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Mitigating carbon footprint: Integrating nitrified urine fertilizer in two hydroponic tomato cultivation systems

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ABSTRACT

The replacement of mineral fertilizers with recycled nutrients from waste streams addresses the pressing need to reduce crop production carbon footprint. Recent research has demonstrated the successful use of nitrified human urine fertilizers in hydroponic tomato cultivation. However, regarding the accumulation of ions such as Na^+ and Cl^-, the feasibility of their use in commercial greenhouse production systems has not yet been evaluated. Our study aimed to assess the influence of nitrified urine fertilizer on plant physiology, product quality, fertilizer use and greenhouse gas emissions from the rootzone for recirculating hydroponic systems. In a two-factorial experiment, tomatoes (*Solanum lycopersicum* cv. 'Avalantino' F1) were cultivated in a recirculated nutrient solution using drip irrigation on rockwool or nutrient film technique. Two nutrient solution treatments were applied: one containing calcium-rich nitrified urine fertilizer along with mineral fertilizers, and another based solely on mineral fertilizers serving as the control treatment.

Our findings demonstrate that tomatoes can be grown in recirculating hydroponic systems using nitrified urine fertilizer without experiencing significant yield reductions. The urine fertilizer solution was discarded twice at a sodium concentration of 12 mM. No significant differences in nitrous oxide (N_2O) emissions and total contents of secondary metabolites in fruit were observed between the fertilizer treatments. In the nitrified urine treatments, high sodium and calcium concentrations did not affect potassium nutrition, but high calcium concentrations necessitated higher magnesium supply. 94% of nitrogen, 13% of potassium, and 7% of phosphorus were provided by the nitrified urine. Depending on emissions related to urine fertilizer production, this may result in a reduction of overall production carbon footprint.

Our study supports the feasibility of nitrified urine fertilizer utilization in commercial hydroponic tomato production, offering a sustainable and environmentally friendly approach to reduce the carbon footprint associated with conventional mineral fertilizers.

1. Introduction

The United Nations Sustainable Development Goals [1] encompass promoting sustainable agricultural production systems while ensuring food security. The cultivation of most food crops relies heavily on the use of mineral fertilizers. The conventional production of nitrogen fertilizers requires natural gas as raw material and energy source and contributes significantly to the carbon footprint of agricultural production systems [2]. Modern catalyst technology for emission reduction is available but is only implemented in Europe. A high threat to food security is the depletion of phosphorus fertilizers, which are mined from non-renewable phosphate rock [3]. At the same time, nitrogen and phosphorus, which enter urban wastewaters mostly through human excreta, have to be removed in energy intensive processes in wastewater treatment plants [4]. Furthermore, substantial nitrous oxide emissions occur during the treatment process [5]. Nutrients, pharmaceuticals and

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hormones are incompletely eliminated from the effluent [6] and thus contribute to eutrophication and pollution of water bodies. Compared to mineral fertilizers and sewage sludge, urine contains only low levels of heavy metals [7], but provides 80% of the nitrogen, 40% of the phosphorus, and 70% of the potassium in domestic wastewaters [8]. Since the concept of source-separation of urine and the use of the nutrients as recycling fertilizers were suggested, many nutrient recovery technologies from urine have been developed and tested [9]. For hydroponic crop production, urine fertilizer products with high solubility of nutrients in the solution are essential. For nitrogen (N), nitrate (NO_3^-) is favored as the more stable form. Nitrate is produced by nitrification treatment processes resulting in a liquid urine fertilizer, for example the uC.R.O.P. fertilizer developed by DLR [10] or the Aurin fertilizer developed by VUNA [11].

Fresh urine and consequently nitrified urine fertilizers contain sodium and chloride (e. g. 0.2-0.3 mol Na + or Cl-per mol dissolved nitrogen [7,12]). These ions can accumulate in plant nutrient solutions and lead to salt stress caused by ion-toxicity or overall salt-build up. Crop salt tolerance is influenced by species, cultivar, growth stage and growing system. For tomatoes, different characterizations regarding salt tolerance have been described, from moderately salt sensitive to moderately tolerant [13]. For example, Li et al. [14] found that yield decreased by 5.1% for each dS m^{-1} in excess of 2 dS m^{-1} overall nutrient solution salinity. And Chrétien et al. [15], found that tomato fruit size was reduced at NaCl concentration of 12 mM.

Nutrients from nitrified urine have been shown to be readily plant available [16]. A study by Halbert-Howard et al. [17] evaluated the nitrified urine fertilizers uC.R.O.*P. and* Aurin compared to other fertilizers in a hydroponic tomato experiment. The authors have shown that tomatoes can be grown with uC.R.O.P. fertilizer in nutrient-film technique (NFT), replacing 100% of nitrogen, 22% of potassium and 35% of phosphorus with nitrified urine without significant yield reduction (~2.5 kg $plant^{-1}$). The experiment was conducted in a recirculating NFT system. However, nutrient solution was replaced weekly, thus examining accumulation effects of Na^+, Cl^- or SO_4^{2-} and increased overall salinity only to a very limited extent. Furthermore, the culture was grown in NFT, thus not examining the feasibility of the application of uC.R.O.P. fertilizer for drip irrigation.

Considering product quality, previous studies hypothesized, that plants fertilized with nitrified urine instead of mineral fertilizers potentially accumulate fewer heavy metals in plant tissue. Chromium is a naturally occurring heavy metal found in trace amounts in many agricultural products, including tomatoes. The chromium found in tomatoes and other foods is typically in the form of trivalent chromium (chromium-3), which is considered safe and even beneficial for humans in small amounts. However, there are different forms of chromium, and some forms can be toxic at high levels. Copper is an essential micronutrient for plants (and humans). However, as high copper intake can have detrimental health effects in humans, the consolidated EU Commission Regulation 396/2005 [18] states a maximum Copper content of 5 mg/kg fresh weight for tomatoes. The EU Commission Regulation 2023/915 [19] determines maximum levels of lead and cadmium in fruit vegetables.

Another important aspect considering product quality and safety are pharmaceutical and hormone residues. Not all pharmaceuticals and hormones in household wastewaters are eliminated from the effluent of wastewater treatment plants [6] and thus contribute to pollution of water bodies. Field-grown vegetables irrigated with treated municipal wastewater have been shown to accumulate metabolites of pharmaceuticals [20]. If collected separately, urine contains on average 64% of those substances [21]. These are also mentioned in a recent product specification for "quality assurance of recycling products from dry toilets for use in horticulture" [22], which was developed by a consortium of experts in resource-based sanitation for the German Institute for Standardization. Pharmaceuticals and hormones can be effectively removed from nitrified urine by activated carbon filtration [23,24]. For this reason, the nitrified urine fertilizer, Aurin [11], the production process of which uses this technology, was approved for horticultural applications in Switzerland, Liechtenstein and Austria. De Boer et al. [25] found bioaccumulation in tomato fruits to be lower than 0.0003%, and metabolites were shown to be mainly stored in tomato leaves [26]. For this reason, accumulation of pharmaceuticals and hormones was not tested in the present study.

In this study, tomato plants were cultivated in two different cultivation systems applying nitrified urine as nutrient source. The nitrification process took place in the presence of calcium carbonate, which acted as a buffer thus increasing nitrate content. After nitrification the urine was sterilized and filtered with activated carbon. The resulting nutrient solution was recirculated in the cultivation systems over several weeks.

