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Authors: Van Eerd, Laura L., Zhou, Yangxue, Turnbull, Amy L., Johnston-Monje, David, Lazarovits, George, et al.

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ARTICLE

Managing tomato vine decline with soil amendments and transplant treatments: fruit yield, quality, and plant-associated microbial communities[1]

Laura L. Van Eerd, Yangxue Zhou, Amy L. Turnbull, David Johnston-Monje, George Lazarovits, and Steven A. Loewen

Abstract: Tomato vine decline (TVD) disease complex results in fruit yield loss, but what soil management strategies might mitigate it? In commercial fields with a history of TVD, five approaches (soil organic amendments and transplant treatments) were evaluated for their impact on fruit yield, fruit quality, and microbial abundance or diversity at four site-years. One site-year had very high TVD pressure and high variability with no yield differences, thus efforts focused on the remaining site-years. Marketable yield was not different among treatments but numerically followed a trend similar to total yield. Amending soil with poultry manure delayed maturity (i.e., increased proportion of green fruit) and had the greatest total yield increases of 17.2%, congruent with decreased abundance of root pathogens (*Verticillium dahliae*, *Rhizopicnis vagum*). Microbial DNA fingerprinting data of rhizospheres, roots and (or) stems suggested treatments did not significantly shift the total diversity fungal nor bacterial populations, but the aforementioned pathogen loads were reduced with the application of organic amendments relative to the untreated control. While drenching tomato transplants with pseudomonad culture increased their presence in roots, pathogen load was not reduced relative to the untreated control. Overall, these results show that soil organic amendments were able to improve tomato total yield in two of four site-years without reducing fruit quality (i.e., soluble solids, pH, colour), perhaps, in part, due to their ability to suppress specific root pathogens in commercial fields.

Key words: plant health, disease complex, plant pathogen, rhizosphere, compost, manure, *Pseudomonas*, terminal restriction fragment length polymorphism (TRFLP), DNA fingerprinting, soluble solids, transplant conditioning.

Résumé : Le déclin de la tomate (DT) est une maladie complexe qui entraîne une baisse de rendement, mais par quelles méthodes de gestion du sol pourrait-on la combattre? Les auteurs ont évalué cinq approches (amendements organiques et traitement des plants repiqués) pendant quatre années-sites, dans des champs commerciaux périodiquement affectés par le problème, en vue d'en établir l'impact sur le rendement, sur la qualité du fruit ainsi que sur l'abondance et la diversité de la microflore tellurique. Une année-site s'est caractérisée par un DT très élevé et une grande variabilité, sans modification du rendement. Les auteurs l'ont donc laissée de côté pour se concentrer sur les autres années-sites. Les traitements n'ont eu aucune incidence sur le rendement en fruits commercialisables, dont le nombre suit une tendance similaire à celle du rendement global. Amender le sol avec du fumier de poulet retarde la maturation (à savoir, proportion accrue de fruits verts), mais engendre la plus forte hausse du rendement global (17,2 %), concomitante avec la plus faible abondance d'agents pathogènes s'attaquant aux racines (*Verticillium dahliae*, *Rhizopicnis vagum*). Les données de la rhizosphère, des racines ou des tiges employées pour profiler l'ADN microbien indiquent que le traitement ne modifie pas de

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L.L. Van Eerd and Y. Zhou. School of Environmental Sciences, University of Guelph, Ridgetown Campus, Ridgetown, ON N0P 2C0, Canada.

A.L. Turnbull,[†] D. Johnston-Monje,[‡] and G. Lazarovits. A&L Biologicals, Agroecology Research Services Centre, London, ON N5V 3P5, Canada.

S.A. Loewen. University of Guelph, Ridgetown Campus, Ridgetown, ON N0P 2C0, Canada.

Corresponding author: Laura L. Van Eerd (email: lvaneerd@uoguelph.ca).

[†]Present address: School of Applied Science and Technology, Fanshawe College, London, ON N5Y 5R6, Canada.

[‡]Present address: Max Planck Tandem Group in Plant Microbial Ecology, Universidad del Valle, Cali, Valle del Cauca, Colombia.

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manière significative la diversité des cryptogames et des bactéries. Cependant, l'application d'un amendement organique réduit la population des agents pathogènes, comparativement à celle relevée dans la parcelle témoin. Si l'arrosage des plants repiqués avec une culture de pseudomonadales accroît la présence de ces bactéries au niveau des racines, on n'assiste pas à une diminution de la concentration de pathogènes, comparativement à la parcelle témoin non traitée. Dans l'ensemble, ces résultats indiquent qu'en bonifiant le sol avec un amendement organique, on a amélioré le rendement global de la tomate deux années-sites sur quatre sans que la qualité du fruit en souffre (à savoir, concentration de solides solubles, pH, couleur), peut-être parce qu'un tel amendement détruit les agents pathogènes spécifiques des racines dans les champs commerciaux. [Traduit par la Rédaction]

Mots-clés : santé des plantes, complexe pathologique, agent pathogène des plantes, rhizosphère, compost, fumier, *Pseudomonas*, TRFLP, profilage de l'ADN, solides solubles, conditionnement au repiquage.

Introduction

In 2009, processing tomato (*Solanum lycopersicum* L.) growers in southwestern Ontario noticed, mid-season, that considerable areas of some fields had poor vegetative growth and premature defoliation. Symptoms included leaf chlorosis and necrosis, stunting, premature plant senescence, root browning and rotting (Supplementary Fig. S1[2]; Johnston-Monje et al. 2017), which resulted in 30% or greater yield losses (LeBoeuf et al. 2010; Trueman et al. 2010; Loewen 2011). Although the extent of the acreage impacted was not estimated, yield losses were severe in 2009 and widespread 2010 (LeBoeuf et al. 2010). The syndrome was termed tomato vine decline (TVD) and had been thought to be associated with a root disease complex that included the tomato plant pathogens, *Pyrenochaeta terrestris, Pyrenochaeta lycopersici, Rhizopycnis vagum, Verticillium dahliae*, and *Fusarium* spp. or *Colletotrichum coccodes* (Trueman et al. 2010), although the obligate biotrophic pathogen *Olpidium virulentus* now appears to play a role as well (Johnston-Monje et al. 2017). Similar symptoms have been observed in California in processing tomato fields (Lieberman 2006; Davis and Miyao 2009). While the field symptoms are similar, it is unknown if premature vine decline in California (Maharaj et al. 2018) and Ontario TVD are the same. Moreover, similar symptoms are observed with aforementioned group of fungal pathogens in melon and potato production (Rowe and Powelson 2002; Cohen et al. 2012). In general, management of this disease complex was expected to be challenging because conditions that suppress one fungal pathogen might amplify others.

Many studies on soil-borne diseases of tomato found soil fumigation, solarization and biosolarization to be suppressive to some extent (Campbell and Schweers 1981; Campbell et al. 1982; Ioannou 2000; Vitale et al. 2011; Diaz-Hernandez et al. 2017). However, results by Trueman et al., (2010) demonstrated limited TVD suppression with soil fumigation (metham) even at the highest rate tested in both commercial fields and micro-plots, as well as in greenhouse screening tests with chemical or biological controls. Shennan et al. (2018) utilized anaerobic soil disinfestation as an alternative to fumigation to suppress *V. dahliae* in strawberry production, but the utility of this approach in processing tomato production is still unknown. To sustain the trend of increasing processing tomato yields in Ontario (42.8 $Mg{\cdot}ha^{-1}$ in 1992 to 86.2 $Mg{\cdot}ha^{-1}$ in 2012), alternative approaches, such as organic amendment or transplant treatments are needed to mitigate the TVD disease complex.

Organic amendments may function not only as a source of nutrients to support crop growth, but also positively enhance soil structure, aggregate stability, and soil hydrology. Compost and manure amendments are routinely applied to agricultural soils or greenhouse rooting media to improve nutrient supply (Maharaj et al. 2018), plant growth (Jack et al. 2011), and health (Cotxarrera et al. 2002). Amendments, which contain organic matter and large populations of microbes, can influence the microbial communities associated with plants (Green et al. 2006; Kokalis-Burelle et al. 2006; Perez-Piqueres et al. 2006; Ros et al. 2006; Saison et al. 2006; Friberg et al. 2009). For instance, *Brassicaceae* seed meal soil amendments have suppressed soilborne pathogens, nematodes, and weeds (Shennan et al. 2018) largely due to high levels of glucosinolates. In addition, incorporation of *Brassicaceae* plant residues can influence the composition of bacterial/fungal communities and therefore, such treatments may be able to reduce pathogen potential of plant-associated microbiomes (Mazzola et al. 2001; Friberg et al. 2009). Similarly, soil organic amendments can influence the composition and activity of soil microbial communities by physically and chemically altering the soil, resulting in direct and indirect influences on plant primary productivity (Ramirez et al. 2012) and plant disease suppression (Hoitink and Fahy 1986; Litterrick et al. 2004 Hashemimajd et al. 2004; Saison et al. 2006; Postma and Nijhuis 2019). Experiments are needed to evaluate the potential of soil organic amendments to mitigate disease complexes, such as TVD, in commercial tomato fields.

