsaicin (CC) and dihydrocapsaicin (DC) contents were determined using high-performance liquid chromatography (HPLC) on a chromatograph (LC-2010, Shimadzu) equipped with a C-18 Kromasil column and a UV detector. The chromatographic method described by Othman et al. (2011) was employed with some modifications. The column temperature was set at 60 °C, and the UV detector was set to a wavelength of 222 nm. The sample temperature was maintained at 20 °C, with an injection volume of 10 µL. The mobile phase consisted of a binary mixture of water and acetonitrile at a volume ratio of 50:50, with a flow rate of 1 mL/min. Concentrations of CC and DC were expressed as parts per million (µg/mL) using a dose-response calibration curve. Total measured capsaicinoids content was calculated using Eq. (2):

$$W = \frac{V}{m} \times \frac{(C_1 + C_2)}{0.9} \quad (2)$$

Where $W$ is the total amount capsaicinoids (mg/ 100 g dry weight), $C_1$ and $C_2$ are the amounts of measured capsaicin and dihydrocapsaicin respectively (µg/mL), $V$ is the volume of the sample extract (mL) and $m$ is the dry mass of the test material (mg). The pungency of chili peppers was measured in Scoville Heat Units (SHU) were calculated multiplying the total amount of measured capsaicinoids by 161 (161 SHU equivalent to 1 mg of capsaicin or dihydrocapsaicin per 100g dry basis) (Meckelmann et al. 2015).

## 2.3 Life cycle assessment

The life cycle assessment (LCA) was conducted in accordance with the ISO 14040-44 standard, following an attributional approach. This LCA encompasses all phases of the life cycle, including the definition of the goal and scope, the life cycle inventory (LCI), the life cycle impact assessment (LCIA), and the interpretation of results, as established by ISO 14040-44 (ISO 14040 2006; ISO-14044 2006).

### 2.3.1 Goal and scope

The goal of this study was to evaluate the influence of key agronomic practices under controlled small-plot conditions —cultivation system (low-tech greenhouse vs. open field), fertilization (organic biochar vs. conventional), and irrigation (normal vs. water stress)—on the yield, bioactive pungency, and environmental footprint of *Capsicum pubescens* (Rocoto).

To achieve this, a comparative, attributional LCA was conducted to quantify and compare the environmental impacts between eight production scenarios, according to ISO 14,044 standards. The analysis employs a cradle-to-gate system boundary, encompassing input materials (seeds, fertilizers, biochar, infrastructure materials), on-farm operations (seedling production, soil conditioning, transplanting, fertilization, irrigation, pest management, and harvest), and use of capital goods (protective structure, irrigation system) over an estimated lifespan of 20 years. The system boundary explicitly excludes post-harvest processing, distribution, consumer use, and end-of-life waste management, as the study focuses on agricultural production efficiency.

Pungency is the key attribute for the commercial and functional quality of peppers, where a level of 70,000 SHU represents a high degree of pungency for rocoto peppers (Duranova et al. 2022). Consequently, the Functional Unit (FU) was defined as the mass of fresh Rocoto fruit required to yield a total pungency of 70,000 SHU. The mass corresponding to 1 FU for each cultivation scenario was calculated using Eq. (3), which effectively normalizes all scenarios outputs to an equivalent pungency value, allowing for a direct comparison of the environmental impact per unit of quality-adjusted yield. In this equation, $MUF_i$ (reference flows) is the corresponding mass to 1 FU for each cultivation scenario $\mathrm{i}$ (kg), $\mathrm{P_i}$ is the average pungency of the fruit obtained for scenario $\mathrm{i}$ (SHU) and $\mathrm{M}$ is the basis flow mass used for calculations (kg). – taken as 1 kg for this study.

$$MUF_i = \frac{70{,}000\, SHU}{\mathrm{P_i}} \times \mathrm{M} \quad (3)$$

The objective is to ascertain the most sustainable and efficient practices that optimize production, quality, and reduce environmental impact. This equation remains valid even for those scenarios where measured pungency is below the 70,000 SHU benchmark. In such cases, it is necessary to use a larger mass per FU in order to meet the same potency standard. This logically and quantitatively reflects the lower bioactive efficiency of that scenario, which is then reflected in its environmental impact per FU. These results offer pertinent information for farmers, producers, the commercial sector, and policymakers. By promoting responsible agricultural practices, the study aim is to enhance the competitiveness of Rocoto peppers in the market. A sensitivity analysis on the FU was conducted, evaluating three distinct pungency levels: 50,000 SHU, 70,000 SHU (baseline), and 100,000 SHU. Table 2 shows the reference flows calculated for the eight scenarios at these three different pungency levels.

**Table 2** Calculated pungency (per kg) of each scenario and reference flows calculated at three different pungency levels

| Scenario | Scenario pungency [SHU] | Reference Flow at different pungency level [kg] | | |
|---|---|---|---|---|
| | | 50,000 [SHU] | 70,000 [SHU] | 100,000 [SHU] |
| 1 | 125,601 | 0.398 | 0.557 | 0.796 |
| 2 | 69,457 | 0.720 | 1.008 | 1.440 |
| 3 | 84,304 | 0.593 | 0.830 | 1.186 |
| 4 | 40,607 | 1.231 | 1.724 | 2.463 |
| 5 | 70,384 | 0.710 | 0.995 | 1.421 |
| 6 | 69,698 | 0.717 | 1.004 | 1.435 |
| 7 | 53,866 | 0.928 | 1.300 | 1.856 |
| 8 | 48,037 | 1.041 | 1.457 | 2.082 |

### 2.3.2 System boundaries

The limits of the system for this study are defined from cradle-to-gate, covering all stages of the production process of the Rocoto chili bell pepper. The system boundaries explicitly exclude upstream process detailed parent plant cultivation for seed production and transportation seedlings to the experimental crop, along with all post-harvest activities as transportation of the harvested fruit, processing, packaging, distribution, consumer use, and end-of-life waste management. This delineation facilitates a comprehensive evaluation of the environmental performance associated with each stage of the agricultural cycle. The selection of these limits is driven by the necessity to accurately identify the environmental impacts generated exclusively during primary production, thereby facilitating an objective comparison of the various cultivation scenarios. To this end, a life-cycle conscious analysis is performed, encompassing all relevant inputs and outputs within the agricultural system up to the point of harvest.

As demonstrated in Fig. 1, these limits and the eight sensitivity scenarios evaluated are illustrated graphically. The eight sensitivity scenarios evaluated combine variations in the type of fertilization, irrigation system, and growing conditions (LTG or open field). These factors significantly influence the environmental performance of fresh chili peppers. A thorough delineation of the agricultural process implemented in this study can be found in Sect. 2.1.

### 2.3.3 Life cycle inventory (LCI)

This section details the life cycle inventory (LCI) data sources, assumptions, and primary data for the core unit processes. The foreground system was constructed using primary data collected over a one-year controlled experimental cycle. The authors provided the data using 1 kg of harvested chili as reference flow for inventory construction in each scenario, subsequently, the reference flow was redefined for all scenarios based on pungency for Life Cycle Impact Assessment. The inventory flows were categorized as reference flow, resources, by-products, waste to treatment, and direct emissions to air, water or soil. All material and energy inputs were normalized per plant and subsequently scaled by scenario-specific yield per plant. The numerical values presented in inventory tables are represented with multiple digits to ensure model reproducibility and transparency; they are not intended to imply significance. Intermediate transportation between co-located production stages was excluded from the system boundaries.

Foreground processes were modeled as follows:

- Seedling production included germination trays (polypropylene), hypochlorite disinfectant, peat substrate, and *Melaleuca alternifolia* fertilizer, with energy and water consumption estimated from primary producer data. Land preparation inputs—including lime, glyphosate, water, and Trichoderma inoculum—were allocated per plant and scaled to the reference flow.
- Foliar fertilizers, insecticides, and fungicides were applied via manual sprayer (no energy consumption); total