We hypothesized that commercial production in recirculating hydroponic systems is feasible if ion accumulation thresholds are considered. We expected a reduction of impacts on fertilizer related emissions in the nitrified urine fertilizer treatments but no difference between the hydroponic systems. These hypotheses were assessed by calculating recycling rates for nitrogen, phosphorus and potassium and by measuring the emissions of the greenhouse gases nitrous oxide, carbon dioxide and methane from the root zone. As the urine fertilizer was obtained from a pilot plant, calculations of greenhouse gas emissions for fertilizer production were not representative. Thus, a comparison with conventional fertilizer production and wastewater plant related greenhouse gas emissions was not performed in this study. We hypothesized that ion accumulation would increase nutrient solution salinity and induce physiological adaptation without causing osmotic stress sufficient to impair marketable fruit yield. Furthermore, we expected this physiological adaptation to be reflected in plant nutrition (mineral elements in root, leaf, and stem tissue and amino acids in leaf tissue) and plant physiology (plant height and leaf area), while product quality (phenolic acids, carotenoids, and heavy metal content) would be positively affected.

2. Materials & methods

2.1. Experimental set-up, plant material, and growing conditions

A two-factorial experiment was established with two nutrient solution treatments and two different hydroponic systems. Each combination was replicated three times with independent hydroponic systems each of which was attached to its own tank. The experimental layout can be found in the supporting information (Fig. S1). The experiment was set up in a 76.8 m^2 cabin of an experimental Venlo-type greenhouse in Berlin, Germany (52° 46′ 82.806″ N, 13° 29′ 88.909″ E). The set-up consisted of 14 channels, but the two edge rows (with 10 plants each) were not evaluated. The 12 remaining channels (with 5 plants each) were equipped alternately with nutrient-film-technique (NFT) systems or drip-irrigation systems (Netafim, Chazerim, Israel) on rockwool (RW) slabs (Cultilene Xfibre Optimaxx, 1000 × 150 × 75 mm, Saint-Gobain Cultilene, Rijen, The Netherlands). All channels were covered with opaque foil and placed on a 1% slope to flow in nutrient solution tanks (90 l) from which nutrient solution was circulated via pumps (Gardena Classic dirty water submersible pump 7000/D, Gardena, Ulm, Germany). In the NFT system channels, water was constantly pumped through a garden hose to the top of the channel at a flow rate of 1 L/min. In the drip-irrigation system nutrient solution was pumped through a tube and delivered through pressure compensated drippers (2 L/h, Netafim, Chazerim, Israel) 10 times per day from 6 a.m. to 7 p.m. CET/CEST with 30% drain.

Tomato plants (*Solanum Lycopersicum* L. cv. 'Avalantino' F1) in small rock wool cubes (100 mm × 100 mm×65 mm) with their first inflorescence visible were obtained from Jungpflanzen Gernert GbR (Albertshofen, Germany) on 29th January 2020 and were placed in the experimental set-up. The experiment started on February 4th, 2020, and

ended June 16th, 2020. The mean air temperature in the greenhouse was 23.0 °C during the day and 19.3 °C at night and the mean relative humidity was 64.5% for the total duration of the experiment. Profiles of temperature and relative humidity inside the greenhouse cabin over the course of the experiment are shown in the supporting information (Fig. S2 + S3). The plants were supported by attachment of the stems to nylon strings. Side shoots were pinched out regularly and leaves growing out of trusses were removed. To prevent mildew infection, bottom leaves hanging into the channels were removed equally for every plant to ensure comparability. Mechanical pollination was performed every other day by vibrating each truss for at least 5 s. Fruit trusses were reduced to 8 fruits per truss by removing fruits developed up to 2 cm diameter.

2.2. Nutrient solution treatments and management

After delivery, plants were first grown in a standard nutrient solution (Phase I in Hochmuth and Hochmuth [27]) diluted to EC 1.8 on NFT channels. In the control treatment (Control) all nutrients were then supplied by commercial mineral fertilizers and in the nitrified-urine fertilizer treatment (CROP + mf) nutrients were supplied by nitrified-urine fertilizer (uC.R.O.P.) from the C.R.O.P.- Project (C.R.O.P. facility, German Areospace Center, Cologne, Germany [10]) supplemented with commercial mineral fertilizers for the lacking macro- and micronutrients (see Table 1). The nutrient solution treatments were prepared with demineralized water and mixed and adjusted according to "Tomato in rockwool: reuse drainage water" [28]. Target concentrations were 164.6 mg/L N, 38.71 mg/L P, 254.15 mg/L K, 110.22 mg/L Ca, 24.3 mg/L Mg, 954 µg/L Fe, 925 µg/L B, and 1.09 mg/L Mn. Nitrogen optimum concentrations were calculated based on total N concentrations. As a result, NH_4-N concentrations deviated from the original recipe. As calcium concentration in uC.R.O.P.-fertilizer was high, calcium levels in the control treatments were adjusted to contain similar levels (160-180% of recipe, 180-200 mg/L). Magnesium target concentration was changed to 60 mg/L Mg for all treatments from 13th April after magnesium deficit was observed in all treatments. Micronutrients were supplied by a premixed cocktail and dosed to achieve optimum Boron concentration. Manganese was added from a stock solution.

The tested nitrified urine fertilizer, uC.R.O.P., was delivered in batches. There is inherent variability in the product and the values shown in Table 2 are averages of all batches used in the experiment. A more detailed list can be found in the supporting information (Table S1).

Nutrient concentrations in each tank were analyzed weekly. Two to three days after sampling, the nutrient concentrations were adjusted to the nutrient solution recipe by filling up the tank to 75 L (70 L in NFT systems) with distilled water and adding nutrients to reach above stated target concentrations. Since the 22nd May 2020, transpiration loss occurred in the gap between sample point and refill and therefore, before refill, distilled water was added to adjust the EC value to the level on the day of sampling. Afterwards, the volume needed for refill was determined. The tank volume was determined by measuring the distance between the top edge and fill level using a previously calibrated scale. Electrical conductivity (EC), pH and nutrient solution temperature were measured daily or every other day with a hand-held device (HI9811-5, Hanna Instruments Deutschland GmbH, Vöhringen, Germany). To maintain the pH at 5.8 – 6.5, phosphoric acid and potassium hydroxide were used. Tanks were emptied and mixed freshly, when CROP + mf treatments reached the threshold of 276 mg Na L^{-1} (12 mM [15]) after 8 and then after 6 weeks (2nd April and 15th May). Discharge of each tank was 75 L for each tank, with the two discharge events making up 12-14% of total water use.

Table 2
Average nutrient concentrations in uC.R.O.P. fertilizer (DLR, Cologne, Germany) batches that were used for the given experiment according to Ion Chromatography (NH_4^+-N, NO_3^--N, Total N, K, Na, S, Cl) and ICP-OES (remaining elements) (values of all batches in Table S1).