While organic amendments can broadly alter soil physical and chemical properties as well as microbial communities, an alternative approach may be to

[2]Supplementary data are available with the article at https://doi.org/10.1139/CJPS-2021-0098.

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manipulate rhizosphere communities directly. It is hypothesized that by inoculating plant roots with disease-suppressive microorganisms before transplanting into the field, these biocontrol microbes will be able to colonize the rhizosphere or endosphere, preventing particular pathogen colonization and mitigate plant disease (Mitter et al. 2021; Tosi et al. 2021). Application of microbial cultures such as *Trichoderma* spp. or *Bacillus* spp. to roots before transplanting has resulted in protection against root diseases (Larkin and Fravel 1998; Porras et al. 2007), however their efficacy in commercial field tomato production is still limited, especially when the disease agents have not yet been identified. In California, several commercial biologicals, such as those containing different microbial species (*Bacillus subtilis* QST 713, SS1, *Streptomyces lydicus* WYEC 108, *Gliocladium virens* strain GL-21), blends of beneficials(*Trichoderma asperellum* strain ICC 012 and *T. gamsii* strain ICC 080) or microbial extracts (*Reynoutria sachalinensis*) have not been able to suppress tomato premature vine decline in commercial fields (Maharaj et al. 2018). Additional experiments using novel biocontrol agents such as soil pseudomonads may yet show utility in TVD management. This culturable genus is known to suppress plant pathogens of tomato including *Fusarium oxysporum* (De Corato, et al. 2020) and *Pythium aphanidermatum* (Postma and Nijhuis 2019). Favourable biocontrol with pseudomonads in the aforementioned greenhouse studies, suggests field testing in TVD environments as a next step.

Given the limited control options available and the devastating effects of TVD on plant productivity, management approaches are needed. Other than Maharaj et al. (2018) no study has evaluated the combined effect of soil organic amendments on tomato rhizosphere and endosphere microbes and their contributions to TVD symptoms under various environmental conditions in commercial field conditions. We conducted experiments in commercial fields that were expected to have high TVD prevalence to evaluate the influence of soil amendments (spent mushroom compost, fresh poultry manure, mustard seed meal, and thermophilic compost) and transplant treatments (pseudomonads and thermophilic compost tea [combined with field application of liquid and solid thermophilic compost]) on tomato fruit production, fruit quality and plant/soil rhizosphere microbiota. The objectives of this study were to evaluate the impact of various soil amendments and transplant treatments on (*i*) processing tomato yield and quality in commercial processing tomato fields with vine-decline disease complex, and (*ii*) tomato stem, root and rhizosphere microbial populations as well as the presence of suspected pathogenic fungi using molecular fingerprints. We hypothesize that soil amendments that increase tomato yield under TVD pressure are able to do so by modifying the species of bacteria and fungi in the rhizosphere, thereby minimizing pathogen colonization and damage of tomato roots.

Materials and Methods

Field experiments

To be representative of the production region, experiments were conducted in commercial fields in Chatham-Kent near Lighthouse Cove in 2011 and 2012, and in Essex County near Leamington in 2012 and 2013. Soil types in the Essex County production area are sandy loam to loamy sand, whereas in Chatham-Kent tomato production is on similar to more loamy soil types. Soil compositional properties are listed in Table 1. Grower cooperators in each region were selected based on a history of TVD in their other tomato fields in 2009 to 2011. The fields selected for this experiment were approximately 40 km away and also contained an experiment evaluating tomato cultivar and grafted root-stock tolerance to TVD (Johnston-Monje et al. 2017).

The field experiment was a randomized complete block design with four blocks consisting of four to five treatment approaches and an untreated control that had no soil amendment nor transplant treatments (Table 2). A systems-based approach was employed as treatments varied greatly in terms of application method (applied to seedling or transplant roots, soil applied with incorporation, foliar drench), timing (early seedling, 2-wks before transplanting, immediately before transplanting, in-season), number (1 to 3 applications) (Table 2). Solid organic amendments were greater than 95% dry matter (Supplementary Table S1[2]). Using Ontario-based prediction equations (OMAFRA 2021), for each amendment the quantity of nitrogen available during the growing season was calculated using N concentration and percent dry matter content (Supplementary Table S1[2]) as well as application method and timing of incorporation. Estimated nitrogen available from the thermophilic compost, was <5 kg N·ha^{-1}, 12 kg N·ha^{-1} for mustard seed meal, 26 kg N·ha^{-1} for spent mushroom compost and 126 kg N·ha^{-1} for poultry manure, due primarily to the high ammonium-N concentration (1694 mg·kg^{-1}).

Two weeks prior to targeted transplanting, soil amendment treatments were established by uniformly hand-applying amendments followed by incorporation to 10 cm depth with two passes of a walk-behind rototiller within 1 hr of application. As per product label instructions, the mustard seed meal was incorporated and within 30 min irrigated with 25 mm using a watering wand with rose nozzle (#059-7176-2 Canadian Tire Corporation LTD, Blenheim, ON) from a water tank of municipal groundwater. Likewise, manufacturer's recommendations for the thermophilic compost treatment also included application of thermophilic compost tea diluted 1 to 500 as a root drench at 1 L per transplant tray (54.61 × 27.94 × 2.54 cm, 288 plug per tray, A.M.A. Horticulture Inc., Kingsville, ON) when seedlings were

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Table 1. Average (SE) surface (15 cm) soil properties[a] collected in the untreated control (n = 4) at each site-year.

Site-year	OM	pH	CEC	P	K	Mg	Ca	S	Na	Zn	Mn	Fe	Cu	B	Al
Lighthouse Cove 2011	3.25 (0.112)	7.27 (0.083)	15.3 (0.47)	67.5 (2.06)	251 (7.49)	357 (10.3)	2250 (618)	24.0 (1.87)	14.5 (2.96)	13.0 (0.91)	18.5 (1.12)	100 (1.9)	4.97 (0.238)	1.70 (0.187)	698 (31.3)
Lighthouse Cove 2012	4.02 (0.536)	7.05 (0.050)	17.6 (1.30)	67.2 (3.42)	316 (43.7)	345 (7.1)	2370 (152.0)	26.5 (5.12)	14.0 (4.30)	14.5 (2.47)	10.0 (0.71)	128 (8.7)	3.90 (0.346)	1.92 (0.192)	438 (25.6)
Leamington 2012	2.90 (0.187)	6.75 (0.250)	10.3 (1.71)	117 (14.9)	168 (21.8)	157 (26.6)	1460 (314.5)	25.8 (4.97)	9.25 (1.920)	16.5 (3.48)	44.7 (19.15)	123 (14.0)	3.25 (0.287)	0.95 (0.269)	1310 (205.4)
Leamington 2013	3.88 (0.708)	6.20 (0.600)	11.9 (3.00)	93.5 (21.66)	244 (16.1)	211 (72.2)	1660 (518.8)	52.0 (18.10)	17.8 (3.83)	14.8 (2.40)	27.8 (17.63)	116 (15.0)	3.45 (0.335)	1.15 (0.610)	1190 (546.6)

[a]OM, organic matter measured by loss of ignition; pH was 1:1 soil:water by volume; CEC, cation exchange capacity was measured by ammonium acetate extraction and pH; P was measured by sodium bicarbonate (Olsen method) and all other elements were extracted with ammonium acetate.

at the three true-leaf stage (i.e., in the greenhouse) and a foliar application 1 mo after transplanting at 56 L·ha^{-1}. The foliar application was applied by hand with a watering can along each row of the entire plot. A fresh poultry manure treatment, applied and incorporated as described above, was included in 2012 and 2013. In addition to the soil amendment treatments, a transplant root drench of 1 L of microbial *Pseudomonas* culture of 10^6 cells·mL^{-1} (A&L Biologicals Inc., London, ON) was applied to transplants 3 d prior to transplanting. Briefly, a broth culture was grown for 48 hours in nutrient broth, then diluted to 0.01 OD (600 nm) with sterile distilled water. One L of culture, per transplant tray of tomatoes, was applied to root media.

