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Microbiological hygiene and food safety assessment of urban aquaponic farming☆

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ABSTRACT

Aquaponic production presents a promising approach in developing sustainable (urban) food systems, through combined production of plants and aquatic organisms for food. A commercial aquaponic farm was subjected to a longitudinal microbiological assessment of hygiene and food safety. Foodborne pathogenic bacteria (*Salmonella* spp., and *Listeria monocytogenes*), indicator bacteria (generic *E. coli*, coliforms, and *Enterobacteriaceae*) and total plate counts were determined during two distinct two-month production periods, focused on basil production from seed to mature plant and all water streams composing the irrigation water. The results indicated no direct food safety concerns to consumers, with neither *Salmonella* spp., nor *Listeria monocytogenes* detected on the ready-to-market basil leaves. The soilless substrate and irrigation water were identified as major risk factors for introducing and spreading foodborne pathogenic bacteria within the aquaponic environment. Overall, *E. coli* was present (LOD 1 CFU/100 mL or 10 CFU/g) in 21.1 % of samples and *Salmonella* spp. was detected in 8 out of 94 analyses. Generic *E. coli* was not a suitable marker for *Salmonella* spp. presence in irrigation water within the aquaponic farm. Strong correlations were found between *Enterobacteriaceae* and coliforms in water samples, however, elevated levels were not linked to positive *Salmonella* spp. detection. To mitigate microbiological food safety risks in aquaponics, the use of fit-for-purpose water, establishing a water quality monitoring plan, implementing effective UV treatment and applying appropriate cleaning and disinfection protocols are recommended. The implementation of tailored good agricultural practices (GAP) is key to ensure safe food production within aquaponic farming.

1. Introduction

Food systems are impacted globally by various drivers of change, such as climate change, scarcity of natural resources, and urbanization (FAO, 2017, 2022). International organisations call for action to build more sustainable food production systems, managed by resilient agricultural practices (FAO, 2021; UN, 2023). In response to these drivers, Controlled Environment Agriculture (CEA) production systems, such as (indoor) vertical farming, hydroponics, and aquaponics, have emerged. Compared with conventional soil-based agriculture, CEA benefits from stable environmental conditions, e.g. light, temperature, and humidity, enabling yield optimization and improved resource efficiency (Benke and Tomkins, 2017).

Aquaponics is defined as a production system of aquatic organisms and plants where the majority (> 50 %) of nutrients sustaining the optimal plant growth derives from waste originating from feeding the aquatic organisms (Palm et al., 2018). In the framework of food production in CEA, aquaponics combines recirculating aquaculture with indoor plant cultivation, relying on three groups of living organisms: aquatic animals, plants, and micro-organisms (Somerville et al., 2014).

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The biological nitrification process and related microbiota, thoroughly described by Kasozi et al. (2021), make the link possible between aquaculture and horticulture units. The aquacultural wastewater contains ammonia compounds (NH_3 and NH_4^+), excreted by fish after ingestion of protein in fish feed, which are converted to nitrate (NO_3^-) and promote plant growth. Aquaponic systems operate either decoupled, when the aquacultural wastewater flows unidirectional from the fish tanks to the horticulture unit, or coupled, when the spent irrigation water is reused in the aquaculture unit (Pérez-Urrestarazu et al., 2019). The latter offers improved water management for both the horticulture and aquaculture units individually, making it increasingly preferred for commercial operations (Tyson, 2017; Yep and Zheng, 2019). As an innovative food production system, aquaponics offers the potential for circularity and resource efficiency (Schoor et al., 2024). If aquaponics is included in urban environments (urban agriculture), it creates local food value chains, fostering community development and creating environmental awareness (Fantini, 2023; Pradhan et al., 2023).

Research on aquaponics has primarily focused on technological aspects like system design and operations, reviewed by e.g. Palm et al. (2018) and Yep and Zheng (2019). However, (microbiological) food safety considerations are of primary importance when (re)designing food production systems (Pearson et al., 2024; van Leeuwen et al., 2024), as recognised among researchers, governments, and industry related stakeholders (Hamilton et al., 2023a). A reduced probability of pathogen contamination is often linked to CEA-grown commodities, compared to conventional soil-based agriculture (Despommier, 2010; Topalcengiz et al., 2024), because increased control (indoor conditions) infers a lower likelihood of plant contact with wildlife or manure (Blidariu et al., 2011; Tyson and Simonne, 2014). However, the controlled humid and warm environment could favour growth of human pathogenic bacteria, if occasionally present (Turner et al., 2020). There might be an increased likelihood for introduction of zoonotic pathogens in aquaponics, due to combined animal- and plant-based production and the use of aquaculture wastewater. Moreover, the integrated and circular design of aquaponics may allow for (pathogenic) bacteria to spread rapidly once introduced (Sela Saldinger et al., 2023).

Limited studies are available on microbiological quality, hygiene or safety of aquaponic systems. Some studies on aquaponics have solely used molecular techniques (amplicon sequencing) to assess the microbiological safety. For instance, Ruiz et al. (2023) found no Amplicon Sequence Variants (ASV) corresponding to *Salmonella* spp. or *Listeria* spp. in three replicate experimental coupled aquaponic systems, and Dong and Feng (2022) could not identify Shiga-Toxin producing *Escherichia coli* (STEC), *Salmonella* spp., or *Listeria monocytogenes* in a commercial aquaponic farm. Although culture-independent microbiological methods are valuable to gain insight in the microbiota composition and diversity, microbiological culture remains necessary when drawing food safety conclusions, enabling appropriate pathogen detection in terms of sensitivity and viability (Gill, 2017).

Other studies on aquaponics use marker organisms, such as *Enterobacteriaceae*, the sub-group of coliforms, and comprised generic *E. coli*, to assess microbiological food safety. Kralik et al. (2023) found no *E. coli* (LOD 1 CFU/5 mL) during a three-year analysis period of a coupled aquaponic system, while Dorick et al. (2021) observed up to 5.3 log CFU/100 mL *E. coli* in the fish tank and derived irrigation water of an experimental decoupled aquaponic system. However, direct pathogen testing is required when determining whether irrigation water or fresh produce is safe (Ceuppens et al., 2014; Holvoet et al., 2014).

Furthermore, small-scale experimental aquaponics are often used to assess the efficacy of food safety interventions, to study the effect of simulated operational conditions on food safety or to gather exploratory data on food safety parameters. Elumalai et al. (2017) have investigated the effect of a post-irrigation UV treatment, as food safety intervention step, on six experimental coupled aquaponic systems. No detectable levels of STEC or *Salmonella* spp. (LOD 1–5 cells/25 g) were found in any of the examined lettuce, basil, or water samples. The issue of pathogen internalization in aquaponics has been addressed, but indicated STEC could only internalize into the edible leaf portion if seedling roots were actively damaged (Wang et al., 2020; Wang et al., 2021). Weller et al. (2020) have studied risk factors for pathogen introduction in three experimental aquaponic systems. Exploratory microbiological analysis resulted in undetected levels of *Salmonella* spp. ($n = 3$, per 25 mL) and *E. coli* ($n = 29$, LOD 1 MPN/100 mL). Given the limited number of samples analysed and the presence of potential risk factors such as public access and the absence of handwashing protocols, further research is warranted to better understand microbiological contamination in commercial-scale aquaponic systems.

At present, only one large survey of *Salmonella* spp., STEC, and *L. monocytogenes* was conducted on a commercial coupled aquaponic system, producing lettuce and tilapia fish. No foodborne pathogens were detected in lettuce leaves, roots, fish, fish faeces, fish tank water and fish tank sponge swabs ($n = 1047$ samples) (Dorick et al., 2024).

