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ORIGINAL ARTICLE

Composts of poultry litter or dairy manure differentially affect survival of enteric bacteria in fields with spinach

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dairy manure, *E. coli*, ecoenzymes, ecosystem stoichiometry, food safety, ITS-1 spacer region, PLFA, poultry manure, V4 region of 16S rRNA, vermicompost.

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Abstract

Aims: The aim was to determine the survival and persistence of *Escherichia coli* in soil amended with compost from different manure sources.
Method and Results: Complex interactions of abiotic and biotic factors on *E. coli* survival were characterized in field experiment plots receiving randomly assigned compost treatments: dairy windrow, dairy vermicompost, poultry windrow or no compost. Biomass, activity and function of indigenous microbial communities in the composts and soils were measured concurrently to determine whether mechanisms of compost were driven by biotic or abiotic properties. *E. coli* persisted in compost containing poultry amendments but not in composts containing dairy or no amendments. Poultry compost contained more NH_4-N and a distinct microbial community compared to dairy and no compost treatments. A laboratory experiment performed on compost extracts suggested that *E. coli* survived better in extracts devoid of indigenous microbes as long as bioavailable nutrients were plentiful.
Conclusions: Dairy-based composts are less likely to support *E. coli* survival than poultry-based composts.
Significance and Impact of the Study: Results aid in risk assessment of the use of different types of manure-based compost and soil amendments in fruit and vegetable production by elucidating the roles of nutrient and microbial community composition on survival of *E. coli* in amended field soils.

Introduction

Vegetable farming represents 14% of the US agricultural market in 2016 (USDA-ERS 2016) and provides nearly double the return per acre than other agricultural operations in the Northeast United States (Chan *et al.* 2011). The total number of vegetable farms is increasing in the Northeast United States and market projections suggest the vegetable market overall and the organic vegetable market will increase in the next decade. Organic vegetable production in the Northeast United States relies on composted manure-based organic amendments to provide plant nutrients and increase soil organic matter content (Goyal *et al.* 2005). The use of compost is safer than raw manure because the practice of composting can reduce enteric bacterial pathogens that were previously present in raw manure (US FDA 2018). Given that there is no enforced regulation of compost quality for agricultural soils, there is a potential for improperly prepared compost to introduce pathogens to soils which can transfer to edible fruits and vegetables (Noble 2011).

Stabilized, mature compost is a product manufactured through a controlled aerobic, microbially driven decomposition process. The US Department of Agriculture (USDA) National Organic Program stipulates that windrow compost piles maintain temperatures between 55 and 77°C for a minimum of 15 days, and turned a minimum of 5 times, to ensure lethal conditions for resident pathogens (Neher *et al.* 2015). However, if compost is allowed to mature and cure, it has functions beyond fertility and carbon sequestration. The composition of carbon compounds in the final product differentially attracts a consortia of micro-organisms which colonize the compost during the cooling phase of the process and are

antagonistic to pathogens (Hadar and Papadopoulou 2012). These microbes have evolved defences (against other microbes) that can be harnessed to target and suppress plant pathogens. These saprophytic microbes in compost may also suppress food-borne pathogens. However, there is a paucity of field experiments to support this claim. Most experiments regarding composts on disease suppression have been conducted on plant pathogens in the laboratory or greenhouse (Noble and Coventry 2005).

Pathogenic strains of *Escherichia coli* can be introduced to vegetable fields through contaminated surface water, ground water or wildlife faeces (Crook and Senior 2017). Multiple outbreaks of *E. coli* O157:H7 have been linked to surface and irrigation water. An investigation concluded that the irrigation water contaminated with feral pig faeces used for the Salinas Valley farms was a likely cause of the 2006 outbreak associated with contaminated spinach (Gelting *et al.* 2011). Improperly managed manure-based compost could facilitate *E. coli* O157:H7 establishment and survival (Fremaux *et al.* 2008), especially if fields are irrigated (Solomon *et al.* 2002).

Mature, dairy manure-based composts with hardwood bark, or those processed as vermicompost are relatively suppressive to copiotrophic soil-borne fungal pathogens, such as *Rhizoctonia solani*, whereas composts made with poultry manure are conducive (Neher *et al.* 2017). Autoclaving soil removed the disease suppression, suggesting the mechanism was microbial in origin. Both of the dairy manure-based compost amendments contained a consortium of saprophytic microbes that produce antimicrobial compounds including Firmicutes (*Bacillus* spp.), γ-proteobacteria (*Pseudomonas* spp.) and Actinobacteria (*Streptomyces* spp.) (Neher *et al.* 2013). Vermicompost of the same origin was used as a reference in this study to determine if suppression of fungal pathogens can also be translated to suppression of copiotrophic bacterial pathogens.

The overall aim of this study was to determine survival and persistence of *E. coli* populations in a field experiment comparing composts from manure sources that demonstrated varying levels of *E. coli* survival in a prior greenhouse pot experiment (Sharma *et al.* 2016). A cocktail of three nonpathogenic *E. coli* isolates were surrogates for food-borne pathogens. If compost introduces or supports human pathogens in soils, they may be transferred to ready-to-eat produce (e.g. spinach) grown in soils and lead to cases of food-borne illness. Compost served as a tool to introduce heterotrophic microbes that would indirectly reduce *E. coli* populations by antibiotic production, siderophores to sequester nutrients or production of enzymes that degrade cell walls (Hadar and Papadopoulou 2012). Ecoenzyme activity determined whether the compost contained adequate energy (carbon), nitrogen and phosphorus for the microbial community to sustain itself (Sinsabaugh *et al.* 2014). The hypothesis tested is that the nutrient content and microbial community composition in different composts would be associated with differences in *E. coli* survival in agricultural fields.

Materials and methods

The main experiment was conducted in the field to consider the complex interactions of abiotic and biotic factors on *E. coli* survival. A secondary experiment was conducted in the laboratory on compost extracts to examine nutrients separately or in combination with the microbial community impact on *E. coli* survival.

Field experimental design

Two fields in South Burlington, Vermont (44°26′37.4″N, 73°11′23.2″W) with sandy loam soil were used for the field trial, from May to November of 2015. Both fields were in hay production for 10 years prior to the study. The 'Lilac' field contained a Hinesburg B fine sandy loam soil with a pH of 6·4 and organic matter content of 2·9%. The 'Wheelock' field contained an Adams B loamy sand soil with a pH of 6·3 and an organic matter content of 2·6%.