Tomato seedlings were commercially grown locally and transplanted on 13 May 2011 and 29 May 2012 at Lighthouse Cove and on 13 May 2012 and 29 May 2013 at Leamington in twin rows 45 cm apart and centered 1.57 m apart with plants spaced 35 cm apart within the row for a population of 29 600 plants·ha^{-1}. Plots were 10 m long by 4.7 m (three twin rows) wide. Growers followed their typical commercial tomato production practices including fertility, irrigation, pest control, as well as nutrients and insecticide in transplant water (OMAFRA 2008). A composite of more than six, 2.5 cm diameter cores per plot of surface (15 cm depth) soil samples were collected, hand homogenized, and sent to a commercial lab (A&L Laboratories, London, ON) to determine select soil properties (Table 1) according to method described by Carter and Gregorich (2008).

To reveal any treatment effects on fruit maturity, a ripening agent (i.e., ethephon) was not applied to the trial area, although this is an industry standard practice. Tomatoes were hand-harvested between 28 Aug. and 22 Sept. 2011–2013 depending on location. All plots were harvested when experimental area was estimated to be over 80% red ripe fruit or shortly after growers machine harvested the rest of the commercial field. The middle 2 m of the center set of twin rows of each plot was harvested, and fruit was graded as marketable according to industry standards (OPVG 2010). Briefly, fruit was marketable if ≥50% of the surface area was at least blush colour and free of defects that would render them culls. Yield of individual categories of unmarketable including culls (rotten or defected) and immature (green) fruits and marketable (red, orange, breakers) were weighed separately. Harvest area and fresh fruit weight were used to calculate fresh yield expressed as Mg·ha^{-1}. To assess impact of treatments on fruit maturity (i.e., immature green fruit as per Thomas et al. (2001), data for culls, immature green, and red-ripe fruit were expressed as a percent of total fruit on a per weight basis.

To assess processing tomato fruit quality, a subsample of approximately 25 randomly selected, red ripe fruit per plot were collected at one site in each year (i.e., fruit quality was not assessed at Leamington in 2012 due to processing capacity constraints). Fruits were washed,

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Table 2. Soil amendment and transplant seedling treatments evaluated in commercial processing tomato field experiments in 2011 to 2013.

Treatment	Phase	Placement	Timing[a]	Rate	Source
Untreated control	—	—	—	—	—
Spent mushroom compost	Solid	Broadcast incorporated	2 wk PPI	22.4 Mg·ha^{-1}	Rol-Land Farms, Blenheim, ON
Fresh poultry manure[b]	Solid	Broadcast incorporated	2 wk PPI	11.2 Mg·ha^{-1}	McKinlay Farms, Thamesville, ON
Mustard seed meal	Solid	Broadcast incorporated and 25 mm simulated sprinkler irrigation	2 wk PPI	4.48 Mg·ha^{-1}	MustGROW™, MPT Mustard Products & Technologies Inc., Saskatoon, SK
Thermophilic compost and tea	(1) Liquid	(1) Seedling root drench	(1) 3 leaf stage PRE	(1) 1 L·tray^{-1}	TerraBioGen Technologies Inc., North Vancouver, BC
	(2) Solid	(2) Broadcast incorporated	(2) 2 wk PPI	(2) 0.45 Mg·ha^{-1}	
	(3) Liquid	(3) Foliar application	(3) 4 wk POST	(3) 56 L·ha^{-1}	
Microbial *Pseudomonad*	Liquid	Transplant root drench	3 d PRE	1 L·tray^{-1} (288 plug tray)	A&L Biologicals, London, ON

[a]PPI, Preplant incorporated treatment broadcast applied at least 2 wk before transplanting and incorporated to 10 cm depth with a rototiller within 1 hr of application; PRE, pre-transplanting; leaf stage; POST, applied after transplanting.
[b]Poultry manure treatment was not included in 2011.

dried and comminuted in a Waring CB6 commercial blender (Waring Commercial, Torrington, CT) on medium speed, under vacuum (88 kPa), for 40 seconds. The tomato pulp was passed through a 27-mesh screen to remove seed particles and peel. Colour was measured using a spectrophotometer (E-5M. Agtron Inc. Reno, NV) calibrated at 48 (Gould 1992; Garcia and Barrett 2006). Screened-pulp pH (Gould 1992; Garcia and Barrett 2006) was measured using a calibrated Orion pH meter (Thermo Fisher Scientific, Nepean, ON). Natural tomato soluble solids (°Brix) were measured on filtered (Fisherbrand P8 coarse porosity filter paper, Fisher Scientific, Pittsburgh, PA) pulp serum using a Palette PR101 temperature-compensated, digital refractometer (Atago USA, Inc., Bellevue, WA).

Microbial community analyses

Microbial community changes were assessed to determine if they were associated with tomato yield. Procedures to characterize microbial communities from rhizosphere, roots and stems were as described in a parallel experiment on root-grafted tomatoes conducted in the same commercial fields (less than 25 m away) (Johnston-Monje et al. 2017). Beginning in 2012, when the experiment was harvested to quantify fruit yield and quality, five randomly selected plants from each treatment were hand-harvested, fruit weighed, roots excavated (20 cm around stem and 20 cm depth) and plants processed to extract stem, root and rhizosphere DNA to compare microbial diversity to fruit yield as described by Johnston-Monje et al. (2017). Briefly, roots removed from the soil were immediately rinsed with tap water and a subsample of soil adhering to roots and roots were collected in a 50 mL Falcon tube. Further sample processing occurred in the lab. A representative sample of fine roots were air dried in a biosafety cabinet to remove excess moisture. Individually roots and stem were cut using a scalpel until a powder was formed. The cells in the sample were lysed by bead beating for 1 min in a Fast Prep (MP Bio) at 6.5 m·s^{-1}, resting 1 min, then beating for an additional minute. DNA was extracted from stems, roots and rhizospheres using Norgen DNA soil isolation kit and 30 ng was used per PCR along with 20 pmol primer. Primer and probe sequences for specific pathogens were proprietary (A&L Biologicals Inc., London, ON). Polymerase chain reaction (PCR) was performed using a Bio-Rad CFX96 Real-Time PCR Systems thermocycler using the following parameters: 45 cycles of 94 °C for 15 s, 60 °C for 30 s, and 72 °C for 15 s. Fluorescence emission was measured at 60 °C during the annealing and extension phase. The 16S rDNA gene was amplified by PCR using primers 63F and 1389R (labelled with 6-FAM dye), while fungal ITS sequences were amplified using primers ITS1F (CTTGGTCATTTAGAGGAAGTAA — labelled with 6-FAM dye) and ITS4 (TCCTCCGCTTATTGATATGC — labelled with Max-550 dye). The PCR conditions used for

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the amplification of bacterial 16S rRNA fragment were 94 °C for 2 min followed by 30 cycles at 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 2 min and a final extension at 72 °C for 10 min. The PCR conditions used for the amplification of the fungal ITS fragment were 96 °C for 3 min, 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min, followed by 72 °C for 7 min. PCR products were verified for successful amplification by visual inspection under ultraviolet (UV) light on an agarose gel stained with GelRed, then purified using a DNA purification kit (DNA Clean & Concentrator, Zymo Research). Restriction digestion of both the bacterial 16S and fungal ITS amplicons was accomplished using 100 ng of purified PCR product, 20 U HhaI (Promega) for 6 h at 37 °C, and heat inactivation. To visualize different sizes of fluorescent fragments, mixtures were sent to Robarts Research at Western University (London, ON) where they were run on a 3730 DNA Analyzer alongside GeneScan 1200 LIZ Size Standards (Applied Biosystems, USA). Terminal restriction fragment length polymorphism (TRFLP) results were analyzed using Peak Scanner Software (ThermoFisher Scientific) on the amplified fragment length polymorphism (AFLP) setting, identifying peaks as usable data (rather than low quality or noise) if they were larger than 40 nucleotides and smaller than 1200 nucleotides in size, and between 50 to 30 000 fluorescent units in intensity. Fragment sizes (6-FAM and Max550) and peak heights were exported to Microsoft Excel. For annotation of fungal TRFLP profiles, ITS sequences KF493901-KF494184 from a related survey of fungi involved in TVD (Johnston-Monje et al. 2017) were used to predict fragment size after digestion with HhaI.

