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Plant-Microbe and Abiotic Factors Influencing *Salmonella* Survival and Growth on Alfalfa Sprouts and Swiss Chard Microgreens

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ABSTRACT Microgreens, like sprouts, are relatively fast-growing products and are generally consumed raw. Moreover, as observed for sprouts, microbial contamination from preharvest sources may also be present in the production of microgreens. In this study, two *Salmonella enterica* serovars (Hartford and Cubana), applied at multiple inoculation levels, were evaluated for survival and growth on alfalfa sprouts and Swiss chard microgreens by using the most-probable-number (MPN) method. Various abiotic factors were also examined for their effects on *Salmonella* survival and growth on sprouts and microgreens. Community-level physiological profiles (CLPPs) of sprout/microgreen rhizospheres with different levels of *S. enterica* inoculation at different growth stages were characterized by use of Biolog EcoPlates. In the seed contamination group, the ability of *S. enterica* to grow on sprouting alfalfa seeds was affected by both seed storage time and inoculation level but not by serovar. However, the growth of *S. enterica* on Swiss chard microgreens was affected by serovar and inoculation level. Seed storage time had little effect on the average level of *Salmonella* populations in microgreens. In the irrigation water contamination group, the growth of *Salmonella* on both alfalfa sprouts and microgreens was largely affected by inoculation level. Surprisingly, the growth medium was found to play an important role in *Salmonella* survival and growth on microgreens. CLPP analysis showed significant changes in the microbial community metabolic diversity during sprouting for alfalfa sprouts, but few temporal changes were seen with microgreens. The data suggest that the change in rhizosphere bacterial functional diversity was dependent on the host but independent of *Salmonella* contamination.

IMPORTANCE Sprouts and microgreens are considered "functional foods," i.e., foods containing health-promoting or disease-preventing properties in addition to normal nutritional values. However, the microbial risk associated with microgreens has not been well studied. This study evaluated *Salmonella* survival and growth on microgreens compared to those on sprouts, as well as other abiotic factors that could affect *Salmonella* survival and growth on microgreens. This work provides baseline data for risk assessment of microbial contamination of sprouts and microgreens. Understanding the risks of *Salmonella* contamination and its effects on rhizosphere microbial communities enables a better understanding of host-pathogen dynamics in sprouts and microgreens. The data also contribute to innovative preventive control strategies for *Salmonella* contamination of sprouts and microgreens.

KEYWORDS growth, alfalfa sprouts, Swiss chard microgreens, *Salmonella enterica*, CLPP

Sprouts, mainly originating from the Leguminosae family, and microgreens, a new class of edible vegetables harvested and consumed at an immature stage, are both gaining popularity across the world as a significant source of vitamins, minerals, and

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phytochemicals (1–4). Thus, they are considered "functional foods," i.e., foods containing health-promoting or disease-preventing properties in addition to normal nutritional values (5, 6). Traditionally, sprouts are produced entirely in water, but they can also be grown in soil and hydroponically. Sprouts are germinated or partially germinated seeds which, depending on the species, are typically eaten with the roots intact. Microgreens are harvested just above the roots when the cotyledons are fully formed or the first true leaves have emerged. They can be grown in soil or soil substitutes or hydroponically and require high-light conditions for efficient growth (7). Microgreens are halfway in size between sprouts and their older counterparts, such as baby spinach, but deliver the most in terms of flavor and nutritional values compared to the other two types of crops (3).

Since 2000, the number of sprout-related foodborne outbreaks has been on the rise, catalyzing a recurrent problem both in the United States and around the world. Between 2000 and 2016, for example, at least 17 salmonellosis outbreaks linked to the consumption of raw sprouts were documented internationally (8; http://www.outbreakdatabase.com; https://www.cdc.gov/foodsafety/outbreaks/index.html), nine of them in the United States. Most involved alfalfa sprouts, but cress, mung bean, and clover sprouts were also implicated. Although various routes of contamination have been noted, contaminated seeds appear to be the source of most sprout-associated foodborne illnesses and are considered the most common source of contamination (9–13). Irrigation water is another potential source of contamination during preharvest growth. Seed sprouting provides an excellent environment for the growth of microorganisms, including foodborne pathogens (14, 15). In fact, a level as low as 4 most-probable-number (MPN) *Salmonella* organisms per kg of seed was determined to be capable of causing a foodborne outbreak associated with sprouts (16). Unlike sprouts, microgreens so far have not been associated with any foodborne outbreaks. Nevertheless, considering that microgreens are consumed raw, like sprouts, and are relatively fast-growing products, similar microbial contamination risks from preharvest or postharvest sources may be present in the cultivation, harvesting, and marketing of microgreens as well. However, the microbial risk associated with microgreens has not been well studied (17, 18).

In order to determine the risk of microbial contamination and the survival of *Salmonella* on microgreens, *Salmonella* growth differences on alfalfa sprouts and Swiss chard microgreens were examined. The effects of *Salmonella enterica* serovar, initial inoculum dose, source of contamination, seed storage time, and growth medium on *Salmonella* levels on sprouts and microgreens were also examined in this study. Finally, community-level physiological profiles (CLPPs) were analyzed and compared to assess differences in microbial richness during the sprouting of alfalfa seeds and production of Swiss chard microgreens.