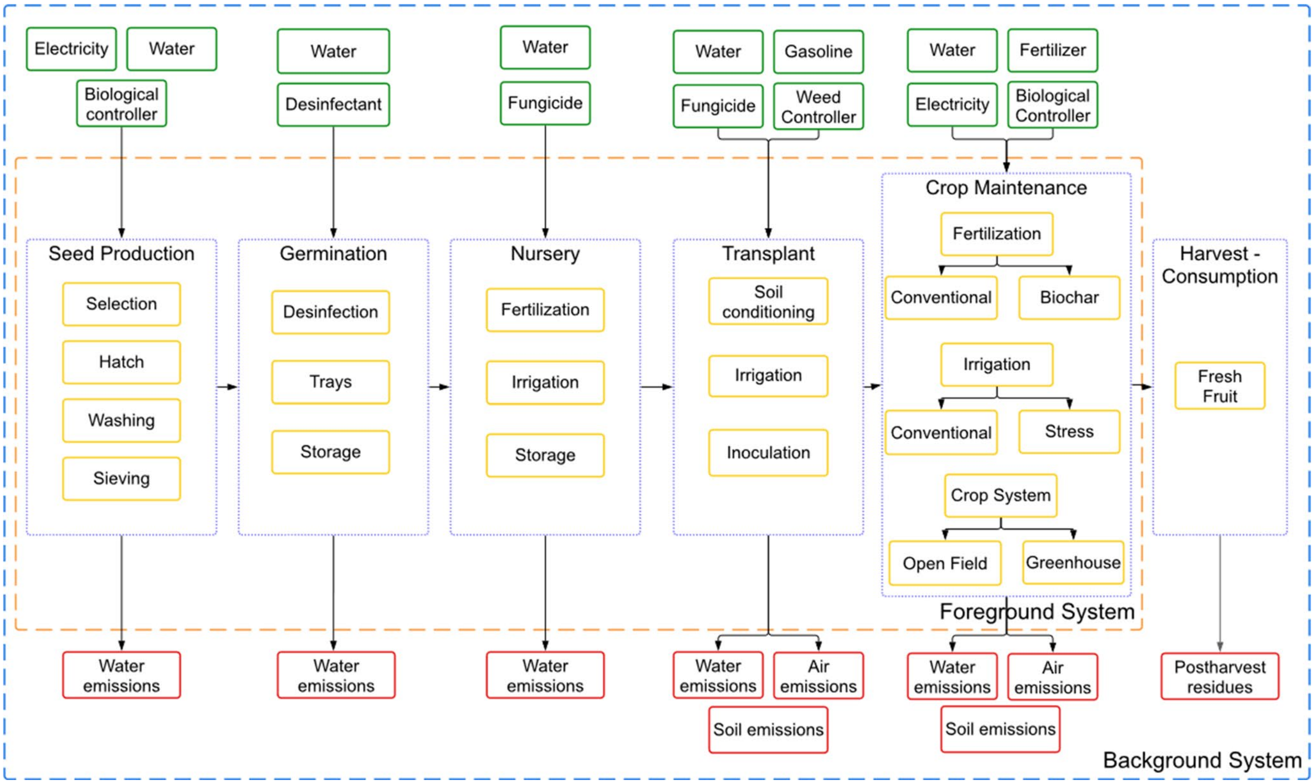

**Fig. 1** System boundaries considered in the LCA for the chili production. Each scenario considers their own inputs and outputs

application was aggregated from dissolution volumes and dosages, then normalized by plant count and yield. Indirect emissions to air, water, and soil from agrochemical use were estimated using linear emission factors (Rodríguez et al. 2020) based on NPK composition derived from technical data sheets.

- Conventional fertilization followed a monthly schedule tailored to phenological stages, with total fertilizer consumption per plant aggregated over the cycle and scaled to yield. Biochar (prepared from vegetable charcoal and pig manure leachate) was modeled using a cut-off approach in accordance with ISO 14,044, entering the system with zero upstream burden. Its fertilizer contribution was modeled based on assumed macronutrient composition (C: 70.31%, N: 2.95%, P: 21.79%, K: 4.93%) from Adebayo (2025), and carbon sequestration was accounted for as negative biogenic $CO_2$ emissions over a 100-year horizon following Adhikari et al. (2024).

Background processes, which encompasses fertilizer and pesticide production, agricultural machinery use, and electricity consumption, reliable secondary sources were used. Specifically, data from the Ecoinvent v.3.8 database was utilized. This database is widely recognized for its role in LCA studies within the agricultural sector. Given the absence of Colombia-specific background inventories in the databases applied, processes were modeled using the Rest of World (RoW) and Global (GLO) geographical schemes, as appropriate. Background datasets were implemented following the default unit process allocation (Alloc Def, U) in accordance with the attributional LCA approach adopted in this study. Whenever available, "*market for*" datasets were used to represent average supply conditions; in cases where these datasets were not available, "*production*" datasets were applied as a methodological approximation.

The integration of primary and secondary data facilitated the construction of a comprehensive and representative inventory of the input and output flows associated with each crop scenario evaluated. An illustrative summary Life Cycle Inventory (LCI), presenting the main input and output flows for Scenarios 1 and 8, is shown in Table 3. The fully documented processes, detailed calculations, underlying assumptions, and the complete LCI table for all eight scenarios are available in the Supplementary Material. LCI data presented in Table A7 includes the infrastructure for the LTG.

#### 2.3.4 Life cycle impact assessment

For the present study, a Life Cycle Assessment was conducted using SimaPro software version 9.3.0.0 (Pre-Sustainability, The Netherlands). To obtain a more comprehensive and substantial perspective on the environmental impact associated with the cultivation of Rocoto peppers, a dual approach was employed, encompassing both midpoint and endpoint assessments. This methodological strategy enabled the analysis of intermediate impacts and their eventual consequences on human health, ecosystems, and resources.

The ReCiPe 2016 methodology (Hierarchist perspective) was employed to assess the environmental impact. It is one of the most updated and widely used methodologies for agricultural LCA studies (Huijbregts et al. 2017). While all 18 midpoint impact categories were calculated, the results and discussion focus on the six categories that exhibited the highest impacts and the most significant variation across the studied scenarios (climate change, human toxicity, metal depletion, agricultural land occupation, water depletion, and fossil depletion). In contrast, the endpoint approach considered all three categories (Human Health, Ecosystems, and Resources).

The findings on protective enclosure systems (LTG) apply specifically to high-altitude regions with mild climates and low-tech management. They should not be extrapolated to energy-intensive, controlled-environment agriculture, which would require a full-chain analysis encompassing industrialized infrastructure.

### 2.4 Statistical analysis

For the characterization assays, each sample was prepared in triplicate and results are expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way unpaired ANOVA, followed by Tukey's multiple comparison test to identify significant differences among treatments. Differences were considered statistically significant at $p \leq 0.05$.

## 3 Results and discussion

### 3.1 Chili yield and bioactive concentration

It is well-established that abiotic stress factors—such as extreme temperatures, drought, nutrient deficiency in fertilization—typically induce metabolic changes in plants, leading to reduced photosynthetic activity, growth rates and productivity (Kopecká et al. 2023). In response to abiotic stress, plants activate complex metabolic and signaling pathways in order to ensure their own survival. Such conditions of stress have been shown to disrupt normal photosynthetic and respiratory processes (Krasensky and Jonak 2012). plants initiate stress pathways involving stomatal closure, increased reactive oxygen species (ROS) production, and

**Table 3** Illustrative life cycle inventory for scenarios 1 and 8 farming data sources to obtain 1 kg of fresh Rocoto chili pepper fruit and emission factors. Inventory data were normalized to the pungency-based functional unit for impact assessment

| | Unit | Scen. 1 | Scen. 8 | Emission Factor (kgCO2eq/Unit) | Reference |
|---|---|---|---|---|---|
| **Formation of chili seedlings** | | | | | |
| Substrate | kg/kg chili | 7.26E-03 | 9.80E-03 | 0.01 | (Nicese et al. 2024) |
| Calcium hypochlorite | kg/kg chili | 1.19E-07 | 1.61E-07 | 0.01 | Calculated (Calc) |
| Polypropylene | kg/kg chili | 5.71E-02 | 7.72E-02 | 0.50 | (Nicese et al. 2024) |
| Nebulizer water | kg/kg chili | 8.69E-08 | 1.17E-07 | | Calc |
| *Melaleuca alternifolia* | kg/kg chili | 4.76E-10 | 6.43E-10 | 0.20 | Calc |
| **Soil adaptation and transplantation** | | | | | |
| Land use | $m^2$/kg/year | 5.95E-01 | 8.04E-01 | | Calc |
| Lime | kg/kg chili | 2.45E-02 | 3.31E-02 | 0.46 | (Eggleston et al. 2006) |
| Water | kg/kg chili | 1.49E-04 | 2.01E-04 | | Calc |
| *Trichoderma* | kg/kg chili | 7.14E-03 | 9.65E-03 | | Calc |
| Organic fertilizer | kg/kg chili | 1.19E-01 | 1.61E-01 | 0.001 | (Graefe et al. 2013) |
| Water for dissolution | kg/kg chili | 1.01E+00 | 7.02E-03 | | Calc |
| **Leaf fertilizer** | | | | | |
| Nitrogen fertilizer | kg/kg chili | 6.11E-03 | 8.25E-03 | 3.97 | (de Jesus Pereira et al. 2021) |
| Phosphate fertilizer | kg/kg chili | 3.71E-03 | 5.00E-03 | 1.13 | (de Jesus Pereira et al. 2021) |
| Potassium fertilizer | kg/kg chili | 1.92E-03 | 2.59E-03 | 0.71 | (de Jesus Pereira et al. 2021) |
| Calcium fertilizer | kg/kg chili | 8.40E-04 | 1.13E-03 | 0.03 | (de Jesus Pereira et al. 2021) |
| Magnesium fertilizer | kg/kg chili | 6.31E-04 | 8.53E-04 | 0.01 | (de Jesus Pereira et al. 2021) |
| **Root fertilizer** | | | | | |
| Biochar + pig manure | kg/kg chili | 8.25E-01 | | 0.001 | (Graefe et al. 2013) |
| Nitrogen fertilizer | kg/kg chili | | 1.36E-01 | 3.97 | (de Jesus Pereira et al. 2021) |
| Phosphate fertilizer | kg/kg chili | | 2.33E-02 | 1.13 | (de Jesus Pereira et al. 2021) |
| Potassium fertilizer | kg/kg chili | | 6.86E-02 | 0.71 | (de Jesus Pereira et al. 2021) |
| Calcium fertilizer | kg/kg chili | | 4.08E-03 | 0.03 | (de Jesus Pereira et al. 2021) |
| Magnesium fertilizer | kg/kg chili | | 1.36E-03 | 0.01 | (de Jesus Pereira et al. 2021) |
| **Pest and disease control** | | | | | |
| **Insecticides** | | | | | |
| Imidacloprid (30.2%) | kg/kg chili | 1.84E-06 | 2.49E-06 | 4.42 | (Ecoinvent Association 2021) |
| Azadiractina (1%) | kg/kg chili | 4.71E-06 | 6.36E-06 | 5.75 | (Ecoinvent Association 2021) |
| Ggarlic extract (100%) | kg/kg chili | 5.46E-09 | 7.37E-09 | 2.10 | (Ecoinvent Association 2021) |
| Spirodiclofen (24.4%) | kg/kg chili | 1.24E-07 | 1.67E-07 | 6.00 | (Ecoinvent Association 2021) |
| **Fungicides** | | | | | |
| Ethylene-bis-dithiocarbamate (75%) | kg/kg chili | 2.03E-06 | 2.75E-06 | 0.75 | (Ecoinvent Association 2021) |
| Prothioconazole (25%) | kg/kg chili | 2.23E-07 | 3.01E-07 | 0.75 | (Ecoinvent Association 2021) |
| **Co-helpers** | | | | | |
| Ethoxylated lauryl alcohol (15%) | kg/kg chili | 8.35E-07 | 1.13E-06 | 2.50 | (Ecoinvent Association 2021) |

