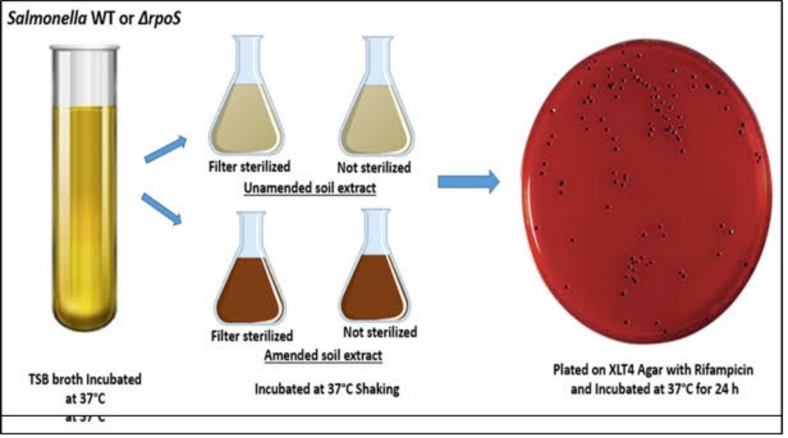

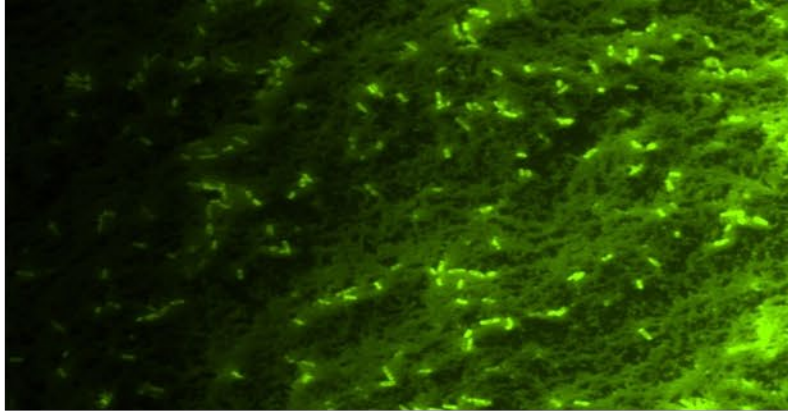

Manan Sharma
Environmental Microbial and Food Safety Laboratory, USDA ARS

February 20, 2019

Characterization and Mitigation of Bacterial Pathogens in the Fresh Produce Production and Processing Continuum

- **Objective 1**: Investigate the mechanism(s) of introduction, transference, and survival of enterohemorrhagic *Escherichia coli* (EHEC), *Salmonella,* and *Listeria* to fresh produce at the farm level.
    - **Sub-objective 1a.** Investigate the population dynamics of non-pathogenic *E. coli* and non-O157 EHEC in soils amended with biological soil amendments (BSA)
    - **Sub-objective 1b.** Determine factors affecting persistence of EHEC, *Salmonella* and *Listeria* in soils amended with BSA

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Public and Environmental Health Microbiology

E. coli survival duration in manure-amended soils is affected by spatiotemporal, agricultural, and weather factors: a multi-season, multi-site field study in the Mid-Atlantic U.S.

Manan Sharma, Patricia D. Millner, Fawzy Hashem, Bryan T. Vinyard, Cheryl L East, Eric T. Handy, Kathryn White, Richard Stonebraker, Corrie P. Cotton