RESULTS

***Salmonella enterica* survival and growth on alfalfa sprouts and Swiss chard microgreens via contaminated seeds.** *S. enterica* serovars, inoculation levels, and seed storage times were examined to determine the factors affecting *Salmonella* growth on alfalfa sprouts and Swiss chard microgreens via contaminated seeds (SC group) (Table 1). Uninoculated sprouts and microgreens were used as plant controls. *S. enterica* serovar Cubana and *S.* Hartford isolates used in this study were also monitored *in vitro* by examining the growth curves for individual isolates at 25°C. No significant growth differences were found between these two isolates. At a seed inoculation level of ~10 CFU/g, *S.* Hartford levels in most sprout samples (6/8 samples) were below the detection level (−0.447 log MPN/g sprouts) after contaminated seeds were stored for 7 days at 4°C. The *Salmonella* population was able to increase by at least 1 log in 1/8 alfalfa sprout samples. Even after the seeds were stored for 28 days at 4°C, 3 of 8 sprout samples were still positive with *S.* Hartford, at ~2 log MPN/g sprouts. Similarly, *S.* Cubana was capable of surviving through 28-day storage and grew to 2 log MPN/g sprouts on germinating alfalfa sprouts in 2/8 sprout samples. As the inoculation level

TABLE 1 *Salmonella enterica* survival and growth on alfalfa sprouts and Swiss chard microgreens via contaminated seeds

Commodity	*S. enterica* serovar	Inoculation level (CFU/g seed)	Contaminated seed storage time (days)	No. of positive samples/total no. of samples	Maximum log MPN/g fresh wt
Alfalfa sprouts	Cubana	10	7	0/8	0
			28	2/8	2.04
		10^2	7	11/16	3.04
			28	5/16	2.04
	Hartford	10	7	2/8	2.04
			28	3/8	2.04
		10^2	7	9/16	2.04
			28	5/16	2.04
Swiss chard microgreens[a]	Cubana	10	7	4/8	2.04
			28	2/8	3.04
		10^2	7	11/12	2.04
			28	11/16	3.04
	Hartford	10	7	8/8	2.04
			28	3/8	3.04
		10^2	7	12/12	2.04
			28	16/16	3.04

[a]Microgreens were grown in soil A.

was increased to ~10^2 CFU/g, the numbers of *S.* Hartford- and *S.* Cubana-positive sprout samples also increased for seeds stored at 4°C for 7 days (9/16 and 11/16 samples, respectively) and 28 days (5/16 and 5/16 samples, respectively). Control sprout samples were all negative for *S. enterica*. Collectively, as shown in Table 2, the mean levels of *Salmonella* populations did not differ significantly between serovars ($P = 0.7631$). However, significant differences in *Salmonella* growth were observed between seed storage times ($P < 0.05$) and between inoculation levels ($P < 0.05$). Analysis of variance (ANOVA) indicated that the differences between the inoculation levels with

TABLE 2 Factors affecting *S. enterica* growth on alfalfa sprouts and Swiss chard microgreens via seed contamination

Plant type and factor(s)	df	Sum of squares	Mean square value	*F* value	*P*(>*F*) value	Statistical significance[a]
Alfalfa sprouts						
Inoculation level	1	4.57	4.572	6.542	0.0122	*
Storage time	1	2.92	2.917	4.173	0.0441	*
Serovar	1	0.06	0.064	0.091	0.7631	
Inoculation level × storage time	1	12.35	12.347	17.667	6.3E−05	***
Inoculation level × serovar	1	0.03	0.028	0.040	0.8427	
Storage time × serovar	1	1.20	1.196	1.711	0.1943	
Inoculation level × storage time × serovar	1	0.05	0.055	0.079	0.7798	
Residuals	88	61.50	0.699			
Swiss chard microgreens						
Inoculation level	1	10.06	10.056	11.375	0.00120	**
Storage time	1	0.10	0.105	0.119	0.73140	
Serovar	1	8.44	8.438	9.544	0.00285	**
Part	1	0.01	0.012	0.014	0.90767	
Inoculation level × storage time	1	1.16	1.163	1.315	0.25521	
Inoculation level × serovar	1	0.07	0.066	0.075	0.78505	
Storage time × serovar	1	1.53	1.529	1.730	0.19261	
Inoculation level × part	1	0.03	0.028	0.031	0.85977	
Storage time × part	1	0.03	0.028	0.031	0.85989	
Serovar × part	1	0.00	0.001	0.001	0.97014	
Inoculation level × storage time × serovar	1	1.42	1.424	1.611	0.20850	
Inoculation level × storage time × part	1	0.32	0.315	0.356	0.55238	
Inoculation level × serovar × part	1	0.67	0.673	0.762	0.38567	
Storage time × serovar × part	1	0.19	0.188	0.212	0.64641	
Inoculation level × storage time × serovar × part	1	0.00	0.000	0.000	0.99396	
Residuals	72	63.65	0.884			

[a]*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TABLE 3 Factors influencing *S. enterica* growth on alfalfa sprouts via irrigation water contamination

Factor(s)	df	Sum of squares	Mean square value	*F* value	*P*(>*F*) value	Statistical significance[a]
Inoculation level	3	177.53	59.18	31.771	5.32E−16	***
Serovar	1	4.49	4.49	2.409	0.1227	
Inoculation level × serovar	3	32.05	10.68	5.735	0.00096	***
Residuals	152	283.13	1.86			

[a]*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

respect to the mean levels of *S. enterica* populations on sprout samples (mean log MPN per gram of sprouts) were dependent on the storage time ($P < 0.001$).