This paper reports on the outcomes of a longitudinal study, investigating a commercial decoupled urban aquaponic farm with a focus on the microbiological hygiene and safety of ready-to-eat basil plants. The objectives of the study were (i) to identify potential pathways for the introduction and spread of foodborne pathogenic bacteria within the aquaponic environment, (ii) to assess the microbiological quality and safety of irrigation water, peat moss substrate, and basil, focusing on selected foodborne pathogens and potential related indicator bacteria, and (iii) to evaluate current system design, water quality management, irrigation and sanitation practices to support good agricultural practices.

2. Materials & methods

2.1. Aquaponic production system

A commercial urban aquaponic farm was available for sampling. According to the terminology as set by the EU Aquaponics Hub, it involved a multiple pass decoupled aquaponic *(s.l.)* system (Palm et al., 2018, 2024), as shown in Fig. 1. The system combines the production of salmon trout *(Arripis trutta)*, in a recirculating aquaculture unit, with basil *(Omicum basilicum)*, in a horticulture unit. External water sources for the aquaponic system include well water and roof-harvested rainfall water. For irrigation, a mixture of aquaculture process water, supplemented with variable ratios of well water, rainfall water, and inorganic fertilisers, is used. The irrigation water circulates in the horticulture unit for a two-month period, after which thorough cleaning and disinfection of the horticulture irrigation tank is performed.

2.2. Experimental set-up

Two sampling rounds were executed, one from October to December 2023 and one from February to April 2024. Sampling focused on basil as aromatic herb and various water streams, being (i) well water (WW), obtained before and after UV, (ii) collected rainfall water (RW), (iii) aquaculture process water (PW), obtained untreated, after biofiltration, and after UV, (iv) drain water (DW), obtained before and after UV, and (v) irrigation water (IW), sampled from the irrigation tank and at the point of irrigation (POI). Rainfall water was not treated using UV disinfection. A semi-continuous workflow is established in the aquaponic farm, with new basil plant batches set up every few days. Each two-month sampling round comprised three basil growth cycles (3 batches). A basil plant growth cycle takes about one month, and sampling events were related to distinct plant growth stadia: (i) basil seeds, (ii) seedlings, (iii) baby leaf basil, and (iv) mature basil, see Fig. 2. In the frame of this research, three basil plant batches were selected to be monitored from start (seeds) to end (mature ready-to-market basil plant) during each sampling round. Basils seeds were sown in peat-based growing medium, amended with bio- and UV filtered PW. Seeds were allowed to germinate for three days in a germination room at 24 °C and

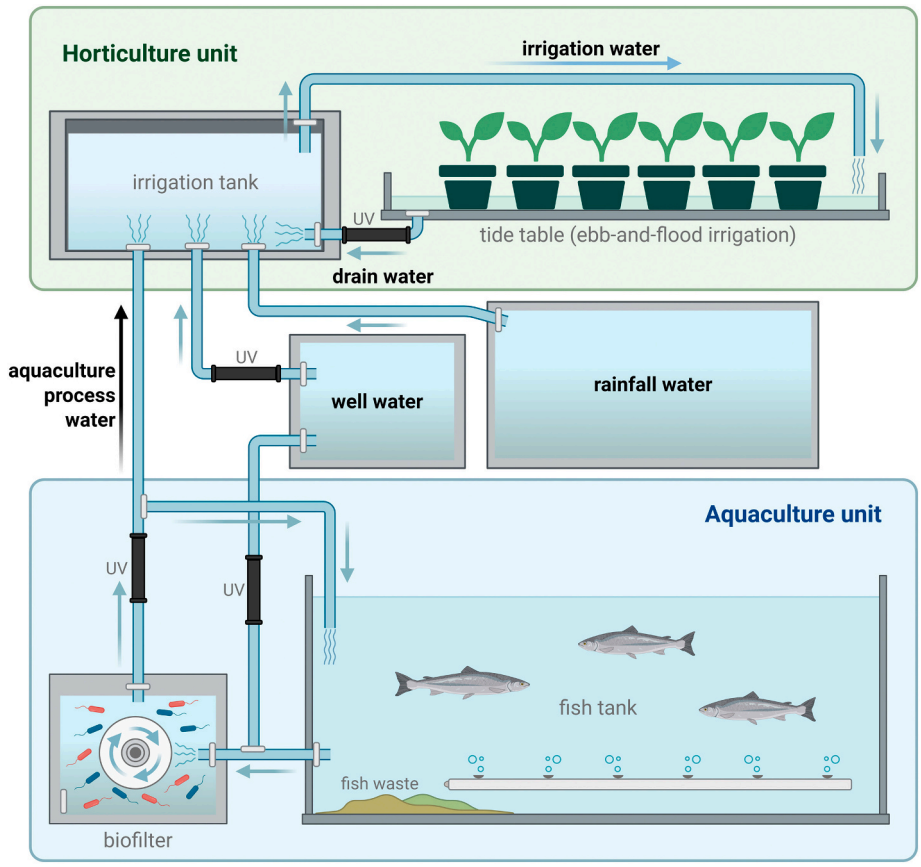

Fig. 1. Overview of the selected commercial decoupled aquaponic *(s.l)* farm, combining a recirculating aquaculture unit (fish tanks, biofiltration, and UV treatment), with a multiple pass horticulture unit (irrigation tank, ebb-and-flood irrigation tide tables, and UV treatment). External water sources include (UV treated) well water and roof-harvested rainfall water. Aquaculture process water couples the aquaculture unit with the horticulture unit. Created with BioRender.com.

> 70 % RH (increased with WW). Seedlings were then stacked on greenhouse trolleys and irrigated (overhead) with RW. Two weeks after sowing, the baby leaf basil plants were spread onto tide tables and irrigated via an ebb-and-flood system, until mature and ready for sale. The indoor temperature (T; °C) was retrieved from the greenhouse monitoring system (Priva) and external climatic data, i.e. temperature (°C) and daily precipitation (mm), were obtained from the Royal Meteorological Institute of Belgium.

2.3. Sampling of water samples

At each sampling event, ten water samples (1.5 L) were collected in sterile HDPE bottles (VWR, Avantor) of 500 and 1000 mL, following ISO 19458:2006 principles. In short, this included subsurface sampling (30 cm) in water tanks whereas at the point of irrigation, water collection occurred after clearing the hose from stagnant water (± 1 min flush time). The temperature of the water was measured directly at sampling. All water samples were transported (± 1 h) on ice in a cool box, stored at 4 °C, and analysed within 18 h.

2.4. Microbiological analysis of water samples

The microbiological parameters for analysis are shown in Table 1, including total plate count (TPC), *Enterobacteriaceae*, coliforms, presumptive *Aeromonas* spp., presumptive *Listeria* spp., generic *E. coli* and foodborne pathogens; *L. monocytogenes* and *Salmonella* spp. Analyses were performed using classical culture methods following either the ISO standard method or using an alternative rapid chromogenic medium with 'NF Validation' certification (ISO 16140).