**Table 3** (continued)

| | Unit | Scen. 1 | Scen. 8 | Emission Factor (kgCO2eq/Unit) | Reference |
|---|---|---|---|---|---|
| **Energy** | | | | | |
| Energy | kwh/kg chili | 1.52E-02 | 8.20E-03 | 0.11 | (UPME 2023) |
| **Irrigation** | | | | | |
| Irrigation | $m^3$/kg chili | 3.54E-01 | 1.91E-01 | | Calc |
| **$CO_2$ Capture** | | | | | |
| Photosynthetic rate | kg/kg chili | 2.07E-02 | 1.98E-02 | | (Sui et al. 2006) |
| Applied biochar* | kg/kg chili | 8.81E-01 | | | (Eggleston et al. 2006; Woolf et al. 2010; Lehmann and Joseph 2015) |
| **Low-tech greenhouse structure** | | | | | |
| Plastic | kg/kg chili | 2.06E-04 | | | Calc |
| Wood | kg/kg chili | 2.48E-05 | | | Calc |
| Metalic elements (screws, nuts, washers, wire and turnbuckles) | kg/kg chili | 1.44E-04 | | | Calc |
| **Irrigation system construction** | | | | | |
| Hose | kg/kg chili | 1.74E-04 | 2.34E-04 | | Calc |
| Irrigation drippers | kg/kg chili | 2.38E-04 | 3.22E-04 | | Calc |
| Valves | kg/kg chili | 6.45E-06 | 8.71E-06 | | Calc |
| Other elements (T-joint, elbow, rubber saddle, connector, male adapter, nail with head, guadua cans, staples, harvester's rope) | kg/kg chili | 2.86E-04 | 3.87E-04 | | Calc |
| **Outputs** | | | | | |
| Ammonia (air) | kg/kg chili | 3.12E-03 | 4.59E-03 | | (Rodríguez et al. 2020) |
| Phosphorous (water) | kg/kg chili | 4.82E-07 | 1.82E-05 | | (Rodríguez et al. 2020) |
| Potassium (water) | kg/kg chili | 6.59E-07 | 1.22E-04 | | (Rodríguez et al. 2020) |
| Calcium (water) | kg/kg chili | 2.74E-07 | 8.50E-06 | | (Rodríguez et al. 2020) |
| Magnesium (water) | kg/kg chili | 6.31E-09 | 1.09E-07 | | (Rodríguez et al. 2020) |
| Carbon dioxide (air) | kg/kg chili | 4.26E-02 | 5.51E-02 | | Calc |
| Postharvest residues organic waste | kg/kg chili | 4.63E-01 | 4.63E-01 | | Calc |

* Modeled as waste with zero upstream burden (cut-off approach)

respiratory adjustments to mitigate oxidative damage. Consequently, plant respiration undergoes a shift to counteract the accumulation of ROS, and energy is redirected toward stress-responsive pathways (Noctor et al. 2018). This results in the induction of secondary metabolite production including phenolics, terpenoids, alkaloids, and capsaicinoids that mitigate oxidative damage and protect against herbivory (Isah 2019). Therefore, plants may demonstrate a decline in productivity while concurrently augmenting the synthesis or accumulation of secondary metabolites of interest. In this study, three key factors with the potential to alter plant metabolism were evaluated, including high exposure to variations in agroclimatic conditions (rainfall and drought periods shown in Fig. 2.), to determine their effects on productivity and concentration of secondary metabolites.

It is hypothesized that open-field cultivation will be more exposed to abiotic stressors, particularly direct solar UV light radiation, and agroclimatic variability including wind exposure, fluctuations in relative humidity, and imbalanced soil humidity, which contribute to imbalances in plant evapotranspiration, and elevate ambient temperature (Rodríguez-Calzada et al. 2019). Conversely, LTG scenarios will moderate these stresses conditions by filtering solar radiation and providing more stable temperature and humidity microenvironment. Soil moisture levels were managed through the irrigation system of the crop and open-field cultivation scenario exhibited a greater reliance on weekly precipitation patterns. In regard to fertilization, traditional methods are recognized for their efficacy in delivering ample nutrients. However, concerns regarding leaching have been observed, necessitating rapid nutrient uptake by plants. In contrast, biochar-based fertilization enables the regulated release of nutrients due to its surface and adsorptive characteristics. Since the same chili pepper variety were used across all scenarios, discrepancies in productivity and bioactive compound concentration are predominantly ascribed to the aforementioned agronomic and environmental factors—or their interaction.

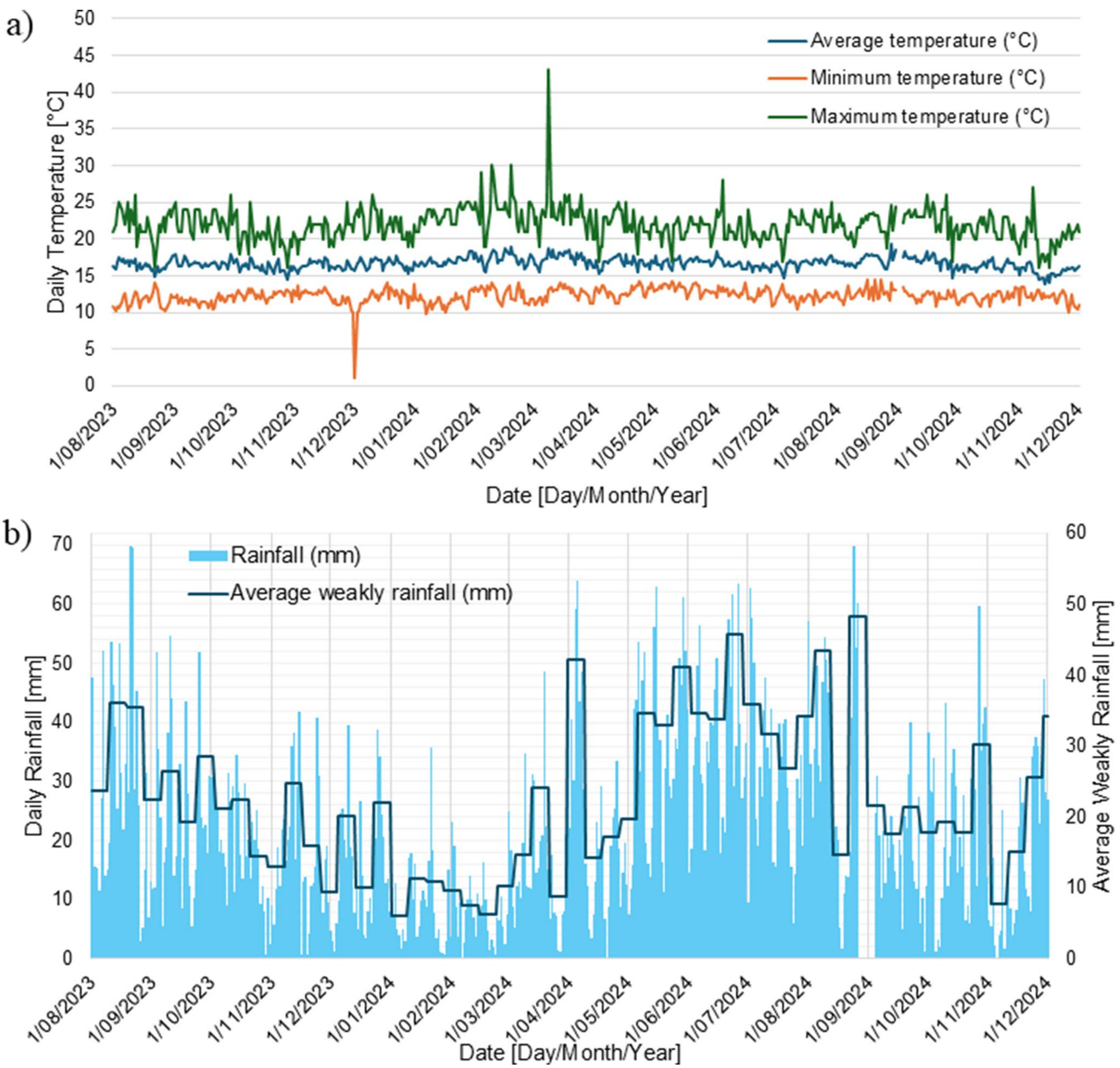

**Fig. 2** Climatic factors variation from crop establishment to the end of its productive cycle (Aug-2023 to Dec-2024). (**a**) Temperatures and (**b**) Precipitation

There is limited information available regarding the commercial productivity of rocoto chili cultivars. In Mexico, commercial yields reported in 2012 ranged from 4.6 to 14.6 tons per hectare (Marina et al. 2020), which is slightly lower than the yields obtained in the present study ranged from 11.48 to 22.19 tons/ha (Table 4). This discrepancy is attributed to planting density as demonstrated by Pérez-Grajales et al. (2009), who reported a yield of 32 tons/ha at a density of 2.5 plants/m². These results are consistent with those obtained in the present study, where planting density used was of 2 plants/m².