In the case of Swiss chard microgreens, mean log MPN of *Salmonella* per gram of microgreens were significantly different between the two serovars ($P < 0.01$) and between inoculation levels ($P < 0.01$). However, seed storage time did not affect *Salmonella* growth on microgreens ($P > 0.05$), and no significant interaction was found among all the factors tested (Table 2). Additionally, at a seed inoculation level of ~10 CFU/g, all eight microgreen samples from the seeds stored for 7 days at 4°C (100%) were positive for *S*. Hartford, while only four samples (50%) were positive for *S*. Cubana. After seeds were stored for 28 days, the percentages of *Salmonella*-positive seeds decreased to similar levels for both serovars. Also, the differences in the percentages of *Salmonella*-positive seeds and the average levels of *Salmonella* populations on microgreens due to serovar were diminished after the seed inoculation level was increased to ~10^2 CFU/g. The control microgreen samples were all negative for *S. enterica*.

***S. enterica* growth on alfalfa sprouts and Swiss chard microgreens via irrigation water contamination.** Various factors, including two *S. enterica* serovars and four inoculation levels, were examined to evaluate *Salmonella* growth on alfalfa sprouts and Swiss chard microgreens via contaminated irrigation water (IC group). In the alfalfa sprout trial (Table 3), similar to the results for *Salmonella* growth in the SC group, the mean levels of *Salmonella* populations in contaminated sprout samples did not differ significantly between serovars ($P > 0.05$). The growth of *S. enterica* on sprouting seeds was largely affected by inoculation level ($P < 0.0001$). However, analysis of variance signaled a significant interaction between inoculation level and serovar with respect to the level of the *S. enterica* population in sprout samples (MPN per gram of sprouts) ($P < 0.001$). Different patterns for the relationship between growth of *Salmonella* and inoculation level were observed for *S*. Hartford and *S*. Cubana, as shown in Fig. 1. It is interesting that at the level of 0.02 CFU/g seed (i.e., 0.002 CFU/ml H_2O), *S*. Hartford was still able to proliferate in sprouts, to up to 3.9 log MPN/g sprouts in 2/12 samples, while *S*. Cubana was only able to grow on sprouts at −0.04 log MPN/g sprouts, in 1/12 samples. When the inoculation level was increased to 0.2 CFU/g seed (i.e., 0.02 CFU/ml), both serovars were able to grow in more than 50% of the sprout samples, and the population surged to up to 4.8 log MPN/g sprouts. At the level of 2 CFU/g seed (i.e., 0.2 CFU/ml), however, *S*. Cubana proliferated on all the sprout samples, and the maximum level of the *Salmonella* population was 5.0 log MPN/g sprouts in 41% of the sprout samples (13/32 samples). In contrast to *S*. Cubana, *S*. Hartford was present in 75% of the sprout samples (18/24 samples), and only 2/24 samples had *Salmonella* populations at 5.0 log MPN/g sprouts.

In the case of Swiss chard microgreens, the growth medium was also examined, along with other factors influencing the growth of *Salmonella* (Table 4). Overall, the growth of *S. enterica* on microgreens was significantly affected by the inoculation level ($P < 0.0001$). However, the difference in inoculation levels with respect to the mean level of *S. enterica* populations in microgreen samples was serovar and growth medium dependent. Specifically, at the level of 0.02 CFU/g seed, *S*. Hartford could not be detected in Swiss chard microgreen samples (i.e., was below the detection limit), while *S*. Cubana was found to be able to survive and grow to up to 2 log MPN/g in soil A. The percentage of *Salmonella*-positive samples largely increased with increases in inoculation level, and the difference between serovars was minimized as well. No significant

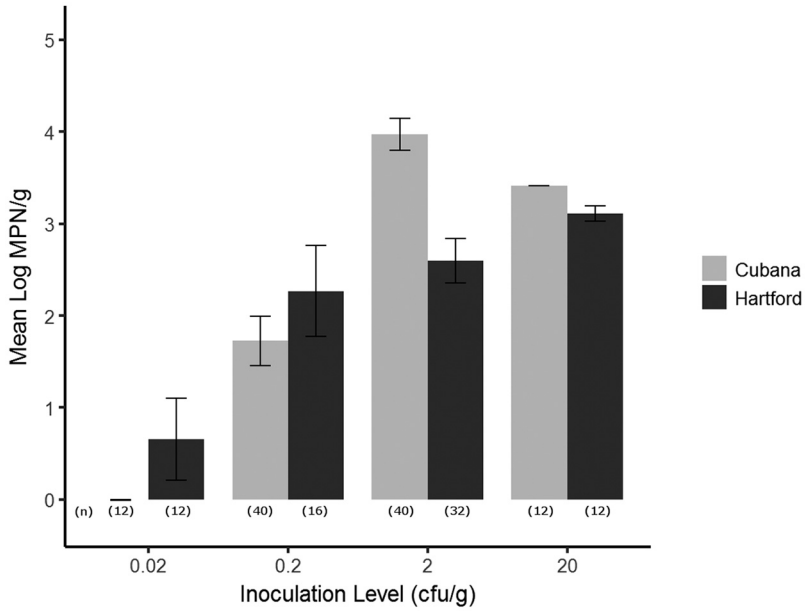

FIG 1 *Salmonella enterica* growth on alfalfa sprouts via irrigation water contamination. Sprouts were grown in a Conviron E7/2 climate-controlled growth chamber for the duration of the experiment. Before germination, growth chamber temperatures were maintained at 24°C (daytime) and 22°C (nighttime) with no light. Temperatures were maintained for 2 days at a constant 22°C day and night, with light, after germination. Growth chambers were kept at a constant relative humidity of 65%. At harvest, the mean log MPN per gram of fresh weight (*y* axis) was calculated against 4 different inoculation levels (*x* axis) for *S. enterica* serovars Cubana (gray) and Hartford (black). Numbers in parentheses show the numbers of replicated samples examined at each inoculation level. The detection limit for *Salmonella* in this experiment was 0.3 MPN/g.