In short, respectively 100 mL and 1000 mL subsamples of the various types of water were filtered over a cellulose nitrate filter (0.45 µm, Sartorius Inc.) for *E. coli* enumeration and *Salmonella* spp. detection. If necessary, more filters were used per sample to prevent filter clogging. For *E. coli* enumeration, the membrane was placed on RAPID'*E.coli* 2 agar (3564024, Bio-Rad). For *Salmonella* spp. detection, the filters were transferred to 50 mL of BPW (CM1049, Oxoid), prior to selective enrichment using RVS (3555773, Bio-Rad) and MKTTn (3556140, Bio-Rad), and selective plating on XLD (CM0469, Oxoid) and RAPID'*Salmonella* (3563962, Bio-Rad). During the first sampling round, *Salmonella* spp. detection was only executed in case *E. coli* (as a faecal indicator organism) was present per 100 mL. In the second sampling round, detection of *Salmonella* spp. was always executed, even when *E. coli* was not detected per 100 mL. A 15 mL subsample was used for pH determination (edge® pH, Hanna Instruments) at 25 °C. Next, water samples were subject to analysis for determination of TPC, *Enterobacteriaceae*, coliforms, presumptive *Aeromonas* spp., and *L. monocytogenes* (Table 1), by preparing a tenfold dilution series and plating on selected agar media. Confirmation of presumptive positive colonies was done on a maximum of four isolates, prior purified by subculture on TSA (CM0131, Oxoid). Furthermore, 1 mL of water was captured in KytoVials (Kytos BV, Belgium) for flow cytometry analysis, determining total cell counts and computing several biological metrics, such as a phenotypic diversity index (arbitrary units, a.u.), productivity (%), and viability (%). The KytoVials contain a small amount of fixative solution to preserve cellular phenotypes at the time of sampling. Briefly, KytoVial samples were diluted in 0.2 µm filtered phosphate-buffered saline (1x, Merck) and stained with 1 vol% nucleic acid stain SYBR® Green I (final concentration: 1:10000) for total cell counts. For viability measurements, a combination of SYBR® Green I (final concentration: 1:10000) and Propidium Iodide (final concentration: 6 µM) was used. The samples

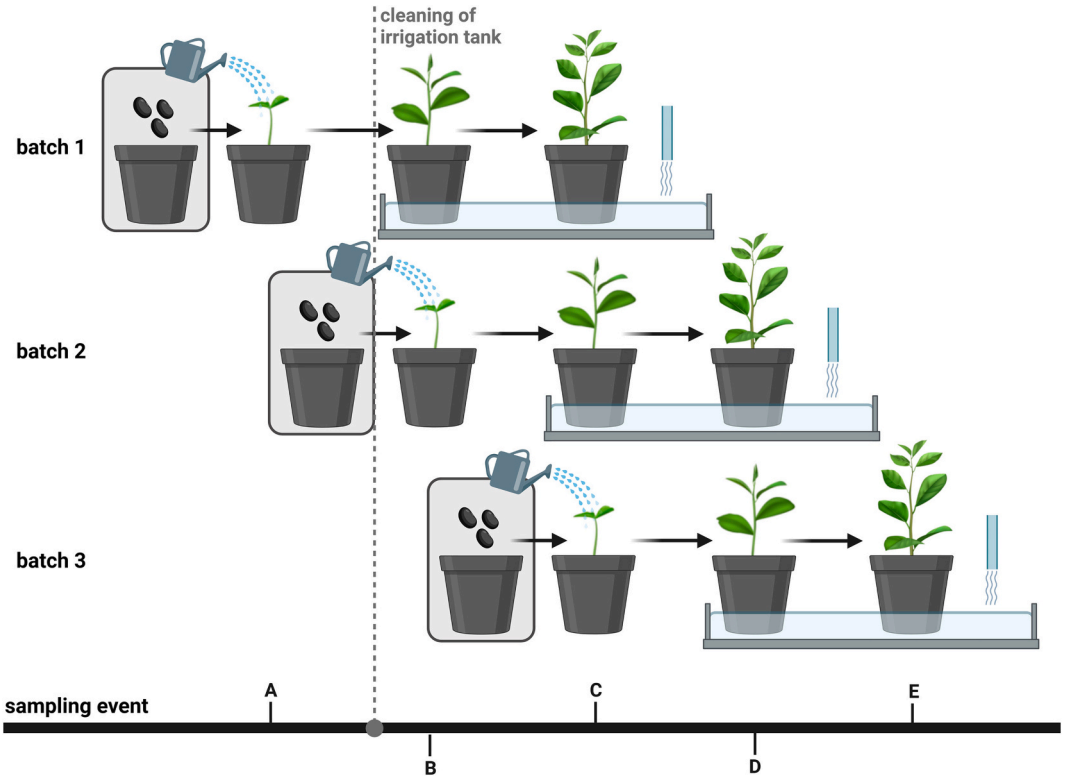

Fig. 2. Experimental set-up of basil sampling, showing the sampling scheme with indication of five sampling events (A-E) over three basil batches (1–3), each showing the basil growth cycle with four plant growth stadia: seeds, seedlings baby leaf basil, and mature basil. Sowing a new batch of basil was always conducted three days prior to the first sampling event of the respective batch. The grey box indicates the germination room (> 70 % RH, increased with well water). The watering can indicates overhead irrigation, using rainfall water, and the tide tables indicate subsurface irrigation with water coming from the horticulture irrigation tank. Before the start of the water tank circulation period in the horticulture unit, the irrigation tank was cleaned, as indicated with the dotted line. Created with BioRender.com.

Table 1
Overview of the microbiological parameters and used methods for their determination in water and other solid (peat moss, seeds, basil) samples.

Parameter	Method	Medium	Incubation conditions		Confirmation	LOD
			Time (h)	Temperature (°C)		
TPC[Ew]	ISO 6222:1999	water PCA (CM1012, Oxoid)	72	22	NA	10 CFU/mL
TPC[Es]	ISO 4833-1:2013	PCA (CM0325, Oxoid)	72	30	NA	10 CFU/g
Enterobacteriaceae[E]	Alternative to ISO 21528-2:2017 (NF validation)	RAPID'*Enterobacteriaceae* (3564004, Bio-Rad)	24	37	NA	10 CFU/g
Coliforms[E]	Alternative to ISO 4832:2006 (NF validation)	RAPID'*E.coli* 2 (3564024, Bio-Rad)	24	37	NA	1 CFU/mL 10 CFU/g
β-glucuronidase-positive *E. coli*[E]	Alternative to ISO 16649 (NF validation)	RAPID'*E.coli* 2 (3564024, Bio-Rad)	24	44	NA	1 CFU/100 mL 10 CFU/g
***Aeromonas* spp.**[Ew]	NEN 6263	Ampicillin dextrin agar + Ampicillin supplement (M1262; FD107A, HiMedia)	24	30	NA	1 CFU/mL
***Salmonella* spp.**[Da]	ISO19250:2010[w] ISO 6579-1:2017[s]	BPW (CM1049, Oxoid); RVS (3555773, Bio-Rad); MKTTn (3556140, Bio-Rad); XLD (CM0469, Oxoid); RAPID'*Salmonella* (3563962, Bio-Rad)	18; 24; 24; 24; 24	37; 41.5; 37; 37; 37	TSI (CM0277); MicroGen MID-64 GN-ID A; MALDI-TOF MS (MALDI Biotyper, Bruker)	detection in 1 L detection in 25 g
***Listeria* spp.**[Es]	ISO 11290–2:2017	ALOA + supplements (3564043; 3564041; 3564042, Bio-Rad)	24	37	NA	10 CFU/g
Listeria monocytogenes[E]	Alternative to ISO 11290–2:2017 (NF validation)	ALOA + supplements (3564043; 3564041; 3564042, Bio-Rad)	48	37	RAPID'*L.mono* (3563694, Bio-Rad)	1 CFU/mL 10 CFU/g

[E] = *enumeration;*
[D] = *detection;*
[w] = *only in case of water samples;*
[s] = *only in case of solid samples;*
[a] = *during the first sampling period, only performed in case of E. coli presence or for mature basil, and during the second sampling period, performed for all samples.*

were then incubated at 37 °C for 20 min to allow the stains to permeate the cells. Following incubation, measurements were conducted using an Attune NxT flow cytometer (Thermo Fisher Scientific). The instrument's performance was monitored daily and calibrated with Attune Performance Tracking Beads (Thermo Fisher Scientific). A blue laser (488 nm) was employed for stain excitation and were analysed with an acquisition volume of 100 μL.