Statistical analysis

All tomato parameter data were subjected to analysis of variance using a mixed-effects model (SAS v9.3) with treatment and site-year as fixed effects (Type III test). Block and block by site-year were treated as random effects. Since the poultry manure treatment was only implemented in 2012, data from 2011, were analyzed separately as a one-way analysis of variance (ANOVA) with treatment as fixed effect and replicate as random effect. Residual plots and the Shapiro–Wilk normality test were used to confirm analysis of variance assumptions. Model fit was tested using Akaike information criterion; for all data convergence criteria were satisfied. Data, where necessary to meet assumptions of ANOVA (fruit quality data only), were subjected to a natural logarithm transformation and results presented in the original scale. Data were reported as least square means with the type I error (α) set at 0.05 and statistical differences among treatments identified with a protected Tukey–Kramer mean separation.

Terminal restriction fragment length polymorphism profiles from five plants per treatment were pooled and converted into binary, presence/absence data; a peak was counted in a pooled treatment profile if it was present in ≥75% of replicates and assigned a value of 1. A value of 0 was assigned if the peak was present in <75% of replicates. This resulted in a matrix of binary data of fragment sizes for samples in each experiment. Principal components analysis (PCA) was performed using binary data for all samples in an experiment with XLSTAT using the covariance matrix. Jaccard coefficient calculations were performed using pooled binary data for each treatment and calculated by dividing the total number of shared peaks in two treatments by the sum of unique peaks in the two samples. The number of unique peaks was calculated as the difference of the total peaks in both samples and the total shared peaks.

Precipitation and temperature

Weather differences among years were mostly due to variations in precipitation (Supplementary Table S2[2]). The 2011 and 2013 growing seasons (May–September) received abundant total rainfall (449 mm and 437 mm, respectively) compared with the 30-yr average of 423 mm (Environment Canada weather station: Ridgetown RCS), whereas the growing season in 2012 was relatively drier with only 362 mm of rainfall. Variations in temperature among years were less extreme than precipitation. The 2011 growing season average temperatures of 18.7 °C was cooler and 2012 warmer (20.6 °C) than 2013 (19.5 °C) and the 30-yr mean of 19.3 °C. Thus, relative to historic average, the growing seasons were slightly wetter and cooler in 2011, drier and slightly warmer in 2012, and near normal in 2013.

Results

Tomato yield

In 2011 at Lighthouse Cove, treatments had no impact on any tomato yield parameters ($P \geq 0.3147$; Supplementary Table S3[2]), likely due to high incidence and field variability of TVD symptoms observed in the experiment and the entire commercial field. Total yield (average across all treatments of 72.2 Mg·ha^{-1}), particularly marketable yield (ave. 42.3 Mg·ha^{-1}), in 2011 (Supplementary Table S3[2]) were considerably lower than other locations (ave. 131 and 112 Mg·ha^{-1}, respectively; Table 3). Although the 2011 Lighthouse Cove experiment was harvested a few days after the commercial field was machine-harvested, the relative proportion of unripe, green fruit was quite high (average across treatments was 39.2% (Fig. 1) and equivalent to red-ripe fruit (ave. 39.8%; Supplementary Table S3[2]). At an average of 6.48%, the percentage of rotten fruit at Lighthouse Cove in 2011 (Supplementary Table S3[2]) was less than the proportions observed at Leamington in 2012 but greater than the other two site-years (Table 3).

Two-way ANOVA of the three other site-years (i.e., Lighthouse Cove and Leamington both in 2012 and Leamington in 2013) revealed that there were no site-year by treatment interactions for all fruit yield

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Table 3. Impact of soil amendments and transplant treatments on processing tomato fruit yield and the percent red-ripe and unmarketable fruits from commercial fields (3 site-years[a]).

	Total	Marketable	Red-ripe	Unmarketable (%)	
Treatment	Mg·ha^{-1}		%	Green	Rotten
Control	122b	109	70.7a	8.35b	3.52
Spent mushroom compost	133ab	112	64.1ab	11.7b	9.09
Poultry manure	143a	111	56.9b	19.9a	5.87
Mustard seed meal	137ab	119	67.2a	11.1b	4.53
Thermophilic compost	129ab	111	64.7a	9.22b	11.9
Microbial *Pseudomonad*	123b	109	70.8a	8.06b	4.24
Standard error	5.83	6.63	1.73	0.897	4.076
Site-year					
Lighthouse Cove 2012	144a	117	60.6b	18.1a	2.14
Leamington 2012	138a	115	54.7c	11.4b	13.8
Leamington 2013	112b	103	81.9a	4.17c	3.60
Standard error	5.29	6.04	0.79	0.821	3.810
Effects			*P*-value		
Treatment	0.0024	0.749	<0.0001	<0.0001	0.5605
Site-year	0.0137	0.1289	<0.0001	0.0002	0.1289
Interaction	0.9769	0.7027	0.3099	0.0042	0.5034

Note:[a–c]Within each column, treatment means with a different letter were significantly different at $P = 0.05$ based on protected Tukey–Kramer means separation test; if not significant, then no letters are given.

[a]Site-year Lighthouse Cove 2011 was not included in analysis as only 5 treatments were included.

parameters ($P \geq 0.1864$), except the percent of immature, green fruit ($P = 0.042$); hence, fixed effects were presented (Table 3). Averaged across these commercial fields, marketable tomato yield was not impacted by treatments but followed a similar trend as total yield. Tomato total yield was greater with poultry manure than treatments without amendments (an increase of 17.2% and 16.2% compared with the control and microbial treatment, respectively) (Table 3). Total yield with all other amendment treatments (i.e., spent mushroom compost, thermophilic compost and mustard meal) was not different than any other treatment. The significant total but not marketable yield increase with poultry manure was attributed to delayed fruit maturity (i.e., greater proportion of green fruit ($P < 0.0001$) compared with other treatments (Table 3) but due to differences in proportion of rotten or defect culls ($P = 0.5605$; Table 3).

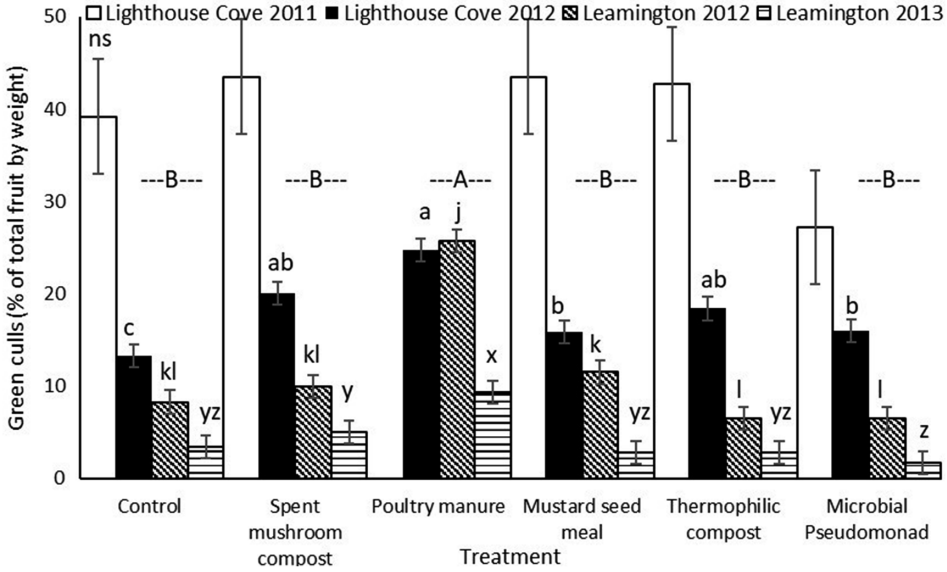
The treatment by site-year interaction observed with the percent green fruit was attributed to a greater magnitude of treatment differences between poultry manure and all other treatments, which were not different from each other (Fig. 1). For instance, in 2012 at Lighthouse Cove, poultry manure had 24.7% immature culls while the next highest treatment had 20.1% (a difference of 4.6). A similar difference was observed at Leamington in 2013 (4.2), while in 2012 at Leamington a difference of 14.2 was observed (green fruit was 25.7% vs. 11.5% with poultry manure and the next highest treatment, respectively). In all 3-site-years, plots without organic amendments applied (i.e., control and microbial) had consistently the least or among the lowest proportion of green fruit (Fig 1). With a ripening agent one would expect immature fruit to mature and be marketable; thus, total yield would be more representative of grower yield. When considering marketable plus immature fruit (i.e., all but rotten, defected fruit), poultry manure increased fruit by 25.4 Mg·ha^{-1} ($P = 0.0237$) at Lighthouse Cove in 2012, and 12.6 Mg·ha^{-1} ($P = 0.2503$) and 24.1 Mg·ha^{-1} ($P = 0.0313$) in Leamington in 2012 and 2013, respectively, compared with the untreated control (Supplementary Table S4[2]). Moreover, in two out of four site-years (both in 2012), all amendments had greater total yields by 9.8% to 19.6% than the unamended control (and significantly greater than the microbial treatment); but in 2011 and 2013 the trend was not significant (Supplementary Table S4[2]).