DOI: 10.1128/AEM.02392-18

Article | Figures & Data | Info & Metrics | PDF

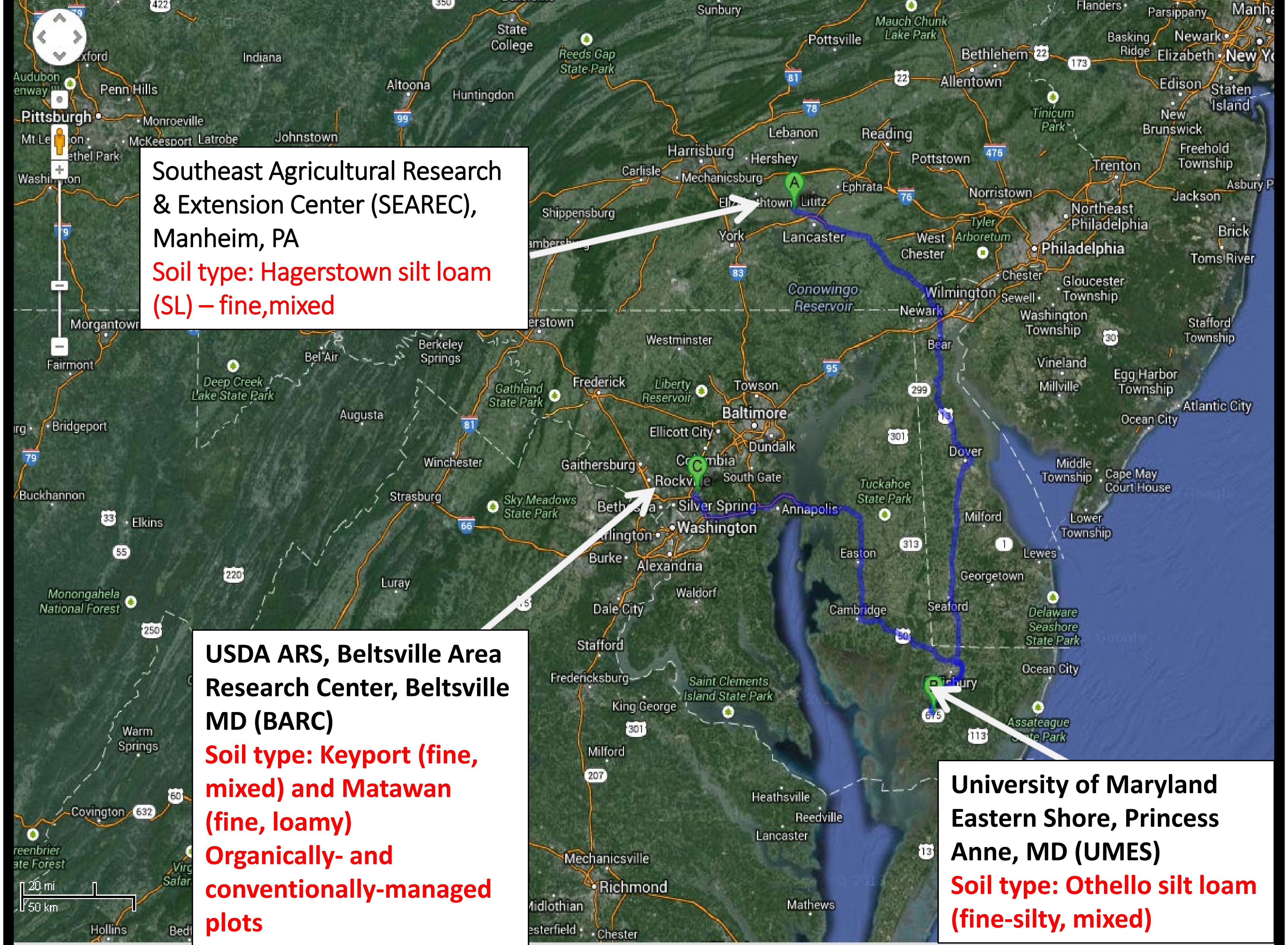
ABSTRACT

Untreated biological soil amendments of animal origin (BSAAO), like manure, are commonly used to fertilize soils for growing fruit and vegetable crops, and can contain enteric bacterial foodborne pathogens. Little is known about the comparative longitudinal survival of pathogens in agricultural fields containing different types of BSAAO, and field data may be useful to determine intervals between manure application and harvest of produce intended for human consumption to minimize foodborne illness. This study generated 324 survival profiles from 12 different field trials at three different sites (UMES, PA, BARC) in the Mid-Atlantic U.S. from 2011-2015 of inoculated non-pathogenic (gEc) and attenuated-O157 *E. coli* (attO157) in soils which were unamended (UN), amended with untreated poultry (PL), horse manure (HM), dairy manure soils (DMS) or liquids (DML). Site, season, inoculum level (low/high), amendment type, management (organic/conventional), and depth (surface/tilled) all significantly ($p < 0.0001$) influenced survival duration

f 4

Manure Pathogen Persistence Study (2011-2018), FDA, USDA-ARS EMFSL, and UMES

- **Agricultural Factors**
  - Amendment type
    - Dairy manure solids
    - Horse manure
    - Poultry litter
    - Unamended
  - Inoculum level
    - Low
    - High
  - Management
    - Organic
    - Conventional
  - Depth
    - Surface
    - Tilled
- **Spatio-temporal factors**
  - Site
    - BARC
    - UMES
    - PA
  - Years
    - 2011
    - 2012
    - 2013
    - 2014
  - Season
    - Spring
    - Fall
- **Weather effects**
  - Air temperature
  - Rainfall
  - Initial soil moisture content



Southeast Agricultural Research & Extension Center (SEAREC), Manheim, PA

Soil type: Hagerstown silt loam (SL) – fine,mixed

USDA ARS, Beltsville Area Research Center, Beltsville MD (BARC)

Soil type: Keyport (fine, mixed) and Matawan (fine, loamy)

Organically- and conventionally-managed plots

University of Maryland Eastern Shore, Princess Anne, MD (UMES)

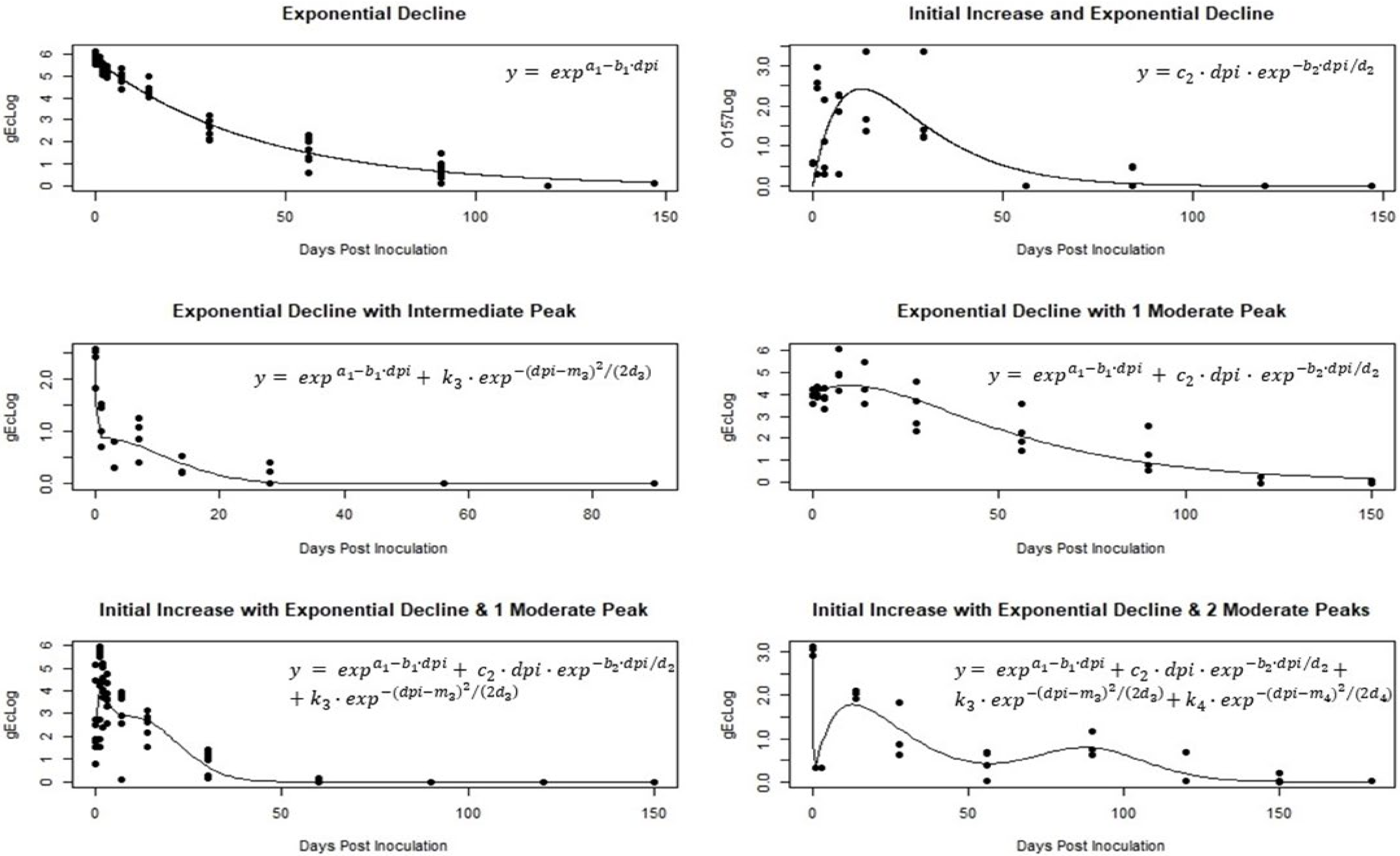
Soil type: Othello silt loam (fine-silty, mixed)