2.5. Sampling of solid samples

At each sampling event, relevant solid samples, being seeds, peat moss substrate and basil plants, were collected according to the plant growth stage. Basil seeds (± 25 g) and substrate (± 50 g) were placed in sterile sampling containers. Basil plant samples consisted of three flowerpots for seedlings, and nine flowerpots for both baby leaf and mature basil plants. The samples were collected as shown in **Fig. S1** and collected in respectively Twirl'EM™ bags (Fisherbrand, Thermo Fisher Scientific) and cardboard boxes. Samples were transported at ambient temperature (approximately 1 h), subsequently stored at 22 °C upon arrival, and analysed within 18 h.

2.6. Microbiological analysis of solid samples

In the laboratory, basil flowerpots ($n = 9$; **Fig. S1**) were first split into plant material (stem and leaves) and substrate (including plant roots), using a sterile scalpel and wearing single-use gloves. The separated plant material and substrate of the nine subsamples were each pooled before analysis. Samples of basil seeds and substrate that were taken from the available stock in the aquaponics company were analysed directly from the sampling containers. Microbiological analysis was performed on a 25 g subsample. The analysed microbiological parameters and respective methods are shown in Table 1. During the first sampling round, *Salmonella* spp. detection was only executed (i) on the pooled sample of the leaves of the mature ready-to-market basil plant (final product), and (ii) in case of *E. coli* presence (LOD 10 CFU/g), as a faecal indicator organism. In the second sampling round, *Salmonella* spp. detection was performed for all samples. 15 g was used for pH determination at 25 °C, based on ISO 10390:2022 principles.

2.7. Evaluation of implemented good agricultural and hygienic practices

To evaluate current implemented good practices, observations during multiple visits of the commercial aquaponic farm were combined with document-analysis, as provided by the company, and in-person interviews. Observations were evaluated against available good agricultural and hygienic practices (GAP; GHP) in Commission notice (EC) 2017/C 163/01, addressing microbiological risks in primary production of fresh fruits and vegetables, focused on controlling organic fertilisers and water for primary production. Available good practices were tailored towards aquaponics and the implementation in the commercial aquaponic farm was assessed.

2.8. Data processing and statistical analysis

General data processing for plate count results, such as log-transforming plate counts and calculating average values with corresponding standard deviations was performed using Microsoft Excel. If no colonies were present for microbiological enumerations (< LOD), the value was replaced by the LOD itself. For flow cytometry data analysis, raw experimental data was collected using the Attune™ Cytometric Software (FCM software, v. 6.2.1). All data processing was executed using Kytos' proprietary data analysis software, KytoFlow (v. 1.9.8). To separate bacterial signals from background noise and to distinguish intact and dead cells, fixed gates were applied as defined by Props et al. (2016). The processed data was subsequently used to calculate total cell concentration, viability, productivity, and phenotypic diversity following the data processing strategy described by Van Nevel et al. (2013).

Statistical analysis was performed in R (v. 4.2.2). The effect of UV treatment was studied using a two-tailed paired *t*-test. The relation between *Salmonella* spp. presence and generic *E. coli* counts or class, having 1.0 and 2.0 log CFU/g as discrete boundaries, were tested respectively with a one-tailed independent t-test and Kendall's Tau-C correlation test. If relevant to the test conditions, the Shapiro-Wilk test was used to confirm the condition of normality and Levene's test was used to verify homoscedasticity. If the assumption of normality was not met, a Wilcoxon signed-rank test or Mann-Whitney *U* test was performed as non-parametric alternative to respectively paired and independent *t*-tests. Correlations between various marker organism were determined by calculating the Spearman's Rho (r_s) coefficient using log-transformed plate count data (non-normal distributed), and by applying the Tobit model (β), censoring the LOD in case of correlations with *E. coli* (Lorimer and Kiermeier, 2007). Correlations between cell loads, determined with flow cytometry, were assessed by the Pearson correlation coefficient (r_p). All statistics were performed at a significance level of 0.05. Next to the statistical significance tests, an equivalency margin of 0.5 log units in plate count results is used to indicate biological relevance (Uyttendaele et al., 2018).

3. Results and discussion

3.1. Microbiological data on water samples

The pH, temperature, and microbiological parameters analysed for the irrigation water (aquaponic nutrient water) and composing water streams (in variable ratios), i.e. well water, rainfall water, process water, and drain water, are shown in Table 2. The measured temperature and pH of the irrigation water and water in the fish tank are able to sustain both optimal plant growth (16–30 °C, pH 5.5–7.5), and cold water fish rearing (10–18 °C, pH 6.0–8.5), according to technical guidelines by Somerville et al. (2014).

Well water and collected rainfall water serve as external water sources in the aquaponic farm. The microbiological contamination of untreated well water was low, with maximum concentrations of TPC ranging within 100–1000 CFU/mL, and no *E. coli* (LOD 1 CFU/100 mL) or *Salmonella* spp. (detection in 1000 mL) were found. These results are consistent with the appointed low-risk level of well water irrigation (FAO/WHO, 2008; Uyttendaele et al., 2015). In contrast, occasional introduction of *E. coli* was found in collected rainfall water during the first sampling round ($n = 3/5$). Roof-harvested rainfall water can become contaminated by excreta of avian species (Ahmed et al., 2016), with the highest contamination typically occurring after extended dry periods (Hamilton et al., 2019; Uyttendaele et al., 2015). The average daily rainfall (mm) during the two sampling rounds is visualized in **Fig. S2**. September 2023 was marked by 25 dry days (< 1 mm daily precipitation), followed by six dry days early October. Hereafter, *E. coli* was found during the first two sampling events in October, but also during the last sampling event in December, when rainfall resumed after five dry days. In the aquaculture unit, the water quality in the fish tanks was comparable to the water post biofilter and post UV, showing similar TPC of respectively 2.8 ± 0.4, 3.2 ± 0.3, and 2.7 ± 0.8 log CFU/mL. Presumptive *Aeromonas* spp. are core to aquatic environments (Lamy et al., 2022) and were present in the fish tanks with an average concentration of 2.0 ± 0.2 log CFU/mL. No *E. coli* was found in the aquaculture unit. Drain water, i.e. spent irrigation water, is circulated in the horticulture unit while mixed in with well water, rainfall water and aquaculture process water to compensate for evaporation and water uptake by the plants. In drain water, *E. coli* was found in 2/10 sampling events (max. 10 CFU/100 mL), however, no *Salmonella* spp. ($n = 0/7$) was detected.

Overall, no *L. monocytogenes* was enumerated in any of the water samples ($n = 100$), while *Salmonella* spp. was detected in three out of 61

Table 2
Results of the microbiological water analyses for the irrigation water, sampled in the tank and at the point of irrigation (POI), and its composing water streams: well water, rainfall water, aquaculture process water, and drain water. Average values and respective standard deviations are calculated over the two sampling rounds, compiling 10 samples (n = 2x5). Results are noted as avg. ± sd. Microbiological parameters are expressed in log CFU/100 mL for *E. coli*, otherwise in log CFU/mL. Temperature is expressed in °C.