For each field, fifteen 1 × 2 m plots were tilled to a depth of 30 cm using a rototiller (Troy Bilt, Cleveland, OH) to prepare the soil. Plots were separated by 1·5-m buffer strips. Five field treatments were employed: (i) *E. coli* with poultry litter compost, (ii) *E. coli* with dairy windrow compost, (iii) *E. coli* with dairy vermicompost, and (iv) *E. coli* without compost, and (v) untreated control with neither *E. coli* nor compost. Treatments were replicated three times per field and arranged in a completely randomized design. For each field, compost amendments were applied first, followed successively by *E. coli* inoculation, and soil retilled to a 10-cm depth using a rototiller. Tilling of inoculated plots occurred in the following order: (i) untreated control; (ii) *E. coli* without compost; (iii) dairy windrow; (iv) dairy vermicompost and (v) poultry. Rototiller blades were surface disinfected with 75% ethanol between each treatment. Finally, spinach was planted, covered with soil that was tamped down to prepare a seed bed. Each step is outlined below.

Compost treatments

Composts were from commercial or research sources that were well-characterized (Table 1). The poultry compost was applied at a rate of 13·4 tons acre^{-1} (30 t ha^{-1}, 2·7 kg plot^{-1}) Dairy manure-based composts were

Table 1 Characteristics of soil (N = 1 per field) and compost (N = 3) treatments in field and laboratory experiment, respectively, and their effect on growth rate (k) of *Escherichia coli* with (non-sterile) or without (sterile) microbes

					Growth rate (k)*		Per cent				mg kg^{-1}	
Field/Compost	Nickname	Field Expt	Starting materials	Processing	Sterile	Nonsterile	C	N	K	P	NH_4-N	NO_3-N
Lilac soil (unamended)	Lilac soil	X	N/A	N/A	N/A	N/A	2·8	N/A	24†	27·6†	3·2	15·8
Wheelock soil (unamended)	Wheelock Soil	X	N/A	N/A	N/A	N/A	2·4	N/A	118·7†	14·8†	3·3	19·5
Dairy windrow mature compost	DWM	X	Dairy manure	Aerated static pile, windrow	$0{\cdot}3198 \pm 1{\cdot}18 \times 10^{-5}$	$0{\cdot}0583 \pm 1{\cdot}00 \times 10^{-9}$	43·6	2·98	1·71	0·35	46·6	1·77
Dairy Vermicompost Mature Compost	DVM	X	Dairy manure	Aerated Static Pile, Vermicompost‡	$0{\cdot}3827 \pm 5{\cdot}75 \times 10^{-7}$	$0{\cdot}05237 \pm 2{\cdot}68 \times 10^{-5}$	39·6	3·82	2·83	0·55	23·5	5711
Poultry Litter Mature Compost	PLM	X	Poultry manure, wood shavings	Windrow	$0{\cdot}5757 \pm 1{\cdot}07 \times 10^{-5}$	$0{\cdot}1046 \pm 7{\cdot}67 \times 10^{-6}$	27·9	3·79	4·66	2·57	1855	1001
Food Poultry Mature Vermicompost	FPVM		Food Scraps picked by poultry	Vermicompost mesophilic	$0{\cdot}3881 \pm 7{\cdot}19 \times 10^{-8}$	$0{\cdot}1566 \pm 2{\cdot}20 \times 10^{-6}$	17·2	1·42	0·8	0·4	0·74	1093
Food Poultry Immature Vermicompost	FPVI		Food scraps picked by poultry	Vermicompost mesophilic	$0{\cdot}3461 \pm 3{\cdot}72 \times 10^{-7}$	$0{\cdot}1471 \pm 2{\cdot}77 \times 10^{-6}$	23·8	1·73	1·06	0·44	2·42	1845
Food Poultry compost	FPW		Food scraps, poultry manure, poultry bedding	Aerated static pile	$0{\cdot}3063 \pm 9{\cdot}26 \times 10^{-6}$	$0{\cdot}163 \pm 1{\cdot}89 \times 10^{-6}$	28·5	2·15	0·96	0·88	6·08	671
Mixed Mature compost	MM		Dairy manure, poultry manure, poultry butchering products	Windrow	$0{\cdot}3903 \pm 1{\cdot}36 \times 10^{-7}$	$0{\cdot}1736 \pm 1{\cdot}47 \times 10^{-6}$	16	1·66	0·76	1·01	2·44	1497
Mixed Immature compost	MI		Dairy manure, poultry manure, poultry butchering products	Windrow	$0{\cdot}3961 \pm 2{\cdot}57 \times 10^{-8}$	$0{\cdot}1318 \pm 4{\cdot}00 \times 10^{-6}$	18·9	1·52	0·51	0·6	1·7	433

*Growth rate constant (k) of the log phase (0–24 h for nonsterile, 0–50 h for sterile) for compost extract treatments in lab experiment. k-Values were determined from exponential growth models fit to the log phase of the mean *E. coli* survival curve for each treatment. All k-value estimates had an R^2 = 1·0.

†mg kg^{-1}.

‡Two-phase treatment: thermophilic windrow followed by vermicompost.

processed as either vermicompost or windrow. Both dairy compost treatments were applied at a rate of 6·72 tons acre^{-1} (15 t ha^{-1}, 1·36 kg plot^{-1}). These application rates were based on preliminary experiments and industry recommendations (Neher *et al.* 2017). Compost was manually spread uniformly across the surface of each plot.

E. coli inoculation

A three-strain inoculum of rifampicin-resistant non-pathogenic *E. coli* (TVS 353, 354 and 355) were used to distinguish them from indigenous microbes in composts or soil (hereafter referenced as 'indigenous'). These strains were isolated from agricultural environments and have been used previously in greenhouse and field studies involving soil amendments (Reynnells *et al.* 2014; Sharma *et al.* 2019). Each *E. coli* strain was grown in tryptic soy broth with 80 μg ml^{-1} rifampicin (TSBR) and adjusted to a final population of 1·67 $\times$ 10^5 CFU per ml. Volumes of cultures for all three strains were then combined in sufficient volume to yield a total inoculum of 5 $\times$ 10^5 CFU per ml. One litre of the *E. coli* inoculum was sprayed per plot, resulting in a total inoculum application of 2·5 $\times$ 10^8 CFU per m^2.

Spinach

Approximately 390 Hybrid savoy-leafed spinach (*Spinacia oleracea*) Reflect F1 seeds from Johnny's Selected Seeds (Windslow, ME) were planted by hand-broadcasting across each plot. In addition to spinach plants, weeds were allowed to grow on all plots to emulate the effect of the plant rhizosphere on soil community dynamics. Although the abundance of weeds was similar among all plots, the species tended to vary between fields. Plots were not irrigated because precipitation met or exceeded crop demand during the duration of the study.

Soil microclimate monitoring

Soil temperature and water potential were recorded every hour in each field at 2 and 10 cm depths during the duration of the field experiment with Campbell Scientific 10x data loggers (Logan, UT). Thermister and Watermark™ probes quantified soil temperature and water matric potential respectively. Rainfall measurements were obtained from the Burlington WSO AP station (6·4 km from field site (US NOAA 2017).

