Growth and survival of aerobic and Gram-negative bacteria on fresh spinach in a Chinese supply chain from harvest through distribution and refrigerated storage

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A B S T R A C T

Spinach is a highly perishable product that degrades over time, including due to bacteria contaminating the product prior to packaging, yet the dynamics of bacterial spoilage and factors that affect it are not well understood. Notably, while China is the top producer of spinach globally, there is limited available microbiological data in the literature for spinach supply chains in China. The overall goal of this foundational study was to establish a baseline understanding of bacterial population dynamics on spinach from harvest to 10 days postprocessing for a Chinese supply chain that includes distribution via traditional grocery (a local physical store) and eCommerce (an online store). To this end, organic spinach samples were collected at different stages in a Chinese supply chain by following the same 3 lots, starting at point-of-harvest through processing and distribution via a local grocery store and eCommerce. After distribution, the same 3 lots were stored at 4 °C with microbiological testing performed on multiple days up to day 10 postprocessing, simulating storage at the point-of-consumer. Results showed aerobic plate counts and total Gram-negative counts did not significantly differ across stages in the supply chain from harvest through processing. However, packaged spinach from the same processing facility and lots, exhibited different patterns in bacterial levels across 0 to 10 days postprocessing, depending on whether it was distributed via the local grocery store or eCommerce. Evaluation of bacterial populations performed on a subset of the packaged spinach samples indicated Gram-negative bacteria, in particular *Pseudomonas*, were predominant across all days of testing (days 0, 3, and 10 postprocessing), with populations differing at the genus level by day. Overall, this study improves our understanding of the dynamics of bacterial populations on spinach and provides baseline data needed for future studies.

1. Introduction

Fresh produce contamination with spoilage-causing bacteria is a problem as these bacteria are able to produce metabolites that may lead to undesirable sensory characteristics and product degradation, thereby limiting shelf-life (Gram et al., 2002; Jacxsens et al., 2003; Lee et al., 2013; Ragaert et al., 2007). Here we refer to shelf-life as the amount of time a food product is considered acceptable for human consumption when stored at the appropriate storage conditions, whereas spoilage refers to a process where a food product becomes undesirable for human consumption. While quantification of total bacteria alone cannot

directly predict the sensory quality of the product, undesirable characteristics may become noticeable to consumers when spoilage-causing bacteria exceed a certain threshold (e.g., >7 $\log_{10}$CFU/g) (Gram et al., 2002; Ragaert et al., 2007; Zhou et al., 2022). Fresh spinach, in particular, is highly perishable and vulnerable to spoilage due to bacteria, especially Gram-negative bacteria (e.g., *Pseudomonas*), based on previous studies using culture-dependent and independent methods (Babic and Watada, 1996; Gu et al., 2018; Lopez-Velasco et al., 2011; Medina et al., 2012; Rosberg et al., 2021; Tudela et al., 2013). However, there is a need to improve understanding of the changes in bacterial levels and populations over time on spinach, from primary production to

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consumption.

The annual worldwide gross production of spinach was ~26 million tonnes in 2018, 90.7% of which was produced in China (Food and Agriculture Organization of the United Nations, 2019). Yet, research on spinach microbial quality and safety has primarily been performed in the United States (U.S.) and Europe, which represent only 1.5% and 2.6% of global spinach production, respectively (Food and Agriculture Organization of the United Nations, 2019). Studies have reported bacterial levels (e.g., aerobic plate counts) on fresh spinach collected at retail in different countries including India (Mritunjay and Kumar, 2017), South Korea (Tango et al., 2014), Spain (Abadias et al., 2008), and the U.S. (Zhang et al., 2018), but not including China. Previous studies have also reported spinach aerobic plate counts on different days of storage postprocessing (Caponigro et al., 2010; Gu et al., 2018; Kase et al., 2012; Zhou et al., 2022); however, fresh spinach evaluated was typically processed (e.g., washed), although spinach is sold to consumers unwashed in many parts of the world (as was the case in our study). Few studies have investigated bacterial populations on unwashed spinach (Gu et al., 2018; Rosberg et al., 2021). Further, previous studies on fresh produce, have been primarily focused on distribution via traditional grocery stores (i.e., physical stores), while online stores (i.e., eCommerce) have become increasingly popular across the world, especially in China (Agriculture and Agri-Food Canada, 2017; Wang et al., 2020; Wang and Somogyi, 2018). There are many challenges to

food quality related to distribution of fresh produce like spinach via eCommerce such as the lack of cold chain infrastructure for direct-to-customer distribution (Jin et al., 2017; Mkansi et al., 2018). Thus, it is important to investigate fresh produce supply chains in China, including distribution via traditional and non-traditional grocery stores (e.g., eCommerce).

In this foundational study, we investigated the microbiological quality of spinach along a Chinese supply chain. In the studied supply chain, spinach was grown in a greenhouse, transported to a separate facility for storage and processing (ending with packaging), and stored until distribution. The distribution either involved (i) transporting packaged spinach to a local grocery store or (ii) allowing customers to purchase packaged spinach online through a WeChat sub-application and then shipping direct-to-consumer. Investigating the changes in bacterial levels and populations over time under controlled storage conditions in different distribution channels, may allow improved understanding of product quality and shelf-life. Additionally, understanding the environmental conditions and bacterial population dynamics on spinach at different stages in the supply chain will allow for identification of strategies for improving microbiological quality. Thus, our aims were to (1) determine bacterial levels on spinach samples collected at different stages in a supply chain, (2) determine bacterial levels and populations on packaged spinach samples that underwent typical consumer distribution pathways either via a local grocery store
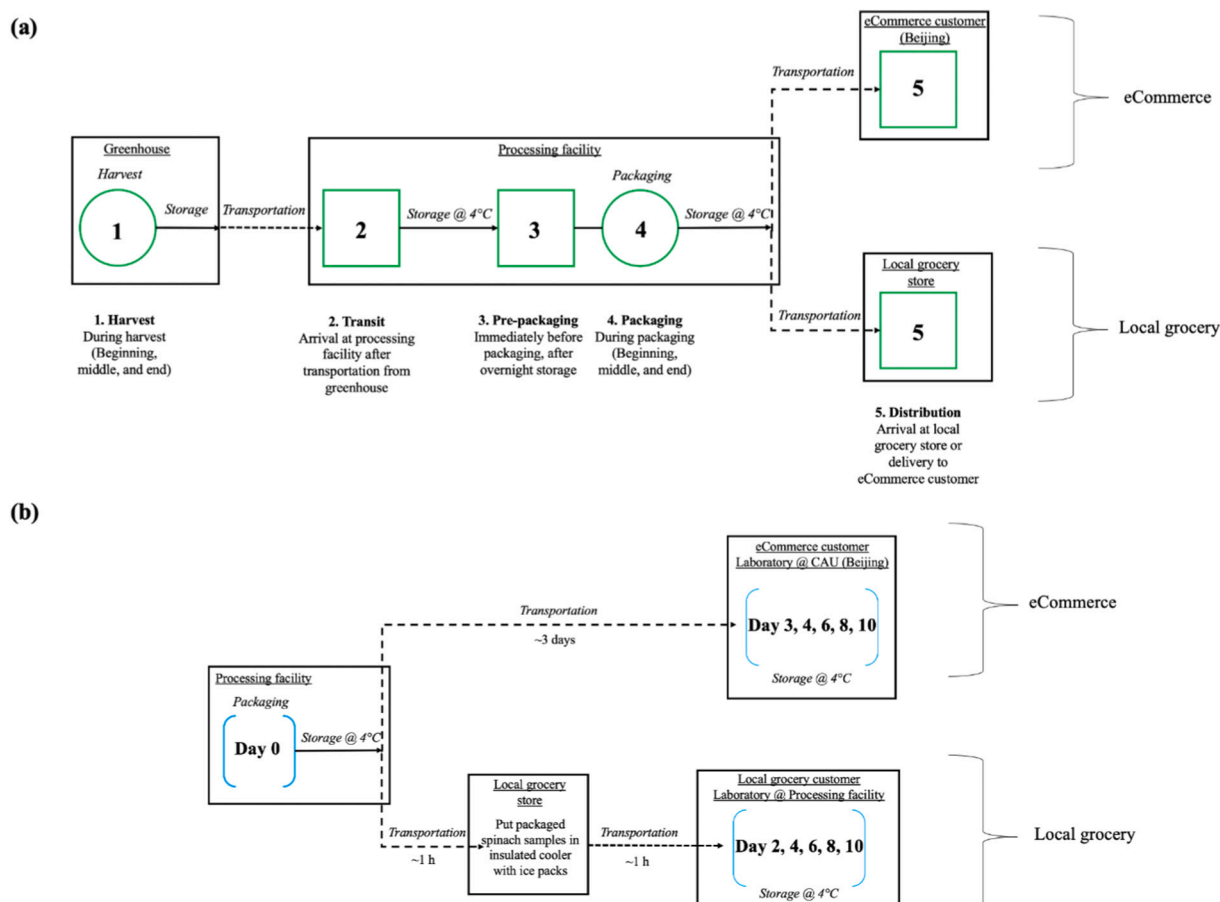