difference ($P > 0.05$) was found between edible and nonedible parts of Swiss chard microgreens. Interestingly, the maximum level of *Salmonella* in the samples varied among different growth meda. For both serovars, compared to the levels in microgreens grown in soil B, the level of *Salmonella* could be 2 to 4 log higher per gram of sample for growth in soil A or hydroponically (Fig. 2).

Microbial richness during sprouting of alfalfa seeds and production of Swiss chard microgreens. Average well color development (AWCD), used as an estimate of

TABLE 4 Factors affecting *S. enterica* growth on Swiss chard microgreens grown hydroponically, in soil A, and in soil B via irrigation water contamination

Growth condition and factor(s)	df	Sum of squares	Mean square value	*F* value	*P*(>*F*) value	Statistical significance[a]
Hydroponic system						
Inoculation level	1	39.56	39.56	211.448	1.42E−14	***
Serovar	1	0.4	0.4	2.132	0.155	
Inoculation level × serovar	1	0.4	0.4	2.132	0.155	
Residuals	28	5.24	0.19			
Soil A						
Inoculation level	2	77.61	38.8	73.397	1.11E−12	***
Serovar	1	2.4	2.4	4.542	0.0409	*
Inoculation level × serovar	1	0.72	0.72	1.363	0.2517	
Residuals	32	16.92	0.53			
Soil B						
Inoculation level	3	77.14	25.712	46.11	5.42E−16	***
Serovar	1	0.2	0.201	0.361	0.55	
Inoculation level × serovar	3	1.15	0.383	0.687	0.563	
Residuals	64	35.69	0.558			