	Well water		Rainwater	Aquaculture process water			Drain water		Irrigation water	
	pre UV	post UV	tank	fish tank	biofilter	post UV	pre UV	post UV	tank	POI
pH	8.2 ± 0.2	8.1 ± 0.4	5.5 ± 0.4	7.9 ± 0.3	8.0 ± 0.2	8.0 ± 0.2	6.4 ± 0.7	5.8 ± 1.6	6.9 ± 0.7	6.9 ± 0.7
Temperature	13.2 ± 0.3	14.2 ± 0.9	16.9 ± 1.7	16.2 ± 0.5	16.3 ± 0.4	16.4 ± 0.5	16.4 ± 2.9	16.1 ± 2.9	19.8 ± 1.4	18.7 ± 1.6
TPC	1.3 ± 0.5[1,2]	2.2 ± 0.9[2]	2.9 ± 0.6[2]	2.8 ± 0.4[2]	3.2 ± 0.3	2.7 ± 0.8[2]	5.1 ± 0.3	4.4 ± 0.7	5.1 ± 0.7	5.4 ± 0.3
Enterobacteriaceae	0.0 ± 0.0[1,2]	0.4 ± 0.6[1,2]	1.4 ± 0.4[2]	1.7 ± 0.3	1.9 ± 0.3	1.5 ± 0.8[2]	3.1 ± 0.4	0.7 ± 0.9[1,2]	2.7 ± 0.9[2]	3.3 ± 0.3
coliforms	< 0.0	0.1 ± 0.2[1,2]	1.2 ± 0.6[2]	1.6 ± 0.3	1.7 ± 0.5	1.6 ± 0.8[2]	3.0 ± 0.5	0.8 ± 0.9[1,2]	2.6 ± 0.9[2]	3.3 ± 0.3
β-glucuronidase-positive *E. coli*										
undetected/100 mL	10/10	10/10	7/10	10/10	10/10	10/10	8/10	9/10	6/10	7/10
≥ 0.0 and < 1.0 log CFU/100 mL	0/10	0/10	2/10	0/10	0/10	0/10	1/10	1/10	4/10	3/10
≥ 1.0 and < 2.0 log CFU/100 mL	0/10	0/10	1/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10
≥ 2.0 log CFU/100 mL	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
***Salmonella* spp.**	0/5	0/5	1/8	1/5	1/5	0/5	0/6	0/6	0/8	0/7
***Aeromonas* spp.**	< 1.0	1.2 ± 0.4[1,2]	1.2 ± 0.5[1,2]	2.0 ± 0.2[2]	2.2 ± 0.5[2]	1.9 ± 1.0[1,2]	2.2 ± 0.5[2]	1.1 ± 0.4[1,2]	2.3 ± 0.5[2]	2.6 ± 0.7[2]
Listeria monocytogenes	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

[1] = *at least one of the plate counts composing the average value was < LOD.*
[2] = *at least one of the plate counts composing the average value was < LOQ.*

analysed samples, introduced trough rainfall water (n = 1/8) and aquaculture process water (n = 2/15), being occasionally detected in water from the fish tanks (n = 1/5) and at the biofilter outlet (n = 1/5). *Salmonella* spp. was not detected at the point of irrigation (n = 0/7). *Salmonella enterica* is not a native resident to cold blooded animals, however, occurrence in aquaculture is described and could arise from the water source, the aquaculture environment and fish feed (Amagliani et al., 2012; Billah and Rahman, 2024; Dalsgaard, 1998; Lunestad et al., 2007).

Besides water acting as introduction source for human pathogenic bacteria, Sela Saldinger et al. (2023) have indicated the risk of water acting as vehicle for spreading human pathogenic bacteria through circulation. Dispersal of *Salmonella* spp. throughout the horticulture unit could cause (i) (cross-)contamination of produce and water contact surfaces, (ii) accumulation in the horticulture irrigation tank, and (iii) formation of or incorporation into biofilms, enabling persistence in the aquaponic environment.

3.2. Microbiological data on solid samples: Basil (seeds and leaves) and peat moss substrate

The results of pH determination and microbiological analyses for basil, from seed to mature plant, and substrate during the basil growth cycle, are shown in Fig. 3. Bacteria are introduced from the basil seed, on average 4.1 ± 0.7 log CFU/g, and reflect the peat moss substrate, TPC 7.6 ± 0.6 log CFU/g, during germination, consistent with Gekenidis et al. (2017). Although seed samples were not of food safety concern in the present study, contaminated seeds are recognised as important vector for transmitting foodborne pathogens, particularly when produce is consumed as sprouts or microgreens (Machado-Moreira et al., 2019; Sela Saldinger et al., 2023). The transmission of STEC, *Salmonella* spp. and *L. monocytogenes* from artificially contaminated seeds to microgreens was previously reported (Işık et al., 2024; Li et al., 2022; Xiao et al., 2015), with decreased growth potential when growing in peat moss substrate compared to hydroponic nutrient solution (Xiao et al., 2015).

During the study of Hamilton et al. (2023a), researchers, industry, and government stakeholders identified the microbiological quality of soilless substrates as a major area of research concern. In the present study, no *L. monocytogenes* was enumerated in peat moss substrate, with a TPC of 7.9 ± 0.6 log CFU/g. However, presumptive *Listeria* spp. was found in concentrations of 3.0–3.5 log CFU/g, remaining stable over time. This could be explained by the saprophytic nature of *Listeria* spp., as persistence of *Listeria* spp. has been reported for high organic carbon substrates, composed of decaying plant material (Misra and Gibson, 2020). A study on hydroponic lettuce did not detect *Listeria* spp. in peat moss substrate, hosting similar TPC of approximately 7.5 log CFU/g (Dankwa et al., 2020). The peat moss substrate was identified as source for *E. coli*, 2.7 ± 0.6 CFU/g, and *Salmonella* spp. (n = 3/6). Awareness on the potential introduction of foodborne pathogens through soilless substrates remains important. In the study of Hamilton et al. (2023b), none of the interviewed CEA growers expressed concern about pathogen transmission through soilless substrates. Previously, the transfer of STEC, *Salmonella* spp., and *L. monocytogenes* was shown from soilless substrates to several microgreens. However, pathogen transfer to the edible plant and subsequent proliferation depend highly on the type of substrate, plant tissue and their ability to support survival or growth of the pathogen. In addition, longer times to harvest and higher loads of competitive microbiota in the substrate were correlated to lower pathogen populations (Deng et al., 2021; Işık et al., 2020; Işık et al., 2024). The additional 2–3 weeks of basil maturation in the present study, from sprouted seed or microgreen onwards, could thus be beneficial for food safety. Moreover, inoculated generic and pathogenic *E. coli*, *Salmonella* spp. and *L. monocytogenes* decayed over time in peat moss substrates (Işık et al., 2020; Misra and Gibson, 2020), similar to the present results for generic *E. coli*, declining at least 1.0 log CFU/g, and *Salmonella* spp., being no longer detected from day 17 onwards. This could be explained by bacterial competition in the substrate and dilution effects through repeated irrigation (wash-out), as noted in *Enterobacteriaceae* which declined about 1 log CFU/g on average during the first 3 days post-sowing.

Combining all analyses on solid samples, no *L. monocytogenes* was enumerated (n = 0/42). *Salmonella* spp. was not detected on seeds (n = 0/3) but detected in the peat moss substrate (n = 5/21). There was no indication of transmission of *Salmonella* spp. from substrate (or contaminated water) to the basil leaves in the present study, as *Salmonella* spp. detection in basil leaves was negative for all analysed samples (n = 0/9).