USDA Agricultural Research Service

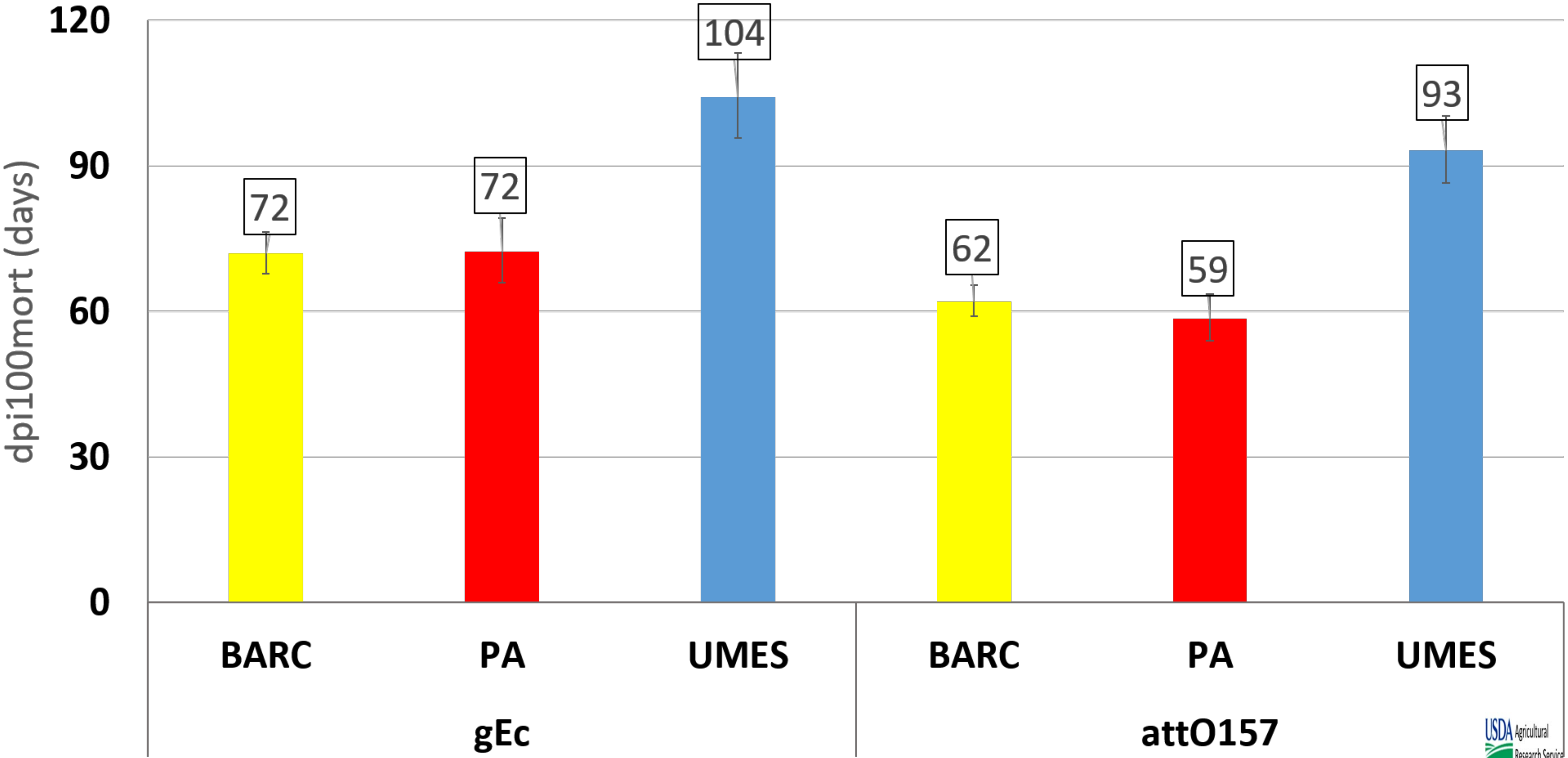
Overall analysis

- 12 separate field trials
- Trials over 4 years leads to:
  - 162 survival profiles for gEC over all sites
  - 162 survival profiles for attO157 over all sites
  - 1296 individual plots
  - 6659 data observations

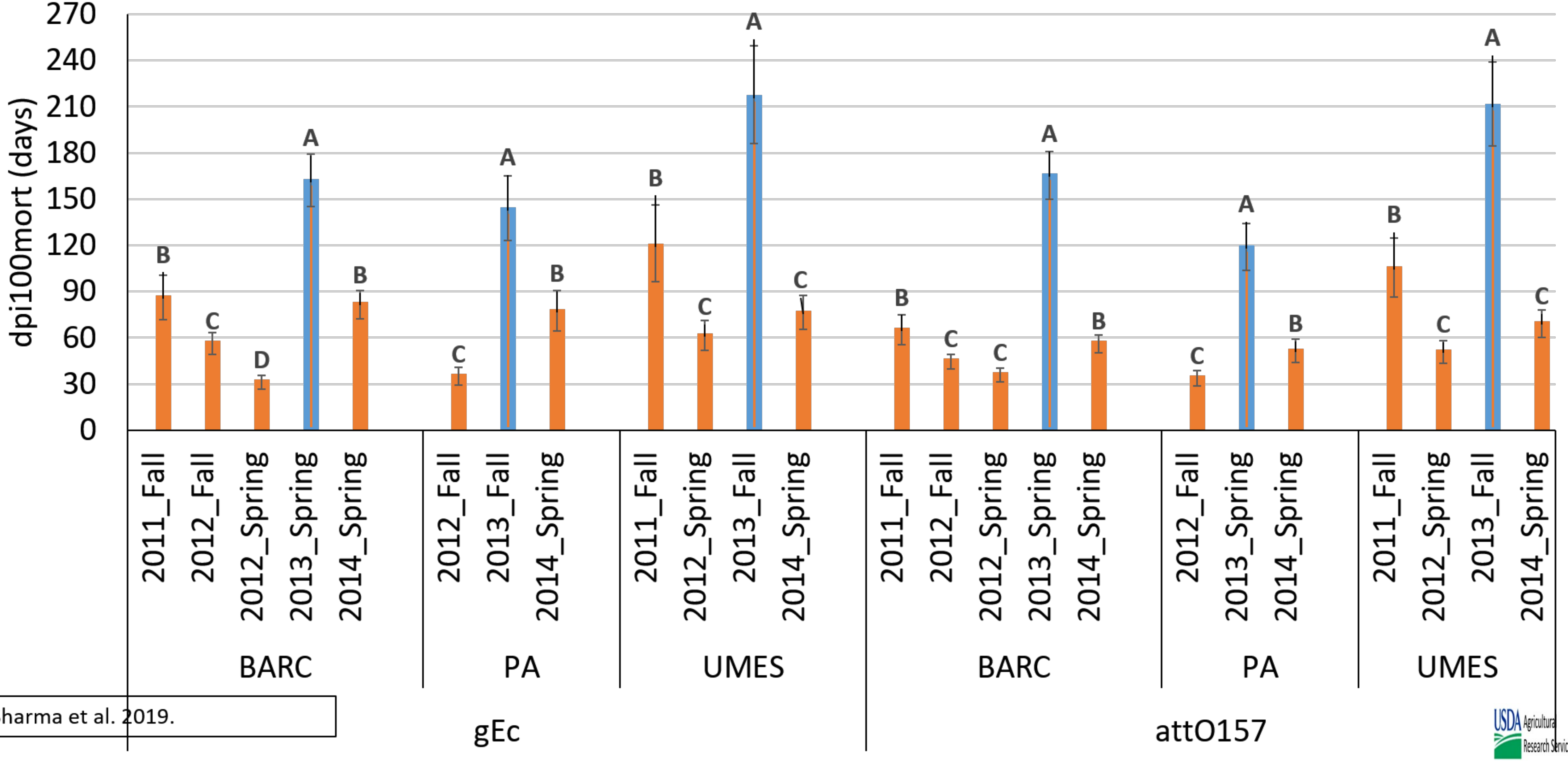
Soil Amendment	Abbreviation
Dairy Manure Liquids	DML
Dairy Manure Solids	DMS
Horse Manure	HM
Poultry Litter	PL
Unamended	UN