The highest yields were recorded in Scenarios 2 and 6, with 22.19 and 18.00 tons per hectare, respectively. These scenarios correspond to conventional fertilization and irrigation practices under LTG and open-field conditions. A comparison of these two cases reveals a decrease in productivity under open-field cultivation, despite identical fertilization and irrigation strategies. This same trend was observed across the other experimental scenarios, suggesting a significant influence of the cultivation environment on productivity outcomes. This phenomenon is attributed to the controlled microclimatic conditions present in LGT

**Table 4** Productivity and bioactive measurements of experimental design scenarios

| Scenario | Productivity [Ton/Ha] | TPC [mg GAE/100 g dw] | FLA [mg QER/100 g dw] | AA [mmol Trolox/ 100 g dw] |
|---|---|---|---|---|
| **1** | 16.80 | 1596.13 ± 38.56 | 222.37 ± 51.42 | 1.14 ± 0.09 |
| **2** | 22.19 | 2054.16 ± 45.16 | 163.70 ± 51.46 | 1.13 ± 0.17 |
| **3** | 13.24 | 1773.71 ± 21.14 | 177.53 ± 55.09 | 0.87 ± 0.16 |
| **4** | 15.84 | 1202.56 ± 120.28 | 133.88 ± 14.09 | 0.71 ± 0.09 |
| **5** | 14.56 | 1868.60 ± 104.93 | 121.42 ± 29.16 | 1.05 ± 0.18 |
| **6** | 18.00 | 2156.15 ± 9.89 | 167.87 ± 45.94 | 1.18 ± 0.03 |
| **7** | 11.48 | 2196.81 ± 133.07 | 118.42 ± 3.33 | 0.85 ± 0.21 |
| **8** | 12.44 | 2056.15 ± 59.83 | 143.75 ± 5.76 | 1.07 ± 0.05 |

[a] dw: dry basis, **TPC**: Total Phenolic Compounds in mg of Gallic Acid Equivalent (GAE), **FLA**: Flavonoids in mg of Quercetin Equivalent (QER), **AA**: Antioxidant Activity in mmol of Trolox Equivalent (Trolox)

[b] **Scenario 1**: LTG + normal irrigation + biochar, **Scenario 2**: LTG + normal irrigation + conventional cultivation, **Scenario 3**: LTG + stress irrigation + biochar, **Scenario 4**: LTG + stress irrigation + conventional cultivation, **Scenario 5**: Open field + normal irrigation + biochar, **Scenario 6**: Open field+ normal irrigation + conventional cultivation, **Scenario 7**: Open field + stress irrigation + biochar, **Scenario 8**: Open field + stress irrigation + conventional cultivation

systems, which serve to mitigate environmental stressors such as wind, excessive heat, and direct solar radiation (Ahmad et al. 2025).

A more pronounced decline in productivity was observed in scenarios involving water stress irrigation (Scenarios 3, 4, 7, and 8). An inadequate water supply has been demonstrated to impede nutrient uptake and compromise plant defenses. Ahmad et al. (2024) reported that inadequate hydration is a significant contributing factor to yield losses in chili crops. Similarly, Zamljen et al. (2020) explained that under prolonged water-stress conditions, plants begin to reallocate sugars from fruits, flowers, and roots to other tissues to maintain cell turgor and prevent wilting or death. It is plausible that these effects will be exacerbated under open-field conditions, where harsher environmental factors—such as higher temperatures, wind exposure, and fluctuating humidity—can intensify water stress and promote stomatal closure, ultimately limiting water availability for physiological processes. These findings serve to reinforce the LGT structure role in alleviating environmental stress through its protective microclimate.

With respect to the last factor, chemical fertilizers consistently yielded the highest results across both cultivation environments. However, the application of organic fertilizer derived from biochar and pig manure under LGT conditions (Scenario 1) exhibited superior yield performance in comparison to scenarios involving water stress in both LGT and open-field conditions. This finding underscores the considerable promise of organic fertilizers in the context of rocoto chili cultivation. As indicated by Khaitov et al. (2019), the cultivation of organic chili has been observed to yield optimal results under controlled conditions. It has been established that the efficacy of organic fertilizers in promoting plant growth and nutrient uptake is contingent upon factors such as nitrogen availability.

Respect to the secondary metabolites in the fruits, the total polyphenol content of the chili was consistent with values reported in previous studies, which ranged from 1,800 to 2,600 milligrams of gallic acid equivalent (GAE) per 100 g of dry chili pepper samples from Peru (Meckelmann et al. 2015). The higher mean total polyphenol levels in field-grown plants (Scenarios 1 to 4; 2069.4 mg GAE/100 g dw) compared to LGT-grown plants (Scenarios 4 to 8; 1656.6 mg GAE/100 g dw) could be attributed to increased UV radiation exposure in the open field. UV radiation is known to stimulate the production of phenolic compounds in plants as a protective mechanism against oxidative stress caused by UV light. Low-tech greenhouse protective conditions, which often filter or reduce UV radiation, may result in lower polyphenol synthesis (Emus-Medina et al. 2023).

A similar rationale is seen respect flavonoid content in LGT grown plants compared to field grown plants. Flavonoids are also part of the plant's defense mechanism against UV radiation, particularly UV-B light stress, which stimulates the biosynthesis of flavonoids in plants as chili (Rodríguez-Calzada et al. 2019; Ferreyra et al. 2021). The present study was conducted in a LGT structure with an Agroclear® plastic cover, which shows high UV-B light blocking capacity. Contrary to expectations, LGT cultivation using UV-blocking plastic resulted in higher flavonoid levels compared to open-field growth, likely due to environmental factors shifting metabolic flux (Ahmad et al. 2025). This is explained by competition between flavonoid and capsaicinoid metabolic pathways, which share common precursors (Maharjan et al. 2024). Furthermore, the incorporation of biochar has been shown to enhance flavonoid content in LGT settings, a finding that aligns with a previous study that demonstrated biochar's capacity to stimulate flavonoid biosynthesis (Yang et al. 2022). Prior studies have demonstrated that organic management practices enhance flavonoid content in crops such as tomatoes (Mitchell et al. 2007) and that mature fruits of other chili varieties exhibit higher polyphenol concentrations under organic cultivation conditions (Ribes-Moya et al. 2018). Similarly, water stress has been observed to induce phytochemical accumulation in peppers (Sarafi et al. 2018). In this study, the highest polyphenol levels were detected in biochar and water stress scenarios, especially in open-field conditions, suggesting that exposure to environmental factors may enhance their synthesis.

## 3.2 Capsaicin, dihydrocapsaicin and pungency

Capsaicinoids are distinctive secondary metabolites within the metabolic profile of chili plants, with capsaicin and dihydrocapsaicin accounting for over 90% of the total pungency (Duranova et al. 2022). These compounds are biosynthesized in the placental tissues of the fruit, enhancing both flavor and heat intensity, and serve as a defense mechanism against herbivores (Maharjan et al. 2024). The dual functionality of capsaicinoids is of particular relevance to the food and pharmaceutical industries, underscoring their significance as a key determinant of commercial chili pepper quality (Stan et al. 2021). Capsaicinoids content and pungency calculated in this study are shown in Fig. 3. The concentrations obtained in this study were 162–585 milligrams per 100 g (mg/100 g) and 90–199 mg/100 g for capsaicin and dihydrocapsaicin, respectively. These concentrations are notably higher than the concentrations reported by Vera-Guzmán et al. (2011), who found concentrations ranging from 12 to 150 mg/100 g and 18–247 mg/100 g, respectively, these discrepancies are ascribed to the influence of sample drying. Previous research has demonstrated that convective drying reduces capsaicinoid content in comparison to levels observed in fresh pulp (Salgado-Aristizabal et al. 2024). The reported pungency value for *Capsicum pubescens* (100,000 SHU) lends to support this hypothesis (Duranova et al. 2022). This value is more consistent with those observed in this study (ranging from 40,000 to 125,000 SHU).