Soil sampling

Samples for *E. coli* population analysis were collected on 0, 1, 3, 6, 10, 15, 23, 29, 37, 49, 63, 78, 105 and 161 days post-inoculation (dpi). Samples for ecoenzymes, respiration, phospholipid fatty analysis (PLFA) and composition of bacterial and fungal communities were taken on different dpi (8, 16, 23, 30, 50, 65 and 105). Each sample was a composite of three 10-cm deep (2·5 cm diameter) soil cores taken in a stratified random sampling pattern per plot. All soil samples were sieved through a 2-mm mesh prior to subsampling for different analyses. *Escherichia coli* populations (20 g) and microbial activity were processed on the same day as samples were collected. Subsamples for PLFA and amplicon sequencing were frozen at −80°C and processed at a later date. A separate subsample of soil was dried at 90°C to compute gravimetric moisture for standardization of abundance and activity measurements as g^{-1} of dry weight of soil (gdw).

Quantification of *E. coli* populations

Escherichia coli populations were monitored by plate counts on MacConkey agar supplemented with 80 μg ml^{-1} rifampicin (MACR) in triplicate until populations declined to 20 CFU per gdw. Rifampicin-resistant colonies were pink/red. Populations as low as −0·24 log MPN per gdw were determined by most probable number (MPN) assay in MacConkey broth supplemented with 80 μg ml^{-1} rifampicin. Six twofold dilutions were each replicated four times in a 48-well plate assay. Wells that turned yellow were considered positive for *E. coli*.

Microbial activity and biomass determination

Reduction of iodonitrotetrazolium chloride (INT) (Sigma-Aldrich, St. Louis, MO) was measured as an indicator of dehydrogenase, a measure of microbial activity (Von Mersi and Schinner 1991). See Supporting information for detailed methods. PLFA was used to determine soil microbial biomass (nmol PLFA per gdw) using a microplate technique (Buyer and Sasser 2012).

Microbial ecoenzymatic activity

Microbial nutrient acquisition was determined by a microplate technique of four substrates labelled with methylumbelliferone (MUB) or methylcoumarin (MC) (Saiya-Cork *et al.* 2002). Substrates were chosen to target cellulose (BG), chitin (NAG), leucine (LUC) or phosphomonoesters (AP) (Table S1). These provide data on the allocation of energy by microbes for the synthesis of particular enzymes relative to microbial limitations of C, N or P. The ratios of BG to AP and BG to (NAG+LUC) were graphed to compare the relative microbial need for acquisition of C, P and N in soil through time (Sinsabaugh *et al.* 2014). Fluorescence was converted to nmols

of substrate used h^{-1} incubated × gdw^{-1} × total PLFA concentration^{-1} to yield enzyme activity h^{-1} gdw^{-1} $PLFA^{-1}$. See supporting information for detailed methods.

Bacterial and fungal community composition

DNA was extracted from 0·5 g of each soil sample using the Qiagen PowerSoil DNA Isolation kit (Germantown, MD) following the manufacturer's instructions with the modifications described by Lauber *et al.* (2009). Extracted DNA was PCR-amplified using 515F/806R primers targeted for the V4 region of the 16S rRNA gene for bacteria and Archaea and ITS-1F/ITS-2R primers to amplify the ITS-1 spacer gene of 18S rRNA for fungi following protocols described previously (Edgar 2013). Sequencing was conducted on an Illumina MiSeq (2 × 150 bp chemistry) at the University of Colorado's Next Generation Sequencing Facility. Reads were merged, demultiplexed and quality-filtered using UPARSE (Emerson *et al.* 2015) following the pipeline described previously (Edgar 2013). Sequences were clustered into operational taxonomic units (OTUs) at the >97% sequence similarity level with the taxonomic identity of each OTU determined using the RDP classifier with a threshold of 0·5 (Wang *et al.* 2007) trained against either the Greengenes database for bacterial and archaeal 16S rRNA gene sequences (McDonald *et al.* 2011) or the UNITE database for fungal ITS sequences (Kõljalg *et al.* 2013). See Supporting information for detailed methods. Raw amplicon sequence data reported are available in the public Figshare database (DOI 0.6084/m9.figshare.7504481 https://figshare.com/s/eb5110f459b726af89bc).

Nutrient analysis

Prior to treatment application, baseline soil samples (10 cm deep) from the two fields and compost samples were analysed for pH and nutrients (Table 1). Briefly, a modified Morgan extract was used for nutrient extraction and pH was tested in a 1 : 1 water solution with Modified Mehlich buffer (NEC-1012 2011). Total carbon (C), potassium (K), nitrogen (N), phosphorus (P), ammonium (NH_4-N) and nitrate (NO_3-N) were determined using the methods of Peters *et al.* (2003).

Laboratory experimental design

Extract preparation

Extracts of **c**ompost and soil (Table 1) were used as a growing medium for *E. coli* to determine the relationship between *E. coli* growth, nutrient levels and the presence of indigenous microbes. To make extracts, samples of each compost and soil sample (250 g) were suspended in distilled water (500 ml), shaken (60 rev min^{-1}) for 24 h at 22°C, and centrifuged at 5000 ***g*** for 20 min. Half (375 ml) of the collected supernatant was reserved as nonsterile extract and the other half was filtered through 0·2-μm pore diameter vacuum filters (Millipore, Billerica, MA) to remove living microbes but retain nutrients ('sterile extracts'). All extracts were stored at −20°C until use.

Escherichia coli inoculation of compost extracts and enumeration

E. coli were prepared as for the field experiment except that log phase *E. coli* isolates were washed and diluted to 10^4 CFU per ml in 0·85% saline. Three replicates of each sterile and nonsterile compost extract were added to test tubes in 5-ml aliquots of TSBR. Each *E. coli* strain was added to each test tube at 10^2 CFU per ml and incubated at 35°C. For sterile extracts, *E. coli* were enumerated at 0, 4, 8, 20, 50, 72, 110 and 150 h after inoculation by plate counts on MACR after incubating at 35°C for 24 h. *Escherichia coli* in nonsterile extracts were enumerated at 0, 24, 72, 110 and 158 h postinoculation.

Statistical analysis

Treatment differences for *E. coli* survival were analysed by a linear mixed model, using treatment and day as fixed effects and plot as a random effect. A linear mixed effect model was used to conduct a repeated measures analysis assessing the effect of compost treatment and time on INT and ecoenzyme levels. All pairwise comparisons were conducted for variables with significant effects using Tukey's multiple comparison test. A linear regression was performed on nutrient content (C, N, K, P, NH_4-N and NO_3-N) as a function of k to determine the effect of discrete nutrients on *E. coli* growth potential. All analyses were conducted in R ver. 3.5 software using the lme4 package for linear mixed models (R Core Team 2016).