[a]*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

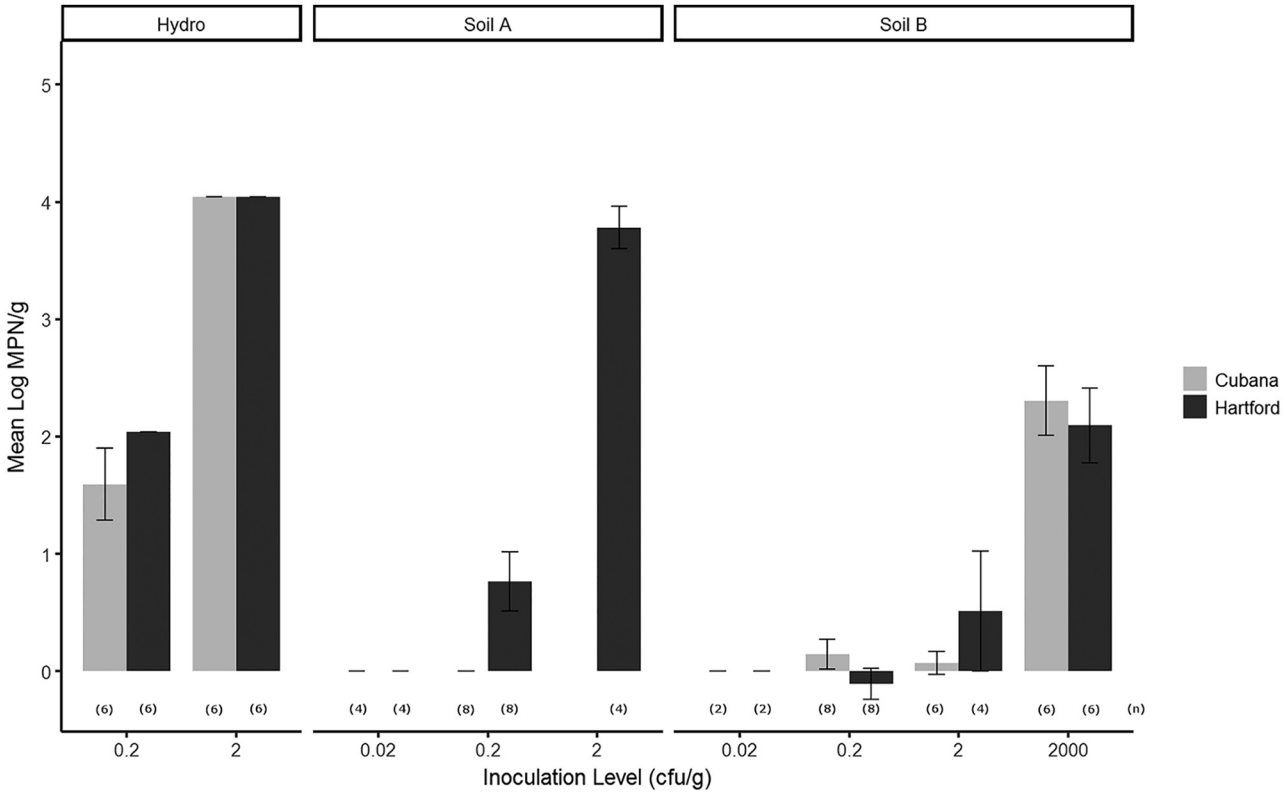

FIG 2 *Salmonella enterica* growth on Swiss chard microgreens via irrigation water contamination. Microgreens were grown in a Conviron E7/2 climate-controlled growth chamber for the duration of the experiment. Before germination, growth chamber temperatures were maintained at 24°C (daytime) and 22°C (nighttime) with no light. Temperatures were maintained for 4 days at a constant 24°C day and night, with light, after germination. Growth chambers were kept at a constant relative humidity of 65%. At harvest, the mean log MPN per gram of fresh weight (*y* axis) was calculated against various inoculation levels (*x* axis) for *S. enterica* serovars Cubana (gray) and Hartford (black) for different growth media, including hydroponic growth, soil A, and soil B. Numbers in parentheses show the numbers of replicated samples examined at each inoculation level. The detection limit for *Salmonella* in this experiment was 0.3 MPN/g.

general bacterial activity, was calculated based on the results obtained with Biolog EcoPlates on day 6, when the saturation point of color development was reached. As shown in Fig. 3A and B, no significant difference in bacterial community metabolic activity was observed among control and different *Salmonella* inoculation levels at any sampling time point for rhizosphere samples from either alfalfa sprouts or Swiss chard microgreens. However, significant differences in bacterial communities during seed sprouting were indicated after comparison of rhizosphere samples pregermination, after light exposure, and at harvest for both control and *Salmonella* treatment groups. This is also clearly shown with a principal coordinate analysis (PCoA) plot (Fig. 4A), in which a total of 12 samples taken pregermination from the three replicates for each control and *Salmonella* inoculation group cluster separately from the 12 samples taken after light exposure or at harvest. This clear temporal shift was diminished for the rhizosphere samples from Swiss chard microgreens, as shown by the reduction of separation along the first principal component, from 70.3% to 26.8% (Fig. 4B).

The microbial community metabolic diversity, as determined by richness (*R*) based on Biolog EcoPlate profiles, showed significant differences between rhizosphere samples from alfalfa sprouts and those from Swiss chard microgreens ($P < 0.05$). Additionally, metabolic diversity was drastically increased in sprouts after germination (Fig. 3C and D), but little change was shown during the production of microgreens.

DISCUSSION

Compared to other fresh produce, sprouts are a unique food safety challenge in that foodborne pathogens on the sprouting seeds may multiply by several logs during the