Fig. 1. Schematic of (a) the supply chain sampling from harvest through distribution and (b) the packaged spinach sampling from day 0 to day 10 postprocessing. For (a), bold numbers centered inside green shapes (circles and squares) indicate stages in the supply chain where spinach samples were collected for the 3 lots. Circles (stages 1 and 4) indicate samples were collected at the beginning, middle, and end of the stage to obtain 3 replicates for the stage, while squares (stages 2, 3, and 5) indicate that all 3 replicate samples for the stage were collected at a single timepoint (for stage 5, samples were collected separately for local grocery and eCommerce channels). For (b), the bold numbers inside blue brackets indicate days postprocessing when packaged spinach samples were collected in triplicate for the 3 lots. For (a) and (b) Labelled boxes indicate location of sampling, where the greenhouse, processing facility, and local grocery store were all located in one city, while the distribution location for eCommerce was another city (i.e., Beijing). Activities are labelled (in italics) above lines with arrows; dashed lines indicate movement of spinach between locations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

or eCommerce and stored up to 10 days postprocessing, and (3) identify factors associated with the dynamics of bacterial populations on spinach along the supply chain and subsequent refrigerated storage.

2. Materials and methods

2.1. Study design overview

This longitudinal study was designed to follow the same lots of organic spinach ("Northeastern Daye" variety) from harvest through distribution to consumers. The spinach supply chain was located in Northern China; all operations (growing, transportation, storage, packaging, purchasing, marketing, etc.), except eCommerce shipping, were managed by a single company. A schematic of the study design is provided in Fig. 1. Sampling was designed such that spinach samples were collected across various stages in the supply chain. Two distribution channels were selected for this study to represent distribution via (i) a local grocery store or (ii) eCommerce. To simulate the distribution to a consumer, packaged spinach samples were transported either to (i) the company's laboratory at the processing facility (via a local grocery store, representing the local grocery-customer end in the supply chain) or (ii) the laboratory of Prof. Li-Qun Zhang at China Agricultural University in Beijing (via the online store, representing the eCommerce-customer end in the supply chain). Immediately upon arrival at the respective laboratories, the spinach packages were stored in a refrigerator, simulating consumer storage. The stored packages were subjected to microbial testing up to 10 days postprocessing, which represented a length of time twice the company's 5 day quality guarantee labelled on the package.

2.1.1. Harvest and transit (1 day before processing)

Organic spinach was grown in 3 m wide by 8 m long plots in a single ~867 m^2 Chinese-style greenhouse (passive solar greenhouse with a thermal blanket that is applied in the nighttime and rolled up during the daytime). Prior to seeding, fertilization of the soil was performed manually using organic sheep manure (a total amount of ~2.6 m^3 for the 1 greenhouse). Pesticides were not used. Well water was applied to the spinach using flood irrigation two times during the crop cycle, once right after the seeding and once in the middle of the crop cycle. After a growth period of ~70 days, spinach was hand-harvested, using a sickle, in different sections of the greenhouse, starting from one side of the greenhouse (closest to the single-entry point) and working across towards the other side of the greenhouse. The harvested spinach was packed in crates and stored in the greenhouse and then transported to the processing facility (~36 km distance) via an enclosed non-refrigerated truck during the same day. Upon arrival at the processing facility (i.e., transit stage; Fig. 1), crates were stored overnight in a cold storage room with a set temperature of 4 °C.

2.1.2. Processing (pre-packaging and packaging) and postprocessing (distribution and refrigerated storage)

Processing: The next day, crates were transferred to the packaging area (i.e., pre-packaging stage; Fig. 1) and then packed in bunches with the root attached. For packaging, bunches were first sorted and trimmed manually (by hand or using a knife) with the purpose of removing leaves that were (i) small, (ii) not tightly attached to a bunch, and/or (iii) visibly defective (e.g., due to insect damage or plant disease). Next, acceptable spinach was packed in plastic wrap (Lejie Trade Co., Ltd., Langfang, China), with each package weighing 300 g. Day of packaging was denoted as day 0 postprocessing, which we describe next.

Postprocessing: Packages of spinach were then stored in crates in a cold storage room with a set temperature of 4 °C. During cold storage, ~30 min prior to scheduled distribution, packages were allocated for either (i) the local grocery store or (ii) eCommerce in Beijing (Fig. 1). Packages destined for the local grocery store were kept in crates, then transported without refrigeration to the store within the same day. Due to logistical constraints, study samples could not be stored in the open

display case at the store, thus upon delivery of product to the grocery store, the allocated spinach packages were immediately transported back to the on-site laboratory at the processing facility, where it was stored in a refrigerator (set at 4 °C) until testing, simulating consumer handling. Orders made through eCommerce were shipped in Styrofoam boxes without refrigeration from the processing facility direct-to-consumer via a commercial service (STO Express Co). Upon arriving at the laboratory in Beijing, packages were also stored in a refrigerator (set at 4 °C) until testing, simulating consumer handling.

2.2. Spinach sample collection and handling

To collect samples, we followed and sampled 3 consecutive lots of spinach (lot was defined as spinach harvested on the same day from the greenhouse) along the organic spinach supply chain. Spinach was harvested on January 6, 7, and 8 for lot 1 (L1), lot 2 (L2), and lot 3 (L3), respectively. For each lot, spinach supply chain samples were collected in triplicate at 5 stages: (1) harvest, (2) transit, (3), pre-packaging, (4) packaging, and (5) distribution for (i) local grocery and (ii) eCommerce (refer to Fig. 1 for a schematic of the sampling design). To obtain a triplicate sample per stage, one sample was collected at each of the beginning, middle, and end of harvest and packaging stages, while for other stages (i.e., transit, pre-packaging, and distribution) the three samples were collected at a single timepoint but each from a separate crate or container, as possible.

For each sample collected prior to packaging (i.e., 3 harvest + 3 transit + 3 pre-packaging = 9 samples per lot), a handful of spinach from each of 4 corners of the crate was collected using a gloved-hand and about 100 g of sample was transferred into a sterile 2.72 L Whirl-Pak bag (Nasco, Fort Atkinson, WI). Gloves were changed and sprayed with 70% ethanol between samples. Samples collected during packaging and distribution (36 samples per lot) were in their original packages, which contained ~300 g of spinach. For L2 and L3, only ~50% of spinach passed the company's routine pre-packaging inspection process, resulting in a reduction in the number of packaged spinach samples collected (27 for L2 and 34 for L3).

Additionally, for each lot, packaged spinach samples were allocated for both the local grocery and eCommerce distribution channels for testing in triplicate every 2 days, until 10 days postprocessing (local grocery and eCommerce packaged spinach studies). The samples collected at packaging represent day 0 postprocessing for both channels. The remaining samples for the eCommerce packaged spinach study were allocated via the company's regular eCommerce process (i.e., ordering through a WeChat program, followed by shipping), ensuring enough samples were shipped for testing in triplicate on each day of testing, while samples for the local grocery packaged spinach study were allocated via the company's daily re-stocking process for the local grocery store and thus collected on-site at the store and then transported back to the laboratory.