The highest concentrations of capsaicin and dihydrocapsaicin, and consequently the highest pungency, were observed in Scenario 1 (585.51 ± 15.28 mg CAP/100 g dw and 199.49 ± 16.72 mg DHC/100 g dw) and Scenario 3 (389.39 ± 12.75 mg CAP/100 g dw and 137.51 ± 12.02 mg DHC/100 g dw). The extant literature supports the notion that the controlled LTG environment effect is a contributing factor to capsaicinoid accumulation. In this study, this phenomenon is likely attributable to the controlled microenvironment provided by LTG structure, which serves to mitigate stress factors and promote balanced plant metabolism (Maharjan et al. 2024; Ahmad et al. 2025). Conversely, open-field scenarios exhibited less variation, with capsaicin ranging between 194 and 315 mg/100 g dw and dihydrocapsaicin between 105 and 164 mg/100 g dw. The treatments in question generally did not exceed 70,000 SHU, and they demonstrated a paucity of significant differences among them. Furthermore, biochar performs differently in both CAP and open-field conditions. This phenomenon is also attributed to the enhanced positive effect of biochar by CAP structure. In open-field systems, elevated levels of solar radiation and loss of moisture generate physiological stress, which in part countervents the biochemical benefits of biochar amendment.

Regardless of soil moisture control, a strong correlation was observed between irrigation regime and capsaicinoid accumulation. All scenarios using normal irrigation (Scenarios 1, 2, 5, and 6) consistently yielded higher concentrations of capsaicin and dihydrocapsaicin than their counterparts under water stress (Scenarios 3, 4, 7, and 8). Although previous studies have reported changes in capsaicinoid content under water stress conditions (Yang et al.

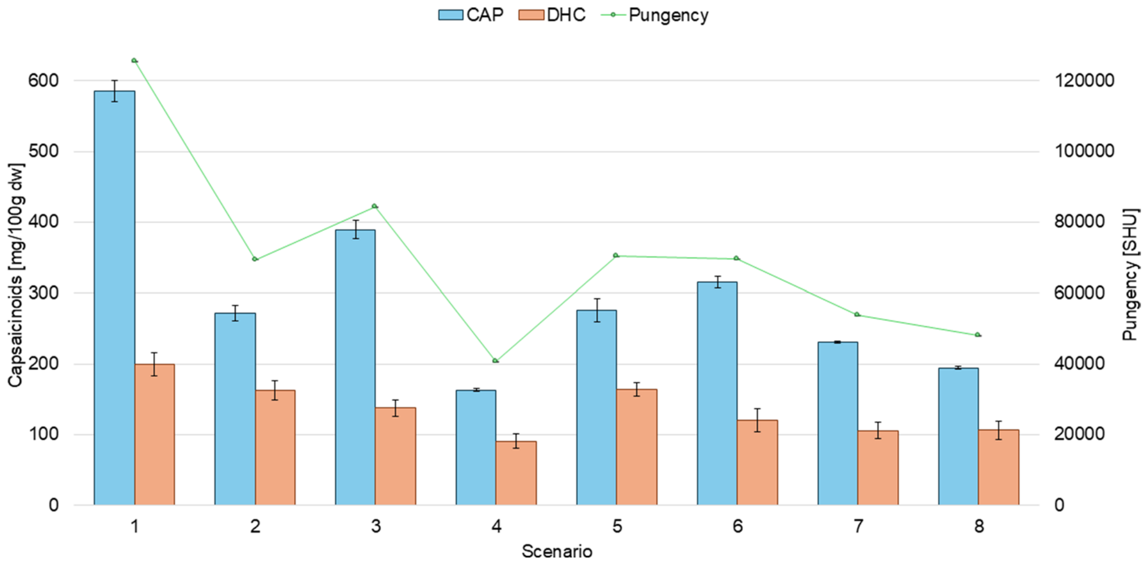

**Fig. 3** Capsaicinoids and pungency measurements of experimental design scenarios. **Scenario 1**: LTG + normal irrigation + biochar, **Scenario 2**: LTG + normal irrigation + conventional cultivation, **Scenario 3**: LTG + stress irrigation + biochar, **Scenario 4**: LTG + stress irrigation + conventional cultivation, **Scenario 5**: Open field + normal irrigation + biochar, **Scenario 6**: Open field+ normal irrigation + conventional cultivation, **Scenario 7**: Open field + stress irrigation + biochar, **Scenario 8**: Open field + stress irrigation + conventional cultivation. **CAP**: Capsaicin, **DHC**: Dihydrocapsaicin

2024), the direction and magnitude of this response appear to be genotype-dependent and context-specific. Initial findings indicate that chili varieties with a high natural pungency are less responsive to water stress in terms of capsaicinoid enhancement, suggesting a robust genotype-Stress interaction (Zamljen et al. 2020; Yang et al. 2024). Furthermore, the temporal dynamics of water-stress in relation to fruit development and maturation stages can exert a substantial influence on metabolite accumulation. This phenomenon exhibits a quadratic trend, wherein capsaicinoid concentration reaches a peak before undergoing a decline (Mahmood et al. 2021). Additionally, previous research indicates that capsaicinoid accumulation may vary according to precursor availability and stress intensity (Kim et al. 2009) highlighting the complexity of environmental regulation in Capsicum spp. Alternatively, the regulation of enzymes may be limited by precursor availability, such as phenylalanine, valine, or intermediate metabolites like phenolic acids (Maharjan et al. 2024). These findings suggest that excessive abiotic stress can suppress, rather than stimulate, capsaicinoid production due to metabolic imbalances.

Furthermore, Haak et al. (2012) reported that increased pungency was associated with higher humidity levels, possibly as a defense mechanism against fungal pathogens. Given that *Capsicum pubescens* is a moderately pungent species adapted to tropical climates, it is plausible that its genetic regulation of capsaicinoid pathways favors humid environments. This finding may provide a rationale for the observed decrease in capsaicinoid levels in water-stressed scenarios within the study. In a similar vein, Mahmood et al. (2021) observed a decline in capsaicinoid content in four chili genotypes under drought conditions, particularly among varieties exhibiting moderate pungency. These results serve to reinforce the hypothesis that moderately pungent genotypes may prioritize defense responses to pathogens rather than drought.

Turning to fertilization strategies, biochar-based organic fertilization demonstrated a positive effect on capsaicinoid content under low-stress conditions. A body of research has demonstrated that biochar has the capacity to enhance nutrient retention, increase nitrogen availability, and reduce leaching, thereby supporting capsaicinoid biosynthesis (Alkharabsheh et al. 2021; Melo et al. 2022). This is of particular relevance given the requirement of phenylalanine, valine, leucine, and an as yet unidentified amino donor for the formation of vanillin in capsaicinoid pathways (Maharjan et al. 2024). Nitrogen availability has been identified as a regulator of capsaicinoid accumulation (Yang et al. 2024). While the application of pig manure alone has been demonstrated to increase nitrogen levels, optimal results are typically obtained when it is combined with NPK fertilizers (Awosika et al. 2014). Although these combinations were not applied in the present study, the controlled release of nutrients from biochar may have modulated nutrient availability in a beneficial way (Marcińczyk and Oleszczuk 2022). However, in open-field conditions under normal irrigation (Scenarios 5 and 6), there were no significant differences in capsaicinoid levels between chemical and biochar fertilization. This finding indicates that the LGT microclimate exerts a more substantial influence on the modulation of capsaicinoid biosynthesis compared to the type of fertilization under field conditions.

In summary, the findings indicate that *Capsicum pubescens* cultivated under open-field conditions demonstrate comparable pungency levels across treatments, likely attributable to uncontrolled environmental variability and abiotic stress. Water stress has been demonstrated to be associated with reduced capsaicinoid accumulation, particularly in this moderately pungent, tropical-adapted species. This pattern is consistent with previous reports indicating that moderately pungent genotypes may respond differently to drought conditions compared to highly pungent varieties (Mahmood et al. 2021), highlighting the importance of genotype–environment interactions. These hypotheses warrant further investigation, as they suggest that rocoto chili represents a valuable system for further research on the environmental modulation of capsaicinoid accumulation. Furthermore, biochar fertilization significantly increased capsaicinoid concentration under low-stress LGT protective conditions, likely associated with improved nutrient retention and nitrogen availability, as documented in previous studies evaluating biochar amendments (Alkharabsheh et al. 2021; Melo et al. 2022). This treatment facilitated an effective trade-off between maintaining acceptable yield levels and enhancing the nutraceutical quality of the fruit through elevated secondary metabolite production.

## 3.3 Life cycle impact analysis

The present study applied the LCA methodology to evaluate the environmental impact of eight production scenarios of *Capsicum pubescens* influenced by the varying agroclimatic and cultivation conditions that were assessed. A significant methodological contribution of this study is the application of a life cycle assessment framework using a pungency-based functional unit for *Capsicum pubescens*. To the best of our knowledge, this is the first LCA for this species and the first within the Capsicum genus to employ a quality-adjusted functional unit. This approach is rooted in primary field data, thereby shifting the assessment from mass output to the efficiency of delivering a key commercial quality trait. The inherent relationship between pungency and capsaicinoid concentration per unit mass enables the integration of yield with biochemical quality. This redefines environmental efficiency in terms that are relevant to growers and supply chains. This framework provides a model for assessing other high-value crops where quality parameters are significant.