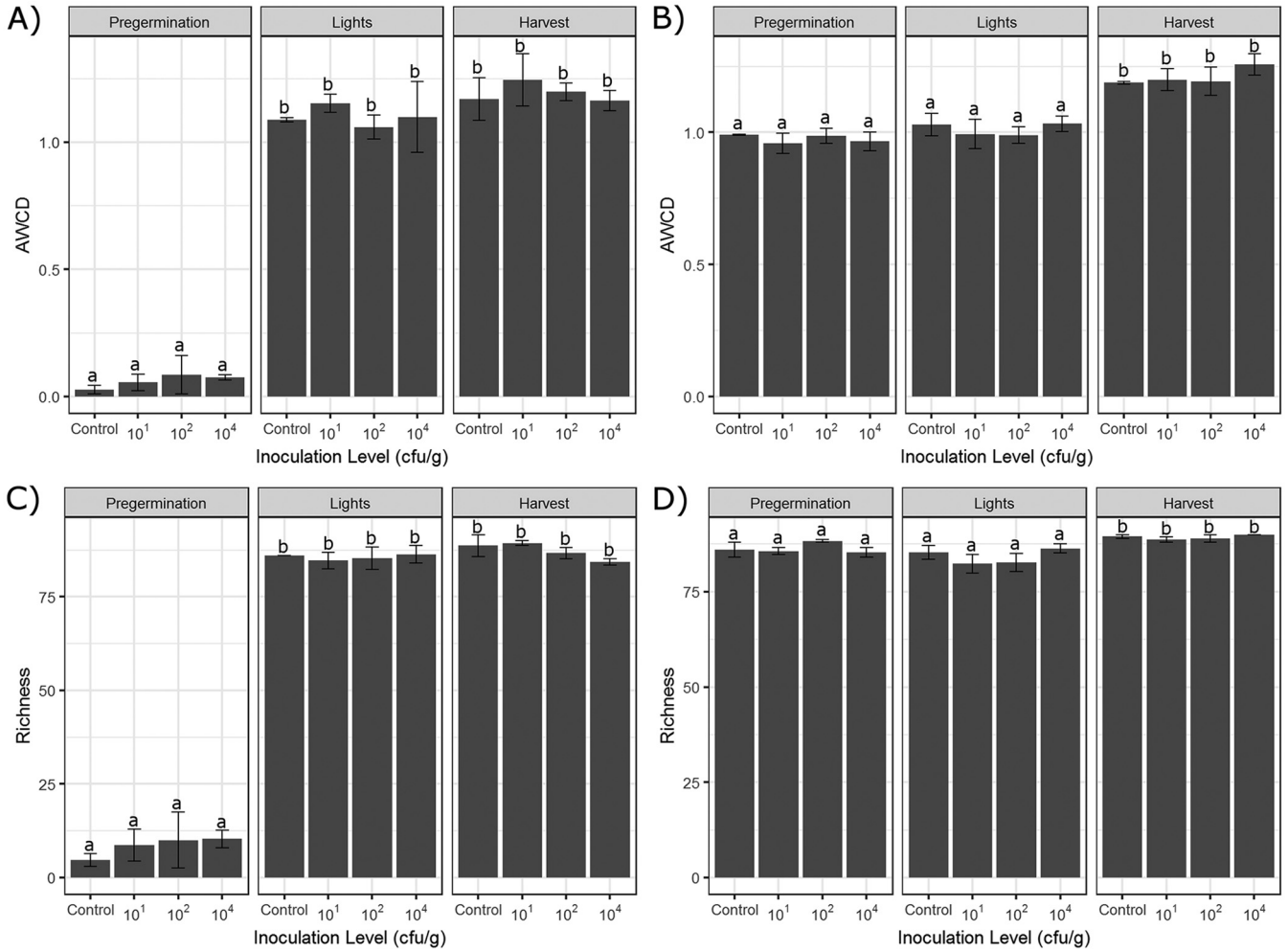

FIG 3 Average well color development (AWCD) (A and B) and richness (*R*) (C and D) of metabolized substrates in Biolog EcoPlates after inoculation with different levels of *S. enterica* pregermination, after light exposure, and at the harvest stage. Data for sprouts are presented in panels A and C, and data for microgreens are presented in panels B and D. Each sample had three replicates. The data shown here are the means of 31 substrate well absorbance values at day 6. Error bars represent standard deviations ($n = 3$), and different letters indicate a significant difference ($P < 0.05$).

short growing period (19, 20). A recent review of sprout-associated outbreaks reported that 85% of the outbreaks in the United States were caused by *S. enterica* (13). Compared to *Escherichia coli*, *S. enterica* was able to grow to significantly higher levels on sprouting seeds (21) and adhered significantly better to alfalfa roots and seed coats (22). Factors that affect the growth of *Salmonella* during sprouting of contaminated seeds were examined by several groups (21, 23–25). The ability of *S. enterica* to grow on sprouting seeds was affected by the initial inoculum dose, incubation temperature, and length of exposure but was independent of the serovar, isolation source, virulence of the strain, and day of exposure to the sprouting seeds. An increase in the frequency of irrigation water exchange will not reduce the levels of *Salmonella*, and a decreased irrigation frequency will increase the *Salmonella* population. In this study, in addition to various levels of initial inoculation, different *Salmonella* serovars, and the time of exposure (seed or irrigation water at day 1), contaminated seed storage time was also examined for its effect on *Salmonella* population levels on sprouting seeds. *Salmonella enterica* serovars Cubana and Hartford, used in this study, belong to different O groups (O13 for *S.* Cubana and O7 for *S.* Hartford) and divergent phylogenetic lineages (26). However, they have both caused salmonellosis outbreaks, associated with alfalfa sprouts and chia seeds, respectively (12, 14). Moreover, *S.* Cubana was the serovar most frequently recovered from sprout samples collected by the U.S. Department of Agri-

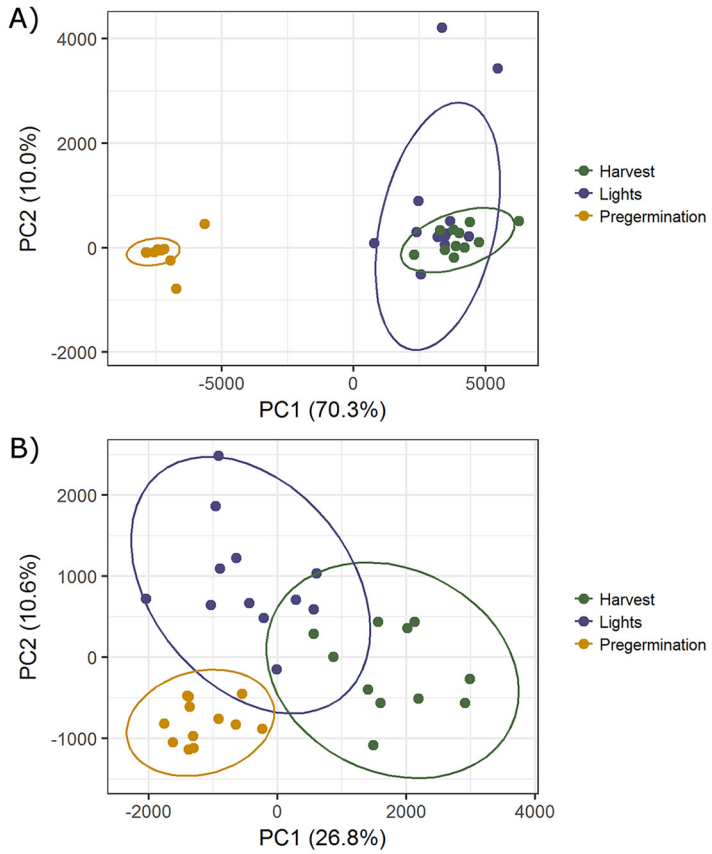

FIG 4 Principal coordinate analysis (PCoA) of bacterial community metabolic profiles for different rhizosphere samples from alfalfa sprouts (A) and Swiss chard microgreens (B). The percentage of total variance explained by each axis is shown parenthetically. All values are based on AWCD data. The various samples from different growth stages are represented by symbols of different colors. Ellipses were drawn with a confidence limit of 0.95.