For both local grocery and eCommerce packaged spinach studies, samples (except day 0) were randomly allocated for testing so that 3 samples were tested on each day of testing. Samples collected at harvest and retail were stored on ice packs in an insulated cooler during transit to the laboratory. Samples collected on-site at the processing facility, were transported to the on-site laboratory immediately after collection. Samples collected prior to packaging, at retail, and the day 0 samples (i.e., point-of-packaging) were tested within 6 h of collection. Packaged spinach samples at the point-of-consumer locations were refrigerated until testing (2, 4, 6, 8, or 10 days postprocessing). Due to ground-shipping delays, testing in the Beijing laboratory started upon delivery on day 3 instead of the originally planned day 2 postprocessing.

2.3. Microbiological evaluation of spinach samples along the supply chain and refrigerated storage

Testing of all spinach samples was performed at the on-site

laboratory at the processing facility except the eCommerce packaged spinach samples, which was performed in the laboratory of Prof. Li-Qun Zhang at China Agricultural University in Beijing.

2.3.1. Bacterial enumeration

For testing each sample, ~25 g of spinach (leaf and stem intact, without root) were transferred to a sterile 1.63 L filter Whirl-Pak bag (Nasco, Fort Atkinson, WI) and suspended in ~100 mL of Butterfield's buffer (Steward Ltd., Worthing, UK) and manually agitated for 120 s. A 10-mL aliquot of each spinach suspension was transferred into an individual sterile 15 mL polypropylene centrifuge tube, which was serially diluted (1:10) with Butterfield's buffer to appropriate levels and plated (1-mL) in duplicate on aerobic count Petrifilm and coliform count Petrifilm (3M, St. Paul, MN). Petrifilms were then incubated at 35 °C for 48 ± 2 h, followed by enumeration for aerobic plate count (APC; APC Petrifilm) and total Gram-negative count (GN; coliform count Petrifilm, enumerating all colonies regardless of gas production to determine GN; the 48 h of incubation allows for break-through growth of non-coliform Gram-negative bacteria on the coliform count Petrifilm thus GN is determined by enumerating all colonies (Rojas et al., 2020)). Additionally, for purposes of isolation for later characterization, for day 3 and day 10 eCommerce samples (but not for day 0 samples, due limited capacity of the on-site laboratory at the processing facility), 100 μL sample aliquots were spread plated onto Brain Heart Infusion (BHI) agar (Difco, BD Franklin Lakes, NJ) plates in duplicate, followed by incubation at 35 °C for 24 and 48 h, followed by enumeration. For serial dilutions and plating, due to lack of a vortex machine at the on-site laboratory, the 15-mL tubes were shaken 25 times to be completed within 7 s (adapted from Frank and Yousef, 2004).

2.3.2. Bacterial isolation and preservation

Bacterial isolates were collected from day 0 samples (Petrifilm tests only), day 3 eCommerce samples (both Petrifilm tests and BHI spread plates), and day 10 eCommerce samples (both Petrifilm tests and BHI spread plates); as the day 0 testing had been performed at the processing facility's on-site laboratory, the Petrifilm tests for the day 0 samples were transported by study personnel to the Beijing laboratory for bacterial isolation. Due to logistical constraints, isolates were not collected from other samples. For each of the tests performed using Petrifilm (i.e., APC and GN), 2 colonies were randomly selected from each plate for isolation (for a total of 4 isolates per sample, per test), as different morphologies on the Petrifilms were not reflective of different taxa. For the BHI spread plates, colony morphologies were observed using a magnifier, and unique colony morphologies were identified based on their size, form, elevation, margin, color, opacity, and surface texture. To maximize the collection of unique taxa that can be found on fresh spinach, 1 colony representing each visually unique colony morphology was selected for isolation after 24 h of incubation and similar intact colonies were marked for later comparison; after 48 h of incubation, colonies were reassessed, and additional unique colonies were selected for isolation. Colonies selected for isolation were sub-streaked from the original plate onto BHI agar, followed by incubation at 32 °C for 24 h. Single colonies were subsequently grown in BHI broth at 32 °C for 18 to 24 h before being frozen and stored in 15% (vol/vol) glycerol at −80 °C.

2.3.3. 16S rRNA gene sequencing

For each sample on each day of isolate collection, isolates were selected for 16S rRNA gene sequencing. Lysates for the selected isolates were prepared by suspending a colony in 100 μL of sterile distilled water (dH$_2$O). Suspensions were heated to 95 °C for 15 min in a thermal cycler and were stored at 4 °C prior to amplification. PCR was performed as previously described (Reichler et al., 2018). An internal fragment of the 16S rRNA gene was amplified with primers 16S-PEU7-F (Rothman et al., 2002) and 16S-DG74-R (Greisen et al., 1994). PCR products were purified using the E.Z.N.A. Gel Extraction Kit (Omega Bio-tek, Inc., Norcross, GA). Sequencing was performed at the GENEWIZ China & Suzhou

Lab (Suzhou, China). Consensus sequences were formed by aligning and proofreading raw sequence data in Sequencher v5.4.6 (Gene Codes Corporation, Ann Arbor, MI). Genus-level identities were obtained by a BLAST search against a local copy (downloaded January 12, 2021) of the Ribosomal Database Project database (Cole et al., 2009). Analyses of sequence data, including assignment of sequence types (ST) and phylogenetic analysis, are described in Supplementary Material 1.

2.4. Environmental and management data collection

Alongside spinach sample collection, relevant management and environmental data were collected throughout the supply chain for each of the 3 sampled lots. Temperature, relative humidity, leaf wetness, and solar radiation data in the center of the greenhouse were collected via a USB micro-station with different sensors (Onset Computer Corporation, Bourne, MA); dew point was calculated using temperature and relative humidity data. Temperature and relative humidity data were collected in the refrigerated open display case located at the local grocery store by affixing a Track-It™RH/Temp Datalogger (Monarch Instrument, Amherst, NH) to the shelf used for displaying packaged spinach to regular customers; these data were collected continuously from this location between January 9th and 17th 2020 (i.e., after the spinach harvest samples were collected). Following the spinach samples from point-of-harvest through the end of distribution stage, temperature and relative humidity data were collected by affixing a Track-It™ RH/Temp Datalogger (Monarch Instrument, Amherst, NH) to samples. Measurements in the greenhouse were recorded by the station every 15 min, while measurements using the portable dataloggers were recorded every 1 min. The timing of each step for a given lot of spinach was also manually recorded (i.e., the start and end time of each supply chain activity).

2.5. Data analyses

Data were compiled and cleaned in R Statistical Programming Environment (version 4.0.5; R Core Team, 2021) and OpenRefine (version 2.7; http://openrefine.org). Bacterial count (CFU/g) data were log$_{10}$-transformed to achieve an approximate normal distribution. Absence of data, due to (i) a recording error for the start time of transportation from the greenhouse to the processing facility for L1 and (ii) a malfunctional datalogger attached to the spinach crate from harvest through transit from the greenhouse to the processing facility for L1, were treated as missing data in analyses.

All analyses were performed in R (R Core Team, 2021). Plots were prepared to visualize study data, using the ggplot2 (Wickham, 2016) and ggpubr (Kassambara, 2020) packages. Summary statistics were calculated for study data, using the dplyr package (Wickham et al., 2021).

In the supply chain analysis, Kruskal-Wallis (KW) tests were performed, using the stats package (R Core Team, 2021), to evaluate (i) whether bacterial levels differ across stages during production or (ii) across beginning, middle, and end replicates within a stage (harvest and packaging). Specifically, stage and replicate (i.e., beginning, middle, and end of the respective stage) were used as the explanatory variables to conduct KW for (i) and (ii), respectively. Subsequently, data from multiple lots were combined across stages for (i) and (ii) since KW did not identify evidence of significant differences among the lots. Here and elsewhere in this study, KW test was chosen because of the very small datasets (McDonald, 2014). Considering the novel nature of the study, correction for multiple testing was not conducted.