Element	Unit	Mean of concentration	Standard Deviation
NO_3-N		4.11	0.22
NH_4-N		0.30	0.11
Total N		4.42	0.15
P		0.07	0.01
K		0.94	0.03
Na	[g L^{-1}]	1.29	0.04
Cl		1.77	0.06
Ca		4.64	0.40
Mg		0.04	0.00
S		0.29	0.03
Fe		0.22	0.07
B		2.29	0.11
Cu		0.18	0.07
Mn	[mg L^{-1}]	0.45	0.03
Mo		0.01	0.01
Zn		0.74	0.43

2.3. Nutrient solution analyses

Nutrient solution samples were taken weekly to ensure the accuracy of treatments. Two samples of each nutrient solution tank were taken and analyzed for nutrient concentrations (B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, NH_4^+-N, NO_3^--N, P, S, Zn and on one occasion also Cd and Pb) via inductively coupled plasma-optical emission spectrometry (ICP-OES)

Table 1
Treatment means of fertilizer amounts applied per tank over total experiment. Manufacturers are named in footers.

Fertilizer	Unit	Control RW	Control NFT	CROP + mf RW	CROP + mf NFT	Product
Nitrified urine fertilizer	[L]	0	0	30.75	32.06	uC.R.O.P. [a]
$CaNO_3$	[g]	727.15	731.33	8.80	12.04	YaraLiva Calcinit [b]
KH_2PO_4	[g]	137.44	135.81	122.41	106.57	YaraTera KRISTA MKP [b]
KNO_3	[g]	249.18	291.91	23.26	25.54	YaraTera KRISTA K PLUS [b]
KSO_4	[g]	210.67	218.06	327.53	354.24	Aqua Light Kaliumsulfat[c]
KAS (76% NH_4NO_3 + 24% $CaCO_3$)	[g]	9.19	16.93	11.66	13.79	Triferto KAS 27% [d]
Yara Krista MgS	[g]	274.43	265.85	270.85	243.02	Yara Krista MgS [b]
Fe-EDTA 13%	[g]	0.20	0.17	0.57	0.39	COMPO Fetrilon 13 [e]
Microelement stock	[g]	25.12	27.69	17.00	16.54	Yara TENSO [b]
$MnSO_4$	[g]	1.20	2.09	1.48	1.96	
KOH 20%	[L]	0.028	0.016	0.035	0.027	Potash lye [f]
H_3PO_4 3%	[L]	0.078	0.128	0.190	0.378	Phosphoric acid [f]

[a] German Aerospace Center, Cologne, Germany.
[b] YARA GmbH & Co KG, Dülmen, Germany.
[c] AQUA-LIGHT GmbH, Bramsche, Germany.
[d] Triferto, Doetinchem, Netherlands.
[e] COMPO GmbH, Münster, Germany.
[f] Carl Roth GmbH & Co KG, Karlsruhe, Germany.

and via continuous flow analysis (NO_3^--N, NH_4^+-N) as previously described in Mauerer et al. [29]. Reference solutions and standard curve are listed in the supporting information (Table S2 + S3). Chloride (Cl^-) concentrations were measured on 31st March, 25th April, 12th May and 16th June with a multi spectral spectrometer (Hach Lange, Düsseldorf/Berlin, Germany) and cuvette tests LCK 330 (Hach Lange). Nutrient concentrations of uC.R.O.P. fertilizer batches (Ca, Cl, K, Na, Mg, NH_4, NO_3, PO_4, SO_4) were measured with an ion exchange chromatograph (Metrohm, Germany: IC-System Professional 850 – Anions + Cations; anion column: Metrosep A Supp 5 – 150 × 4.0 (carbonate eluent), pre-column: Metrosep A Supp 4/5 Guard, Metrosep RP 2 Guard 3.5) and expressed in mg L^{-1}.

2.4. Calculation of fertilizer savings and recycling rates

Nutrients (N, P, K) supplied by fertilizers listed in Table 1 were summed per treatment. Recycling rates (in %) in CROP + mf treatments were calculated according to Halbert-Howard et al. [17]. For the nutrients (*i*) N, P and K the mass of the nutrient applied through uC.R.O.P. fertilizer was divided by the total mass of the nutrient applied in the treatment multiplied by 100.

$$RR_i\ (\%) = \frac{i_{uC.R.O.P.}}{i_{total}} \times 100$$

2.5. Measurement of N_2O, CO_2 and CH_4 emissions

Measurements of N_2O, CO_2 and CH_4 emissions from the root zone were done on 20th February, 27th May and 17th June. Measurement dates and date selection were constrained by COVID-19-related restrictions affecting access to analytical facilities. Gas fluxes were determined using the closed chamber method as described by Karlowsky et al. [30] for hydroponic systems. Briefly, acrylic glass chambers with two openings for tomato stems were placed around the rockwool slabs or on the NFT channels and sealed with foam rubber to obtain a closed headspace with a volume of approximately 16 L (rockwool slabs) or 32 L (NFT channels). Over a period of 1 h after closing, four gas samples were taken in 20 min intervals with a syringe through a sampling port on top of the chamber. The gas samples were analyzed on the same day by a gas chromatograph (GC 2010 Plus, Shimadzu Corporation, Kyoto, Japan) equipped with an electron capture detector (ECD) for N_2O, a thermal conductivity detector (TCD) for CO_2, and a flame ionization detector (FID) for CH_4. For the measured gas concentrations, gas fluxes were calculated using the R package "gasfluxes" [version 0.4–4 [31]], including automatic selection of the most suitable regression method (linear, robust linear, or non-linear HMR model). Input variables used were gas concentration (µmol m^{-3}, converted from ppm values according to the ideal gas law assuming SATP conditions), chamber volume (m^3), area (m^2) and time after closing the chamber (h). Because each chamber measurement always included two plants, the area (*A*) to which the fluxes referred was calculated as: $A = 2 \times Dp^{-1}$, with *Dp* being the actual plant density in the greenhouse (1.38 m^{-2}).

2.6. Determination of physiological plant development and fruit yield

Measurements of plant height and leaf area were performed on 7th February, 13th February, 19th February, 26th February and 4th March. Plant height was also measured on 19th June with a flexible metal measuring tape from the surface of the rockwool cube to the apical meristem at the top of the plant [32]. Area of fully developed leaves (leaf length > 20 cm) was determined by measuring the length (L) and the half width (0.5 W) and calculating the leaf area according to coefficients specifically determined for the location Berlin and the variety Avalantino F1 with the formula

$$\text{leaf area} = -61.6998527909226 + 0.35112554472495605\ (L*W)$$

Harvest started on 14th April and was repeated every six days until 19th June. Fruit yield was determined directly after harvest by weighing all marketable fruits and non-marketable fruits per plant separately. Fruits were declared non-marketable if they were parthenocarpic or showed any deviation such as bruising due to blossom-end rot or calcium oxalate accumulation or if they were still green on the last day of harvest.

2.7. Calculation of nutrient use efficiencies and water use efficiencies

Nutrient use efficiencies were calculated as partial productivity factors [33] for nitrogen, potassium, and phosphorus. For each nutrient and tank, marketable fruit yield [g] was divided by total nutrient mass applied over the experimental period minus the mass of the nutrient that was discharged. Water use efficiencies were calculated by dividing marketable fruit yield per tank [g] by use of demineralized water [L] over the course of the experiment.