culture Microbiological Data Program (MDP) between 2002 and 2012 (27). As shown in previous studies, initial inoculation levels played a significant role in the growth of *Salmonella* on sprouts. Additionally, the overall *Salmonella* positivity rate with the same inoculation levels (20 CFU/g seed and 2 CFU/g seed) was much higher among the sprout samples from the IC group (60/66 samples [90.9%]) than among those from the SC group (37/81 samples [45.7%]), and the presence of *Salmonella* was much more homogeneous in sprout samples from the IC group than in those from the SC group. Furthermore, the relationship between inoculation level and mean *Salmonella* population on alfalfa sprouts was also dependent on seed storage time (in the SC group) and serovar (in the IC group). In general, serovar was not considered to be a factor affecting *Salmonella* growth on germinating alfalfa seeds, as observed in previous studies. However, serovar might play a role in relation to the population of *Salmonella* on sprouts when the contamination is introduced via irrigation water.

CLPP analysis revealed that the change in rhizosphere bacterial functional diversity was host dependent but *Salmonella* contamination independent, which explained why *S. enterica* was found growing epiphytically on sprout surfaces without producing obvious signs of contamination (9, 28). The exponential increase in microbial richness from times pregermination to postgermination and at sprouting, as shown by CLPP analysis, was probably due to the release of micronutrients from germinating seeds. The fairly low microbial richness ($R = 5$ to 10) at the pregermination stage also provided a nice niche in which *Salmonella* could colonize and grow, by up to 4 orders of magnitude.

Compared to their growth on sprouts, *E. coli* O157:H7 and O104:H4 strains have

been shown to grow, but to a lesser extent, on microgreens from contaminated seeds grown in soil and in a hydroponic production system (17, 18). This phenomenon was also observed with *S. enterica* in our current study. The trivial differences in microbial richness between rhizosphere samples from microgreens from times pregermination to postgermination and at harvest may explain why harvested microgreens carried fewer pathogens than those carried by sprouts. Niche competition with other microorganisms in microgreen growth media may also slow down the growth of pathogens. Factors affecting *Salmonella* growth on Swiss chard microgreens were also examined in this study. For the SC group, unlike the results for alfalfa sprouts, in addition to the initial inoculation level, the proliferation of *Salmonella* on Swiss chard microgreens was also serovar dependent. Overall, *S.* Hartford survived and grew better on microgreens than *S.* Cubana when seeds were contaminated at a low level. Although a direct comparison between *S. enterica* and *E. coli* O157:H7 strains cannot be made because the initial inoculation level was much lower in the current study, the increase in the *Salmonella* population from the initial inoculation level was much higher on microgreens than that for *E. coli* O157:H7. For the IC group, consistent with a previous study of *E. coli* O157:H7, the type of growth medium played a significant role in *Salmonella* survival and growth during microgreen production, with the hydroponic system having the highest percentage of *Salmonella*-positive samples and the highest *Salmonella* population level on microgreens (18). This suggests that the microbial risk associated with hydroponic production systems is potentially much higher than those associated with other forms of production. No significant difference was observed between edible and nonedible parts of Swiss chard microgreens, indicating that the contamination was systemic (18), irrespective of the point of contamination introduction. This may potentially pose a serious microbial safety risk for the production of microgreens. Clearly, these data will contribute to new and innovative preventive control strategies for *Salmonella* contamination of sprouts and microgreens.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. enterica* serovar Cubana strain CFSAN055271, isolated from alfalfa sprouts, and *S.* Hartford strain NY20, isolated from chia seeds, were obtained from the stock culture collection of the Division of Microbiology, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD. Stock cultures were stored in brain heart infusion (BHI) broth containing 25% glycerol at −80°C and maintained on tryptic soy agar (TSA) plates.

Germination and growth of alfalfa sprouts and Swiss chard microgreens. Seeds for sprouting were obtained from West Coast Seeds (British Columbia, Canada). For alfalfa seeds, 5 to 6 g of seeds was sown onto a 13-cm by 13-cm Micro-Mats hydroponic growing pad (Handy Pantry) which was fit into a large (250 ml) square weighing boat to create individual hydroponic systems for all the experiments. For Swiss chard microgreen seeds, 5 to 6 g of seeds was sown onto premoistened potting soil and then covered with a paper towel in a G35 (17 × 10 × 5 cm) mini-seed tray (Garland Products Ltd., UK) placed in an aluminum foil folded tray to serve as a water reservoir or onto hydroponic growing pads, based on the experimental design. Potting soil A (75 to 85% sphagnum peat moss, peat humus, perlite, earthworm castings, and dolomitic limestone), which was used for both the SC and IC groups, and potting soil B (70 to 80% Canadian sphagnum peat moss, perlite, dolomite lime, and a wetting agent [yucca extract]), which was used only for the IC group, were purchased from Amazon, Inc. (Seattle, WA). All the trays were placed in a Conviron E7/2 climate-controlled growth chamber for the duration of the study. Before germination, growth chamber temperatures were maintained at 24°C (daytime) and 22°C (nighttime) with no light for both alfalfa sprouts and Swiss chard microgreens. Temperatures were maintained at a constant 22°C for alfalfa sprouts and 24°C for microgreens day and night, with light, after germination (2 days for alfalfa sprouts and 4 days for Swiss chard microgreens). Growth chambers were kept at a constant relative humidity of 65%. Sprouts and microgreens were irrigated from overhead daily with 40 to 50 ml of tap water (to saturation) for a total of 7 days and 14 days, respectively.