In the packaged spinach data analysis, the outcome of interest was bacterial level (log$_{10}$CFU/g) for each of the 2 tests (i.e., APC and GN) on spinach over the 10 days of testing postprocessing in each of 3 lots and 2 locations. Bacterial levels on samples (i.e., APC and GN) on different days were first visually evaluated and then subjected to descriptive statistics. Given the limitations associated with a relatively small sample size, the observed bacterial dynamics across the 0 to 10 days

postprocessing was described with a statistic, "daily rate of change" ($\Delta\log_{10}$CFU/d), which represented the rate of net change in bacterial counts during the phase of apparent constant increase in bacterial counts (hereafter referred to as "constant increase phase"). To achieve this, subsets of data representing that constant increase phase were identified; for the local grocery packaged spinach study these subsets were L1 (days 2 to 6), L2 (days 2 to 6) and L3 (days 2 to 6); for the eCommerce packaged spinach study these subsets were L1 (days 4 to 8), L2 (days 4 to 6), and L3 (days 4 to 8). Next, to calculate $\Delta\log_{10}$CFU/d, the starting concentration in a subset (i.e., mean bacterial level for the first day of the phase) was subtracted from the last concentration in the subset (i.e., mean bacterial level for the last day of the phase) and divided by the number of days (i.e., duration of the phase). The reason for taking this simplified approach was that fitting growth models (e.g., Baranyi and Gompertz growth models; (Micha and Corradini, 2011)) was considered inappropriate, given spinach samples were naturally contaminated (i.e., not controlled growth experiments with inoculated samples) and the bacterial growth likely represented multiple different organisms. The estimated $\Delta\log_{10}$CFU/d for the packaged spinach studies were subjected to summary statistics (mean, minimum, and maximum). Subsequently, we fit a linear regression model, using the "lm" function in the lme4 package (Bates et al., 2015), to determine whether $\Delta\log_{10}$CFU/d (outcome) differs by location or bacterial test (independent fixed effects). In all analyses, statistical significance testing was conducted at the significance level of $P < 0.05$.

For the isolate data, the R package vegan was used to determine the number of unique genera detected in each sample (Oksanen et al., 2020). Using only data from the Petrifilms, Fisher's Exact tests were performed using the R package stats (R Core Team, 2021), to evaluate whether genus-level populations differed by (i) test, (ii) lot, and (iii) testing day. Separately, Fisher's Exact tests were performed using data from the BHI spread plates, to evaluate whether genus-level populations differed by (i) lot and (ii) testing day. This analysis was performed separately for the Petrifilm and BHI isolate data due to methodological differences: (i) data were not available from BHI for day 0 and (ii) a different isolate selection process was used (selecting all visually unique colonies from BHI spread plates, compared to selecting 2 colonies from each Petrifilm).

3. Results

3.1. Spinach sampling and environmental data collection highlighted variations in supply chain logistics and environmental conditions

Comparison of timing of supply chain logistics among the 3 lots showed that, except for the end of transit from the greenhouse to the processing facility, the time of day that each specific activity occurred (i.e., start or end time of each activity) was within 1.5 h among the 3 lots (Table S1). In particular, distribution activities (i.e., transportation of packaged spinach from the processing facility to the local grocery store and Beijing) had consistent timing among the 3 lots, relative to activities upstream in the supply chain (e.g., harvest) that were more variable (Table S1). This indicates that harvest and production scheduling may vary more day-to-day, compared to distribution activities. For all supply chain activities involving transportation, duration of transportation activities varied across the 3 lots (Table 1); transportation was especially affected by poor driving conditions and increased traffic due to bad air quality (smog) or weather events (e.g., snowfall). Notably, shipping to Beijing took 3 days instead of the typical 2 days for all 3 lots in this study, suggesting that the weather conditions were abnormally difficult for transportation.

Environmental conditions in the greenhouse showed distinct daily trends that may be explained by diurnal variation as well as the design of the greenhouse (Fig. S1). Summary statistics for the 5 environmental variables showed that conditions in the greenhouse in the 24 h prior to spinach harvest varied among the 3 lots (Table 2).

Table 1
Summary of temperature and relative humidity data collected via dataloggers attached to spinach samples throughout the supply chain for 3 lots.

Lot	Activity	Duration (minutes)	Temperature (°C)				Relative humidity (%)			
			Mean	Q1[c]	Median	Q3[d]	Mean	Q1[c]	Median	Q3[d]
L1	Harvest	40	NA[a]	NA	NA	NA	NA	NA	NA	NA
	Storage in greenhouse	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Transit from greenhouse to processing facility	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Cold storage	1197	2.3	2.2	2.5	2.6	86.2	84.5	88.4	89.0
	Packaging	71	8.9	8.9	9.0	9.2	83.7	82.0	82.8	84.5
	Cold storage for both local grocery and eCommerce packages	178	3.1	2.2	2.4	3.2	87.0	86.2	87.8	89.4
	Cold storage for eCommerce packages only[b]	101	3.0	2.6	2.6	3.1	75.0	72.4	76.6	78.7
	Transit from processing facility to local grocery store	42	3.1	3.0	3.1	3.1	89.3	88.8	89.6	89.6
	Transit from processing facility to eCommerce customer in Beijing	4010	2.8	2.5	3.0	3.2	91.7	90.4	92.4	93.5
L2	Harvest	17	9.2	7.6	8.9	11.1	54.4	50.6	55.2	59.1
	Storage in greenhouse	294	5.5	4.9	5.3	6.1	75.2	74.5	76.3	77.0
	Transit from greenhouse to processing facility	86	3.9	3.1	4.1	4.6	82.6	78.9	85.7	85.8
	Cold storage	1160	3.1	3.1	3.1	3.2	87.7	85.9	88.0	89.6
	Packaging	56	7.7	7.6	7.8	7.9	91.7	91.2	91.6	91.9
	Cold storage for both local grocery and eCommerce packages	106	5.0	4.2	4.3	5.4	87.4	86.8	88.3	88.7
	Cold storage for eCommerce packages only[b]	136	5.0	4.1	4.4	4.9	67.4	65.2	70.9	73.9
	Transit from processing facility to local grocery store	69	4.8	3.9	4.7	5.7	87.3	85.9	87.4	88.6
	Transit from processing facility to eCommerce customer in Beijing	4005	3.0	2.4	3.0	3.5	91.3	91.3	92.7	93.4
L3	Harvest	22	9.0	7.4	7.8	10.6	53.5	47.7	54.9	58.2
	Storage in greenhouse	324	7.8	6.4	8.4	9.3	78.5	78.2	80.0	81.1
	Transit from greenhouse to processing facility	102	4.4	3.9	4.3	4.4	83.5	82.5	85.8	86.5
	Cold storage	1017	3.9	3.8	3.9	4.1	91.0	89.9	91.4	92.6
	Packaging	75	6.2	5.9	6.4	7.0	93.7	93.6	93.7	93.8
	Cold storage for both local grocery and eCommerce packages	185	4.2	3.8	4.2	4.4	90.4	90.6	91.1	91.5
	Cold storage for eCommerce packages only[b]	74	5.5	5.0	5.2	5.8	70.8	68.7	71.5	73.7
	Transit from processing facility to local grocery store	53	5.2	4.3	5.2	5.8	89.3	89.1	89.3	89.8
	Transit from processing facility to eCommerce customer in Beijing	4066	2.2	1.6	2.4	2.7	90.2	88.7	91.0	93.2

[a] NA = not available due to malfunctional datalogger.
[b] Includes only the remaining storage for the eCommerce packages, after the local grocery packages had been removed from the cold storage room.
[c] Q1 = 25th percentile.
[d] Q3 = 75th percentile.

Table 2

Summary of environmental conditions in the greenhouse and the distribution stage for the 24 h prior to the start of spinach harvest for each of the 3 lots. Spinach was harvested for lot 1 on January 6th (starting at 10:13 am), lot 2 on January 7th (starting at 9:08 am), and lot 3 on January 8th 2020 (starting at 9:25 am).