2.8. Analysis of mineral elements, heavy metals and secondary compounds in plant tissue

For leaf tissue mineral content analysis, leaf samples of the three center plants of each row were taken on 31st March, 9th May, 16th June. Two leaves per plant were removed from the bottom of the canopy (16th-20th leaf). Fruit samples were taken on 14th April, 2nd May, 19th May and 19th June. Samples of roots from NFT channels and 1 m of stem (1 – 2 m from apical meristem) of all treatments were taken on 19th June. For all samples fresh weight was determined, samples were oven dried at 80 °C until constant weight, weighed again and ground to a fine powder using a ball mill (MM 400, Retsch, Haan, Germany). Ca, Cd, Cr, Cu, Fe, K, Mg, Na, Ni, P, Pb, S, and Zn analysis was then performed via microwave digestion and ICP-OES as previously described in Mauerer et al. [29] (for reference solution concentrations and measurement specifics see Table S4). The same applies to carbon and nitrogen content analysis via elemental analyzer.

For amino acid analysis, three leaves were taken from each of the three center plants per channel. Leaves were taken from the top of the plant, starting from the youngest leaf that reached a petiole length above 30 cm, and were immediately frozen in liquid nitrogen. Freeze-dried and ground (Retsch, Haan, Germany) samples (10 mg) were extracted with 250 µl of 70% methanol. 5 µl of Norleucin as internal standard was added. Samples were sonicated for 10 min on ice and centrifuged at 10.000 rpm at 4 °C. Supernatant was collected in a new reaction tube. The remaining pellet was twice re-extracted with 100 µl 70% methanol and centrifuged. The combined extracts were filled up to 400 µl and 100 µl protein precipitation solution was added (membraPure GmbH, Henningsdorf, Germany). After incubation for 60 min at 4 °C the samples were centrifuged at 12.000 rpm and 4 °C. The supernatant was transferred to Spin-X filter and centrifuged at 3.000 rpm. The flow through was analyzed with an ARACUS amino acid analyzer (membraPure GmbH, Henningsdorf, Germany) according to the manufacture protocol/program of 70 min. Peak identification and quantification of single amino acids was done by using the internal standard as reference.

Fruit samples for secondary compound analysis were taken on 19th May and 18th June, weighed and immediately frozen in liquid nitrogen. Samples were then freeze-dried, weighed again after constant weight was reached and ground to a fine powder (MM400, Retsch, Haan, Germany). Flavonoids and phenolic acids, and also carotenoids, were extracted and analyzed via ultra-high performance liquid chromatography as previously described in Mauerer et al. [29] with the modification that reference compounds for carotenoid analysis were trans-lycopene, trans-β-carotene, α-carotene, cis-β-carotene and lutein.

2.9. Statistical analysis

Except for gas flux measurements, all data were analyzed with SAS

9.4 software for Windows (SAS Institute Inc., Cary, NC, USA). Each of the 12 nutrient solution tanks was counted as a biological replicate and represented a combination of the two factors tested (n = 3). The statistical model for analysis of variances considered the nutrient solution treatment (Control, CROP + mf) and the hydroponic system (RW, NFT) and their interaction as fixed treatment factors and the random variation between tanks. Normal distribution of residuals was tested with Shapiro-Wilk test and graphic representation of residuals. Homogeneity of residual variances was evaluated by comparing models with a single residual variance for all groups to models allowing separate residual variances for different nutrient solution treatments or hydroponic systems. Model selection was based on Akaike's Information Criterion (AIC). For each response variable, the variance structure with the lowest AIC was retained for inference. For each trait, least squares means and standard errors of the means were estimated, and comparisons between treatments were performed with Tukey's HSD ($\alpha \leq 0.05$).

Statistical analyses on gas fluxes were performed in the R software (version 3.6.3). Linear mixed-effects models (LMMs) were done using the R package "lme4" [version 1.1–21 [34]]. LMMs on gas fluxes included the hydroponic system (rockwool or NFT), the nutrient solution treatment (CROP + mf or Control) and the sampling date as fixed factors, while the nutrient solution tank was set as random intercept. If a significant effect was found in one LMM, a Tukey post-hoc test was performed on its results using the R package "emmeans" [version 1.6.0 [35]], with a level of significance of $\alpha = 0.05$ and the Holm correction for multiple comparisons.

3. Results

3.1. Recycling rates and environmental impact

Recycling rates in CROP + mf treatments calculated for the experiment are for nitrogen 94.65% in RW system, and 93.99% in NFT system, for potassium 12.45% in RW and 12.65% in NFT and for phosphorus 6.79% in RW and 7.05% in NFT (calculation data in supporting information, Table S5). The calculated nutrient use efficiencies for these nutrients were comparable or higher for the CROP + mf treatment (results in supporting information, Table S6). The average pH, which was constantly adapted during the experiment was comparable for all treatments (Control RW: 6.03 ± 0.01, Control NFT: 6.1 ± 0.03; CROP + mf RW: 6.04 ± 0.01; CROP + mf NFT: 6.13 ± 0.04).

Fig. 1 shows nitrous oxide (N_2O) emissions for all treatments at three dates during the experiment. No significant differences were observed between nutrient solution treatments (Control and CROP + mf), but the means of RW treatments were consistenly higher than of NFT treatments.

CO_2 emissions show no clear pattern on three different sampling dates and significant differences only on the last measuring date, with Control RW having the highest emissions and both NFT treatments equally low emissions (see supporting information, Fig. S4). CH_4 emissions were not detected. Water usage was increased for NFT treatments and thus the respective water use efficiencies were lower but did not differ between nutrient solution treatments (see supporting information, Table S7).

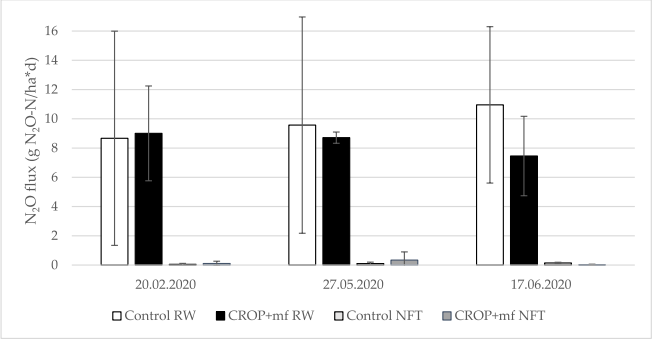

Fig. 1. Nitrous oxide emissions on three sampling dates. White bars show means for Control RW replications, black bars CROP + mf RW, light grey bars Control NFT and dark grey bars CROP + mf NFT respectively. Error bars indicate standard deviation. A significant difference ($p \leq 0.05$) was observed between RW and NFT treatments but not between nutrient solution treatments.

3.2. Nutrient solution salinity

Average EC values were higher in NFT treatments and within the cultivation system they were higher in CROP + mf treatments (Control RW: 2.29 dS m^{-1}; CROP + mf RW: 2.61 dS m^{-1}; Control NFT: 2.74 dS m^{-1}; CROP + mf NFT 3.29 dS m^{-1}). More extensive data are found in supporting information (Fig. S5). Higher EC values correlate with water usage.

Sodium accumulation is shown in Fig. 2. Chloride accumulation in CROP + mf tanks showed levels of up to 176 mg Cl^- L^{-1} (5 mM) and were on average much higher in CROP + mf treatments (87.2 mg L^{-1}) than in Control treatments (1.5 mg L^{-1}) (data in supporting information, Table S8). Sulphur also accumulated in the nutrient solution up to a level of 649 mg L^{-1} with the highest levels found in CROP + mf NFT (in supporting information, Fig. S6).