To exemplify this situation, a commercial requirement of 70,000 SHU is considered, in which a fruit with 80,000 SHU would require approximately 12.5% less mass compared to one with 60,000 SHU to achieve the same level of pungency. This reduction in the physical amount of product required to provide equivalent quality value leads to a significant decrease in environmental impact per FU. These findings are consistent with research reported by Svanes and Johnsen (2019), who evidenced that using functional units associated with nutritional attributes, such as vitamin C content, results in higher and more realistic environmental impact estimates compared to using mass-based units. The choice of purely mass-based functional units in the assessment of the environmental impact of different cultivation techniques could introduce biases in the analyses, underestimating the efficiency of systems with higher concentration of compounds of interest.

As illustrated in Table 5, the results of the six categories of greatest impact for the eight scenarios are presented based on the FU (pepper to achieve 70,000 SHU). A notable finding is the presence of substantial variations in the environmental impacts among the different scenarios. The most sustainable scenario was scenario 1, which obtained the lowest values in five of the six impact categories analyzed, including climate change (0.092 kg $CO_2$ eq/FU) and agricultural land use (0.348 $m^2$/FU), in contrast the less sustainable scenario was scenario 8 (0.553 kg $CO_2$ eq/FU). From a comparative perspective, these results are consistent with published LCA studies on other Andean fruit crops. For instance, Elorrieta-Mendoza et al. (2025) report Climate Change impacts ranging from 0.156 to 0.934 kg $CO_2$ eq per kilogram of avocado, depending on cultivar, cultivation system, and ecological conditions. A comparison of scenarios reveals that scenario 1 achieved an 83.4% reduction in CO2eq emissions and a 76.6% reduction in agricultural land use, as compared to scenario 8, demonstrating the adverse environmental impact of synthetic fertilizers and the absence of controlled microclimate provided by LTG structures over agro-climatic variables, such as water evaporation or exposure to pollutants.

A sensitivity analysis was conducted to assess the reliability of the results in the presence of variations in the pungency attribute (Scoville Heat Units) embedded within the FU definition. Quantitatively, a lower-pungency FU (50,000 SHU) requires an average of 0.79 kg of fresh chili pepper to meet the specified potency, corresponding to a mean reduction of approximately 28–29% in environmental impacts relative to the baseline FU (70,000 SHU; 1.11 kg on average reference flow). In contrast, a higher-pungency FU (100,000 SHU) necessitates approximately 1.58 kg of fresh fruit, resulting in an average increase of 42–44% across all six impact categories examined (Climate Change, Human Toxicity, Metal Depletion, Agricultural Land Occupation, Water Depletion, and Fossil Depletion) (see Table 5).

While modifying the pungency threshold alters the absolute impact values, the relative ranking of the eight

**Table 5** Total environmental impact of the scenarios to principal midpoint categories and sensitivity of environmental impact results to functional unit pungency level (50,000; 70,000; 100,000 SHU)

| Category | Unidad | SHU | Sc1 | Sc2 | Sc3 | Sc4 | Sc5 | Sc6 | Sc7 | Sc8 |
|---|---|---|---|---|---|---|---|---|---|---|
| Climate change | kg CO2 eq | 50,000 | 0.066 | 0.191 | 0.098 | 0.368 | 0.097 | 0.257 | 0.128 | 0.395 |
| | | 70,000 | 0.092 | 0.267 | 0.137 | 0.515 | 0.136 | 0.360 | 0.179 | 0.553 |
| | | 100,000 | 0.131 | 0.381 | 0.196 | 0.736 | 0.195 | 0.514 | 0.256 | 0.790 |
| Human toxicity | kg 1,4-DB eq | 50,000 | 0.071 | 0.226 | 0.107 | 0.305 | 0.045 | 0.207 | 0.063 | 0.342 |
| | | 70,000 | 0.099 | 0.316 | 0.150 | 0.427 | 0.063 | 0.290 | 0.088 | 0.479 |
| | | 100,000 | 0.141 | 0.452 | 0.214 | 0.610 | 0.090 | 0.414 | 0.126 | 0.685 |
| Metal depletion | kg Fe eq | 50,000 | 0.068 | 0.124 | 0.105 | 0.222 | 0.029 | 0.038 | 0.044 | 0.062 |
| | | 70,000 | 0.095 | 0.173 | 0.147 | 0.311 | 0.041 | 0.053 | 0.061 | 0.087 |
| | | 100,000 | 0.136 | 0.247 | 0.210 | 0.445 | 0.058 | 0.076 | 0.087 | 0.124 |
| Agricultural land occupation | m2a | 50,000 | 0.249 | 0.418 | 0.462 | 0.926 | 0.511 | 0.526 | 0.834 | 1.062 |
| | | 70,000 | 0.348 | 0.586 | 0.647 | 1.297 | 0.716 | 0.737 | 1.168 | 1.486 |
| | | 100,000 | 0.497 | 0.837 | 0.925 | 1.853 | 1.023 | 1.053 | 1.667 | 2.124 |
| Water depletion | m3 | 50,000 | 0.144 | 0.205 | 0.110 | 0.203 | 0.294 | 0.251 | 0.196 | 0.224 |
| | | 70,000 | 0.201 | 0.287 | 0.155 | 0.284 | 0.411 | 0.353 | 0.275 | 0.313 |
| | | 100,000 | 0.287 | 0.410 | 0.222 | 0.405 | 0.587 | 0.504 | 0.392 | 0.447 |
| Fossil depletion | kg oil eq | 50,000 | 0.010 | 0.033 | 0.013 | 0.057 | 0.018 | 0.053 | 0.019 | 0.069 |
| | | 70,000 | 0.014 | 0.047 | 0.018 | 0.080 | 0.025 | 0.074 | 0.027 | 0.096 |
| | | 100,000 | 0.020 | 0.067 | 0.025 | 0.115 | 0.036 | 0.106 | 0.039 | 0.137 |

**Nota:** Reference flows to achive 70,000 SHU in each scenario are **Sc1:** 0.557 kg, **Sc2:** 1.008 kg, **Sc3:** 0.830 kg, **Sc4:** 1.724 kg, **Sc5:** 0.995 kg, **Sc6:** 1.004 kg, **Sc7:** 1.300 kg y **Sc8:** 1.457 kg cultivation scenarios remains unchanged across all impact categories and SHU levels analyzed. This finding suggests that alterations in the FU definition impact all scenarios in a proportional manner, thereby preserving the comparative order of environmental performance. Scenarios with consistently higher or lower impacts maintain their relative positions irrespective of the specific pungency value selected. For instance, in the Climate Change category, Scenario 4 consistently exhibits the highest impacts, while Scenario 1 shows the lowest. This pattern is consistently mirrored across all evaluated categories.

These findings confirm that, while the choice of FU influences absolute impact magnitudes, the study's comparative conclusions—particularly the identification of the most and least environmentally favorable cultivation systems—are robust to uncertainty arising from biological variability in chili pungency within the tested range. Consequently, the utilization of an integrated functional unit that incorporates mass and pungency facilitates a equitable and consistent comparison across production systems that exhibit markedly divergent agronomic and biochemical characteristics.

As illustrated in Fig. 4, the analysis of the crop variables within the six categories reveals their contribution. The stepwise analysis revealed that in protected systems infrastructure (LGT) represented the primary contributor to the impact in the metal depletion category (more than 75%), due to the intensive use of metallic materials and plastics. Conversely, synthetic fertilizers were identified as the primary contributors to climate change and human toxicity in open field systems, accounting for up to 80% of the total impact in climate change. These results are consistent with the extant literature, which has identified conventional fertilizers as responsible for between 64% and 72% of greenhouse gas emissions in agricultural systems (Wang et al. 2018). The high contribution of agriculture to climate change has a deleterious effect on crop yields and the biosynthesis of bioactive compounds (Zhou et al. 2024). The increasing global temperatures and atmospheric carbon dioxide concentrations have led to a more complex web of interactions between crops and pests. This has often resulted in an increased reliance on agrochemicals. These dynamics compromise the environmental sustainability of the agricultural system and can negatively impact the functional quality of products such as rocoto (Mitra et al. 2024).

The extensive utilization of agrochemicals in open fields contributes considerably to environmental contamination. Tudi et al. (2021) documented that conventionally fertilized systems demonstrate a higher ecotoxicological impact due to the utilization of chemical pesticides and fungicides. Despite the presentation of certain organic fertilizers (such as swine manure) as safer alternatives, recent studies have cautioned that these fertilizers may potentially introduce heavy metals into the soil. However, it has been reported the potential of biochar to adsorb these elements, thereby reducing their potential toxicity (Qaswar et al. 2020; Beily et al. 2023). Notwithstanding the promotion of biochar as a means to enhance water use efficiency, its integration with conventional irrigation methods has the potential to augment apparent consumption. However, it should be noted that a portion of the water content of biochar is already present within the system, thereby negating the necessity to consider biochar as an additional water input (Oldfield et al. 2018).

From a functional quality perspective, scenarios that included biochar exhibited not only reduced environmental impact, but also elevated concentrations of total phenolic compounds and antioxidant activity. This dual effect suggests that biochar-based systems may generate synergistic benefits, simultaneously improving soil functionality and stimulating secondary metabolite synthesis in rocoto fruits. The environmental benefits observed in biochar treatments are primarily associated with a reduced reliance on synthetic fertilizers and enhanced nutrient retention in soil. This contributes to reduced emissions related to fertilizer production and nitrogen losses. Concurrently, biochar amendments have been shown to enhance soil physicochemical properties, microbial activity, and nutrient-use efficiency. These effects may potentially induce moderate physiological stress or improved nutrient signaling pathways that promote the biosynthesis of phenolic compounds and antioxidants. This congruence between diminished environmental loads and augmented phytochemical content underscores a pivotal nexus between agroecological management and product functional quality.