Lot	Variable (units)	Mean	Q1[b]	Median	Q3[c]
L1[a]	Leaf wetness (%)	0.3	0.0	0.0	0.6
	Solar radiation (watts/m^2)	4.9	0.6	0.6	4.4
	Temperature (°C)	3.7	3.3	3.4	3.9
	Relative humidity (%)	97.9	96.9	98.8	99.9
	Dew point (°C)	3.3	3.1	3.2	3.4
L2	Leaf wetness (%)	1.5	0.0	1.8	2.4
	Solar radiation (watts/m^2)	16.9	0.6	0.6	37.2
	Temperature (°C)	5.7	4.7	5.3	6.4
	Relative humidity (%)	99.3	98.8	100.0	100.0
	Dew point (°C)	5.6	4.7	5.3	6.3
L3	Leaf wetness (%)	1.8	1.2	2.4	2.4
	Solar radiation (watts/m^2)	13.3	0.6	0.6	28.4
	Temperature (°C)	2.0	0.1	1.8	4.0
	Relative humidity (%)	100.0	100.0	100.0	100.0
	Dew point (°C)	2.0	0.1	1.8	4.0

[a] Due to the timing of launching the environmental station, L1 includes 23 h, rather than 24 h of data.

[b] Q1 = 25th percentile.

[c] Q3 = 75th percentile.

Temperature and relative humidity during spinach handling from harvest through packaging and distribution showed similar patterns among the 3 lots and illustrate varying levels of environmental control for spinach across the steps in the supply chain (interrupted cold chain) (Table 1). Among all supply chain activities, greenhouse activities (harvest and storage) and packaging had the highest mean temperatures for the 3 lots, while transportation to Beijing and cold storage prior to packaging had the lowest mean temperatures for the 3 lots. Relative humidity was consistently below 95% (>95% has been suggested to be optimal relative humidity for maintaining spinach quality) (López Camelo, 2004) across all activities and lots. Retail display data showed continually fluctuating conditions (Fig. S2); overall, temperature ranged from 5.9 to 16.5 °C (mean of 8.4 °C) and relative humidity ranged from 33.9 to 71.6% (mean of 59.7%).

3.2. Spinach from different stages across the supply chain did not have significantly different bacterial levels

Overall, spinach at harvest had a mean ± standard deviation (SD) across all 9 samples of 6.4 ± 0.6 log$_{10}$CFU/g for APC and 5.9 ± 0.6 log$_{10}$CFU/g for GN (Table S2). Among all supply chain samples, APC ranged from 5.6 to 7.4 log$_{10}$CFU/g and GN ranged from 5.1 to 6.7 log$_{10}$CFU/g (Table S2). Visualization of microbiological data across the stages of the supply chain for the 3 lots indicated that there did not appear to be a systematic difference in bacterial levels between lots or stages (Fig. S3).

Results of KW tests performed separately for APC and GN support that there was no significant difference in bacterial levels among the stages for supply chain data and among replicates representing the beginning, middle, and end of packaging and harvest stages ($P > 0.05$). In lots L1 and L3, bacterial levels at the end of harvest were numerically higher relative to those collected at the beginning or middle of harvest, whereas for L2, samples collected at the middle and end of harvest had numerically higher bacterial levels relative to those at the beginning (Fig. S4). However, results of KW tests performed separately for APC ($P = 0.07$) and GN ($P = 0.07$) each showed only borderline evidence for association between replicate and bacterial level for harvest data.

3.3. Bacterial population dynamics on packaged spinach over 10 days postprocessing differed by location and lot

Visualization of microbiological count data from the packaged spinach studies showed patterns in bacterial levels over time that differed among locations and lots (Fig. 2). The pattern (relative levels and direction of change) of mean bacterial levels over days of testing was similar for APC and GN, indicating Gram-negative bacteria, rather than Gram-positive bacteria, were the predominant aerobic bacteria on spinach samples (Fig. 2); the dominance of Gram-negative bacteria was also supported by the sequencing data. With a few exceptions, mean bacterial levels were lowest on day 0 postprocessing for both APC and GN, supporting that the microbial population on spinach samples included psychrotrophic bacteria (Fig. 2, Table S3). Notably, for the eCommerce packaged spinach study, mean bacterial levels were instead lowest on day 3 (GN for L2) or day 4 (APC for L2, L3), which could be due to chance or may suggest environmental conditions during shipping to Beijing or handling upon arrival at the laboratory in Beijing that may have been unfavorable for bacterial growth or survival (Fig. 2, Table S3). Mean bacterial levels consistently reached their maximum before the last day of testing (i.e., day 10 postprocessing), either on day 6 (L1, L2, L3 local grocery; L2 eCommerce) or day 8 (L1, L2 eCommerce) for APC and similarly either on day 6 (L1, L3 local grocery; L2 eCommerce) or day 8 for GN (L2 local grocery; L1, L3 eCommerce) (Fig. 2, Table S3).

We identified that the duration of the phase marked by an apparent constant net increase of mean bacterial levels per day was 4 days for both APC and GN, except L2 eCommerce which only included 2 days (from day 4 to day 6) as mean bacterial levels appeared to either plateau (APC) or decline (GN) between day 6 and day 8 (Fig. 2). It should be noted that these estimates of the duration of this constant increase phase were conservative because testing for the packaged spinach studies was limited to once every two days for each location. For the local grocery packaged spinach study, Δlog$_{10}$CFU/d ranged from 0.07 to 0.15 log$_{10}$CFU/g per day (mean of 0.12 log$_{10}$CFU/g per day) for APC and from 0.08 to 0.14 log$_{10}$CFU/g per day (mean of 0.11 log$_{10}$CFU/g per day) for GN. In comparison, Δlog$_{10}$CFU/d for the eCommerce packaged spinach study ranged from 0.19 to 0.29 log$_{10}$CFU/g per day (mean of 0.23 log$_{10}$CFU/g per day) for APC and from 0.11 to 0.22 log$_{10}$CFU/g per day (mean of 0.18 log$_{10}$CFU/g per day) for GN. Results from fitting linear regression models with the Δlog$_{10}$CFU/d estimates showed (i) location was significantly associated with Δlog$_{10}$CFU/d, where Δlog$_{10}$CFU/d from the eCommerce study was faster than Δlog$_{10}$CFU/d from the local grocery study by an effect size of 0.09 log$_{10}$CFU/g per day (95% Confidence Interval [CI]: 0.03, 0.15; $P = 0.008$) (Table 3), but (ii) bacterial test (APC, GN) was not significantly associated with Δlog$_{10}$CFU/d ($P > 0.05$); thus, the final model included only location as a fixed effect (Table 3).

3.4. Genus-level populations differed by day

In total, we successfully sequenced 456 bacterial isolates collected from the subset of 27 spinach samples; these isolates represented 127 unique 16S rRNA gene ST. Overall, the 16S rRNA data classified isolates into 4 phyla: Proteobacteria (88%; 401/456 isolates), Actinobacteria (8%; 35 isolates), Bacteroidetes (3%; 15 isolates), and Firmicutes (1%; 5 isolates) (Fig. S5). Within Proteobacteria, the majority of all isolates were classified into the class Gammaproteobacteria (375 isolates, representing 88 unique ST), which includes *Pseudomonas* (224 isolates, 29 ST), *Pantoea* (72 isolates, 27 ST), *Erwinia* (32 isolates, 5 ST), and *Rheinheimera* (19 isolates, 8 ST), as well as 12 other genera (28 isolates, 19 ST).