16S rRNA and ITS gene sequences were rarefied to a depth of 19 600 and 18 012 reads per sample respectively. OTU abundance was converted to a proportion of the total number of sequences per sample. Pairwise dissimilarity (Bray–Curtis) matrices were analysed by principal coordinate analysis using PRIMER ver. 6 software (PRIMER-E, Plymouth, WA). Differences in community composition among treatments through time were analysed using principal response curves (PRC) following the approach of Neher *et al.* (2005). PRC is a multivariate method for the analysis of repeated measurement design where statistical significance is computed by Monte Carlo permutation of both first ordination axis and all axes together using CANOCO ver. 5 software (ter Braak and Šmilauer 2012).

Results

Effect of compost on *E. coli* survival in field

Escherichia coli survival trends were similar in both field sites (Fig. 1). There was an initial exponential decay, some stabilization at 50 dpi, followed by a second rapid decay until extinction. The exception was poultry composts ($P = 0{\cdot}0005$). In soil amended with poultry compost, *E. coli* populations increased initially and then declined exponentially until 105 dpi after which the populations stabilized. *Escherichia coli* was absent in noninoculated plots, verifying that there was no cross contamination between plots and no rifampicin-resistant *E. coli* indigenous to the soil.

Microbial community activity, community composition, and ecosystem function

Microbial activity (INT) tended to be greater in poultry than dairy composts (Fig. S1) and followed the temporal trends of *E. coli* survival. The temporal occurrence of the minimum value of INT corresponded with a similar minimum value of both AP and BG. Ecoenzyme activity was most variable among all five treatments at 8 dpi, shortly after composts were added (Fig. S2). The treatment without compost was more C limited (smaller values on *y*-axis) than treatments with compost (Fig. 2). The C limitation varied by sample date, but generally increased through time. Ecoenzyme ratio BG:AP values declined (a limitation of P) as the experiment progressed. The ratio BG:(NAG+LUC) remained relatively constant (no limitation of N).

Community composition of bacteria and fungi was distinct for each field location (Fig. 3) and clustered by compost treatment within fields (Figs 4 and 5). Poultry compost amendment had a sustained effect on bacterial and archaeal community composition compared to that of the dairy compost treatments and treatment without compost for the duration of the 160-dpi experiment (Fig. 4). The composition of the bacterial community of the two dairy compost treatments started to converge (become more similar) towards the community of untreated control within 6 months. Compared to the soil without compost or amended with dairy composts, soil amended with poultry compost was distinguished by a relative abundance of Sphingobacteria (Bacteroidetes) and Acidobacteria (orders RB41 or iii1–15) in both fields. In fungal communities, the distinguishing taxa were field specific. All compost amendments reduced the relative abundance of *Mortierella camargensis* (Zygomycota) in Lilac, and *Ascobolus* sp. (Ascomycota) distinguished poultry from dairy composts in Wheelock (Fig. 5).

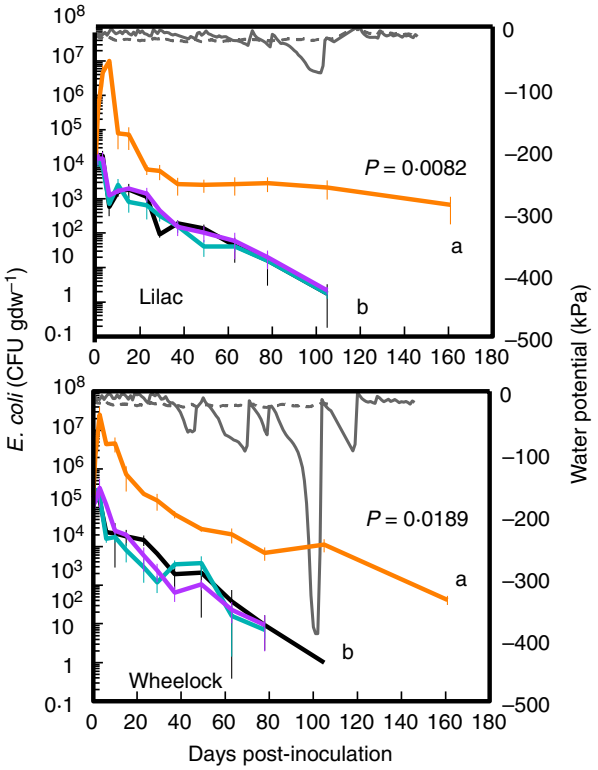


Figure 1 Abundance of *Escherichia coli* through time (left *y*-axis) and water potential through time (grey lines, right *y*-axis) in Lilac (top) and Wheelock (bottom). Solid lines represent water potential taken at a 2-cm depth and dashed lines represent water potential taken at a 10-cm depth. Note that field capacity is −30 kPa. Means ± 1 SE ($N = 3$) are illustrated for untreated (black), WP vermicompost (blue), WP windrow (purple) and poultry windrow (orange) compost at various days after inoculation. Contrasting lowercase letters represent statistical significance ($P \leq 0{\cdot}05$). [Colour figure can be viewed at wile yonlinelibrary.com]

Separating the effect of nutrients and microbial community on *E. coli* survival

The presence of indigenous microbes in nonsterile compost extracts markedly limited growth of *E. coli* populations compared to sterile extracts (Fig. 6). In contrast, *E. coli* populations exhibited exponential growth in sterile compost extracts during the first 50 h after *in vitro* inoculation, and sustained a population of 10^8–10^{14} CFU per ml for the remainder of the experiment. Nonsterile extracts of poultry composts sustained *E. coli* populations at higher populations than nonsterile dairy compost extracts. There was a positive association of NH_4-N, P and K content of compost extracts with increased growth rate (k) of *E. coli* when indigenous microbes were absent ($P < 0{\cdot}05$, Table S2). All three of these nutrients were more abundant in poultry than dairy-based compost (Table 1). Levels of bioavailable nutrients in the sterile