3.3. Yield and morphological parameters

Marketable fruit yield did not differ between any of the treatments on any harvest date or in total (Fig. 3). The same was true for yield of green fruits at the end of the experiment and non-marketable fruit yield (Table 3). Plant height was higher in CROP + mf treatment after one month, on March 4th 2020, but not in the first month of the experiment or at the end of the experiment (supporting information, Fig. S7+S8). No significant differences in calculated leaf area were detected during the first month of the experiment (supporting information Figure S9).

3.4. Mineral elements in plant tissue

Mineral element contents in fruit tissue are shown in Table 4. A lower N content in fruit tissue in CROP + mf plants was observed on three of four sampling dates, but no significant differences were found regarding N content in leaf, stem, or root tissue (data found in the supporting information, Table S9 + S10). The same is true for phosphorus levels and for sulphur levels, which both are also significantly lower in three sampling dates in CROP + mf treatment. Sodium levels are consistently elevated in CROP + mf fruits on all four sampling dates. Zink levels are higher in NFT system fruits on all sampling dates.

Results of metal content analysis regarding chromium, copper, lead and cadmium can be found in the supplementary material (Tables S9 + S11). A significant effect was only observed for nutrient solution treatment in root tissue, where copper and cadmium contents were higher in

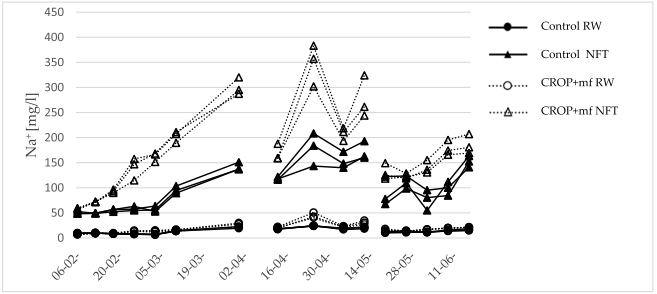

Fig. 2. Sodium accumulation in nutrient tanks over the course of the experiment (triangle: CROP + mf, circle: Control, dotted lines: NFT, full lines: RW). Nutrient solution was replaced on April 2nd and May 15th.

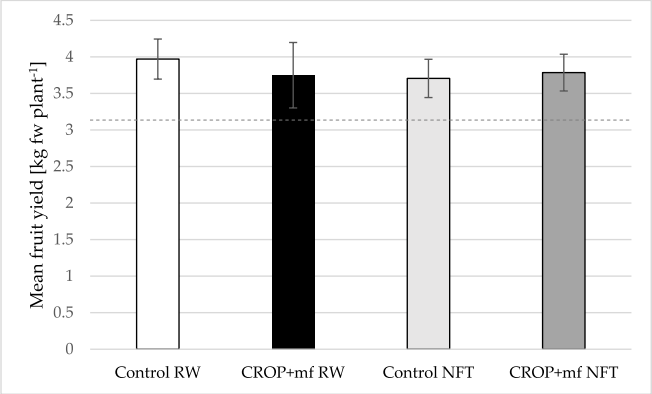

Fig. 3. Means of marketable fruit yield per plant. White bars show means for Control RW replications, black bars CROP + mf RW, light grey bars Control NFT and dark grey bars CROP + mf NFT respectively. Error bars indicate standard deviation, dashed line indicates Control RW mean – HSD of Tukey's HSD. No significant differences ($p \leq 0.05$) after Tukey's HSD.

Table 3
Means of marketable fruit yield, green fruit at the end of experiment and non-marketable fruit yield per plant with standard deviations.

Treatment	Marketable fruit yield		Green fruit at end of experiment		Non-marketable fruit yield	
	[kg fw plant^{-1}]					
	Mean	SD	Mean	SD	Mean	SD
Control RW	3.97	0.27	2.16	0.23	0.10	0.11
CROP + mf RW	3.71	0.26	2.06	0.19	0.04	0.05
Control NFT	3.75	0.45	2.25	0.29	0.04	0.03
CROP + mf NFT	3.79	0.25	2.19	0.07	0.02	0.01

Control treatment. Cadmium could not be detected in other plant tissues and copper levels were not significantly different in other tissues. Chromium levels did not differ in root, leaf or fruit tissue, except for one date where NFT plants showed significantly higher levels in leaf tissue. Nickel was not detected in plant tissues except the roots and lead content did not differ in leaf tissue and it was not detected in fruit tissue.

3.5. Secondary metabolites and amino acids

Carotenoids detected in fruit tissue were lycopene, lutein, α-carotene, trans-β-carotene, cis-β-carotene and trans-lycopene (Table S12). No significant differences were found in carotenoid contents for nutrient solution treatments or hydroponic systems, except for trans-β-carotene contents. On the first sampling date, levels of trans-β-carotene were significantly higher in Control treatments (Control RW: 319.72 ± 39.28 µmol g^{-1} dw; Control NFT: 303.35 ± 41.41 µmol g^{-1} dw; CROP + mf RW: 265.87 ± 32.30 µmol g^{-1} dw; CROP + mf NFT: 260.64 ± 15.05 µmol g^{-1} dw), which was not observed on the second sampling date, where levels in CROP + mf RW were elevated in CROP + mf NFT treatment (Control RW: 299.66 ± 4.00 µmol g^{-1} dw, Control NFT: 290.38 ± 28.75 µmol g^{-1} dw; CROP + mf RW: 309.15 ± 15.47 µmol g^{-1} dw; CROP + mf NFT: 255.44 ± 4.79 µmol g^{-1} dw).

Flavonoids and phenolic acids detected in fruit tissue and their contents in fruit tissue are listed in the supporting information (Table S13). Caffeic acid hexoside, ferulic acid hexoside, quercetin rutinoside and naringenin derivate contents were significantly higher in CROP + mf fruit tissue, with some significant effects of interaction between the two treatments factors. Contents of caffeoyl quinic acid hexoside and caffeoyl quinic acid dihexoside were significantly higher in Control fruit tissue on both sampling dates, again with some significant effects of interaction between the two treatments factors. Other significant effects could not be observed on both sampling dates.

Amino acids and their contents detected in leaf tissue are shown in the supporting information (Table S14). Phosphoethanolamine contents were elevated in leaf tissue of Control plants than in CROP + mf plants (Control RW: 1.25 ± 0.19 µmol g^{-1} dw; Control NFT: 1.25 ± 0.24 µmol g^{-1} dw; CROP + mf RW: 0.98 ± 0.17 µmol g^{-1} dw; CROP + mf NFT: 0.98 ± 0.09 µmol g^{-1} dw). Serine contents were significantly higher in CROP + mf RW plants than in Control NFT plants (Control RW: 4.35 ± 0.54 µmol g^{-1} dw, Control NFT: 3.60 ± 0.68 µmol g^{-1} dw; CROP + mf RW: 6.68 ± 1.68 µmol g^{-1} dw; CROP + mf NFT: 5.80 ± 0.66 µmol g^{-1} dw). Lysine content was increased in leaf tissue of NFT plants (Control RW: 0.48 ± 0.11 µmol g^{-1} dw, Control NFT: 0.68 ± 0.27 µmol g^{-1} dw; CROP + mf RW: 0.29 ± 0.26 µmol g^{-1} dw; CROP + mf NFT: 0.70 ± 0.24 µmol g^{-1} dw).