Among the subset of 27 spinach samples that underwent characterization of representative bacterial isolates using 16S rRNA gene sequencing, all (100%) yielded at least 1 isolate identified as *Pseudomonas*, and 22 and 14 samples yielded at least 1 isolate representing the genera *Pantoea* and *Erwinia*, respectively. For the 14 samples
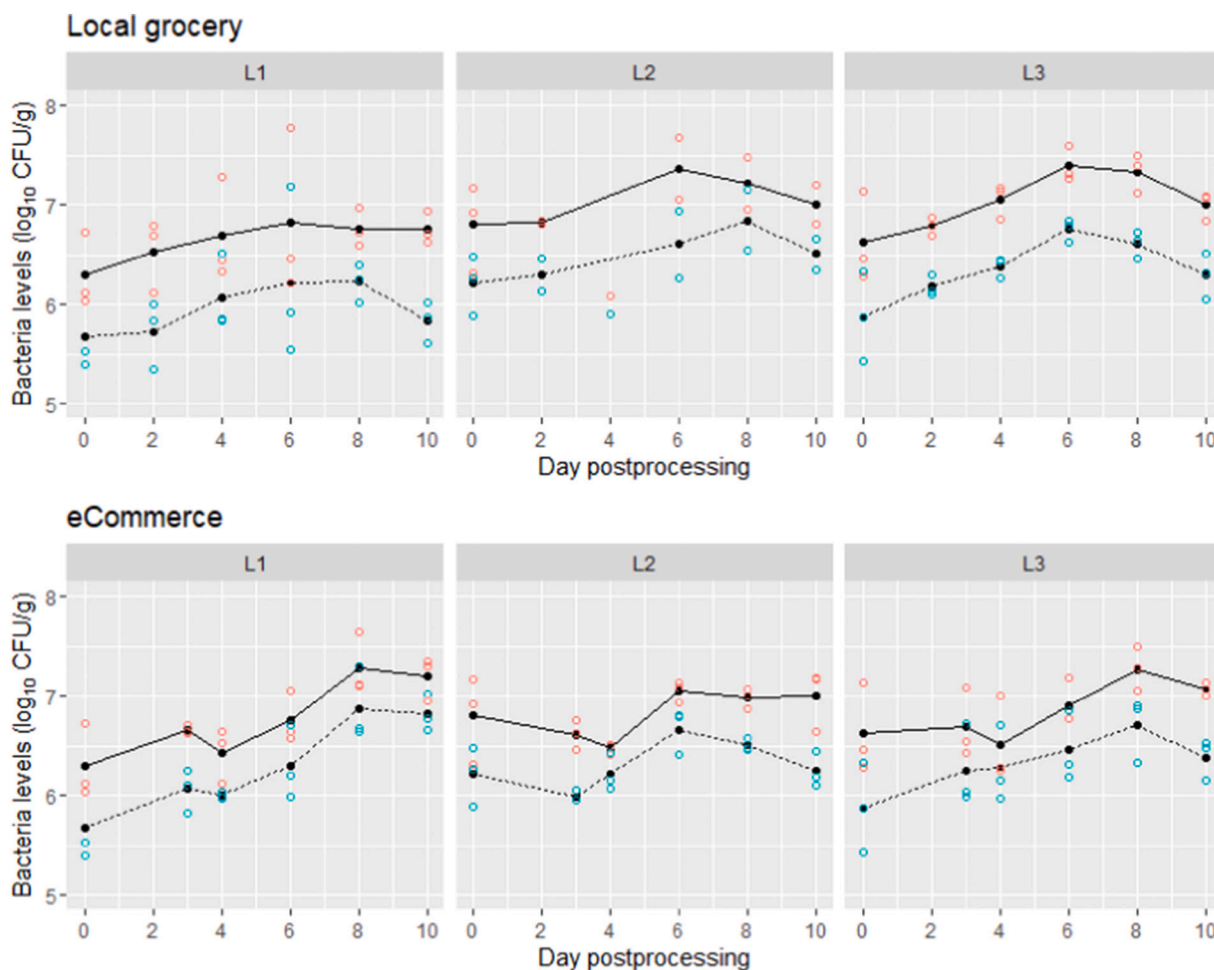
Fig. 2. Bacterial levels on spinach ($\log_{10}$ CFU/g) across each day of testing performed for the 3 lots (L1, L2, and L3) for each of the local grocery and eCommerce distribution and refrigerated storage chains. The black points connected with lines depict mean bacterial levels of the sample replicates for aerobic plate count (solid lines) and total Gram-negative count (dashed lines), respectively; also found in Supplemental Table S5. Mean aerobic plate count and total Gram-negative count are not included for L2 day 4 because only 1 sample was collected. Data from day 0 were equivalent for the local grocery and eCommerce packaged spinach studies as these samples were collected at packaging. The red and blue points overlaid represent individual data for each spinach sample and postprocessing day for aerobic plate count and total Gram-negative count, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3

Final linear regression model for predictors of net change of mean bacterial levels per day ($\Delta\log_{10}$CFU/g per day) during the apparent constant increase phase.

Factor	Level	Coefficient	95% CI[a]	P-value
Location of packaged spinach study	Local grocery (intercept)	0.12	(0.07, 0.16)	<0.001
	eCommerce	0.09	(0.03, 0.15)	0.008

[a] 95% CI = 95% confidence interval.

contaminated with *Erwinia*, *Pseudomonas* and *Pantoea* were also detected. Among the 127 unique ST, 16 (13%) were isolated from at least 1 sample for all 3 lots, with the majority (69%; 11/16 ST) representing the genus *Pseudomonas* (Fig. S6).

Results of Fisher's Exact tests, performed only using data from the Petrifilms (i.e., isolates selected from APC and GN Petrifilms for day 0 samples as well as day 3 and 10 eCommerce samples), showed that genus-level populations are significantly associated with type of Petrifilm test (APC, GN) ($P \leq 0.001$); this finding was expected and justified performing subsequent analyses separately for APC and GN data. More unique genera were detected on APC, compared to GN, including Gram-

negative genera (e.g., *Rheinheimera* and *Sphingobacterium*) that were only obtained from APC Petrifilms; this finding is attributed to differences in the media types. Results from Fisher's Exact tests, performed separately for APC and GN data, showed genus-level populations are significantly associated with lot (L1, L2, L3) for APC ($P = 0.04$), but not for GN ($P > 0.05$); this may suggest that APC is a better choice than GN for observing lot-to-lot variability in genus-level populations. Notably, genus-level populations were also found to be significantly associated with day of testing (day 0, 3, 10 postprocessing) for APC ($P \leq 0.001$) and for GN ($P \leq 0.001$). Visualization of genus-level isolate identities for each APC and GN, across days of testing, shows mixed bacterial populations predominated by *Pseudomonas*, followed by *Pantoea* and *Erwinia* (Fig. 3).

The BHI methodology used for day 3 and day 10 samples demonstrated the richness of populations on the spinach, detecting approximately twice the number of genera compared to APC and GN methods, respectively (Fig. S7); this was expected given isolates were selected for all observed distinct morphologies from BHI and at random from the Petrifilms (selecting 2 colonies per plate), leading to a mean of 3.9 and 3.8 times as many characterized isolates per sample for BHI, compared to APC and GN Petrifilms, respectively. In total (including APC, GN, and BHI), 24.7 isolates for day 3 and 18.8 isolates for day 10 were
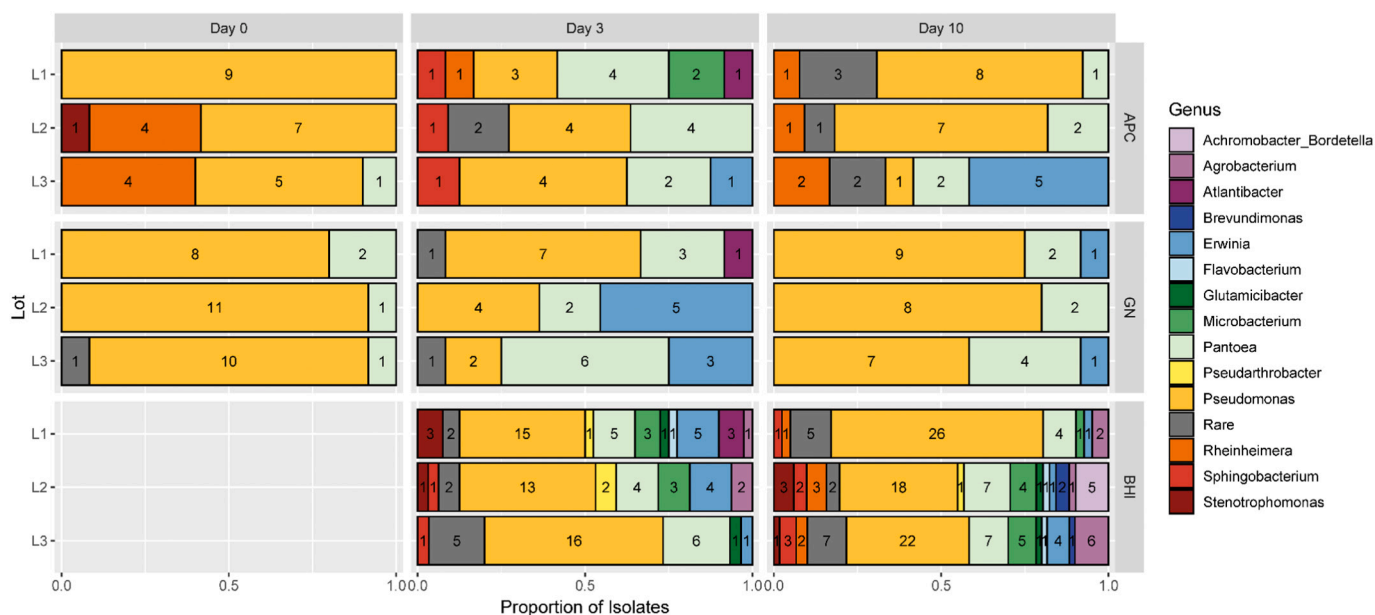
Fig. 3. Barplots of the genus-level identity of sequenced isolates, faceted by day of testing and microbiological test from which the isolates were selected; APC = aerobic plate count [Petrifilm], GN = total Gram-negative count [Petrifilm], and BHI = Brain Heart Infusion spread plate. The x-axis represents the proportion of isolates that belong to a particular genus on a given day, and they y-axis represents lot. The text within the bars represents the number of isolates by genus, test, day, and lot. Genera with less than 3 isolates were represented as "Rare", and include: *Achromobacter, Acinetobacter, Brevibacterium, Cellulosimicrobium, Comamonas, Corynebacterium, Curtobacterium, Cytobacillus, Delftia, Dyadobacter, Enterobacter, Halomonas, Leclercia, Obesumbacterium, Paenarthrobacter, Paenibacillus, Paraburkholderia, Pectobacterium, Planococcus, Pseudoclavibacter, Rahnella, Sanguibacter, Serratia, Sphingomonas, Staphylococcus, Vibrio,* and *Zhihengliuella*. One of the "Rare" isolates had identical BLAST matches with isolates in three genera (*Enterobacter, Leclercia,* and *Lelliottia*) and could not be assigned to a single genus.