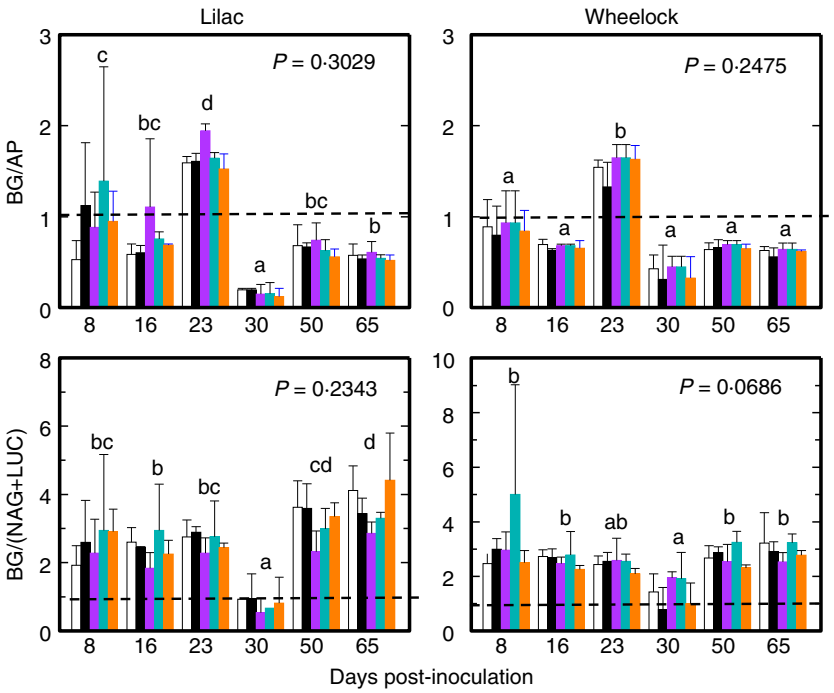


Figure 2 Ratios of ecoenzymes reflecting relative acquisition of C : P (top row) and C : N (bottom row) for Lilac (left column) and Wheelock (right column) sites. C : P represents the ratio of β-1,4-glucosidase/phosphatase (BG/AP), and C : N represents the ratio of β-1,4-glucosidase to β-1,4-*N*-acetylglucosaminidase+leucine (BG/(NAG+LUC). The dashed line represents a 1 : 1 ratio. Treatments are represented by fill colour (white: no *Escherichia coli*, no compost; black: *E. coli*, no compost; purple: *E. coli*, dairy windrow compost; turquoise: *E. coli*, dairy windrow compost; orange: *E. coli*, poultry compost). $N = 3$ per treatment at each sampling date. Contrasting lowercase letters represent statistical significance between time points ($P \leq 0{\cdot}05$). Treatment differences were not significant for BG/AP ($P = 0{\cdot}3029$ and 0·2475 for Lilac and Wheelock respectively) or for BG/(NAG + LUC) ($P = 0{\cdot}2343$ and 0·0686 for Lilac and Wheelock respectively). [Colour figure can be viewed at wileyonlinelibrary.com]

soil extract (without compost) were inadequate to sustain *E. coli* populations.

Soil temperature and soil moisture

The first 50 dpi were unusually wet and rainfall exceeded 30 cm, followed by a relatively dry period (cumulative total rainfall of 7 cm) from 51 to 160 dpi when soils drained (Fig. 1). Despite the fields containing fast-draining sandy soils, the sites were waterlogged at 50 dpi. The soil temperature remained relatively constant up to 100 dpi and quickly dropped as autumn began. Both *E. coli* survival and general microbial respiration mirrored soil moisture dynamics.

Discussion

Greater survival of these *E. coli* strains in soils amended with poultry litter than dairy manure-based soil amendments was observed previously in a greenhouse setting (Sharma *et al.* 2016) and in field trials conducted in the Mid-Atlantic United States (Sharma *et al.* 2019). Soil amended with poultry compost contained more NH_4-N and a distinct composition of microbial taxa compared to dairy compost or no compost. Sphingobacteria, Acidobacteria and Opitutales respond to N input and are associated with N_2O fluxes (Hester *et al.* 2018). Both Sphingobacteriales and Opitutales increase with high N availability and Acidobacteria order RB41 increases with low N availability (Ramirez *et al.* 2012). Acidobacteria grow best on low nutrient media, but can tolerate rich media, defining their classification as facultative copiotrophs (Eichorst *et al.* 2007). It is impossible to separate the effect of readily available NH_4-N and microbial community composition in poultry composts. In the current study, the positive correlation between poultry compost treatment and Sphingobacteria, Acidobacteria and Opitutales reinforces the observations of high N amendments reported previously (Ward *et al.* 2009; Ramirez *et al.* 2012). However, this study demonstrated a greater response to NH_4-N than NO_3-N.

Fungal groups associated with poultry compost also relate to N availability. *Ascobolus* sp. (Ascomycota) are coprophilous, and sometimes referred to as ammonia fungi, because they develop reproductive structures exclusively or relatively abundantly in response to the sudden addition of ammonia that occurs after urea applications (Suzuki *et al.* 2002). Our results suggest that N availability and microbial communities are inseparable in the field experiment making it impossible to identify direct mechanisms favouring the survival of *E. coli* by poultry compost.

Nutrient analysis of soil in both fields indicated that N and P content of soil in both fields was within an ideal range for crop growth before compost treatments were added. Therefore, any macronutrients added to these soils from the compost amendments would reflect microbial demand and activity rather than a competition between crops and microbes. Ecoenzymes are only secreted by