This finding indicates the presence of potential benefits beyond pungency, which were not previously considered in the definition of the functional unit. This finding presents a promising avenue for future research, which may include the integration of multiple functional dimensions, such as pungency, antioxidant activity, and nutritional value, into environmental analyses. The results therefore suggest that future LCA frameworks for specialty crops could benefit from integrating multiple functional performance indicators into the functional unit or decision-support metrics. For instance, composite functional units incorporating pungency, antioxidant capacity, or bioactive density per kilogram could provide a more comprehensive representation of product value relative to environmental burdens.

Finally, Fig. 5 presents the outcomes of the environmental impact analysis employing the ReCiPe Endpoint (H) methodology. In the majority of the scenarios examined, the resource use category emerged as the predominant contributor to the aggregate impact, particularly in the LGT systems (scenarios 1 to 4), where it constituted approximately 50%

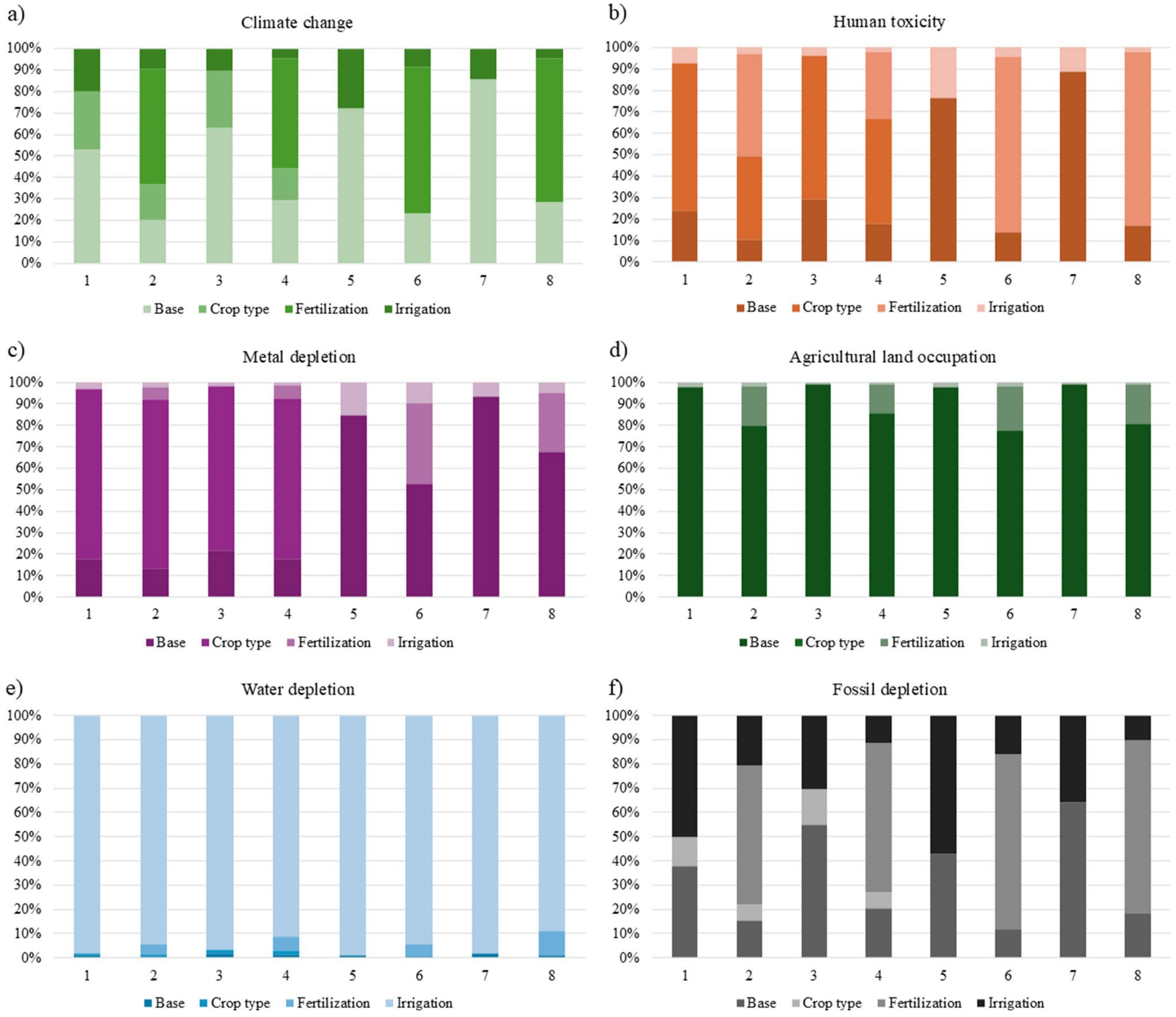

**Fig. 4** Contribution of studied variables to (**a**) climate change, (**b**) human toxicity, (**c**) metal depletion, (**d**) agricultural land occupation, (**e**) water depletion, and (**f**) fossil depletion, based on a functional unit of 70,000 SHU. **Base**: Common processes shared by all scenarios. **Crop type**: Cultivation method (LGT or open field). **Fertilization**: Fertilizer type (conventional or organic biochar). **Irrigation**: Water application regime (normal or stress). **Scenario 1**: LTG + normal irrigation + biochar, **Scenario 2**: LTG + normal irrigation + conventional cultivation, **Scenario 3**: LTG + stress irrigation + biochar, **Scenario 4**: LTG + stress irrigation + conventional cultivation, **Scenario 5**: Open field + normal irrigation + biochar, **Scenario 6**: Open field+ normal irrigation + conventional cultivation, **Scenario 7**: Open field + stress irrigation + biochar, **Scenario 8**: Open field + stress irrigation + conventional cultivation

of the total impact. In the open field systems (scenarios 5 to 8), this category ranged from 35% to 40%. These discrepancies are indicative of the augmented input demands inherent to protected agriculture, which encompass all the structure materials for its construction. This phenomenon also underscores the pivotal role of agriculture in the global consumption of natural resources, particularly water and energy, and emphasizes the challenges associated with productive intensification. Moreover, mounting pressures on water resources are attributable to urban expansion, industrialization, and climate change, thereby underscoring the imperative for sustainable management strategies, particularly within technified contexts (Kourgialas 2021).

In terms of ecosystem impacts, scenario 8 exhibited the most unfavorable results, with high levels of impacts attributable to the intensive use of synthetic fertilizers, which cause soil degradation, loss of biodiversity, and accumulation of pollutants. According to Wowra et al. (2021), the utilization of nitrogen fertilizers has been demonstrated to exert a substantial environmental impact across diverse contexts, contributing to the processes of eutrophication and

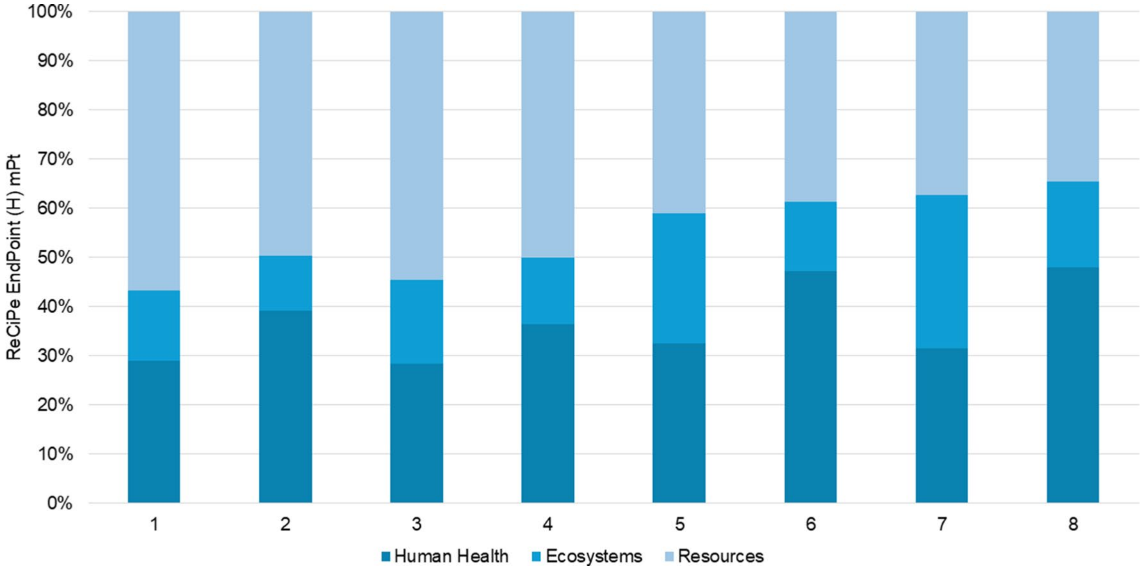

**Fig. 5** Contribution of life cycle stages to the total scores of eight scenarios. **Scenario 1**: LTG + normal irrigation + biochar, **Scenario 2**: LTG + normal irrigation + conventional cultivation, **Scenario 3**: LTG + stress irrigation + biochar, **Scenario 4**: LTG + stress irrigation + conventional cultivation, **Scenario 5**: Open field + normal irrigation + biochar, **Scenario 6**: Open field+ normal irrigation + conventional cultivation, **Scenario 7**: Open field + stress irrigation + biochar, **Scenario 8**: Open field + stress irrigation + conventional cultivation

acidification in terrestrial and aquatic ecosystems. This, in turn, has a direct and observable effect on biodiversity loss.