characterized per sample; a mean of 7.2 isolates were characterized per sample for day 0 (included only APC and GN). Plating on BHI also allowed for selection of more Gram-positive bacteria, compared to APC and GN Petrifilm methods. However, visualization of genus-level isolate identities for BHI also supports that Gram-negative bacteria, mostly *Pseudomonas* were predominant on both day 3 and day 10 (Fig. 3). Results of the Fisher's Exact tests performed only using data from BHI (isolates selected from BHI spread plates for day 3 and 10 eCommerce samples) showed genus-level populations were not significantly associated with lot ($P = 0.26$) and showed only a borderline association of genera identified with day of testing ($P = 0.06$).

4. Discussion

Our study showed bacterial levels were >6 log$_{10}$CFU/g APC on the spinach collected during harvest and did not change significantly across the stages of the supply chain. Overall, mean APC levels on unwashed spinach from our study were consistent with those reported in previous studies, ranging from mean ± SD APC of 5.36 ± 0.12 log$_{10}$CFU/g (Tango et al., 2014) to 7.3 ± 0.8 log$_{10}$CFU/g (Mritunjay and Kumar, 2017). Bacterial levels on fresh produce throughout the supply chain may change or remain unchanged depending on the structure of the supply chain, which includes processing and handling practices, facility, and type of produce, as supported by our study along with previous studies (Ailes et al., 2008; Johnston et al., 2006, 2005; Van Dyk et al., 2016). Risk of bacterial contamination is the highest when the product is exposed to the environment, which may occur at any stages of the supply chain from preharvest to retail (Dallaire et al., 2006). Packaging, in particular, is an important barrier for preventing contamination (Cutter, 2002). Spinach in our study was sold in packages and thus after packaging there would not have been additional vulnerabilities for contamination events if packaging was intact, whereas loose spinach would still have the potential to be contaminated with bacteria during handling by workers or customers.

Our data support that primary bacterial contamination on spinach

occurred preharvest. Due to the nature of spinach production (i.e., cultivated in soil), all spinach is likely contaminated with bacteria at preharvest. However, the extent of contamination would vary depending on various preharvest sources and factors, such as soil, irrigation water, manure used for fertilization, wildlife, equipment, farm workers, and weather conditions (Alegbeleye et al., 2018; Castro-Ibáñez et al., 2015; Machado-Moreira et al., 2019; Marine et al., 2015; Truchado et al., 2019). Accordingly, spinach grown in protected environments, such as the enclosed greenhouse used in our study, may be less likely to be affected by certain sources such as wildlife and factors such as wind speed, compared to spinach grown in open fields. We recommend future research should investigate the relative importance of sources and factors influencing bacterial population dynamics on fresh produce grown in protected environments.

In this study, there was no significant increase in bacterial levels postharvest. Multiple studies have identified that, relative to other processing steps, cross-contamination primarily occurs during the wash step (Danyluk and Schaffner, 2011; Mokhtari et al., 2018). Although spinach processing in our study did not include a wash step, other stages in the supply chain might have allowed difficult to detect cross-contamination events. For example, spinach in our study was manually harvested and then stored in open crates from point-of-harvest until packaging. Also, packaging included opportunities for contamination via equipment and workers directly handling the spinach. That being said, our study identified a potential relationship between timing of sample collection during harvest and the contamination level. Specifically, for 2 of the 3 lots, samples collected at the end of harvest had higher bacterial levels (though not statistically significant) than those collected at the beginning or middle. This potential relationship may be explained by observations we made during harvest. Specifically, we had observed some of the farmers sorted spinach continuously and transferred spinach to crates, while others prepared piles of spinach prior to sorting, where spinach was in direct contact with soil. Altogether, these findings highlight the complexity of describing cross-contamination events and mechanisms of transmission overall, as practices are not

always consistently implemented.

Our findings also support that the supply chain lacked any effective approaches to reduce microbial load on spinach postharvest. Screening (i.e., visual inspection and removal of spinach that does not meet quality standards) performed prior to packaging may have the potential to reduce microbial load. The impact of screening, however, appeared to be minimal, as our data showed there was no significant decrease between spinach pre-packaging (before screening) and after packaging (after screening and packing). While several strategies and technologies aimed at reducing microbial load on spinach and fresh produce have been developed for implementation prior to packaging, these approaches may be cost-prohibitive for some producers and may have limited efficacy. Advantages and disadvantages of available decontamination technologies for fresh produce have been reviewed previously (Goodburn and Wallace, 2013; Mir et al., 2018; Ramos et al., 2013), however the reports on these technologies primarily consider contamination with foodborne pathogens and typically do not considering spoilage bacteria. There remains a need to identify cost-benefits of available decontamination approaches to aid producers in decision-making, including considerations for producers that may have different constraints such as organic, small-scale, or rural producers that may have limited access to resources (e.g., freshwater, energy).

Overall, the bacterial populations identified on the packaged spinach samples were consistent with previous studies; both where our study and others found Proteobacteria were the dominant phyla and *Pseudomonas* were the dominant genus on unwashed spinach (Gu et al., 2018; Rosberg et al., 2021; Tenzin et al., 2020). Also consistent with our study, previous studies have reported differences in bacterial populations on packaged spinach (washed and unwashed) on different days of refrigerated storage (Gu et al., 2018; Lopez-Velasco et al., 2011). Specifically, over time, under storage at refrigeration temperatures, heterogeneity of the bacterial population on the packaged spinach reduces as genera associated with psychrotolerant growth (e.g., *Pseudomonas*, *Erwinia*), typically become predominant (Gu et al., 2018; Tatsika et al., 2019; Tenzin et al., 2020). As such, bacteria predominant on spinach after prolonged storage (e.g., 10 days) may be attributed to causing product spoilage, however the relationship between the bacterial dynamics (levels and populations) and sensory defects resulting in spoilage requires further investigation. Additionally, our findings suggest bacterial populations may have differed across lots; given that all lots were from the same greenhouse, this finding suggests that spinach handling postharvest may have affected bacterial populations on the spinach. This could also simply be due to the fact that lots were harvested on different days and may have experienced slightly different conditions during that time. The approach used here may be an option for establishing a preliminary understanding of bacterial populations and obtaining isolates for further characterization.