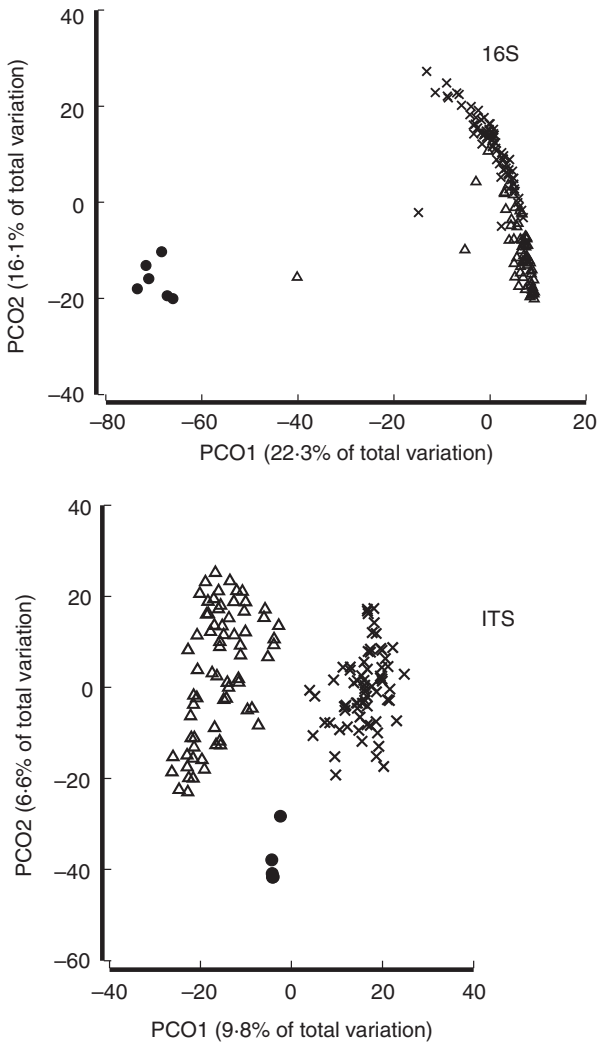


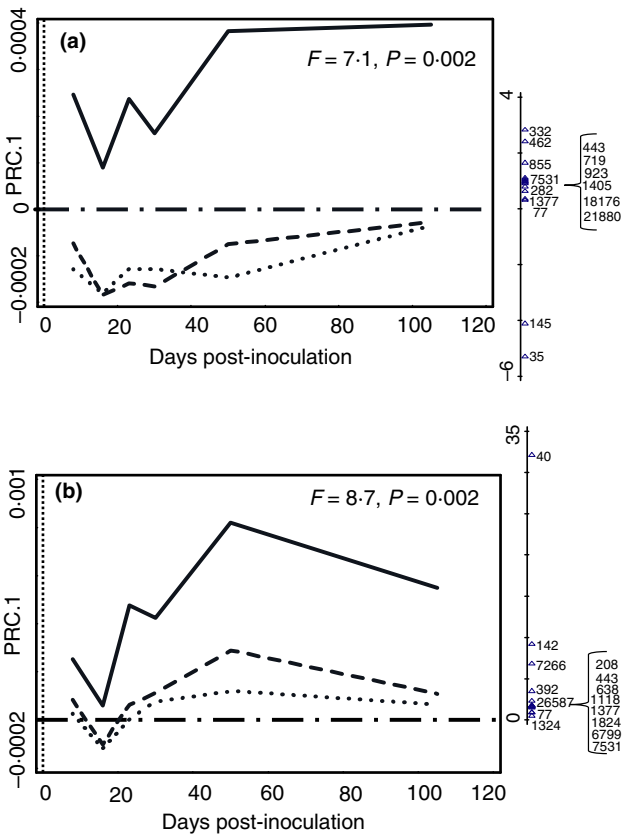
Figure 3 Principal coordinate analysis for all samples ($N = 150$) of 16S sequences for bacteria and Archaea (top) and ITS sequences for fungi (bottom) for all compost-amended soil treatments (poultry, dairy windrow, dairy vermicompost, none) combined within field, and composts alone. Symbols represent Bray–Curtis dissimilarity values between pairwise samples. Fields are labelled as Lilac soil (open triangle), Wheelock soil (X), and compost treatments (solid circle).

microbes in soils when nutrients are scarce because protein synthesis is an energy-intensive process (Sinsabaugh *et al.* 2014). Thus, ecoenzyme activity in soil provides insight into the relative nutrient availability for the microbial community. Variability in ecoenzyme activity at 8 dpi likely reflects a response to disturbance caused by the initial cultivation (Liu *et al.* 2016). The remaining time points had more uniformity among treatments, indicating that microbial activity was controlled by other factors. The ecoenzyme ratio, BG:AP, declined to values less than 1·0 as the experiment progressed, suggesting that soils in this study became P limited for soil microbes (Sinsabaugh *et al.* 2014). Verifying this conclusion is the concurrence of *Mortierella elongata* in compost-amended soils, associated with increased activities of soil AP (Liu *et al.* 2016). *Mortierella* metabolizes readily accessible carbohydrates including chitin, but excluding cellulose, thus increasing shortly after carbon additions (Werner *et al.* 2016). In contrast, facultative copiotrophic Acidobacteria respond similarly to oligotrophs in response to organic soil amendments (Fierer *et al.* 2007).

We did not directly measure oxygen, but there are multiple indications that the soil became anaerobic during the beginning of the study. For example, both respiration and ecoenzyme activity of the compost microbes were lower at 8, 16, 23 and 30 dpi compared to that at 50 or 65 dpi, suggesting that growth of compost microbes was inhibited by saturated soils. After an initial exponential decay phase up to 7 dpi, *E. coli* decay slowed in all treatments in conjunction with lower microbial respiration suggesting that microbial competition became limited in the water saturated soils. After 50 dpi, rain was less frequent and the soils drained, *E. coli* populations quickly fell below the level of detection (0·36 CFU per gdw) except in the poultry compost treatment. The positive correlation between *E. coli* and Sphingobacteriales indicates that poultry compost favours copiotrophic communities. This finding corroborates previous suggestions of copiotrophic strategy among the Sphingobacteriales, and suggests that survival of *E. coli* in poultry compost-amended plots during drying/rewetting events was reflective of the survival of the greater copiotrophic community at large.

Soil temperature and soil moisture

The response of a microbe to soil moisture dynamics could be a direct effect of the organism's adaptive responses or an indirect response from moisture's effect on nutrient availability. Optimum soil moisture can be described at the phylum level. For example, soil moisture optima are drier for Acidobacteria than Proteobacteria and Bacteroidetes (Lennon *et al.* 2012); Zygomycota are less tolerant to dry conditions than Ascomycota and Basidiomycota (Dix and Webster 1995). Some microbe taxa may be able to coexist by partitioning the moisture niche axis, for example, dry-adapted generalists who tolerate a broad range of water potentials and wet-adapted specialists whose metabolism is restricted to wet soils (Lennon *et al.* 2012). Changes in microbial community composition vary by hysteresis, with differences depending on whether soils are draining or wetting (Barnard *et al.* 2013). Both Acidobacteria and Verrucomicrobia (Opitutales) increased in wet soils. Formerly considered an obligate anaerobe, *Opitutus* (Verrucomicrobia: Opitutaceae) is classified as a