	Seeds	Seedlings	Baby leaf basil	Mature basil
pH	-	-	-	6.2 ± 0.4
TPC	4.1 ± 0.7	-	5.8 ± 0.9	5.8 ± 1.6
Enterobacteriaceae	1.2 ± 0.4[1,2]	-	4.7 ± 0.2	3.8 ± 1.7[2]
coliforms	1.1 ± 0.2[1,2]	-	4.6 ± 0.3	3.7 ± 1.6[2]
β-glucuronidase-positive *E. coli*		-		
< 1.0 log CFU/g	6/6	-	6/6	6/6
≥ 1.0 and < 2.0 log CFU/g	0/6	-	0/6	0/6
≥ 2.0 log CFU/g	0/6	-	0/6	0/6
***Salmonella* spp.**	0/3	-	0/3	0/6
***Listeria* spp.**	1.5 ± 0.4[1,2]	-	1.2 ± 0.3[1,2]	1.6 ± 1.3[1,2]
Listeria monocytogenes	< 1.0	-	< 1.0	< 1.0

	Substrate (day 0)	Substrate (day 3)	Substrate (day 17)	Substrate (day 31)
pH	6.6 ± 0.1	6.7 ± 0.2	6.3 ± 0.1	6.3 ± 0.2
TPC	7.9 ± 0.6	7.6 ± 0.6	7.8 ± 0.9	7.2 ± 0.7
Enterobacteriaceae	6.9 ± 0.5	5.9 ± 0.3	5.7 ± 0.5	5.7 ± 0.2
coliforms	6.8 ± 0.5	5.8 ± 0.3	5.4 ± 0.3	5.9 ± 0.3
β-glucuronidase-positive *E. coli*	2.7 ± 0.6			
< 1.0 log CFU/g	0/6	1/6	1/6	5/6
≥ 1.0 and < 2.0 log CFU/g	0/6	5/6	5/6	1/6
≥ 2.0 log CFU/g	6/6	0/6	0/6	0/6
***Salmonella* spp.**	3/6	2/5	0/6	0/4
***Listeria* spp.**	3.5 ± 1.2	3.1 ± 0.8	3.0 ± 0.7	3.4 ± 0.9
Listeria monocytogenes	< 1.0	< 1.0	< 1.0	< 1.0

Fig. 3. Results of the pH and microbiological analyses of solid samples. Average values and respective standard deviations are calculated over the two sampling rounds, compiling 6 batches of basil ($n = 6$) from seed to mature plant. Results are noted as avg. ± sd. Microbiological parameters are expressed in log CFU/g. Illustrations created with BioRender.com.
[1] = at least one of the plate counts composing the average value was < LOD.
[2] = at least one of the plate counts composing the average value was < LOQ.

Mature basil had a TPC of 5.8 ± 1.6 log CFU/g, which remained stable during the last two weeks of development. Fresh produce grown in CEA is often linked to a 1–2 log CFU/g lower bacterial count compared to their field-grown counterparts (Bączek et al., 2019; Dong and Feng, 2022; Neto et al., 2012; Sathyanarayana et al., 2021; Sirsat and Neal, 2013; Tham et al., 2021; Weller et al., 2020; Williams and Marco, 2014). Reduced total counts in aquaponics are sometimes associated with statements about safety, e.g. lower risk for foodborne illness, and quality, e.g. improved shelf-life (Nissen et al., 2021; Sirsat and Neal, 2013). Although observations thereof are possible, it cannot be seen as unambiguous cause-effect relationships. Regarding safety, fresh produce with lower total counts cannot be linked directly to reduced pathogen presence, nor the lower incidence of foodborne disease (NSW Food Authority, 2009). Considering quality, the shelf-life of edible plants and spoilage are not only in function of microbiological counts, but the result of the interrelated physiological, enzymatic and microbiological activity (Brackett, 1993; Ragaert et al., 2007).

3.3. Use of marker organisms in verification and monitoring for aquaponics

Both the EU (EU 2020/741 and EC 2017/C_163/01) and U.S. (Food and Drug Administration Produce Safety Rule) established *E. coli* threshold values to monitor and evaluate irrigation water quality. In this study, the irrigation water contained <1–10 CFU/100 mL *E. coli*, well below the 1000 CFU/100 mL limit set by regulation (EU) 2020/741 on water reuse. The level of 1000 CFU/100 mL in irrigation water is selected here because basil is grown above ground, is consumed raw, and subsurface irrigation avoids direct contact with the edible basil leaves. Although regulation (EU) 2020/741 does not apply for aquaculture wastewater, it provides useful guidelines for irrigation in aquaponics. Generic *E. coli* is used as index organism for faecal contamination

and can be associated with an increased likelihood of occurrence of ecologically related enteric pathogenic species, such as *Salmonella* spp., if present in elevated numbers (EFSA Panel on Biological Hazards (BIOHAZ), 2014; Mossel et al., 1995). Therefore, *Salmonella* spp. detection was only performed when *E. coli* was enumerated during the first sampling round. However, no *Salmonella* spp. was detected in 11 water samples containing *E. coli* (max. 1.53 log CFU/100 mL in rainfall water). During the second sampling round, *Salmonella* spp. detection was always executed, resulting in three positive water samples ($n = 3/50$). None of the three *Salmonella*-positive samples showed detection of *E. coli* per 100 mL, thus detracting from *E. coli* as suitable marker organism for *Salmonella* spp. presence in water within this aquaponic farm. The relationship between elevated levels of generic *E. coli* and the presence of *Salmonella* spp. was previously shown for well water and surface water (Ceuppens et al., 2014; Gu et al., 2021; Havelaar et al., 2017; Truchado et al., 2018), but conflicting results are also available (Ahmed et al., 2010; Benjamin et al., 2013). Regarding solid samples, no associations could be assessed for seeds and basil leaves as no *E. coli* or *Salmonella* spp. were found during the study period. Generic *E. coli* counts were significantly higher ($p < 0.05$) in *Salmonella*-positive peat moss substrate samples, and *Salmonella* spp. presence was significantly correlated with the *E. coli* class (Kendall's Tau-C = 0.44; $p < 0.05$), and concentration ($\beta = 1.27$, SE = 0.24, $p < 0.001$) (Fig. 3). Ceuppens et al. (2014) indicated that the association between *E. coli* and *Salmonella* spp. in fresh produce production relies on multiple factors, such as climate and seasonality, here the indoor controlled environment, and the level of control and assurance activities in place, linked to the presence of risk factors for *Salmonella* spp.

In contrast to *E. coli*, *Enterobacteriaceae* and comprised coliforms are ubiquitous in the environment and not limited to faecal origin. A strong correlation was found between *Enterobacteriaceae* and coliforms, both in water samples ($n = 100$, $r_s = 0.96$, $p < 0.001$) and solid samples ($n = 42$, $r_s = 0.94$, $p < 0.001$). As expected, concentrations of *E. coli* in water samples were not significantly associated with *Enterobacteriaceae* ($\beta = 0.52$, SE = 0.32, $p = 0.10$) or coliforms ($\beta = 0.55$, SE = 0.31, $p = 0.07$). No significant higher levels of coliforms ($\Delta_{P50} = 0.5$, $p = 0.12$) or *Enterobacteriaceae* ($\Delta_{P50} = 0.4$, $p = 0.14$) were linked to water samples when *Salmonella* spp. was detected. In peat moss substrate, concentrations of *E. coli* were moderately associated to coliforms ($n = 24$, $r_s = 0.66$, $p < 0.001$; $\beta = 1.27$, SE = 0.24, $p < 0.001$), with significant higher levels of coliforms ($\Delta_m = 0.6$ log CFU/mL, $p < 0.05$) and *Enterobacteriaceae* ($\Delta_{P50} = 1.0$ log CFU/mL, $p < 0.01$) in *Salmonella*-positive samples. As mentioned earlier, both dilution and competitive effects might have occurred over time in the peat moss substrate, causing distortion in the statistical tests of various peat moss substrate samples at different time points. In basil, previous research indicated no significant relation between the level of coliforms and presence of *Salmonella* spp. (de Bruin et al., 2016). These results question the suitability of *Enterobacteriaceae* and coliforms as marker organisms for *Salmonella* spp.