Exponential Decline
$y = exp^{a_1 - b_1 \cdot dpi}$
gEcLog
Days Post Inoculation
Initial Increase and Exponential Decline
$y = c_2 \cdot dpi \cdot exp^{-b_2 \cdot dpi/d_2}$
O157Log
Days Post Inoculation
Exponential Decline with Intermediate Peak
$y = exp^{a_1 - b_1 \cdot dpi} + k_3 \cdot exp^{-(dpi - m_3)^2/(2d_3)}$
gEcLog
Days Post Inoculation
Exponential Decline with 1 Moderate Peak
$y = exp^{a_1 - b_1 \cdot dpi} + c_2 \cdot dpi \cdot exp^{-b_2 \cdot dpi/d_2}$
gEcLog
Days Post Inoculation
Initial Increase with Exponential Decline & 1 Moderate Peak
$y = exp^{a_1 - b_1 \cdot dpi} + c_2 \cdot dpi \cdot exp^{-b_2 \cdot dpi/d_2} + k_3 \cdot exp^{-(dpi - m_3)^2/(2d_3)}$
gEcLog
Days Post Inoculation
Initial Increase with Exponential Decline & 2 Moderate Peaks
$y = exp^{a_1 - b_1 \cdot dpi} + c_2 \cdot dpi \cdot exp^{-b_2 \cdot dpi/d_2} + k_3 \cdot exp^{-(dpi - m_3)^2/(2d_3)} + k_4 \cdot exp^{-(dpi - m_4)^2/(2d_4)}$
gEcLog
Days Post Inoculation