Inoculation of seeds and irrigation water. A single colony of each culture was transferred to 5 ml of tryptic soy broth (TSB) and grown with shaking at 36°C for 18 to 20 h. Each culture was harvested by centrifugation at 5,000 × *g* for 10 min, followed by washing with 0.01 M phosphate-buffered saline (PBS) (pH 7.2) three times and then resuspension in 5 ml of TSB. For seed inocula, the culture was further diluted in sterile double-distilled water (ddH_2O) to two desired levels (~10^1 and ~10^2 CFU/g seed). Fifty grams of seeds was soaked in 200 ml inoculum for 20 min, drained, and allowed to air dry at room temperature in a Lumina hood. Seeds were stored at 4°C until use. For irrigation water inocula, the culture suspended in PBS was further diluted to five different levels (~0.02, ~0.2, ~2, ~20, and ~2,000 CFU/g seed). A one-time inoculation was made through initial irrigation with a 50-ml inoculum. The inoculation levels for each strain were determined by plate counts immediately following inoculation.

Recovery and enumeration of *Salmonella* from alfalfa sprouts and Swiss chard microgreens. Alfalfa sprouts and Swiss chard microgreens were harvested on day 7 and day 14, respectively. Sprouts were sampled whole, and each tray of sprouts was split into two sprout samples by weight. The microgreens were cut above the soil line with a pair of sterilized scissors. Both edible and nonedible parts (i.e., rhizosphere samples) of the microgreens were harvested for evaluation. *Salmonella* was recovered from the samples and enumerated by use of a miniature three-tube MPN procedure (29), with incorporation of standard enrichment and selection procedures for the pathogen. In short, the sampled sprouts and microgreens were weighed and submerged in modified buffered peptone water (mBPW) at a 1:3 sample-to-broth ratio in individual sterile Whirl-Pak filter bags (Fort Atkinson, WI). Sample bags were mixed for 1 min at 230 rpm with a Seward stomacher 400 circulator, and 4 ml of the mixed solution was then transferred to a sterile 5-ml 48-deep-well plate (Axygen Scientific, Tewksbury, MA), with each sample run in triplicate. An aliquot of 0.4 ml of the original mixed sample was added to a well in the second row of 6-well series, which was prefilled with 3.6 ml of mBPW. The well in the second row was mixed using a disposable serological pipette before transfer of 0.4 ml of sample to a well with 3.6 ml mBPW in the third row. This process was repeated, with changing of pipettes between each transfer, to create a serial 10-fold dilution series. The 48-well plate was then incubated for 24 h at 35°C. An aliquot of 0.1 ml from each well was transferred to 10 ml of Rappaport-Vassiliadis (RV) broth for incubation at 42°C. After 24 h, the selective enrichment tubes were each streaked (10 μl) onto XLT-4 agar plates by use of sterile loops. Plates were incubated for 24 h at 35°C. The presence of colonies with morphologies typical of *S. enterica* on XLT-4 agar was considered to be a positive result, and random colonies were chosen for confirmation as *Salmonella* by use of somatic group antisera (Statens Serum Institute, Copenhagen, Denmark) and a Vitek 2 system (bioMérieux, Hazelwood, MO). MPN calculations were performed as previously described (30).

Community-level physiological profiling assay. Community-level physiological profiles (CLPPs) of each rhizosphere were obtained by use of Biolog EcoPlates (Biolog, Inc., Hayward, CA) as previously described (31), with some modifications. Briefly, 3-g samples of rhizospheres from different growth stages of sprouts and microgreens were transferred to individual sterile Whirl-Pak filter bags filled with 30 ml of saline solution (0.85% NaCl). Sample bags were mixed for 2 min at 200 rpm by use of a stomacher, and then 25 ml of mixed sample was transferred to a 50-ml sterile centrifuge tube. Every suspension was centrifuged at 50 $\times$ *g* for 5 min, and the supernatant was then transferred to a sterile 50-ml centrifuge tube and centrifuged again at 130 $\times$ *g* for 5 min. Three EcoPlates per treatment and one for each replicate sample were filled with 100 μl of suspension per well, followed by incubation at 23°C. The rate of utilization of the carbon sources was monitored using MicroLog 3.0 software with a Biolog MicroStation reader (Biolog, Inc., Hayward, CA). Color development was analyzed at days 0, 1, 3, and 6, when the curve of average well color development (AWCD) reached the saturation point.

Statistical analysis. All *Salmonella* growth data were analyzed using analysis of variance (ANOVA) and Tukey's mean separation test, with *P* values of <0.05 denoting significance. Most-probable-number (MPN) data were logarithmically transformed prior to statistical analysis.

For CLPP data, AWCD was calculated as the sum of the corrected optical density for each well divided by the number of carbon substrates, and richness (*R*) was calculated as any oxidized carbon substrate. CLPPs were analyzed by principal coordinate analysis (PCoA) to determine the levels of similarity between treatments and time points. Data on AWCD and *R* were also analyzed by ANOVA, with *P* values of <0.05 denoting significance.

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