Tomato fruit quality

As expected, there were significant differences ($P < 0.0001$) among years in fruit quality (Agtron colour, soluble solids, pH) (Supplementary Table S5[2]). Soluble solids were greater in 2013 (4.55) than 2011 and 2012 (4.22 and 4.35, respectively), which were equivalent.

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Fig. 1. Impact of soil amendments and transplant treatments on the percentage of total fruits that were green (immature), at four commercial fields in 2011 to 2013. Poultry manure treatment was not included in 2011 and therefore this site-year was analyzed separately. Treatment means with different lower-case letters were significantly different at $P < 0.05$ based on protected Tukey–Kramer means separation test. Upper case letters indicate treatment differences across the three site-years (i.e., excludes 2011). ns, not significant.



Likewise, pH was greater in 2013 (4.17) than the other 2 yr (4.13 and 4.12). Agtron colour values were similar in 2012 and 2013 (24.6 and 23.1, respectively) and greater than 2011 (19.4). There was no treatment effect nor treatment by site-year interaction ($P \geq 0.0523$) as none of the treatments impacted any of the measured fruit quality parameters in all site-years (Supplementary Table S5[2]). Across all treatments, mean value of Agtron colour was 22.5, natural tomato soluble solids was 4.4 °Brix and pH was 4.14 and within Ontario processing industry standards (OPVG 2010).

Rhizosphere and root microbial communities

To further understand factors that might influence tomato production, we performed principal component analysis on TRFLP data from root and rhizosphere microbial communities of tomato plants from field experiments. At Leamington and Lighthouse Cove, PCA ordination of root fungal community diversity was able to account for 60.6% and 58.7%, respectively of variation in the data (Fig. 2). Similar variation was accounted for in fungal and bacterial rhizosphere community diversity 66.09% and 66.82%, respectively (Supplementary Fig. S2[2]). Using PCA to analyze TRFLP data and drawing 95% confidence ellipses around groups, we observed no significant nor consistent treatment-induced shifts (i.e., separation of non-overlapping ellipses) in community diversity of bacterial and fungal populations in tomato stems, roots, and rhizospheres (Fig. 3). At both locations, tomato stem, root, and rhizosphere microbial communities of plants grown in amended soil or from inoculated transplants showed considerable overlap with the untreated controls, suggesting no treatment acted broadly and strongly enough on soil microbial diversity to cause significant shifts in the community structure.

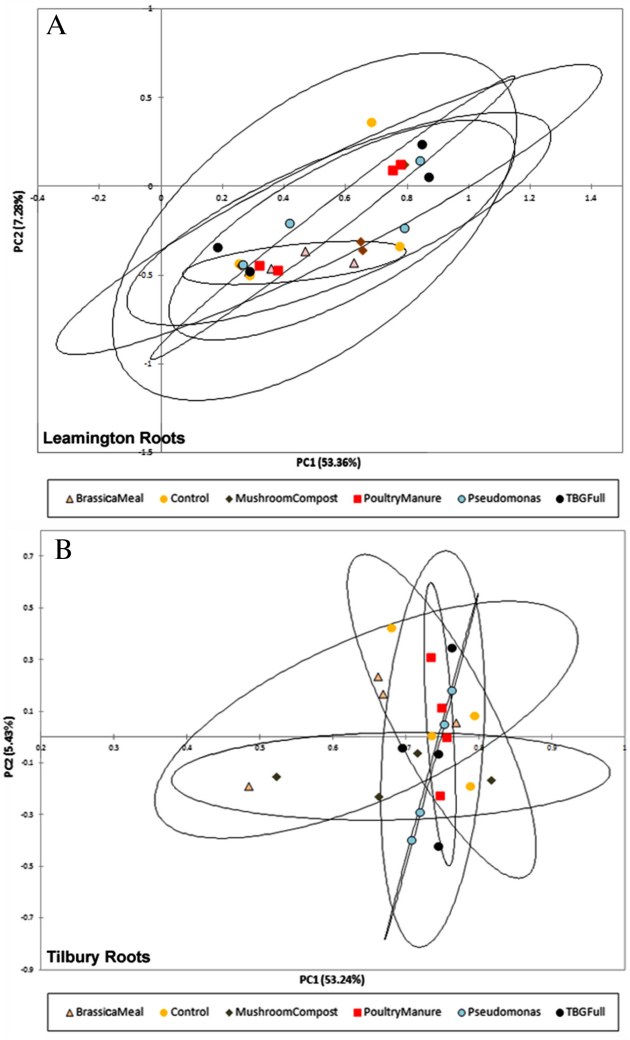
Microbial diversity and population analyses

Overall, poultry manure treatment in 2012 at Lighthouse Cove resulted in the lowest detected amount of DNA for three out of four tested fungal pathogens (*P. lycopersisi, P. terrestris, R. vagum* but not *V. dahliae*) in root tissues (Fig. 4A); where the relatively lower pathogen levels corresponded with the greatest tomato yield. In contrast, in 2012 at Leamington, thermophilic compost resulted in the greatest tomato yield, and also the lowest plant pathogen load for *P. lycopersisi, P. terrestris,* and *V. dahliae* (Fig. 4B). Interestingly, at both site-years the greatest pathogen loads (tested by real time PCR) were observed in plants that received the pseudomonad culture root drench before transplanting, perhaps explaining why there was a lack of yield effect. The lack of significant differences among microbial communities as detected by TRFLP (Fig. 3), the reduction of fungal pathogen loads among treatments as detected by real time PCR (Fig. 4), and the corresponding increases in levels of plant productivity, suggest that soil amendments may be functioning divergently amongst microbial species.

In 2013 at Leamington, the rhizosphere fungal profiles showed similarity to root profiles, whereas stems were vastly different (Supplementary Fig. S4[2]). Microbial profiles from roots were of special interest to us, as we speculated root pathogens may be contributing to TVD symptoms. To annotate fungal TRFLP profiles, we

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Fig. 2. Impact of soil organic amendments and transplant treatments on root fungal communities from tomato plants grown in commercial fields at Leamington (A) and Lighthouse Cove (B) in 2012 as shown by principal component analysis (PCA) of DNA fingerprinting data. A treatment may be considered to significantly impact bacterial or fungal populations if grouping circles (95% confidence intervals) do not overlap the untreated control group (yellow dots). (TBG Full: thermophilic compost)



matched fragments to peaks within a few basepairs based on the sequence data available, which included *Fusarium solani* (93 bp), *Verticillium dahliae* (103 bp), *Pyrenochaeta lycopersici* (105 bp), *Rhizopicnis vagum* (118 or 119 bp), *Plectosphaerella cucumerina* (140 bp), *Fusarium oxysporum* or *Fusarium chlamydosporum* (253 bp), *Colletotrichum coccodes* (258 or 269 bp), *Bionectria ochroleuca* (269 bp), *Gibellulopsis nigrescens* (274 bp), or *Olpidium virulentus* (357 bp). Root profiles appeared to be dominated by *V. dahliae* (100–103 bp), *P. cucumerina* (136–138 bp) and likely either *C. coccodes* or *B. ochroleuca* (265–269 bp) (Supplementary Fig. S4[2]). Of the other originally suspected fungal pathogens, only *R. vagum* (118–119 bp) appeared to be present in root samples at detectable levels. Based on TRFLP data, there was no consistent or discernable effect between the two evaluated amendments (thermophilic compost and poultry manure) on the abundance of fungi in these plant tissues.