4. Discussion

4.1. Nitrified urine fertilizer management and influence on yield and plant physiological development

In our study, we found no significant differences in marketable and total yield and plant physiological development when replacing 94.65% and 93.99% of nitrogen from mineral fertilizer with nitrogen from nitrified urine fertilizer in the RW and the NFT system, respectively. Due to the composition of the tested nitrified urine fertilizer uC.R.O.P., this included a replacement of 12.45% of potassium in RW and 12.65% of potassium in NFT and for phosphorus 6.70% in RW and 7.05% in NFT. As nutrient use efficiencies for these nutrients were comparable or higher for the CROP + mf treatment, we assume these recycling factors to be reliable. uC.R.O.P. fertilizer is very high in calcium, which leads to a higher precipitation of phosphate during its production process and thus to a lower relative phosphorus content than other nitrified urine fertilizers. For example, Aurin fertilizer has a N:P ratio of ~22 [12], while in the uC.R.O.P. batches we found a N:P ratio of ~60. On the other hand, the high grade of nitrification, which is achieved by the addition of calcium bicarbonate, increases storability and diminishes the risk of ammonium toxicity in tomato production. The results of this study were achieved by adjusting also the calcium concentration of the Control treatment to 160-180% of recipe (180-200 mg Ca L^{-1}) and increasing the magnesium nutrition to 60 mg/L to avoid magnesium deficit due to high calcium content. The carbon footprint for Mg from mineral fertilizer is estimated to be a third of the carbon footprint for N from mineral fertilizer [36,37] and the N:Mg ratio in the nutrient solution recipe is 6.8. However, this 2.5-fold increase in magnesium fertilizer usage should be considered when comparing overall carbon footprints of nutrient solution treatments. Furthermore, according to the threshold found by Chrétien et al. [15], where fruit size was reduced at NaCl concentration of 12 mmol, the nutrient solution drain was exchanged twice during the experiment, when sodium levels reached this threshold (276 mg Na^+ L^{-1}) and sulphur levels of 12.5 mM (400 mg L^{-1}) in CROP + mf NFT treatments. Sulfate levels were so high, as potassium sulfate was used instead of potassium nitrate. The increased use of potassium sulfate should also be considered when estimating carbon footprints of the fertilizer treatments. According to Brentrup et al. [2] the reference carbon footprint for potassium sulfate in Europe is 0.12 kg CO_2-eq kg K_2SO_4 as opposed to 0.25 kg CO2-eq kg KCl for potassium chloride, which is used in the production of potassium nitrate.

Regarding overall salinity, Li et al. [14] found that yield decreased by 5.1% for each dS m^{-1} in excess of 2 dS m^{-1}. This relationship was not observed in the present study in which higher transpiration (indicated by increased water usage) in NFT treatments increased average EC values to 3.29 dS m^{-1}. Assuming a 5.1% yield decrease for each dS m^{-1}, a yield decrease of 1.6%, 2.3% and 5.3% would have been expected for CROP + mf RW, Control NFT and CROP + mf NFT treatments respectively in comparison to the Control RW treatment. Even if a trend of yield reduction can be observed in the corresponding mean values, no

Table 4
Mineral elements in fruit tissue according to ICP-OES and (mean + - standard deviation, letters indicate significant effects of interactions according to Tukey's HSD ($p \leq 0.05$) within sampling date). If present, significant effects of nutrient solution treatments or cultivation system are highlighted below each date and parameter.

		14.04.20				02.05.20				19.05.20				19.06.20			
Element	Unit	Control RW	CROP+mf RW	Control NFT	CROP+mf NFT	Control RW	CROP+mf RW	Control NFT	CROP+mf NFT	Control RW	CROP+mf RW	Control NFT	CROP+mf NFT	Control RW	CROP+mf RW	Control NFT	CROP+mf NFT
C	[%]	46.60 ±0.16 **a**	46.30 ±0.15 **a**	46.48 ±0.10 **a**	46.17 ±0.23 **a**	45.91 ±0.56 **a**	46.33 ±0.30 **a**	46.03 ±0.46 **a**	46.05 ±0.28 **a**	46.11 ±0.26 **a**	46.03 ±0.46 **a**	46.20 ±0.38 **a**	45.72 ±0.51 **a**	46.46 ±0.73 **a**	45.85 ±0.68 **a**	46.19 ±0.24 **a**	46.40 ±0.39 **a**
		Control: a, CROP+mf: b															
N		2.96 ±0.14 **ab**	2.84 ±0.08 **ab**	3.08 ±0.10 **a**	2.77 ±0.05 **b**	2.77 ±0.22 **a**	2.77 ±0.13 **a**	2.86 ±0.16 **a**	2.56 ±0.02 **a**	2.59 ±0.14 **ab**	2.43 ±0.14 **ab**	2.68 ±0.07 **a**	2.32 ±0.09 **b**	2.08 ±0.04 **a**	2.05 ±0.10 **a**	2.05 ±0.12 **a**	1.78 ±0.11 **b**
		Control: a, CROP+mf: b								**Control: a, CROP+mf: b**				**Control: a, CROP+mf: b RW: a, NFT: b**			
P	[g kg^{-1} dw^{-1}]	7.29 ±0.24 **a**	6.85 ±0.16 **ab**	7.27 ±0.06 **a**	6.54 ±0.16 **b**	6.57 ±0.41 **ab**	5.96 ±0.22 **b**	6.47 ±0.25 **a**	5.71 ±0.23 **b**	6.12 ±0.35 **a**	5.25 ±0.29 **ab**	6.07 ±0.45 **a**	4.98 ±0.19 **b**	4.52 ±0.19 **a**	4.36 ±0.33 **a**	4.47 ±0.13 **a**	4.34 ±0.05 **a**
		Control: a, CROP+mf: b				**Control: a, CROP+mf: b**				**Control: a, CROP+mf: b**							
K		54.41 ±1.56 **a**	55.62 ±2.70 **a**	54.67 ±0.80 **a**	54.48 ±1.41 **a**	51.68 ±1.55 **a**	52.79 ±1.94 **a**	51.68 ±0.72 **a**	52.06 ±1.17 **a**	50.36 ±0.96 **a**	50.86 ±2.18 **a**	50.95 ±2.07 **a**	49.15 ±0.60 **a**	45.32 ±0.77 **a**	46.56 ±0.41 **a**	45.56 ±0.73 **a**	44.95 ±0.84 **a**
Na		0.15 ±0.01 **b**	0.34 ±0.02 **a**	0.14 ±0.03 **b**	0.34 ±0.02 **a**	0.17 ±0.03 **b**	0.42 ±0.01 **a**	0.16 ±0.01 **b**	0.44 ±0.04 **a**	0.18 ±0.01 **b**	0.44 ±0.02 **a**	0.17 ±0.02 **b**	0.49 ±0.04 **a**	0.20 ±0.01 **b**	0.59 ±0.08 **a**	0.26 ±0.12 **b**	0.57 ±0.08 **a**
		Control: b, CROP+mf: a				**Control: b, CROP+mf: a**				**Control: b, CROP+mf: a**				**Control: b, CROP+mf: a**			
Ca		0.61 ±0.04 **a**	0.72 ±0.11 **a**	0.69 ±0.12 **a**	0.82 ±0.07 **a**	0.60 ±0.04 **a**	0.78 ±0.21 **a**	0.73 ±0.14 **a**	0.76 ±0.07 **a**	0.85 ±0.15 **a**	0.95 ±0.07 **a**	0.83 ±0.09 **a**	0.87 ±0.01 **a**	0.85 ±0.13 **b**	0.92 ±0.03 **b**	1.01 ±0.10 **ab**	1.17 ±0.07 **a**
														RW: b, NFT: a			
Mg		1.59 ±0.01 **a**	1.54 ±0.06 **a**	1.63 ±0.06 **a**	1.57 ±0.06 **a**	1.72 ±0.22 **a**	1.59 ±0.10 **a**	1.77 ±0.13 **a**	1.55 ±0.06 **a**	1.86 ±0.21 **ab**	1.59 ±0.12 **ab**	1.82 ±0.08 **a**	1.55 ±0.07 **b**	1.50 ±0.04 **a**	1.46 ±0.12 **a**	1.56 ±0.03 **a**	1.50 ±0.004 **a**
										Control: a, CROP+mf: b							
S		2.15 ±0.02 **a**	1.97 ±0.01 **b**	2.18 ±0.005 **a**	1.90 ±0.02 **c**	2.07 ±0.15 **ab**	1.90 ±0.05 **ab**	2.16 ±0.10 **a**	1.83 ±0.05 **b**	2.03 ±0.18 **a**	1.75 ±0.10 **a**	2.11 ±0.18 **a**	1.72 ±0.04 **a**	1.69 ±0.06 **a**	1.63 ±0.17 **a**	1.77 ±0.06 **a**	1.78 ±0.07 **a**
		Control: a, CROP+mf: b RW: a, NFT: b				**Control: a, CROP+mf: b**				**Control: a, CROP+mf: b**							
Fe	[mg kg^{-1} dw^{-1}]	67.36 ±5.76 **a**	58.87 ±5.44 **a**	86.64 ±40.03 **a**	55.69 ±3.69 **a**	53.37 ±9.83 **a**	51.34 ±4.79 **a**	56.99 ±10.74 **a**	43.89 ±3.25 **a**	44.97 ±4.86 **a**	44.57 ±7.11 **a**	43.46 ±3.96 **a**	55.35 ±46.59 **a**	49.28 ±12.80 **a**	58.52 ±19.55 **a**	54.37 ±31.95 **a**	40.63 ±1.50 **a**
Zn		27.76 ±0.84 **b**	26.64 ±1.34 **ab**	33.39 ±1.10 **a**	32.75 ±4.13 **ab**	21.24 ±2.10 **b**	20.30 ±1.37 **b**	26.96 ±2.17 **a**	26.26 ±1.30 **a**	19.46 ±3.10 **ab**	16.11 ±2.47 **b**	23.71 ±3.59 **a**	21.25 ±1.63 **ab**	14.12 ±0.58 **a**	14.45 ±0.71 **a**	20.24 ±3.83 **a**	17.00 ±0.60 **a**
		RW: b, NFT: a				**RW: b, NFT: a**				**RW: b, NFT: a**				**RW: b, NFT: a**			