Spatiotemporal factors: Survival duration (dpi100mort) at sites

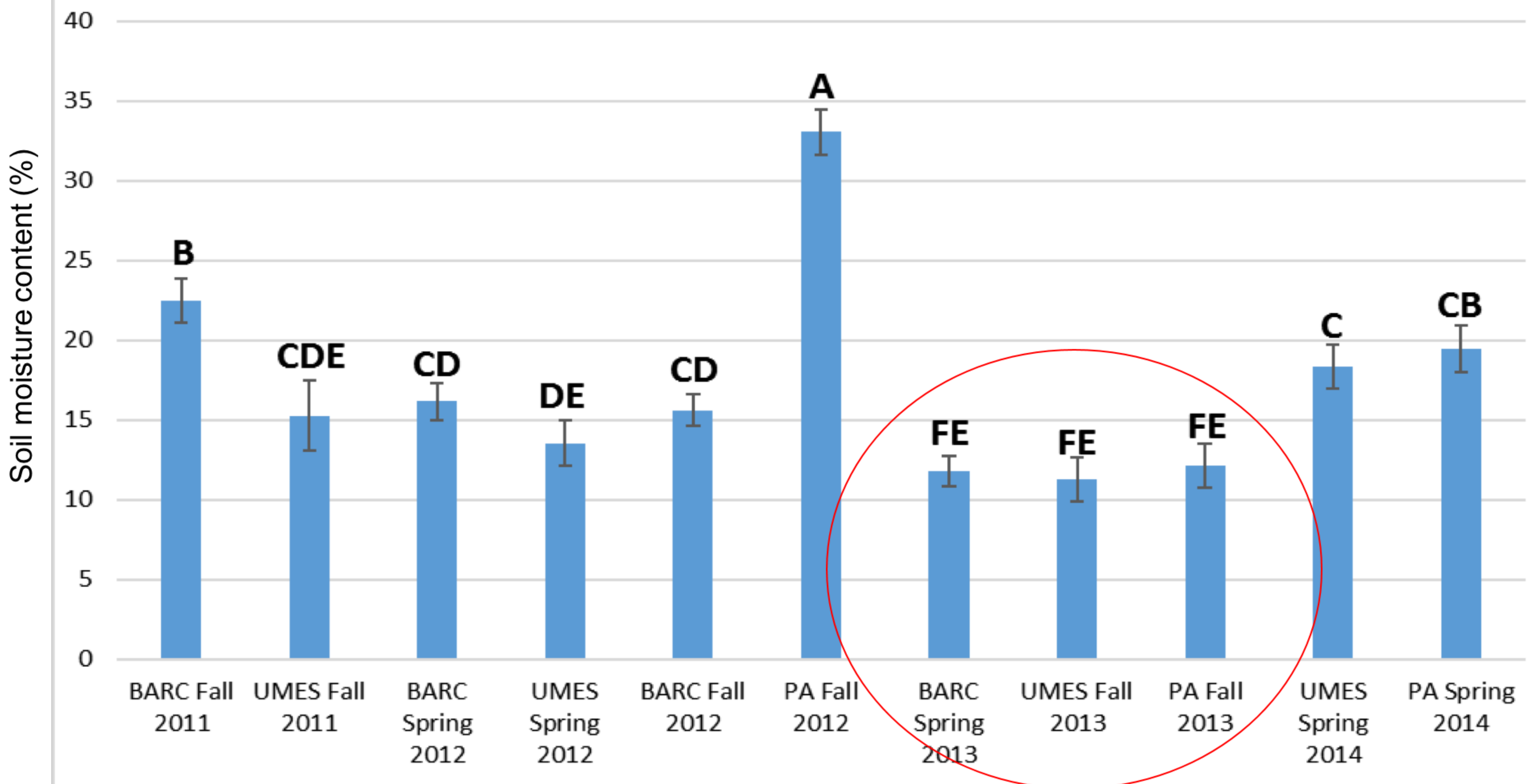


Spatiotemporal factors: Survival duration (dpi100mort) by seasons

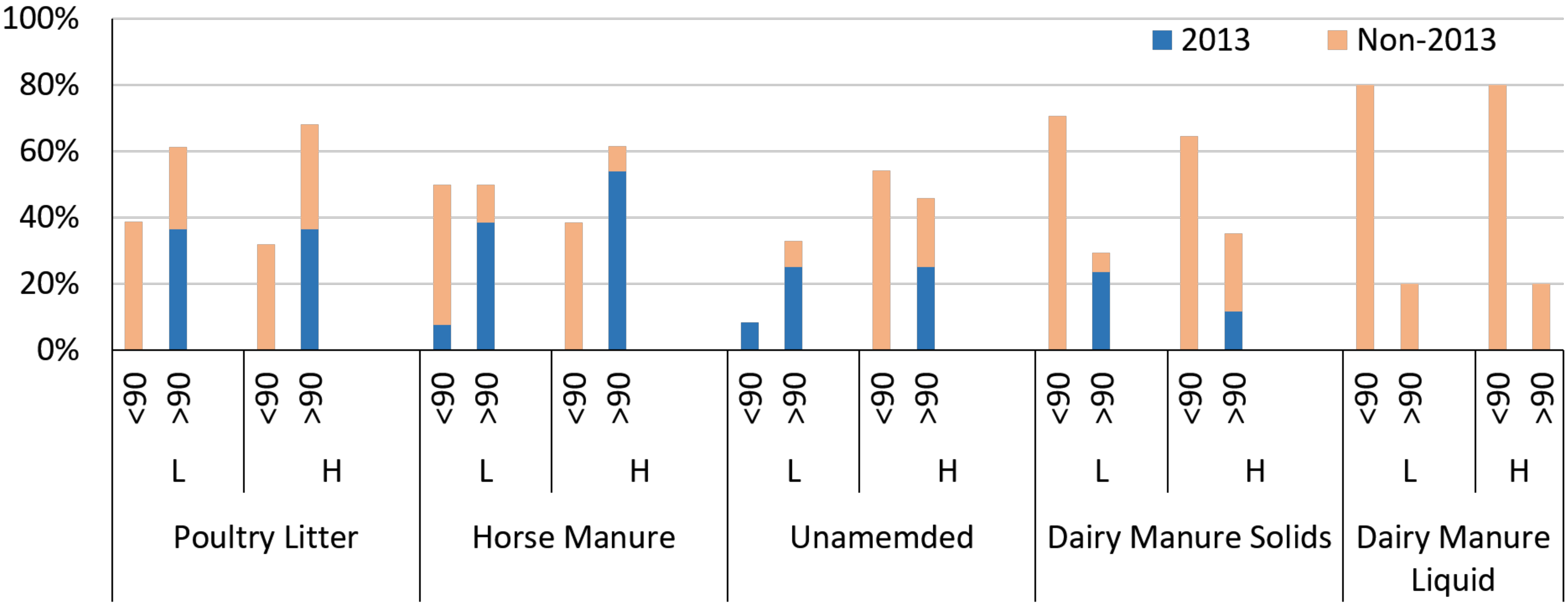


Sharma et al. 2019.

Weather effects: Initial soil moisture content (day 0)



Agricultural factor: % of survival profiles for amendment / inoculum level combinations which survive for < 90 or > 90 days



	BARC Fall 2011		UMES Fall 2011		BARC Spring 2012		UMES Spring 2012		BARC Fall 2012		PA Fall 2012		BARC Spring 2013		UMES Fall 2013		PA Fall 2013		BARC Spring 2014		UMES Spring 2014		PA Spring 2014	
	gEc	O157	gEc	O157	gEc	O157	gEc	O157	gEc	O157	gEc	O157	gEc	O157	gEc	O157	gEc	O157	gEc	O157	gEc	O157	gEc	O157
H PL C S	97	81	>147 0.42	>147 0.58	38	41	>154 0.31	62	>219 0.17	>219 1.6	63	61	>219 0.54	>180 0.45	>219 2.31	>219 2.32	>219 1.08	>219 0.25	>180 0.36	160	59	40		
L PL C S	86	48	154	131	31	21	>154 0.48	>154 0.31	103	74	22	19	155	>180 0.12	>219 1.20	>219 1.22	>90 0.64	>90 0.64	41	69	48	72		
H PL C T													>219 0.52	>180 0.21	>219 2.12	>219 1.72	>219 2.76	>90 0.79	72	50	62	81	>219 0.18	64
L PL C T													156	153	>219 1.29	>219 0.84	>90 2.09	>219 0.37	57	72	126	76	>219 0.24	100
H PL O S									>154 0.72	>154 1.71			>180 0.21	118					>180 0.32	137				
L PL O S									>120 0.12	>120 0.78			>180 0.16	164					>180 0.20	43				
H PL O T													>180 0.49	>180 0.49					180	65				
L PL O T													124	135					45	73				
H UN C S			140	123	13	33	6	6	61	71	49	23	157	>180 0.15	>219 0.12	219	>180 0.12	>219 0.14	>180 0.15	47	68	64	36	48
L UN C S	12		49	51	>14 0.20	>14 0.21	6	6	>14 0.20	5	12	29	158	>180 0.11	>219 0.70	169	27	24	33	31	71	62	60	59
H UN C T													147	128	>219 0.15	>219 0.14	>90 0.29	>219 0.10	74	50	83	86	43	61
L UN C T													142	>180 0.14	170	171	10	12	61	51	66	47	31	30
H UN O S	114	101			10	30			56	73			137	138					>180 0.20	49				
L UN O S	45	38			>28 0.23	5			41	5			126	155					42	24				
H UN O T													141	126					74	43				
L UN O T													161	146					55	73				
H HM C S					51	41	>154 0.47	>154 0.46	67	76	40	53	>180 0.51	>180 0.14	>219 1.30	>219 1.10	>219 0.22	>90 0.15			74	65		
L HM C S					25	16	122	125	41	17	29	23	135	130	>219 0.52	>219 0.58	>90 0.17	58			83	73		
H HM C T													123	147	>219 1.34	>219 1.97	>90 0.66	>90 0.26			112	84		
L HM C T													136	129	>219 0.56	>219 0.38	>90 0.36	82			89	93		
H HM O S									>219 0.12	63														
L HM O S									18	5														
H DMS C S	111	101	>147 0.35	100	14	30	>154 0.35	154	73	82	36	41	>180 0.38	>180 0.23					>180 0.23	56			62	36
L DMS C S	93	52	38	72	30	28	6	6	25	27	23	28	124	>180 0.08					69	43			46	25
H DMS C T													>180 0.28	162					31	54			66	50
L DMS C T													116	131					69	78			31	30
H DMS O S	98	82			13	31			71	53									75	32				
L DMS O S	39	29			29	56			21	26			124	126										
H DMS O T																			66	46				
L DMS O T													132	149										
H DML C S	114	98			36	27	51	50	57	76	84	68												
L DML C S	93	34			15	12	>154 0.14	124	25	34	38	25												

KEY (dpi100mort)
1
20
40
60
90
110
130
150
170
190
219

Abbreviations		
Inoculum level	H	High
	L	Low
Amendment	PL	Poultry Litter
	UN	Unamended
	HM	Horse Manure
	DMS	Dairy Manure Solids
	DML	Dairy Manure Liquids
Management	C	Conventional
	O	Organic
Depth	S	Surface
	T	Tilled

Proportion of explained variance individual ANOVA models can account for (R^2, %)

ANOVA Model	R^2 gEc (%)	R^2 attO157 (%)
Temperature and Rainfall	10.1	10.8
Initial Moisture Content	29.6	33.6
Inoculum level, Amendment, Management, and Depth	22	26.7
Temperature, Rainfall, and Initial Moisture Content	37.2	41.8
Inoculum level, Amendment, Management, Depth and Initial Moisture	43.5	49.4
Inoculum level, Amendment, Management, Depth and Initial Moisture, Temperature and Rainfall	50	57.3
Site, Year, Season	40.4	33.1
Site, Year, Season, Initial Moisture Content	52.4	49.4
Site, Year, Season, Initial Moisture Content, Inoculum level, Amendment, Management, and Depth	63.6	64.5
Total Variance % Explainable by Saturated Model Including all Nested Effects	97.2	97.3

Physical and chemical characteristics of manure amendments used in greenhouse experiments.			
Characteristic	Poultry Litter	Dairy Manure Liquids	Horse Manure
Phosphorus (P_2O_5),%[1]	**2**	**1**	**0.04**
Moisture (%)[2,]	**18**	**99**	**54.9**
Dry matter (%)	**82**	**1**	**45.1**
Total Nitrogen (mg/kg)[3,]	**3800**	**860**	**2600**
Ammonium Nitrogen (NH_4N), (mg/kg)[4]	**400**	**720**	**660**
pH	**8.30**	**8.49**	**9**

[1]Phosphorous was determined by EPA Method 3015A .