In routine monitoring and verification of established Good Agricultural Practices (GAP) to influence food safety, such as UV treatment, *Enterobacteriaceae* is the preferred indicator over the ambiguous defined group of coliforms, without established relation to the potential presence of human pathogenic bacteria (Mossel et al., 1995). A significant build-up of TPC ($\Delta_m = 0.9$ log CFU/mL, $p < 0.05$) was observed pre- and post UV treatment of well water. The build-up of bacteria could be explained by the distance between the UV unit and the well, located outside of the aquaponic farm. Transport of water trough pipes results in biofilms, which also significantly release bacteria, as studied in drinking water distribution systems (Chan et al., 2019). No significant effects of UV treatment were observed in the aquaculture unit for TPC ($\Delta_m = -0.1$ log CFU/mL, $p = 0.82$), *Enterobacteriaceae* ($\Delta_{P50} = -0.2$ log CFU/mL, $p = 0.28$), coliforms ($\Delta_{P50} = -0.2$ log CFU/mL, $p = 0.56$), or presumptive *Aeromonas* spp. ($\Delta_m = -0.3$ log CFU/mL, $p = 0.32$). This could be explained by the circular design of the aquaculture unit with continuous UV disinfection. In contrast, UV disinfection had a significant effect on various of Gram-negative bacteria in drain water, with significant reductions for *Enterobacteriaceae* ($p < 0.001$) and coliforms ($p < 0.001$) of approximately 2.5 log CFU/mL, and 1 log CFU/mL for presumptive *Aeromonas* spp. ($p < 0.05$). Other studies on UV treatment in aquaponics had variable outcomes, showing either no significant reductions in TPC or coliforms (Elumalai et al., 2017) or being effective in reducing coliforms (Pantanella et al., 2015). Differing results could be explained by variation in water quality (concentration of suspended solids and organic matter) or design and operations of the UV system (van Haute et al., 2015).

3.4. Water quality monitoring using flow cytometry

Apart from monitoring indicator bacteria using standard microbiological plate counting methods, water quality monitoring using flow cytometry is valuable to rapidly gain insight in the characteristics and dynamics of the water microbial community (Favere et al., 2020; Gabrielli et al., 2021; Vergine et al., 2020). Therefore, water samples were not only assessed based on their concentration of various microbiological parameters (plate counts), but also on (i) changes in abundance and composition of microbial communities, and (ii) by determined flow cytometry metrics, such as phenotypic diversity (Props et al., 2016), nucleic acid content as measure for bacterial activity (productivity) (Props et al., 2018b; Safford and Bischel, 2019) and viability (Gillespie et al., 2014; Hammes et al., 2010; Safford and Bischel, 2019) of the present bacteria in the water streams (Fig. 4).

Flow cytometry resulted in significantly higher viable cell loads compared to plate count results, as described by the great plate count anomaly (Van Nevel et al., 2017). The difference was on average 2.5 ± 0.7 and 1.1 ± 0.7 log cells or CFU/mL, respectively for (i) lower-nutrient water streams: well water, rainfall water and aquaculture process water, and (ii) high-nutrient water streams: drain water and irrigation water. The difference is often explained by the presence of bacteria in a viable-but-non-culturable (VBNC) state in response to oligotroph or stressful (disinfection, temperature, etc.) environments (Wingender and Flemming, 2011).

The low-nutrient water streams had viable cell densities, measured by flow cytometry, of respectively 4.3 ± 0.4, 5.4 ± 0.3, and 5.4 ± 0.3 log cells/mL, being low and stable over the two sampling rounds. On average 98.5 %, 78.2 % and 57.5 % intact cells were present (viability). The phenotypic diversity in aquaculture process water was on average 1862.8 ± 326.9 a.u. (arbitrary units), which is lower compared to drinking water in a study of Props et al. (2018a), indicating both lower richness and evenness of the microbial community (Props et al., 2016). The bacterial community is potentially dominated by oligotroph bacteria (Schmautz et al., 2022) and specific nitrifying bacteria, which originate from the biofilter (Schreier et al., 2010) and are selected by continuous circulation including biofiltration and UV treatment.

In the high-nutrient water streams, the cell load of the irrigation water, at the point of irrigation, was the highest, 5.9 ± 0.4 log cells/mL, and followed the dynamics of the incoming drain water ($r_p = 0.73$, $p < 0.05$), which hosted 5.8 ± 0.5 log cells/mL (post UV treatment). The irrigation water in the horticulture unit is circulated for approximately two months, whereafter cleaning and disinfection takes place. Apart from build-up of organic material and peat moss substrate in the irrigation tank, which is introduced through drain water, bacteria could also accumulate. The effect of cleaning and disinfection, executed two-monthly in the irrigation tank, was observed in the flow cytometry metrics productivity and phenotypic diversity, while a consistent reduction in total count could not be detected by standard plate counting (Fig. 4). This highlights the additional value provided by flow cytometry to monitor water quality and get insight in cleaning and disinfection strategies. After three days post cleaning and disinfection, the bacterial activity and phenotypic diversity in the irrigation tank dropped compared to the previous sampling event. High nutrient levels became rapidly available in the tank, mainly introduced through drain