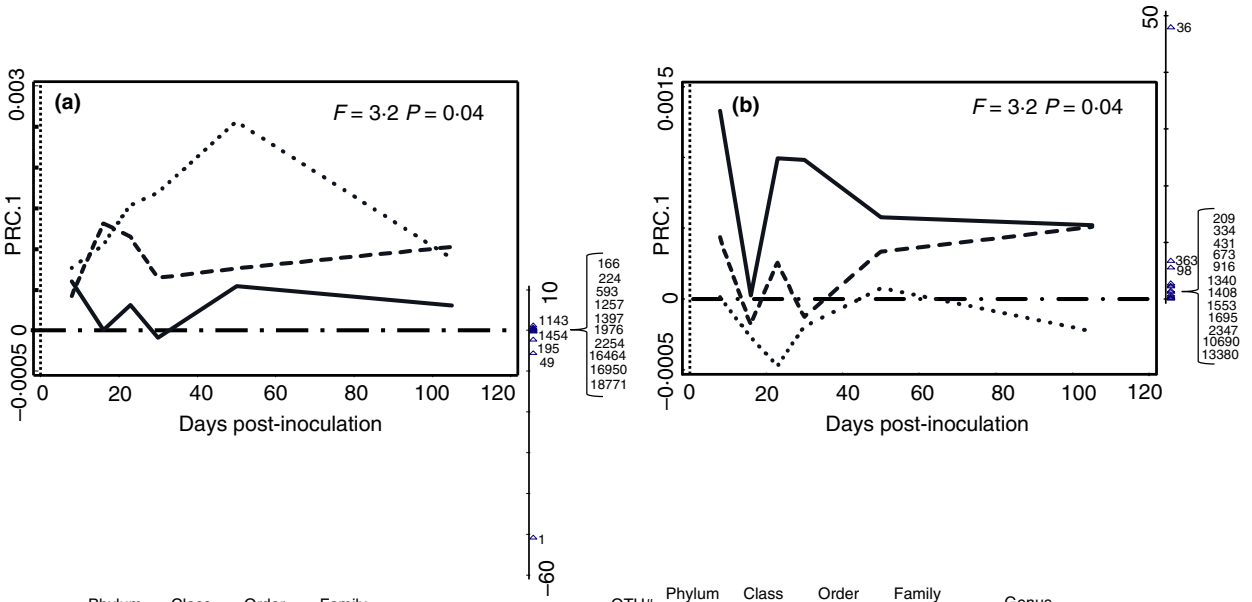


OTU #	Phylum	Class	Order (-ales)	Family (-aceae)	Genus
35	Proteobacteria	γ-proteobacteria	Xanthomonad	Sinobacter	na
40	Bacteroidetes	Sphingobacteriia	Sphingobacteri	na	na
77	Firmicutes	Bacilli	Bacill	Bacill	na
142	Bacteroidetes	Sphingobacteriia	Sphingobacteri	na	na
145	Proteobacteria	β-proteobacteria	MND1	na	na
208	Proteobacteria	γ-proteobacteria	na	na	na
282	Acidobacteria	[Chloracidobacteria]	RB41	Ellin6075	na
332	Proteobacteria_	γ-proteobacteria	Xanthomonad	Sinobacter	na
392	Bacteroidetes	[Saprospirae]	[Saprospir]	Chitinophag	na
443	Verrucomicrobia	[Pedosphaerae]	[Pedosphaer]	Ellin517	na
462	Verrucomicrobia	Opitutae	Opitut	Opitut	*Opitutus*
638	Proteobacteria	α-proteobacteria	Ellin329	na	na
719	Proteobacteria	α-proteobacteria	Sphingomonad	Sphingomonad	*Novosphingobium*
855	Acidobacteria	[Chloracidobacteria]	RB41	Ellin6075	na
923	Proteobacteria	α-proteobacteria	Sphingomonad	Sphingomonad	na
1118	Proteobacteria	γ-proteobacteria	Alteromonad	Alteromonad	na
1324	Fibrobacteres	Fibrobacteria	258ds10	na	na
1377	Verrucomicrobia	Opitutae	Opitut	Opitut	*Opitutus*
1405	Bacteroidetes	Sphingobacteriia	Sphingobacteri	Sphingobacteri	na
1824	Proteobacteria	α-proteobacteria	Sphingomonad	Sphingomonad	*Kaistobacter*
6799	Bacteroidetes	[Saprospirae]	[Saprospir]	Chitinophag	na
7266	Acidobacteria	Acidobacteria-6	iii1-15	na	na
7531	Bacteroidetes	Saprospirae	Saprospir	Chitinophag	*Ferruginibacter lapsinanis*
18176	Bacteroidetes	Sphingobacteriia	Sphingobacteri	na	na
21880	Firmicutes	Bacilli	Bacill	Bacill	*Virgibacillus*
26587	Proteobacteria	α-proteobacteria	Ellin329	na	na

Figure 4 First principal response curve coefficient (PRC1) 16S sequences for (a) Lilac, (b) Wheelock. Curves represent deviation between a compost treatment from untreated soil (untreated: dash-dot; solid: poultry; dashed: dairy vermicompost; dotted: dairy windrow) as a function of days post-inoculation. The weights of the 15 best fit OTUs are shown on the right axis. Missing taxonomic information occurs if higher resolution was not available for the OTU (na). Monte Carlo permutation tests permuting whole time series were applied to compute statistical significance. $N = 144$ total samples.

facultative anaerobe with both fermentative and respiratory metabolism. Its growth is stimulated by the presence of oxygen up to 2% and it tolerates up to 16% oxygen (Tegtmeier *et al.* 2018). At the class level, iii1–15,4 (Acidobacteria) reportedly increased in response to wetting of soil (Barnard *et al.* 2013). Because fungi have long hyphal extensions, they are less limited by the immobility of nutrients and the availability of water during dry periods compared to bacteria (Orchard and Cook 2008). Similarly, the level of resuscitation of *E. coli* after irrigation or rewetting depends on the specific soil amendment used (Sharma and Reynnells 2016). A comprehensive review of survival studies verifies that dry soils support slower declines of *E. coli* population compared to wet soils (Park *et al.* 2016). As a single factor, initial soil moisture content (measured gravimetrically) accounted for the greatest proportion of variability in *E. coli* survival durations in manure-amended soils, with survival duration greater in initially drier soils (Sharma *et al.* 2019). In the current study, soil moisture (water potential) was monitored throughout the duration of the study rather than solely at the outset, and different amendment (manure *vs* compost), soil types and climatic conditions may have led to different relationships between moisture content and *E. coli* survival between the current study and previous ones. Other investigators have shown that the role of moisture in the recovery of *E. coli* differs based on soil type, with high moisture levels leading to higher prevalence in soils from croplands and grassland, but not in soils from wooded or pastured lands (Dusek *et al.* 2018).

Soil moisture differentially affects availability of N, especially in sandy soils such as our field study site. NH_4-N is converted readily to NO_3-N in warm moist soils through nitrification (Stark and Firestone 1995). N as NO_3-N is more likely to leach than NH_4-N because of its ionic binding with clay and organic matter (Paul 2015). Leucine amino-peptidase activity per unit biomass did not vary among treatments and continued to decline for the duration of the experiment validating that N was in ample supply for all treatments.