Discussion

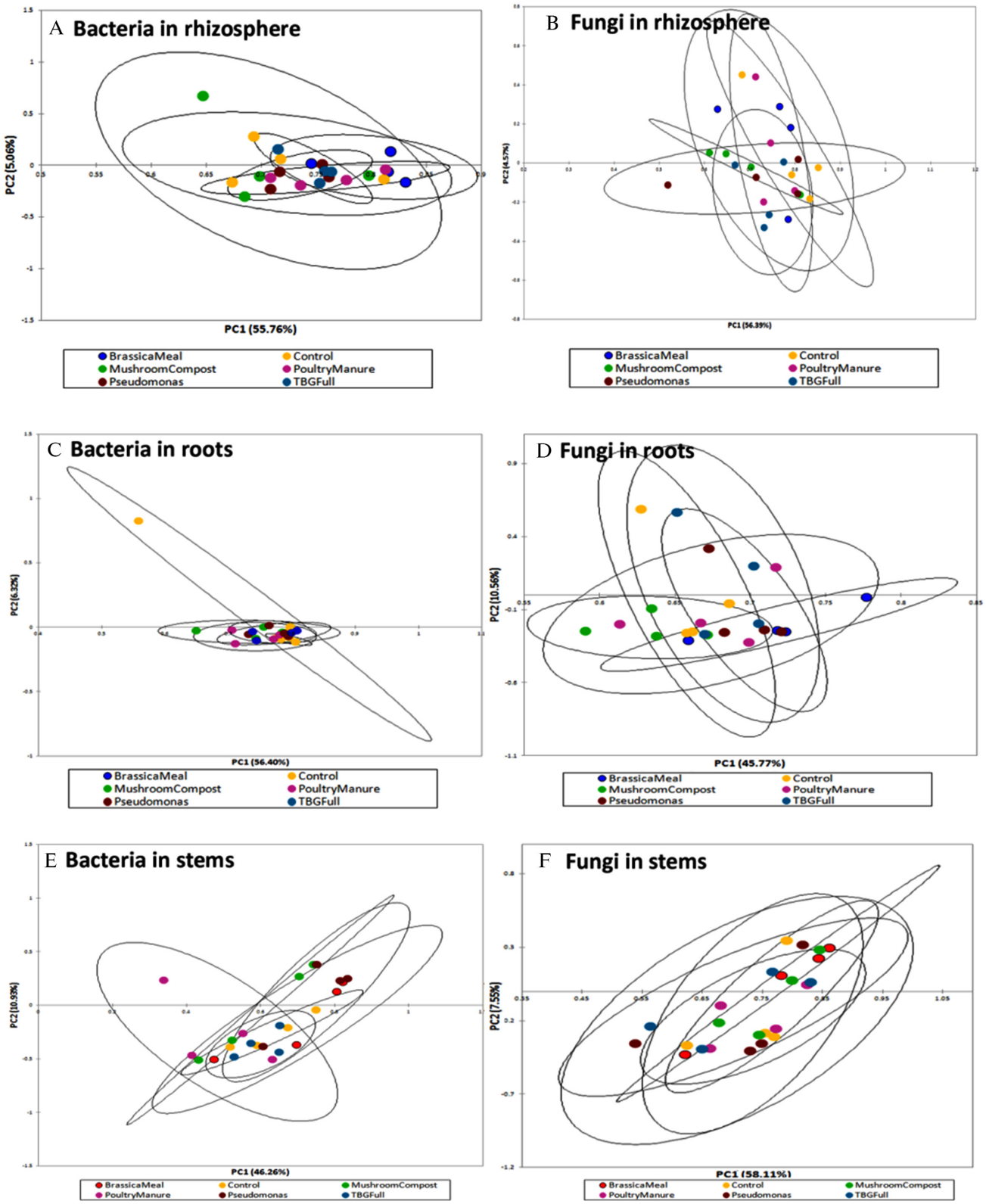
Except for 2011, yields at all site-years were representative or greater than industry expectations of 91.6 Mg ha^{-1} (OPVG 2014) and considerably greater than 2008 to 2012 reported provincial averages (78.7 Mg·ha^{-1}; Mailvaganam 2018). In 2011, a split fruit set occurred (abortion of fruit along the vine for a short time followed by a period of improved weather conditions resulting in an abnormally extended flowering and fruit setting time) and partially explains the observed low in marketable fruit yield and large percentage of immature, green fruit (ave. 39%; Fig. 1). Given this, the lack of poultry manure treatment, and the lack of treatment effects on yield parameters in 2011, the discussion focuses largely on the remaining three site-years.

Soil amendments and transplant treatments were evaluated as possible strategies to manage for TVD in commercial processing tomato production. Marketable yield was not different among treatments but numerically followed similar trend of total yield (Table 3). Across the three site-years, total yield of processing tomato with poultry manure was 16.2% and 17.2% greater than the unamended treatments (i.e., untreated control and microbial treatment, respectively). At both site-years in 2012, all organic amendments had greater total yield than the unamended treatments (averages of 151 Mg·ha^{-1} compared with 135 Mg·ha^{-1}, respectively). This result was consistent with others that have observed a positive effect of soil organic amendments on crop productivity (Pieper and Barrett 2008; Oldfield et al. 2018; Brunetti et al. 2019). In agreement, Maharaj et al. (2018) observed application of composted poultry manure increased tomato yield in half of the tested fields in California and was associated with suppression of premature vine decline.

While poultry manure provided greater tomato yield, the greater percentage of immature green fruit (i.e., 19.9% with poultry manure compared with 8.06% to 11.7% in all other treatments) suggests that fruit maturity was delayed, which may be rectified only partly with application of ethephon. The delayed maturity was attributed to the greater nitrogen fertility with poultry manure compared with other treatments (predicted available nitrogen of 126 kg N·ha^{-1} with poultry manure versus <5 to 26 kg N·ha^{-1} with all other amendments vs. none in the control and microbial treatment). Similarly, in Ontario tomato production systems, the amount of green tomato fruit increased by 46% to 58% with application of inorganic fertilizer nitrogen (Van Eerd et al. 2015). The impact of nitrogen on proportion of green fruit was

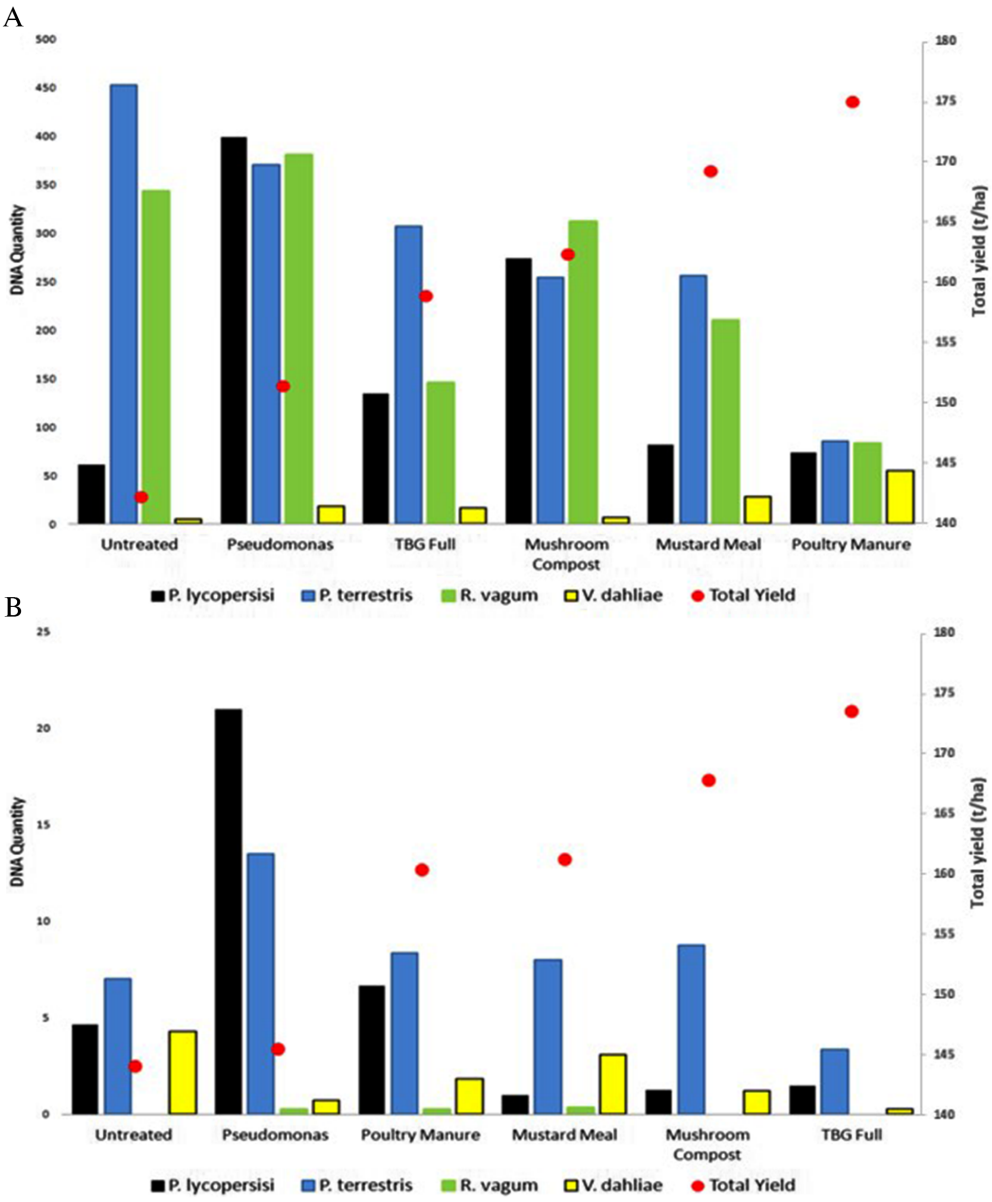
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Fig. 3. Impact of soil organic amendments and transplant treatment on bacterial (left) or fungal (right) diversity from rhizosphere (A,B), roots (C,D), and stems (E,F) of tomato plants grown in a commercial field at Leamington in 2013 as shown by principal component analysis (PCA) of TRFLP data. A treatment may be considered to significantly impact bacterial or fungal populations if grouping circles (95% confidence intervals) do not overlap the untreated control group (yellow dots). (TBG Full: thermophilic compost)