significant effects on yield were found.

We expected the amino acid proline to be upregulated as a marker for osmotic stress, which was not confirmed. Looking at amino acids in leaf tissue, Serine content was increased in CROP + mf RW plants in comparison to Control NFT plants. Apart from being relevant for protein synthesis, serine formation and transformation are key steps in the photorespiratory pathway. Serine can furthermore contribute to the synthesis of compatible solutes like glycine betaine, which help in osmoregulation of cells. Phosphoethanolamine was detected in amino acid analysis and significantly higher in leaf tissue of Control plants than in CROP + mf plants. Phosphoethanolamine is a precursor in the synthesis of phosphatidylethanolamine, a phospholipid and thus contributes to structural integrity and fluidity of cell membranes it is also a signalling molecule in cell growth, differentiation, and death. In conclusion, it appears that CROP + mf plants adapted to higher salt concentrations by a change in the amino acid profile but did not experience osmotic stress which would have led to proline upregulation.

4.2. Carbon footprint

The recycling rates calculated from saved mineral fertilizer translate to a reduction in carbon emission. For this evaluation, the carbon footprint associated with nitrified urine fertilizer production should be compared with the carbon footprint of treating the urine in a wastewater treatment plant. In addition, the avoided emissions from mineral fertilizer production should be considered, as demonstrated in a previous study [29]. As the nitrified urine fertilizer uC.R.O.P. was obtained from a lab scale plant, a thorough analysis of production carbon footprint, as e. g. in Faust et al. [38], was not possible because many factors such as scaling of production and transport of the liquid fertilizer influence this evaluation. These calculations should be performed as soon as data are available.

Regarding greenhouse gas emissions from the rootzone, it can be concluded that the use of nitrified urine fertilizer does not have a significant impact on the emissions of nitrous oxide (N_2O), carbon dioxide (CO_2) or methane (CH_4) from the root zone. However, a significant impact has been observed for RW treatments, which consistently

showed higher N_2O emissions than NFT treatments. This is likely caused by nitrification and denitrification processes by microorganisms on the high surface area of the rockwool substrate, providing favorable micro-niches with varying oxygen concentrations. The observed magnitude of N_2O emissions from RW is well in line with previous findings for hydroponic cultivation of tomato on rockwool during the spring season [30].

4.3. Nutrient management in different hydroponic systems

It was observed that accumulation of Na^+ was higher in NFT systems, both regarding concentration and absolute content, while accumulation of Cl^- ions was higher in rockwool substrate. The higher sodium accumulation in NFT systems can be explained by the cation exchange capacity (CEC) of the rockwool substrate, which, even if lower than soil, leads to adsorption of cations [39]. The higher chloride concentration and content in drip-irrigation systems, could either be caused by a release of chloride ions from rockwool substrate from the manufacturing process. Another explanation could be a higher uptake of chloride by plants in NFT systems. Unfortunately, it was not possible to analyze chloride content in plant tissue in this study. As sodium accumulation was the limiting factor of nutrient solution recirculation in this study, it can be assumed that growth substrates act as a buffer in this context, but accumulation in the substrate has to be considered when reusing or recycling the substrate. Concentrations of SO_4^- ions was also higher in NFT systems, but mainly due to possible evaporation from these systems. Absolute SO_4^- contents per nutrient solution tank were not affected by hydroponic system type.