In the human health category, the greatest impact was recorded in scenario 6 (open field with conventional irrigation and chemical fertilization), due to the intensive use of synthetic agrochemicals, offset by low productivity and commercial quality of the fruit. These substances pose a dual threat: they endanger consumers and repeatedly expose agricultural workers to toxic compounds.

On the contrary, the scenarios that incorporated biochar, especially scenarios 5 and 7, presented a more balanced distribution of environmental impacts among the different categories. This is attributed to its beneficial properties, such as improved nutrient use efficiency, improved soil physicochemical properties and reduced greenhouse gas emissions.

This study lends support to the necessity of formulating environmental analysis instruments that encourage functional units centered on quality and functionality attributes. The study's findings demonstrate the potential for these attributes to be associated with crop productivity, extending beyond conventional metrics that solely consider productivity per unit area. Notwithstanding, some limitations of the present study include the use of a cradle-to-gate system boundary, which excludes post-harvest stages. This boundary was selected to concentrate the assessment on primary agricultural practices, without the confounding variables of downstream processes. Furthermore, the study is based on data from a single, controlled one-year experimental plot at a specific location (Manizales, Colombia). While this design ensures a precise comparison of the agronomic treatments, the absolute results are influenced by the local high-altitude climate and may not be directly scalable to large-scale commercial production without accounting for logistical efficiencies and inter-annual variability. Consequently, the findings are most representative of smallholder systems under similar agroclimatic conditions. Future LCA research would benefit from incorporating inventory data across multiple farms and growing seasons. This approach would enhance the robustness of the research and allow for the capture of broader temporal and spatial variability.

## 3.4 Overall system analysis

This study adopts a novel perspective to address the complex challenges involved in identifying more sustainable agricultural practices. Conventional agricultural practices are commonly linked to substantial crop productivity, yet they are also associated with considerable environmental repercussions, largely attributable to the application of chemical fertilizers. Conversely, organic agriculture has been demonstrated to curtail the environmental impact of farming. However, it frequently culminates in diminished crop yields. This trade-off poses a significant challenge for environmental impact assessments based on LCA methodologies, which typically rely on mass-based allocation to normalize the impacts across different scenarios. However, this study adopted a functional unit based on nutritional quality that allows us to balance yield and quality, and facilitates a more equitable comparison between different production systems. This alternative approach overcomes the limitations of mass-based units by integrating key commercial attributes, especially in an increasingly food quality-oriented market.

It has been already demonstrated that pungency (related to capsaicinoids content) is a more suitable allocation factor, as it more effectively integrates productivity with commercial quality and environmental impact in the assessment of chili production systems. As previously discussed, capsaicinoids synthesis can be stimulated by abiotic stress factors, such as extreme temperatures, drought, or nutrient deficiency. However, while stress-induced metabolic changes have been shown to enhance the accumulation of bioactive compounds, they frequently result in a reduction in yield. The controlled exposure to such stressors may offer a promising strategy to balance productivity, the bioactive quality of the fruits, and environmental impacts of farming.

From a productive standpoint, the low-tech greenhouse scenarios with normal irrigation and conventional fertilization (scenario 2) demonstrated the optimal agronomic performance (22.19 Ton/Ha), which is consistent with the findings of Valiente-Banuet and Gutiérrez-Ochoa (2016) on the benefits of the controlled microclimate in the physiology of Capsicum. This represents a competitive advantage for farmers, despite requiring a higher initial investment and operating costs. A comparative economic analysis reveals that LGT systems possess considerably higher fixed assets (12.86% of total costs) compared to open-field systems (4–5%), primarily due to infrastructure, with labor costs constituting approximately 41% of total production costs, as documented in studies of similar crops in southeastern Spain (García García and García García 2022). However, this higher investment is offset by significantly higher productivity, consistent with economic trends reported by Khan et al. (2017). Biochar scenarios as a soil amendment have been shown to offer economic advantages in the medium and long term by reducing fertilization costs (15–25%) and progressively improving soil health (Barłóg et al. 2022). In relation to the content of bioactive compounds, open field treatment under water stress and fertilization with biochar (Scenario 7) led to the maximal synthesis of polyphenolic compounds. The pungency reached its maximum expression (125,601 SHU) under low-tech greenhouse conditions with normal irrigation and biochar fertilization, coinciding with the observations made by López Puc et al. (2020) who determined that a controlled microclimate improve the capsaicinoid content. Life Cycle Assessment demonstrated that systems incorporating biochar consistently exhibited a reduced carbon footprint, underscoring scenario 1 with minimal environmental impact (9.17E-02 kg CO2 eq). This outcome is attributable to the capacity of biochar to sequester carbon and reduce reliance on synthetic inputs (Lehmann et al. 2011).

**Table 6** Normalized performance indicators and integrated score

| Scenario | Yield | SHU | CC | Integrated Score |
|---|---|---|---|---|
| 1 | 0.497 | 1.000 | 1.00 | **0.832** |
| 2 | 1.000 | 0.335 | 0.62 | 0.652 |
| 3 | 0.165 | 0.514 | 0.902 | 0.527 |
| 4 | 0.408 | 0.000 | 0.082 | 0.163 |
| 5 | 0.288 | 0.335 | 0.904 | 0.509 |
| 6 | 0.610 | 0.311 | 0.418 | 0.446 |
| 7 | 0.000 | 0.120 | 0.810 | 0.310 |
| 8 | 0.090 | 0.072 | 0.000 | 0.054 |

A comprehensive comparison of the evaluated systems reveals inherent trade-offs among productivity, phytochemical composition, organoleptic quality, and environmental performance. While certain scenarios maximize individual indicators, none simultaneously achieves superior performance across all dimensions. In this context, Scenario 5 emerges as a noteworthy alternative due to its reduced environmental footprint and lower infrastructure requirements, while maintaining acceptable productivity levels (14.56 tons per hectare). These multidimensional differences highlight the need for an integrated assessment capable of simultaneously considering productivity, pungency, and environmental impact in order to identify the most balanced configuration.

To provide a structured and transparent comparison of the evaluated scenarios, productivity (Ton ha-1), pungency (SHU), and climate change impact (kg CO2 eq per FU) were normalized using a min-max scaling approach. The scale for yield and pungency was such that higher values corresponded to better performance. Inversely, the scale for climate change impact was such that lower emissions resulted in higher normalized scores. The three normalized indicators were subsequently averaged without weighting in order to avoid introducing subjective assumptions regarding their relative importance. This procedure generated an integrated performance score for each scenario, as presented in Table 6.

According to the integrated normalized performance score (Table 6), Scenario 1 (LGT, normal irrigation, biochar) exhibited the highest overall performance among the evaluated systems, reflecting a balanced configuration in terms of productivity (16.80 tons ha$^{-1}$), superior nutraceutical quality (maximum pungency and moderate-high polyphenol content), and reduced climate change impact. Consequently, Scenario 1 is recommended as the best available practice for growers seeking to maximize both quality and environmental performance under protected cultivation. Despite most of CAP scenarios performance the best integrated scores, in scenarios where investment in CAP infrastructure is limited, Scenario 5 (open field, normal irrigation, biochar) presents a promising lower-capital alternative. Despite lower absolute yields, this system achieved an acceptable level of nutraceutical quality, reduced its carbon footprint relative to conventional open-field practices, and benefited from the same biochar-mediated environmental improvements.

## 4 Conclusions

The present study offers an integrated agronomic, biochemical, and environmental analysis of eight cultivation scenarios for Rocoto chili pepper. Within the specific context of high-altitude Andean agriculture, the use of low-tech protective enclosures for organic rocoto production—requiring no operational energy for climate control—demonstrates favorable environmental performance per unit of delivered pungency compared to open-field systems in the same region, primarily due to significant yield increases under similar input regimes. Specifically, Scenario 2 (LGT, conventional fertilization, normal irrigation) yielded the highest harvest (22.19 tons/ha), yet it exhibited a more substantial environmental impact. In contrast, Scenario 1 (LGT, biochar, normal irrigation) emerged as the most balanced system, offering the highest pungency (125,601 SHU), a robust yield of 16.80 tons/ha, and the lowest environmental impact. From an environmental perspective, biochar-based systems have been demonstrated to be effective in mitigating environmental impacts. In general, performance metrics indicated that open-field conditions resulted in suboptimal outcomes. However, Scenario 5 (open field, biochar, normal irrigation) presented a viable lower-investment alternative with an acceptable yield (14.56 tons/ha) and a reduced carbon footprint. These findings establish a clear, evidence-based framework for growers to align productivity goals with quality and environmental sustainability in Rocoto chili cultivation.

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**Data availability** Data will be made available on a reasonable request.

## Declarations

**AI-assisted technologies in the writing process** During the preparation of this work the authors used DeepL Write AI in order to polishing and style correction. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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