[2] Moisture and Dry matter were measured according to standard methods.

[3] Total Nitrogen was determined by combustion (Dumas method).

[4]Ammoniacal Nitrogen was measured using flow injection analysis of water extracts.

Summary and Conclusions

- Twelve separate field trials conducted in the mid-Atlantic U.S., showed that spatiotemporal factors (site, year, and season) affect survival durations of *E. coli* in manure-amended soils more than agricultural factors (manure type, organic or conventional management of soils, and depth of application) or weather effects.
- Poultry litter frequently supported longer survival durations compared to other manure types, and lower initial soil moisture values were associated with longer survival durations of *E. coli* in manure-amended soils.

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The effect of heat treated poultry pellets on *Salmonella* Newport survival in soils

- Evaluate the role that *rpoS* gene plays in survival of *Salmonella* Newport in HTTP-amended soils
- Evaluate if the presence / absence of spinach plants affects survival
- Does irrigation influence changes in *S.* Newport populations in soils

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Research Note

Survival and Growth of Wild-Type and *rpoS*-Deficient *Salmonella* Newport Strains in Soil Extracts Prepared with Heat-Treated Poultry Pellets

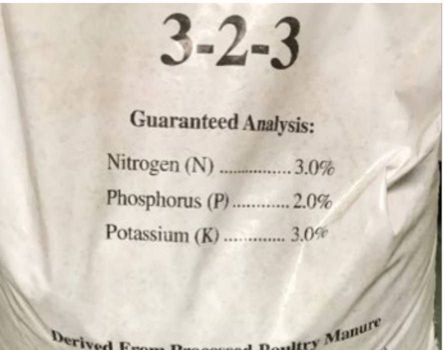
MANOJ K. SHAH,[1]† RHODEL BRADSHAW,[2] ESMOND NYARKO,[2] PATRICIA D. MILLNER,[2] DEBORAH NEHER,[3] THOMAS WEICHT,[3] TERESA M. BERGHOLZ,[1]† AND MANAN SHARMA[2]*†

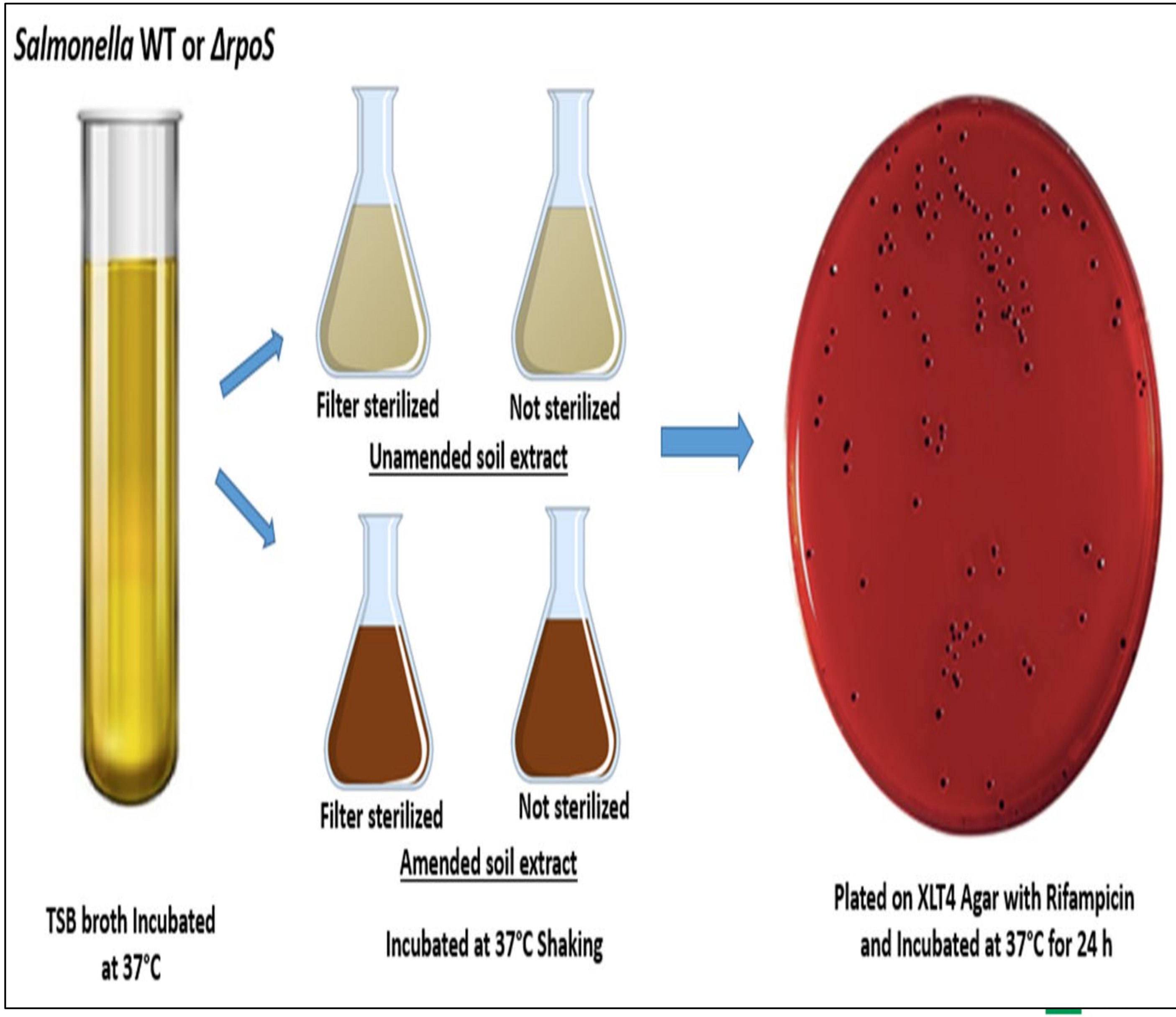
[1]*Department of Microbiological Sciences, North Dakota State University, Fargo, ND 58102, USA;* [2]*Environmental Microbial Food Safety Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, Northeast Area, U.S. Department of Agriculture, Beltsville, MD 20705 (ORCID: http://orcid.org/000-0002-8585-0308 [M.S.]); and* [3]*Department of Plant and Soil Science, University of Vermont, Burlington, VT 05405*

MS 18-465: Received 27 September 2018/Accepted 26 November 2018/Published Online 0 Month 2019