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Fig. 4. Impact of soil organic amendments and transplant treatments on the total fruit yield and root pathogen abundance (measured by real time PCR of fungal ITS DNA) of individual tomato plants grown at commercial fields at (A) Lighthouse Cove and (B) Leamington in 2012. (TBG Full: thermophilic compost)



consistent with Belfry et al. (2017) but other research showed no effect (Seliga and Shattuck 1995).

The lack of effect on tomato yield with the microbial pseudomonad transplant root drench was the opposite to the promising results observed during lab trials in a controlled environment. Although DNA fingerprinting showed greater pseudomonads in tomato roots, the contrasting results in commercial production field to controlled environments was not surprising (see reviews by Mitter et al. (2021) and Tosi et al. (2021)). There was no evidence to suggest a detrimental effect of this treatment in commercial tomato fields. In 2011 where TVD pressure was high, the microbial pseudomonad treatment had the numerically, but not significantly, greatest total yields (22.5% greater compared with the control; Supplementary Table S3[2]). Further research on optimizing the method and timing of application and (or) formulation is recommended to improve efficacy (Mitter et al. 2021; Tosi et al. 2021) of the microbial pseudomonad transplant root drench on TVD.

Although only significant in half the site-years, increases of 5.7% to 12% in total yield compared with the unamended control observed with the other organic amendments (i.e., poultry manure had 17.2% greater total yields) warrants further research. This is particularly true given that applying organic amendments has been a long-recognized regenerative agricultural practice to enhance soil health (Francis et al. 1986;

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Sherwood and Uphoff 2000). It is worth considering that the mustard seed meal and thermophilic compost used in this study were generated in Saskatchewan and British Columbia, respectively, which may present challenges in terms of availability and anticipated high transportation costs for Ontario tomato growers. Poultry manure and spent mushroom compost are more available locally. Moreover, there would be added expenses with some approaches, such as irrigating the mustard seed meal and multiple applications for the thermophilic compost treatment.

In contrast to yield, the quality of processing fruit was not impacted by treatment, which was consistent with other Ontario research (Seliga and Shattuck 1995; Thomas et al. 2001; Loewen 2011; Belfry et al. 2017; Chahal and Van Eerd 2021) and elsewhere (Brunetti et al. 2019). Cultivar, fruit maturity at harvest (Anthon et al. 2011), and incidence of precipitation or irrigation between the onset of ripening and harvest (Johnstone et al. 2005), are known to strongly influence fruit quality; hence, the lack of treatment effect on fruit quality was expected. All fruit quality parameters were within industry standards (Gould 1992). Thus, the results were consistent with those reported in the literature and suggest there is no impact of single applications of organic amendments nor microbial pseudomonad treatments on fruit processing quality.

Although not significant ($P = 0.1249$), the overall trend of soluble solids over the four site-years was poultry manure > all other treatments > untreated control (4.63 >> 4.36 > 4.30 °Brix, respectively). Processing efficiency of tomato paste products improves with tomato soluble solids levels. For example, in early 2000s in California, for each 0.1 °Brix decrease in soluble solids there was an additional cost of $1.30 USD per Mg of tomatoes processed (Linden 2004). Applied to Ontario, a 0.1 °Brix decrease would equate to additional processing costs of $440 000 USD based on the quantity of tomatoes processed in 2014 (OPVG 2014). Given the aforementioned trend in soluble solids and greater yields of amendments, real gains in soluble solids yield per hectare would be realized (poultry manure > all other treatments >> untreated control (5.22 > 5.13 >> 4.74 Mg sugar per hectare, respectively). This quantity of soluble solids yield per hectare was equivalent to those observed in California (Johnstone et al. 2005). Although, it is the concentration of soluble solids that drives processing costs, our results suggest that the application of organic amendments, particularly poultry manure may provide significant gains for the processing tomato industry.

No change to microbial communities in stem, roots and rhizosphere detected

To better understand TVD and impact of various organic amendments and transplant treatments on primary productivity, microbial populations in tomato stems, roots and rhizosphere were characterized. There was no global shift in bacterial and fungal population structure. In contrast, where organic amendments altered the rhizosphere microbiome (Deng et al. 2020), bacteria were identified as important for suppressing tomato bacterial wilt [*Ralstonia solanacearum* (Smith)]. The positive effect of organic amendments on microbial populations in Deng et al. (2020) but not our study was attributed to system differences, a soil-grown tomato greenhouse with many years of tomato monoculture compared with our commercial fields with at crop rotation.

The lack of effect on microbial populations was not surprising given the complexity and variability of soil biological, chemical, and physical properties in commercial fields. This was particularly true when TVD pressure was high (2011), as there was considerable field variability in yield and symptoms. Lack of treatment effect in the clustering of microbial community diversity in PCA might also be explained by dilution of amendments within surface soil due to tillage and time lapsed from application (2 wk before transplanting) to sampling at tomato maturity.

The lack of shifts in microbial communities in stems, roots, and rhizosphere indicates that the treatments did not have strong, global effects on either bacterial or fungal populations associated with tomato plants. It is also possible the methodology used for TRFLP and its statistical analysis (PCA) were either not sensitive enough or flawed in their ability to resolve differences between microbial communities. In growth room studies, shifts in tomato-associated fungal populations can be subtle despite the use of amendments applied at much greater concentrations with thorough mixing into soil (Supplementary Fig. S3[2]; Johnston-Monje et al. 2017). Similarly, high application rates of organic amendment were necessary to influence the establishment of beneficial bacterial populations in greenhouse experiments (Inbar et al. 2005; Postma and Nijhuis 2019). Thus, the lack of changes in soil microbial populations was not unexpected and may have been due the sampling at one time point (i.e., fruit harvest).

Evidence of pathogen suppression with amendments but not with pseudomonad transplant treatment

Realtime PCR was conducted to determine the effectiveness of treatments on reducing the abundance of four soilborne fungal pathogens associated with tomato roots. *P. terrestris* and (or) *R. vagum* pathogens were most strongly suppressed by poultry manure in one trial and thermophilic compost in another trial, which is similar to other studies that did not find strong and consistent correlations between pathogen levels and yield (Maharaj et al. 2018, Deng et al. 2020; De Corato et al. 2020). For instance, Maharaj et al. (2018) found that soil amendments of poultry manure to low-cation exchange capacity (CEC) (but not high-CEC) fields, resulted in higher fruit yield and less leaf necrosis but inconsistent

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and insignificant control of Verticillium and Fusarium wilt, root rot or corky root. Dramatic reductions in pathogen levels were not observed in the other treatments. Variability in treatment effects from field to field might be explained by differences in soil chemistry and soil microbiology, but without greater repetition across years, it is difficult to speculate why treatments behaved differently.

The survey of fungal populations found *V. dahliae* as a possible suspect pathogen involved in root infections, which is in agreement with Johnston-Monje et al. (2017) who suggested *V. dahliae* and *O. virulentus* contribute to TVD disease complex in the same commercial tomato fields as this experiment. Similarly, in California, *V. dahliae* has been associated with a premature vine decline in commercial tomato fields (Davis and Miyao 2009; Maharaj et al. 2018). Moreover, enhanced crop performance (avg. 17.2% total yield increase,) under poultry manure treatment corresponded with decreased targeted total fungal pathogen DNA. Further research is needed to understand the association and potential mechanism of fungal pathogen suppression (*V. dahliae* among them) with organic amendments and impact on tomato yields. Observed differences in suppressive effects on fungal populations depended on the treatment, field location and the pathogen species detected. Future research focusing on assessing pathogen and microbial communities at key points during the growing season (i.e., flowering, and fruit set) may be useful to better understand the influence of organic amendments on tomato productivity.