4.4. Product quality

Looking at the mineral contents in fruits, the increased sodium levels in CROP + mf fruits were expected, as sodium level in the nutrient solution was also elevated (Fig. 2). Sodium is an essential nutrient for humans. Na^+ and Cl^- ions in combination improve the sensory properties of food consumed by humans [40]. As long as marketable yield is not reduced, an elevated concentration of sodium and chloride in tomato fruit could thus be desirable. This speculated effect on fruit taste needs to be evaluated in further studies. Despite high concentrations of sodium and calcium in the CROP + mf nutrient solution and a significant reduction of potassium content in root tissue, no influence on potassium nutrition was observed in other plant tissues. However, although nitrogen (N) contents in root, stem, and leaf tissue indicate no significant difference, the lower N content in CROP + mf fruit tissue hints at a possibly reduced N uptake by these plants. N contents in leaf tissue in all treatments are within sufficiency ranges [41], except CROP + mf NFT and Control NFT on one date, yet at the lower end. Sulphur (S) levels were also often lower in CROP + mf fruits, even though sulfate (SO_4^-) concentrations in nutrients solutions were generally higher in CROP + mf treatments (Fig. S4) due to the increased use of sulfate fertilizers. Leaf S content was not affected by the treatments (Table S9), and sulphur levels in leaves were much higher than sufficiency range of 4-8 g/kg dw [41] in all treatments. Phosphorus levels in CROP + mf treatments were also often significantly lower than in Control fruits and sometimes leaves, although sufficiency levels in leaves [41] were reached in all treatments. Nitrate, sulfate and phosphate uptake and translocation to the fruit might have been influenced by higher chloride uptake of CROP + mf fruits, which could not be analyzed in this study. This is supported by the higher chloride contents in CROP + mf nutrient solutions (Table S8) and the knowledge that N, P and S levels were the same or even higher in CROP + mf treatments. Zink contents in fruit tissue of NFT system fruit were significantly higher than in rockwool, but the same effect could not be shown for leaf tissue, except on one of three sampling dates. This might be due to a fixation of zinc ions in the rockwool substrate, which can be explained by its CEC already mentioned in section 4.3. We suspect that the effect might be more prominent for zinc due to its chemical divalence paired with low concentration in the nutrient solution.

Flavonoids and phenolic acid contents and their composition are relevant for the taste of tomato fruits and can also provide health benefits due to their antioxidant activity. While total flavonoid and phenolic acid content in fruit tissues did not differ, the observed difference in secondary metabolite profiles in the fruit tissue of the nutrient solution treatments is very prominent. The higher contents of caffeic acid hexoside, ferulic acid hexoside, quercetin rutinoside and naringenin derivate in CROP + mf fruit tissue indicate that these plants experienced an environmental stress. It has been shown that the synthesis of hydroxycinnamic acid derivatives, such as caffeic acid and ferulic acid, increases under stress conditions [42]. Quercetin rutinoside contributes to the greatest extent to the antioxidant capacity of tomatoes [43]. And naringenin has been shown to alleviate short-term osmotic and salinity stress in bean (*Phaseolus vulgaris*) plants [44]. Thus, as already mentioned in section 4.1 the higher salinity has probably caused a metabolic shift in CROP + mf plants. Carotenoids are also antioxidants and thus beneficial to human health. For carotenoid contents no significant effects that were repeatable on both sampling dates were found.

One secondary objective of this study was to evaluate whether tomato plants fertilized with nitrified urine accumulate lower concentrations of heavy metals than plants fertilized with mineral fertilizers. Chromium contents in root, leaf and fruit tissue were not found to be different between the treatments. Levels of copper detected in fruit tissue were far below the maximum copper content of 5 mg/kg fresh weight for tomatoes [19] and no significant effects were found between treatments both in leaf and in fruit tissue. Lead contents did not differ in leave tissue and it was not detectable in fruit tissue. Cadmium was not detected in leaf or fruit tissue. However, copper and cadmium contents in root tissue showed a significantly higher accumulation in Control nutrient solution treatment. Results regarding lead contents were inconclusive even though the same tendency was observed. Our findings suggest that the mineral fertilizers, which were applied in higher doses to the Control nutrient solution treatment contain copper, cadmium and lead. This has been shown for phosphate fertilizers [45], 8% of which were replaced in this study. Apparently, a low translocation of lead and cadmium in tomatoes prevents an accumulation in leaf and fruit tissue. Still, especially when nutrients are recycled from biomass, a bioaccumulation of these heavy metals seems possible and further investigations are needed. Our results show that the use of nitrified urine fertilizers may reduce the introduction of the heavy metals cadmium and lead into the agricultural production chain.

4.5. Study limitations and future research

Several limitations of this study should be considered when interpreting the results. First, the experiment was conducted with three independent nutrient solution tanks per combination of nutrient solution treatment and hydroponic system, which limits the statistical power. While each tank with its 5 tomato plants represents the appropriate biological replicate in recirculating hydroponic systems, larger-scale experiments with additional independent systems would strengthen the robustness of the findings.

Second, the uC.R.O.P. fertilizer used in this study originated from a pilot-scale production facility. Consequently, greenhouse gas emissions and economic costs associated with fertilizer production are not directly representative of commercial-scale implementation. Future work should therefore include full life cycle assessments and techno-economic analyses based on industrial-scale fertilizer production and distribution scenarios.

Third, the experiment focused on a single and shortened cultivation cycle. Although sodium accumulation thresholds and nutrient solution management strategies were evaluated, longer-term studies are required to assess repeated nutrient solution reuse in tomato cultivation and also the possible reuse or recycling of growth substrates, including the

potential accumulation of sodium, chloride and other ions in rockwool or alternative substrates.

Further studies are needed to compare N_2O emissions from NFT and RW systems and to analyze the underlying factors for the significant effects observed in this study.

Finally, product quality assessment was limited to mineral composition and selected secondary metabolites. Future studies should investigate consumer-relevant quality traits such as sensory properties and taste perception, particularly because elevated sodium concentrations in fruit may influence flavour. Accumulation of pharmaceuticals and hormones was not tested in the present study. Source separation and activated-carbon treatment of urine fertilizers could reduce the release of pharmaceuticals and hormones into water bodies and limit their transfer into agricultural production systems. However, when using the nitrified urine fertilizers in a circular system where biomass and nutrients are directly recycled, bioaccumulation of traces of these compounds should be also further investigated.

5. Conclusion

With the results of this study, we show that nitrified urine fertilizers have great potential to reduce the mineral fertilizer consumption of hydroponic tomato production. This type of recycling fertilizer can be a suitable and sustainable alternative for hydroponic tomato producers as soon as it is legally approved in the country of production. The accumulation of undesirable ions, mainly Na^+ from nitrified urine and possibly SO_4^- from mineral fertilizers, has to be considered when managing nutrient solutions containing nitrified urine fertilizers. Marketable yield and quality of product in this study showed no significant reduction, when sodium accumulation was monitored and did not surpass a threshold of 12 mM. Depending on the calcium content of the fertilizer, cation nutrient ion levels in the nutrient solution, such as magnesium levels, might have to be adapted. More research is necessary to determine at which dimension of scaling, production and use of this nitrified urine fertilizer can reduce overall production carbon dioxide emissions. Yet, by source-separating urine and local use of the resulting fertilizer in hydroponic tomato production, an overall reduction of carbon footprint in greenhouse vegetable production is likely. In addition, a reduction of heavy metals entering the agricultural chain and a reduction of pharmaceuticals and hormones polluting water bodies can be expected.

Regarding the comparison of NFT with drip-irrigation on rockwool-substrate, we found that the buffering capacity, but also possible release of chloride ions of rockwool substrates as well as the higher evaporation in NFT systems have to be considered when working with nitrified urine fertilizers.

CRediT authorship contribution statement

Mareike Mauerer: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **T. Rocksch:** Conceptualization, Methodology, Supervision, Writing – review & editing. **S. Karlowsky:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **I. Mewis:** Data curation, Formal analysis, Methodology, Writing – review & editing. **G. Bornemann:** Data curation, Formal analysis, Funding acquisition, Resources, Writing – review & editing. **U. Schmidt:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mareike Mauerer reports financial support was provided by Stiftung Industrieforschung. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2026.103178.

Data availability

Data will be made available on request.

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