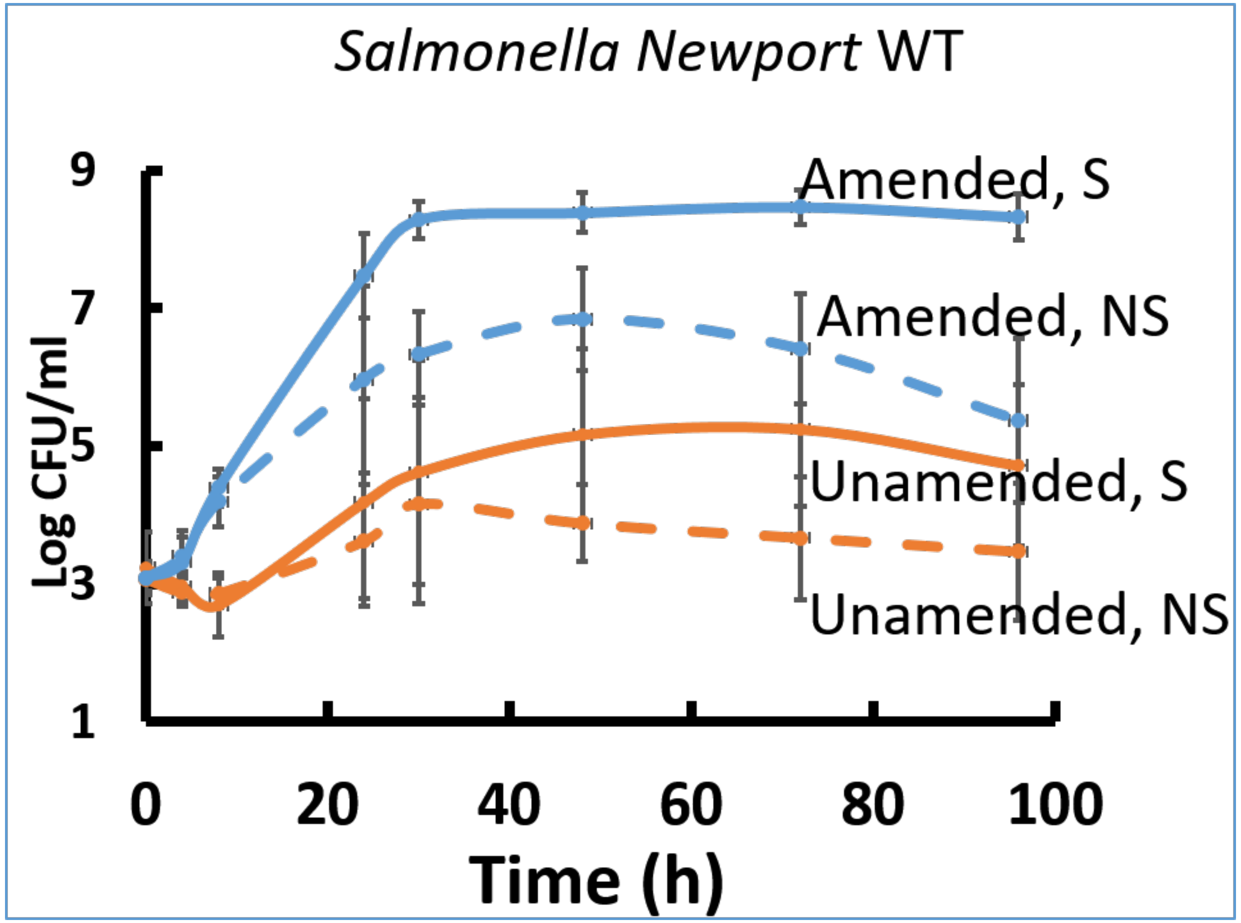
Can enteric pathogens grow in these environments?

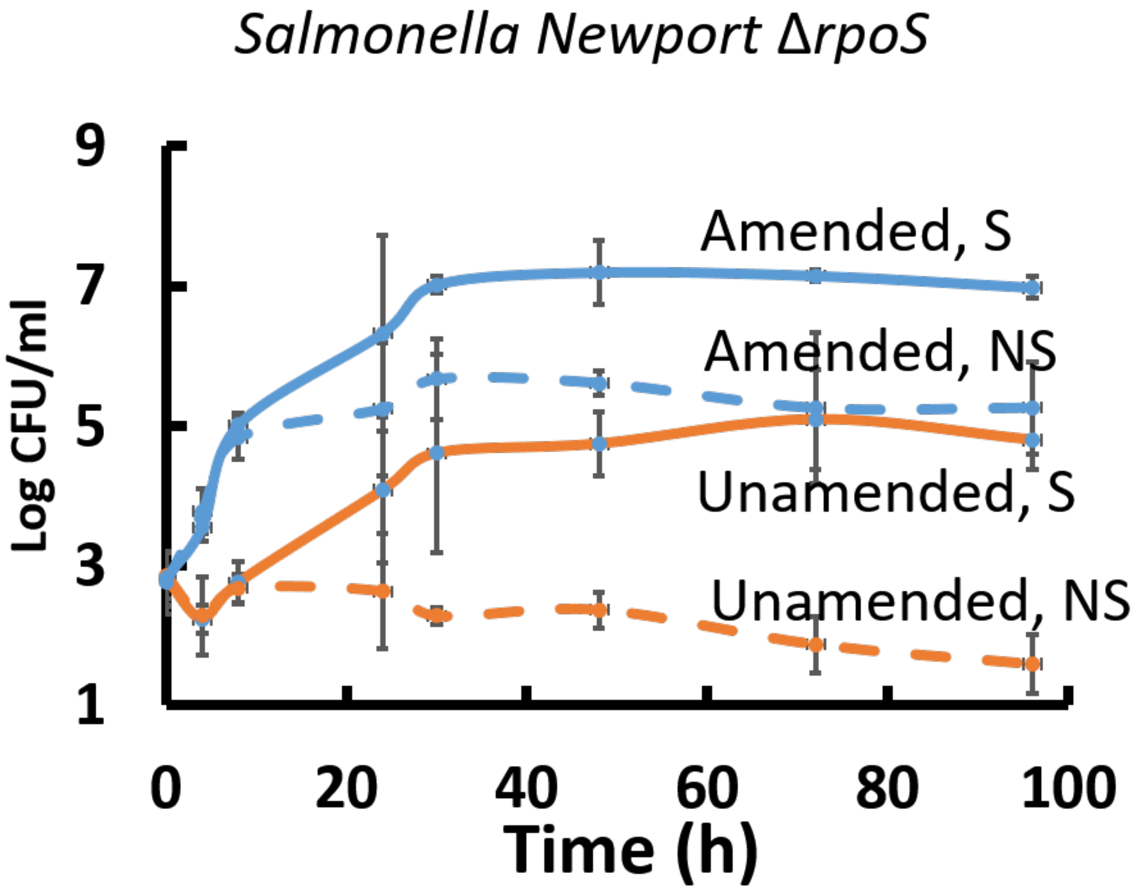
- Evaluate survival and growth of *Salmonella* Newport (WT and *rpoS*-deficient strains) in simulated manure-runoff
  - Soil extracts amended with biological soil amendments (heat-treated poultry pellets)





Growth of wild-type and *rpoS*-deficient *S.* Newport in sterile (S) or non-sterile (NS) soil extracts amended or not amended with HTPP (25^{o}C, 96 h)



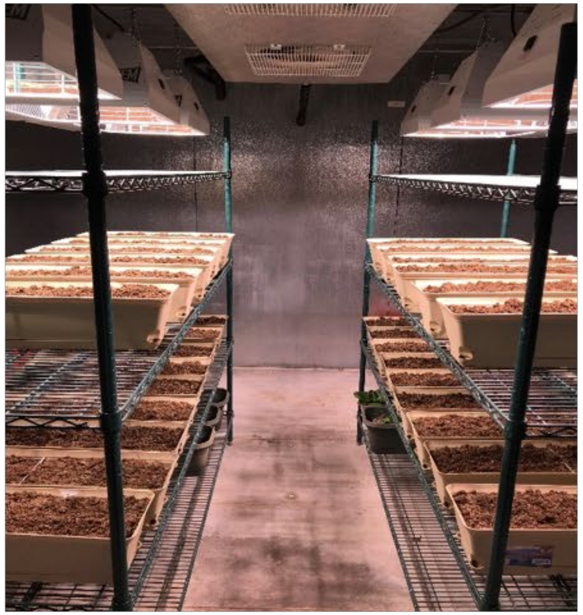


Shah et al. 2019. *J Food Prot.* 82: 501-506.