OTU#	Phylum (-mycota)	Class (-mycetes)	Order (-ales)	Family (-aceae)	Genus
1	Zygo	Incertae sedis	Mortierell	Mortierell	*Mortierella camargensis*
36	Asco	Pezizo	Peziz	Ascobo	*Ascobolus* sp
49	Uncl	Uncl	Uncl	Uncl	Uncl
98	Basidio	Uncl	Uncl	Uncl	Uncl
166	Asco	Sordario	Microasc	Microasc	Uncl
195	Asco	Uncl	Uncl	Uncl	Uncl
209	Basidio	Agarico	Uncl	Uncl	Uncl
224	Asco	Uncl	Uncl	Uncl	uncl
334	Uncl	Uncl	Uncl	Uncl	Uncl
363	Asco	Sordario	Sordari	Lasiosphaeri	Uncl
431	Uncl	Uncl	Uncl	Uncl	Uncl
593	Uncl	Uncl	Uncl	Uncl	Uncl
673	Asco	Uncl	Uncl	Uncl	Uncl
916	Uncl	Uncl	Uncl	Uncl	Uncl

OTU#	Phylum (-mycota)	Class (-mycetes)	Order (-ales)	Family (-aceae)	Genus
1143	Basidio	Uncl	Uncl	Uncl	Uncl
1257	Uncl	Uncl	Uncl	Uncl	Uncl
1340	Asco	Uncl	Uncl	Uncl	Uncl
1397	Asco	Uncl	Uncl	Uncl	Uncl
1408	Basidio	Agarico	Agaric	Psathyrell	Uncl
1454	Asco	Sordario	Xylar	Incertae sedis	*Monographella nivalis*
1553	Basidio	Uncl	Uncl	Uncl	Uncl
1695	Uncl	Uncl	Uncl	Uncl	Uncl
1976	Uncl	Uncl	Uncl	Uncl	Uncl
2254	Uncl	Uncl	Uncl	Uncl	Uncl
2347	Uncl	Uncl	Uncl	Uncl	Uncl
10690	Asco	Pezizo	Peziz	Ascobol	*Ascobolus* sp
13380	Basidio	Uncl	Uncl	Uncl	Uncl
16464	Zygo	Incertae sedis	Mortierell	Mortierell	*Mortierella camargensis*
16950	Zygo	Incertae sedis	Mortierell	Mortierell	*Mortierella camargensis*
18771	Zygo	Incertae sedis	Mortierell	Mortierell	*Mortierella camargensis*

Figure 5 First principal response curve coefficient (PRC1) ITS sequences for (a) Lilac, (b) Wheelock. Curves represent deviation between a compost treatment from untreated soil (untreated: dash-dot; solid: poultry; dashed: dairy vermicompost; dotted: dairy windrow) as a function of days post-inoculation. The weights of the 15 best fit OTUS are shown on the right axis. Missing taxonomic information occurs if higher resolution was not available for the OTU (Unclassified (Uncl)). Monte Carlo permutation tests permuting whole time series were applied to compute statistical significance. $N = 144$ total samples. [Colour figure can be viewed at wileyonlinelibrary.com]

Separating the effect of nutrients and microbial community

Correlations among abiotic factors, community composition and *E. coli* survival reveal insights into the complex relationships that occur in actively managed agricultural soil environments.

In fresh poultry manure, 60–80% of N is in organic form as proteins and amino acids (Kelleher *et al.* 2002). Throughout the composting process, a large fraction of the organic N is converted to NH_3, NH_4-N and NO_3-N (DeLaune *et al.* 2004). High concentrations of mineralized N are desirable for compost, because NH_4-N, and NO_3-N represent plant-available forms. Others suggest that *E. coli* prefers NH_4-N over NO_3-N (Reitzer 2003). Total N was similar among compost types but the predominant form was NH_4-N in poultry and NO_3-N in dairy compost, similar to other studies (Jack *et al.* 2011). This study suggests that the NH_4-N of poultry amendment allows soil to support high levels of naturally occurring, nonpathogenic *E. coli*.

Filter sterilization of compost extracts eliminates direct competition, antagonism and predation from indigenous soil microbes, providing *E. coli* full access to existing nutrients. In the absence of compost microbes, bioavailabile nutrients were inadequate to sustain the *E. coli* population beyond 50 h. In contrast, *E. coli* populations were sustained in nonsterile soil extract for an additional 108 h. Perhaps the enzymatic activity of the indigenous oligotrophic soil organisms released nutrients for *E. coli* survival that were otherwise not available in the sterile soil extracts (Allison 2005). If nutrient levels are sufficiently high, *E. coli* survives in soil environments despite indigenous microbes. Including additional compost sources in the laboratory experiment, than used in the field experiment, suggest that results from the field experiment in

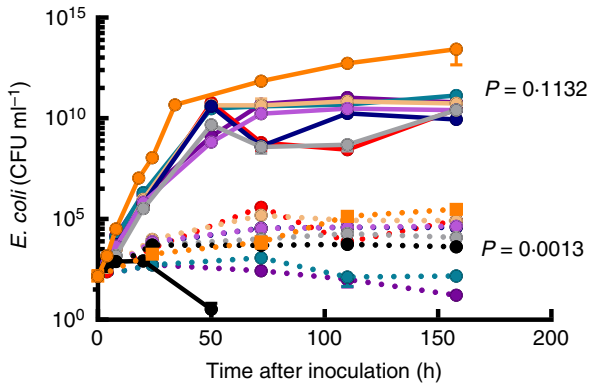


Figure 6 *Escherichia coli* growth in nonsterile (broken lines) and sterile (solid lines) compost and soil extracts incubated at 35°C for the duration of the 150-h experiment. Orange = PLM, grey = FPVI, light purple = MM, navy blue = FPVM, flesh = FPW, red = MI, turquoise = DVM, purple = DWM, black = soil composite from Wheelock and Lilac field sites (N = 3 replicates per sample type). Standard error bars are included, but are too small to see with the exception of the sterile poultry extract in the last time point. Sample abbreviations are defined in Table 1. [Colour figure can be viewed at wileyonlinelibrary.com]

this study may also apply to other commercially available compost products.

The results of this study on the ecology of *E. coli* survival in soils provide knowledge useful to US regulators and vegetable growers as they consider future recommendations for farming practices to reduce the risk for contamination of produce commodities with bacterial pathogens. This study suggests that dairy-based composts are less likely to support *E. coli* survival than poultry-based composts. The mechanism(s) relate(s) to inseparable effects of nutrient availability (NH_4-N) and microbial community.

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Conflict of Interest

No conflict of interest declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Detailed methods for (a) iodonitrotetrazolium chloride for measure of microbial respiration, (b) ecoenzyme assays for measure of ecosystem stoichiometry, (c) metagenomics for characterization of bacterial/Archaea and fungal community composition.

Table S1. Ecoenzymes tested and associated soil substrates, experimental substrates and positive controls.

Table S2. Linear regression between the growth rate constants (*k*-value) and the nutrient content of sterile compost extracts.

Figure S1. Soil respiration measured as iodonitrotetrazolium chloride reduction through time (μg INT gdw^{-1} h^{-1}).

Figure S2. Temporal dynamics of ecoenzyme activity (mmol h^{-1} gdw^{-1} $PLFA^{-1}$).