Although *Pseudomonas* were present in tomato roots, there was no effect of the microbial treatment (i.e., pseudomonad application to tomato transplants) on tomato yield and pathogen suppression observed. This result was consistent with other research evaluating the introduction of microbes as biocontrol products in the field (Marahaj et al. 2018) and greenhouse (Giotis et al. 2009). Jack et al. (2011) reported that a bacterial community introduced via transplant media colonized roots at rates multiple times greater than indigenous soil taxa. However, the success of induced or introduced microbial species/communities depends on specific plant species and soil environment (Tosi et al. 2021), and reflects the rhizosphere buffering theory (Weller et al. 2002). Refinement and standardization of inoculation procedures are needed (Tosi et al. 2021) and may enhance the applicability of microbial treatments to suppress pathogens.

Possible mechanisms of action

Organic amendments may have potential in suppressing soil borne plant pathogens. This has been attributed to two major mechanisms that limit plant pathogens: (*i*) through the introduction or induction of other microbes and (or) (*ii*) by altering soil conditions (e.g., an increase the available energy and nutrients along with soil aeration during incorporation). In addition to the complex microbial communities within soil amendments, poultry manure also adds readily available nutrients (especially ammonia, P, K, Na, and S, (data not shown), which partially explains its enhanced effect on tomato yield. Poultry manure decreased premature vine decline symptoms (reduced leaf necrosis and sunburn damage to fruit) in commercial processing tomato field in California, that had lower CEC along with higher potassium content (Maharaj et al. 2018). Greater soil nitrate concentration boosts tomato seedling growth, giving plants opportunity to develop substantial resilience towards root diseases (Jack et al. 2011).

A potential mechanism is nutrient competition between biological control organism, plant pathogens, and plant host (Hoitink and Fahy 1986; Larkin and Fravel 1999), where greater nutrients applied in the form of the organic amendments, particularly poultry manure, probably enhanced plant growth concomitantly with plant pathogen suppression. In a disease with similar symptoms to TVD, corky root was less severe in organic than conventional tomato production systems and this was related to N concentration (especially ammonia content) in soil (Workneh and van Bruggen 1994a; Bailey and Lazarovits 2003). Snyder et al. (2009) also demonstrated that seed meals from Brassicaceae oilseed crops tend to be phytotoxic in the short term (i.e., days) at high rates and provide plant available N during the growing season. The toxicity of ammonia to soilborne organisms is well documented. For instance, the content of ammonia in manures reduced viability of *V. dahliae* microsclerotia in soil and wilt symptoms in potatoes (Conn and Lazarovits 1999). An increase in ammonia concentration and enhanced soil nutrients provides a partial explanation of how poultry manure (e.g., ammonium-N was 1694 $mg \cdot kg^{-1}$) suppressed soil-borne pathogens and resulted in better crop performance.

Concomitantly with nutrient release, decomposition of organic amendments releases various organic and amino acids as well as carbohydrates and sugars that influence microbial communities and suppress pathogens (Donaldson and Deacon 1993). A comparison of organic and conventional farms showed that, enhanced actinomycete rhizosphere populations in organic production fields contributed to greater crop yield with greater proportions of starch hydrolysing bacteria and chitinolytic fungi (Workneh and van Bruggen 1994*b*). Decomposition of organic amendments alters other soil chemical properties such as pH, which in turn influences pathogen incidence. For instance, Bailey and Lazarovits (2003) reported elevated nitrogen in amendments may reduce soil-borne plant pathogens of potatoes [*Bipolaris sorokiniana* (Sacc.), *Verticillium* spp.] due to lowering of soil pH and the release of chemicals released such as ammonia, organic acids and volatile fatty acid compounds during decomposition of manures. Research has shown

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that lower pH would likely reduce plant root rot by *Phytophthora cinnamomi* (Blaker and MacDonald 1983), and also reduces sporangium formation, zoospore release, and motility (Blaker and MacDonald 1983; Hoitink and Fahy 1986). This provides a partial explanation for observed results with poultry manure in commercial field experiments.

Additionally, induced resistance and antibiosis are thought to be the main mechanisms of disease suppression of introduced microorganisms (Litterick et al. 2004). Organic amendments such as manure and compost have diverse microbial populations (Bailey and Lazarovits 2003; Perez-Piqueres et al. 2006; Jack et al. 2011) and their incorporation into soil and subsequent establishment in the rhizosphere may act to suppress plant pathogen development and colonization of plant roots (Litterick et al. 2004; Deng et al. 2020). Irrespective of the source of introduced or induced microorganisms (i.e., applied as a commercial biocontrol product, a root drench, or present in organic amendments), the survival and colonization in the rhizosphere and (or) root is a key factor for efficacy (Romano et al. 2020; Mitter et al. 2021; Tosi et al. 2021). Maintaining root health is critical to enhancing primary productivity (Cook 1986). The proportion of disease-suppressive to pathogenic microorganisms in the soil profile and rhizosphere is of great importance when evaluating the direct and indirect effects of organic amendments (Deng et al. 2020). Plant rhizosphere communities are established quickly upon transplanting and remain intact despite introduction of new microbes (Turnbull et al. 2014). Thus, despite a lack of changes in rhizosphere microbial community, by enhancing soil conditions at transplanting with incorporation of organic amendments, soil conditions may have reduced pathogen pressure and enhanced yield.

The comparison of TRFLP fingerprints among the rhizosphere, roots and stems suggests that soil pathogens do not penetrate all the way to the stem, but mainly function in roots. Specifically, *V. dahliae* was detected in roots but less so in stems and rhizosphere. Suppression of *V. dahliae* and 3 other pathogens (*P. lycopersisi, P. terrestris, Rhizopicnis vagum*) in tomato roots when organic amendments were applied, suggests a possible mechanism of action for soil amendments in suppressing TVD.

Regardless of the mechanism of disease suppression, the potential exists to reduce disease pressure in fields with organic amendments (e.g., review by Litterick et al. 2004) as study results suggest. With any animal-based organic amendment, human pathogens may be a concern. In this study organic amendments were applied and incorporated 2 wk before transplanting, which one would expect to mitigate human pathogens load on fruit by harvest; however, future research should quantify the potential food-borne human pathogen risk, particularly for fresh market tomatoes. An alternative solution to overcome TVD is to avoid fields with previously observed symptoms; which would increase costs, such as land rental fees and moving equipment and people to new fields. This practice has been employed by some Ontario growers by renting land where Solanaceous crops have not been grown before. Irrespective of TVD, growing tomatoes on these fields is expected to result in yield gains of 10% (Van Eerd et al. 2015). These fields offer an opportunity for further research to compare microbial soil and rhizosphere communities and explore causal agents of TVD.

Conclusions

Experiments were conducted in four commercial fields selected based on an expected high prevalence of TVD. Tomato vine decline symptoms were present in all fields but high pressure was observed in one site-year (Lighthouse Cove 2011). Marketable yield and fruit quality (i.e., Agtron colour, pH, soluble solids) were not impacted by treatments. In the three site-years where poultry manure was evaluated, total fruit yields were greater than the untreated control. Without the use of ripening agent (ethephon), the greater incidence of immature green fruit indicated that fruit maturity was delayed with poultry manure, which was attributed to greater available nitrogen compared with all other treatments. In two of four site-years, total yield was greater under all soil amendment treatments compared with both non- amended treatments (i.e., untreated control and the pseudomonad microbial transplant root drench treatment), which were not different from each other.

It was not readily apparent how (or if) rhizosphere, root or stem microbial populations were affected by the four tested organic amendments in this commercial field experiment. There were high populations of *V. dahliae* and *R. vagum* inside roots of both control and pseudomonad treated plants, with observed greater populations of these and other fungal pathogens in untreated control plants. Consistent with earlier research, soil-borne fungal root pathogens seem to be associated with the disease complex resulting in tomato vine decline, or with reducing tomato yield. A single, preplant application of organic amendment enhanced processing tomato yield but did not affect quality parameters and appeared to decrease fungal pathogens in roots. The hypothesis was that these treatments act by modifying the diversity of bacteria and fungi in the tomato rhizosphere, however no evidence of this was found using TRFLP. In contrast, it was shown that some organic soil amendments, but not the pseudomonad transplant treatment, were able to reduce the abundance of some known fungal pathogens colonizing tomato roots, likely to the advantage of crop yield. The mechanism(s) of action of amendments remain unclear, which is not surprising, given the multitude of soil, plant, and weather variables that influence disease suppression in commercial tomato fields and provides an opportunity for future research.

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