Survival of S. Newport in HTPP-amended soil

Crop field soil

Planters

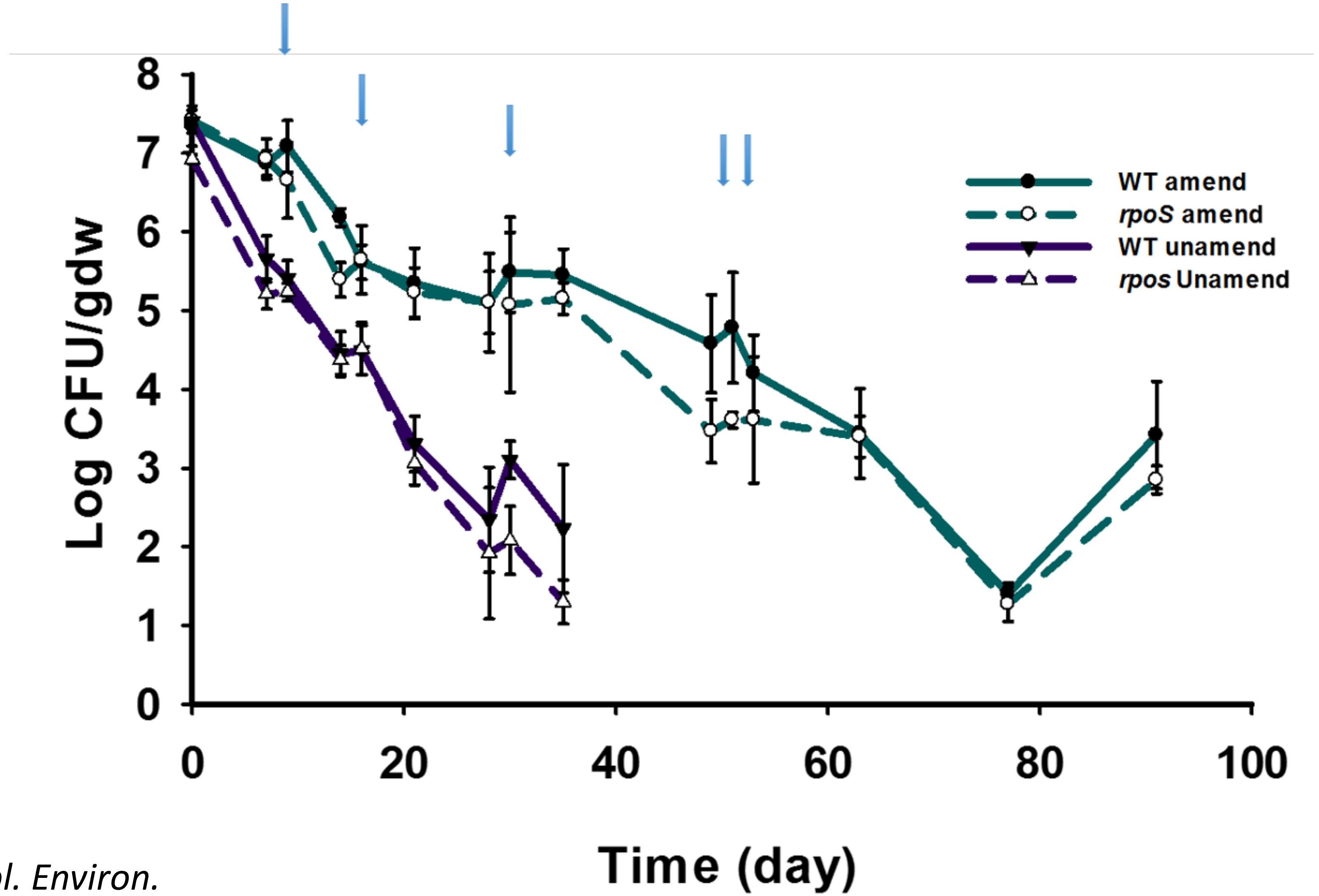


Growth Chamber

Amended with HTPP

***S*. Newport WT and Δ*rpoS* strains**
(Drip inoculated with water-culture mixture)
8 log CFU/gram dry weight

S. Newport declined more slowly and survived for longer durations in HTPP-amended soil vs unamended soils



Shah et al. 2019. *Appl. Environ. Microbiol*. e00334-19.

Inactivation rate (log CFU/g/day) of *S.* Newport was significantly greater in unamended soils vs amended soils.

Strain	Amendment	Spinach	K_{max}
WT	yes	yes	0.10±0.01^A
WT	yes	no	0.12±0.02^A
WT	no	yes	0.36±0.03^B
WT	no	no	0.33±0.04^B
ΔrpoS	yes	yes	0.10±0.02^A
ΔrpoS	yes	no	0.13±0.01^A
ΔrpoS	no	yes	0.38±0.03^B
ΔrpoS	no	no	0.36±0.03^B

Soil characteristics which significantly influenced inactivation rate (K_{max}) of *S.* Newport in amended and unamended soils.

	r^2	Pr > F
Moisture	0.8479	<0.0001
NH_4N+NO_3N	0.9991	<0.0001
Total Organic Carbon (Water extractable)	1.0000	<0.0001

Potential routes of contamination for enteric pathogens to fruits and vegetables

Summary and Conclusions

- Soils fertilized with heat-treated poultry pellets supported longer durations of survival of *Salmonella* Newport than unfertilized soils.
- Higher levels of *S.* Newport from HTTP-amended soil transferred to and survived on spinach leaves for longer periods of time compared to *S.* Newport from unamended soil ($p<0.05$).
- Higher levels of water-extractable carbon, soil moisture, and total nitrogen in soils slowed the decline of *S.* Newport in soils.

Funding sources

- USDA ARS Project Plan
  - "Characterization and Mitigation of Bacterial Pathogens in the Fresh Produce Production and Processing Continuum"
- Inter-Agency Agreement – FDA / ARS - "Continuation and Expansion and Development and Analysis of a Protocol to Assess Survival of Fecal Organisms in Agricultural Water or Agricultural Soils Amended with Raw Manure"
- Specific Cooperative Agreement, University of Maryland Eastern Shore, *"Survival of Fecal Organisms and Indicators in Agricultural Soils Amended with Raw Manure"*
- Specific Cooperative Agreement, University of Delaware, "Persistence of Enteric Pathogens in Manure-amended Soils in Northeast U.S. Produce-Growing Environments"

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  - Kathryn White
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  - Manoj Shah
  - Teresa Bergholz
- University of Maryland Eastern Shore
  - Fawzy Hashem
  - Corrie P Cotton
- Penn State
  - Alyssa Collins

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