Data from our packaged spinach studies showed different patterns of bacterial levels on spinach over time between the local grocery and eCommerce distribution channels. We also found that the net change of mean bacterial levels per day ($\Delta\log_{10}$CFU/d) during the apparent constant increase phase were faster for the eCommerce distributed spinach, compared to spinach distributed via the local grocery store. Previous studies showed that environmental conditions, especially temperature, influence the ability of bacteria to survive and grow on fresh produce, including during transportation (Lopez-Velasco et al., 2011; Zhou et al., 2022; Zoellner et al., 2016). Accordingly, differences in bacterial populations on spinach between the two distribution channels may be explained by the transit time and temperature differences. Specifically, refrigerated storage started on day 0 postprocessing for the local grocery packaged spinach study, whereas for the eCommerce study, spinach shipped to Beijing was exposed to lower and more variable temperatures during the 3 days of shipping prior to being stored under refrigeration upon delivery. Given our study was conducted in January in Northern China (i.e., during winter), environmental conditions during the various stages of the supply chain likely prevented or slowed growth of bacteria

and likely selected for psychrotolerant bacteria, even prior to the refrigerated storage. It is possible some psychrotolerant bacteria may only grow below 35 °C and thus the bacterial enumeration tests in our study (APC, GN) may have underestimated the total bacterial populations. Psychrotolerant counts (PC) can be assessed, however the methods typically involve incubation at 4–6 °C for 7–10 days, which is time-consuming and requires a separate incubator, and thus was not feasible for our study. Previous studies on packaged spinach that determined both APC and PC, have reported PC are similar to or lower than APC on packaged spinach and show similar growth kinetics (Luo et al., 2009; Mritunjay and Kumar, 2017; Zhou et al., 2022). Notably, external conditions affected the duration of transportation activities in our study as smog and snowfall led to traffic jams and road closures. For example, transportation delays added an extra day for eCommerce shipping to Beijing. Overall, our findings support that duration and environmental conditions during distribution influence bacterial growth patterns and populations on spinach; follow-up studies investigating distribution with varying distances and practices, should be performed. Additional studies should also be performed to further improve understanding of supply chain factors influencing bacterial population dynamics on spinach, to facilitate identification of strategies aimed at delaying spoilage.

Cold chain is typically maintained throughout the fresh produce supply chain as temperature abuses (i.e., exceeding required or optimal temperature) result in quality defects (Mercier et al., 2017; Thompson et al., 2001). Temperatures for fresh spinach to maintain product quality for optimal shelf-life has been suggested to be from 0 to 5 °C (Gil and Garrido, 2020). However, the supply chain evaluated in our study did not maintain cold chain and cooling was limited to the cold storage room located at the processing facility and the retail display case. In the cold storage room located at the processing facility, temperatures often exceeded 4 °C and temperatures in the retail display case were even higher with an average >8 °C. Overall, our findings of challenges with maintaining cold chain were not surprising as it has been estimated previously that in China 95% of fruits and vegetables are transported by trucks without refrigeration (Zhao et al., 2018). Further, temperature abuse (i.e., exceeding required or optimal temperature) of product in supply chains has been reported in a number of studies, where abuse occurs often during transportation and retail display (Mercier et al., 2017; Ndraha et al., 2018). In addition to problems related to maintaining suggested temperatures in the supply chain, there were also delays between harvest and the start of cold storage (i.e., precooling using the room cooling method), ranging from ~3 to 6 h, hence, sometimes exceeding the recommended maximum delay for spinach of 4 h (Thompson et al., 2001). However, there may not have been an impact to spinach quality caused by a delay in precooling because the temperature at harvest was similar to that reached during precooling (Garrido et al., 2015). Our study provided important information on a fresh produce supply chain in China, as data on cold chains and temperature conditions across supply chains are mostly limited to North American and European countries (Mercier et al., 2017; Ndraha et al., 2018). Alternative ways to optimize supply chains without sufficient access to cold storage should be considered. For example, depending on weather, same-day shipping or even multi-day shipping may not require cold chain. Additionally, distribution centers, self-serve kiosks, or physical retail stores could be set up in strategic locations (e.g., closer to existing or potential customers) to reduce shipping times.

While temperature is the primary environmental factor influencing spinach quality and shelf-life, relative humidity may also influence spinach quality. For example, Medina et al. (2012) found relative humidity differences impacted leaf water content, where spinach stored in low relative humidity had increased water loss compared to spinach stored in high relative humidity. Interestingly, some studies have reported no difference or minimal differences in bacterial levels on leafy greens stored under different humidity conditions (Agüero et al., 2011; Medina et al., 2012), however this may depend on the organism.

While this study generated novel findings regarding spinach followed through a supply chain and refrigerated storage, it has several important limitations. The study involved collection of a small number of spinach samples at multiple stages in the production chain. This was a compromise that allowed us to collect data from the whole supply chain. Additionally, spinach samples were collected during a narrow time frame and findings may not be generalizable to other times in the year with different environmental conditions (e.g., during seasons other than winter); a follow-up study over a longer time period will be required. The scope of the study was by design limited to a single supply chain with two representative distribution channels, to allow a more detailed investigation within the chain, but because of that generalizability of findings to other spinach supply chains in China or other countries may not be appropriate. Also, all spinach in this study was grown in a greenhouse and may not represent field grown spinach. There were no preliminary data to allow power-based calculations of sample size for the supply chain of interest. The possibility of information bias in terms of inaccurate measurements is unlikely but could not be excluded; however, if it did exist it is unlikely to have been differential, meaning that it would have introduced noise into the data but no systematic effects. Additionally, the design of the packaged spinach studies consisted of testing approximately every 2 days until 10 days postprocessing; thus, changes in bacterial levels and populations between days of testing and after the 10 days were not captured. Further, the approach we used to assess bacterial populations in this study has limited resolution, especially relative to culture-independent metagenomics approaches. While not unexpected when conducting research involving field work, our study did encounter challenges that required adaptation to the study design including (i) limited laboratory capacity, especially at the on-site laboratory at the processing facility, and (ii) logistical constraints related to investigating spinach across a real supply chain. Altogether, we wish to strongly emphasize that this study will require follow-up studies to confirm repeatability and generalizability of our findings.

5. Conclusions

Overall, our findings highlight the importance of investigating fresh produce from point-of-harvest through shelf-life to understanding the full spectrum of bacterial population dynamics. Interestingly, our data support that relative to initial contamination in the greenhouse, bacterial levels on spinach did not appear to change significantly across supply chain stages. However, we also identified differences in distribution via local grocery compared to via eCommerce, including environmental conditions and duration of distribution, that may have influenced growth and survival of bacterial populations on the spinach samples. Given these findings, future research should focus on improving upon current understanding of the role of contaminating bacteria in spinach spoilage and elucidating the relative contribution of factors influencing spoilage, in order to allow for identification of interventions aimed at extending shelf-life. Importantly, our study contributes a baseline dataset and initial understanding of changes in bacterial levels and populations on fresh spinach along a supply chain located in Northern China. In particular, our study highlighted a need to identify ways to improve product quality for food systems in areas with limited cold storage. Based on the insights provided from our study, we recommend supply chains in different types of food systems should be investigated to better understand diversity, challenges, and opportunities for food quality and safety worldwide.

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Data availability

Data and code generated for this study can be accessed online at https://github.com/IvanekLab/SpinachSpoilage.

CRediT authorship contribution statement

Sarah Ingersoll Murphy: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Project administration. **Ruixi Chen**: Methodology, Investigation, Data curation, Writing – review & editing, Project administration. **Alexandra Marie Belias**: Methodology, Investigation, Writing – review & editing. **Wei Chen**: Investigation, Data curation. **Li-Qun Zhang**: Investigation, Resources, Project administration. **Sriya Sunil**: Data curation, Formal analysis, Visualization, Writing – review & editing. **Ece Bulut**: Writing – review & editing. **Yirui Li**: Investigation. **Martin Wiedmann**: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Renata Ivanek**: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

MW serves as a paid consultant for 3M. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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