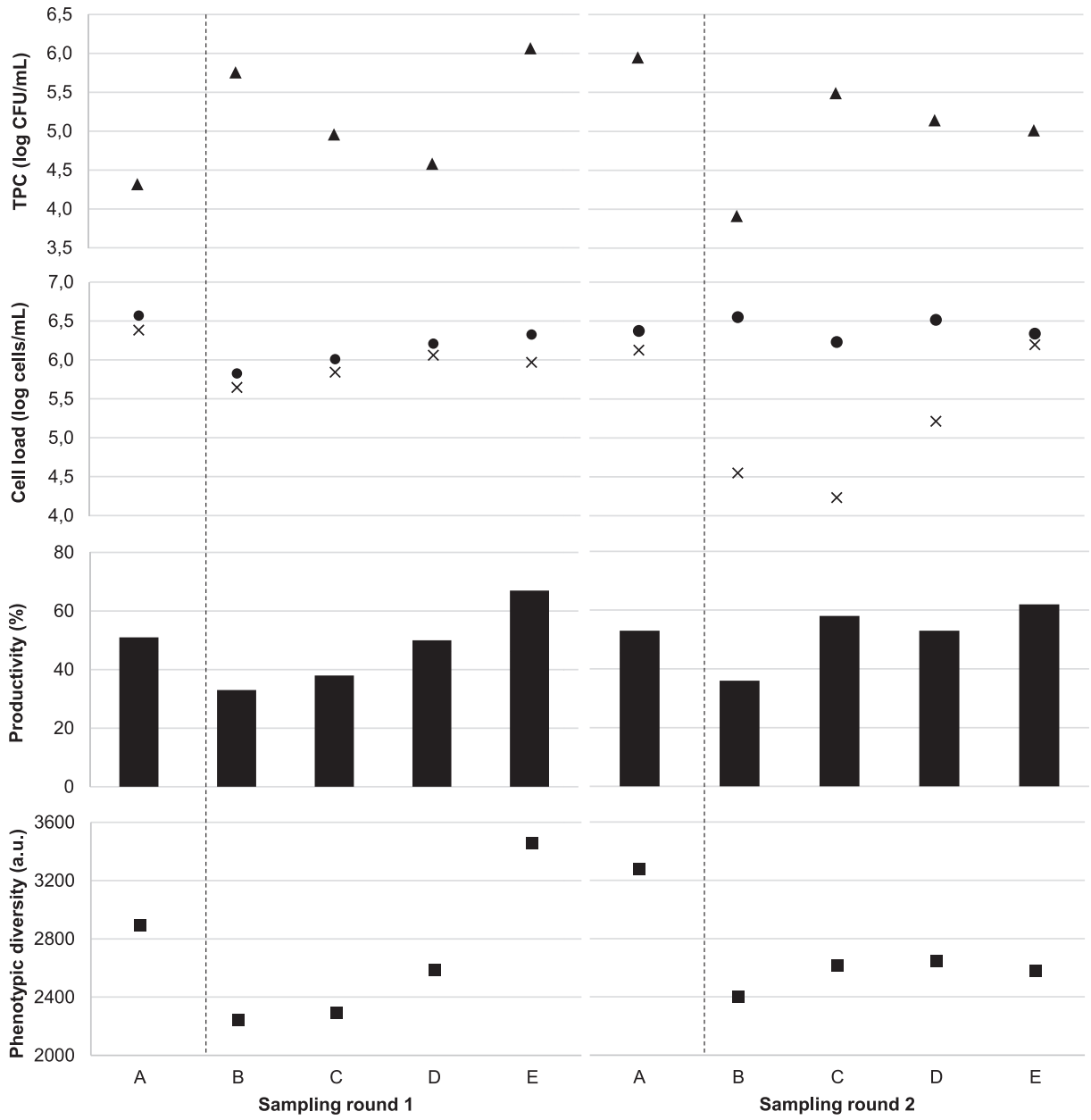

Fig. 4. The effect of cleaning and disinfection in the horticulture irrigation tank, as indicated with the vertical dotted line, on the TPC (log CFU/mL; ▲) and the flow cytometry metrics total cell load (log cells/mL; ●), total viable cell load (log cells/mL; x), productivity (%; bar plot) and phenotypic diversity (a.u.; ■).

water, and allowed regrowth and accumulation of bacteria during the further water tank circulation period (Rojas-Tirado et al., 2017). Mixing of several water streams may have facilitated this bacterial regrowth as the various water streams provide different (limiting) nutrients. Similar effects were previously observed in drinking water distribution systems (Favere et al., 2020; Niquette et al., 2001). At the last sampling event in both sampling rounds, the bacterial activity became high (> 60 %), indicating a high nucleic acid content bacterial population which can rapidly respond to nutrients (Claveau et al., 2024). Despite the increased diversity (enabling microbiome resilience), excess of nutrients, especially easily biodegradable carbon, could enhance proliferation of introduced fast-growing (pathogenic) bacteria (Fossmark et al., 2020; Rojas-Tirado et al., 2017). This indicates that the (arbitrary chosen) two-month circulation period serves its purpose in operation of the aquaponic farm.

3.5. Good agricultural and hygienic practices in aquaponics

Appropriate implementation of GAP and GHP in aquaponics, as part of food safety management, is of primary importance to address microbiological food safety (EFSA Panel on Biological Hazards (BIOHAZ), 2014). Combining the laboratory analyses with meta-analysis of existing good practices in Commission notice (EC) 2017/C 163/01, tailored good practices to prevent the introduction and persistence of foodborne pathogens in the commercial aquaponic farm were elaborated, as described in Table 3. Considering the aquaponic process water, used as organic fertiliser, a physical separation between the aquaculture and horticulture unit is advised, combined with an appropriate manure management plan, which determines the use of aquaculture wastewater for irrigation. Other water streams should be used 'fit-for-purpose', for example, executing overhead irrigation only using low-risk water sources, during the early stages of basil growth, while using aquaculture wastewater only with subsurface irrigation. Both in relation to irrigation

Table 3
Overview of key GAP and GHP, identified in Commission notice (EC) 2017/C_163/01, which can be tailored to the introduction and persistence of foodborne pathogens in aquaponics, and an observation of implementation in the commercial urban aquaponic farm, yes (+) or no (−).

GHP/GAP (EC) 2017/C_163/01	Tailored GHP/GAP	Observed implementation
Control of organic fertilisers:	In aquaponics, aquaculture process water (containing fish waste) is used as organic fertiliser:	
Development of a manure management plan: identifying where and when manure can and cannot be applied.	Development of a manure management plan: identifying where and when aquaculture wastewater can be applied for crop irrigation, taking into account already implemented measures (wastewater treatment: biofilter and UV) and present alternatives.	+
Avoid locating treatment or storage areas for organic fertilisers near cropping areas.	Physical separation of the decoupled aquaculture and horticulture units should be implemented to reduce (cross)-contamination.	+
Any equipment that has come into contact with organic fertilisers should be thoroughly cleaned and sanitised before being used again.	Apart from cleaning and disinfection of equipment, there should be a clear separation of equipment for use in the aquaculture and horticulture unit.	+
Personnel handling organic fertilisers should practice good personal hygiene, wear appropriate personal protection equipment.	Personnel working in the aquaculture unit should practice good personal hygiene and wear appropriate personal protection equipment, and take care of appropriate disposal, cleaning and disinfection after leaving the unit, especially before entering the horticulture unit.	+; Additional separation of personnel working in the aquaculture and horticulture unit.
Control of water for primary production:		
For overhead irrigation, a higher quality of water should be used as it comes into direct contact with the edible parts of the plant and if possible, only at the early stages of plant growth. A time interval between irrigation period and harvest can be applied. This is the case for all products eaten raw.	Overhead irrigation should use a low-risk water source (e.g. well water or treated rainfall water), only during the early stages of basil growth (first two weeks). Later-on, subsurface irrigation (last two weeks of basil development) is preferred, while limiting (accidental) splashing or irrigation water to the basil leaves.	+
The quality of the water used for soil-less systems should be regularly checked and should be changed frequently, or if recycled, it should be treated to minimize microbial contamination. If no compliance with indicators is observed, mitigation strategies mostly based on water treatment technique should be put in place.	Water quality monitoring, including water testing, of all water streams composing the irrigation water should be systematically implemented. Effective UV treatment of all water streams, and thorough cleaning and disinfection procedures (a frequency of once every two months in the present study) should be implemented.	+

water and the peat moss substrate, it is important to limit both contact with the substrate and (accidental) splashing of irrigation water to the edible basil leaves. Finally, a water quality monitoring plan should be established, in which flow cytometry seems to be an appropriate, simple and quick analytical method, combined with effective UV treatment, also implemented for rainfall water, and appropriate cleaning and disinfection procedures for pipes, water tanks, and the broader aquaponic environment.

4. Conclusions

CEA production systems, including aquaponics, are often referred to as safer compared to conventional, soil-based agriculture because of the lower introduction stress of foodborne pathogenic bacteria from faecal origin (of warm-blooded animals). In the present commercial urban aquaponic farm, no food safety issues were identified for the ready-to-market basil plants. No *L. monocytogenes* was enumerated in any of the water, substrate or plant analyses ($n = 142$), and no *Salmonella* spp. was detected, neither in basil leaves ($n = 6$), nor in peat moss substrate ($n = 4$) of mature basil plants. However, introduction of foodborne pathogenic bacteria remains possible, and the initial quality of introduced water streams, seeds, and soilless substrate play a major role in food safety governance (Topalcengiz et al., 2024). *Salmonella* spp. was introduced through the peat moss substrate ($n = 3/6$), rainfall water ($n = 1/8$), and aquaponic process water ($n = 2/15$), potentially driving the distribution and detected occurrence ($n = 8/94$) throughout the system. The implementation of appropriate GAP and GHP, tailored to aquaponics, are fundamental to address the microbiological food safety challenges in aquaponics.

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CRediT authorship contribution statement

Mathis Vermeersch: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Liesbeth Jacxsens:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Tara Baele:** Writing – review & editing, Investigation. **Inge Van Damme:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Bavo Verhaegen:** Investigation, Formal analysis, Conceptualization. **Nico Boon:** Writing – review & editing, Conceptualization. **Mieke Uyttendaele:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mathis Vermeersch reports financial support was provided by European Union. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have

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Data availability

The data that has been used is confidential.

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