Taylor & Francis
Taylor & Francis Group

Check for updates

Ecoenzymes as Indicators of Compost to Suppress *Rhizoctonia Solani*

Deborah A. Neher[a], Lynn Fang[a,b], and Thomas R. Weicht[a]

[a]Department of Plant and Soil Science, University of Vermont, Burlington, Vermont; [b]Soil & Compost Consulting & Education at The Growing Club, Diamond Bar, California

ABSTRACT

Reports of disease suppression by compost are inconsistent likely because there are no established standards for feedstock material, maturity age for application, and application rate. The overall goal of the study was to evaluate a suite of biological indicators for their ability to predict disease suppression. Indicators included both commercial available methods for compost stability (Solvita[TM], respiration) and metrics of soil ecology not yet adopted by the compost industry (e.g., ecoenzymes, nematode community index). Damping-off by *Rhizoctonia solani* on radish was chosen as a model system given its global importance, competitiveness affected by carbon quality, and lack of disease management options for organic production. Biological indicators were evaluated for their ability to consistently differentiate among curing process, maturity, and feedstock material as a function of disease severity of a seedling bioassay and a compost extract assay to test competition with *R. solani* growth. Compost processed as vermicompost and anaerobic digestate were more suppressive against *R. solani* than windrow or aerated static pile. Mature composts were more suppressive than immature components. Feedstocks containing dairy manure and/or hardwood bark tended to have suppressive qualities. In contrast, poultry manure-based components were conducive to disease. Microbial ecoenzymes active on chitin and cellulose and nematode community indices were better predictors of disease suppressiveness than microbial respiration. These indicators are quicker than plant bioassays and could be adopted as tools to certify commercial products.

Introduction

Finished compost should be stable and mature, two terms that are often used interchangeably, so it can be safely packaged and transported, and not cause adverse effects during its end use (Wichuk and McCartney 2010). *Stability* is a resistance to decomposition and is usually determined using indices of microbial activity. Commonly recommended of the stability tests is some variation of a compost self-heating test through respirometry (Gómez, Lima, and Ferrer 2006) or the 8-point color scale Solvita[TM] test produced by Woods End Laboratory (Brinton et al. 1995). Solvita[TM] is simple and practical but has been criticized for its imprecision and cost. In contrast, *maturity* infers that the material is ready for a particular use, and, for horticultural purposes, is determined by plant germination and growth assays. Plant bioassays are a gold standard because they empirically determine whether there are any detriments to plant

growth or development (Wichuk and McCartney 2010). Effective plant bioassays are standardized by plant cultivar and environmental conditions, but are time consuming (2–4 weeks) to complete, which may be longer than desired. Comparably robust, but quicker (1–2 day) assays would be ideal for quality control and quarantine programs.

Vegetable farming represented 14% of the US agricultural market in 2016 (USDA-ERS 2016) and provided nearly double the return per acre than other agricultural operations in the Northeast (Chan et al. 2011). The total number of vegetable farms is increasing in the Northeast and market projections suggest the vegetable market overall and the organic vegetable market will increase in the coming decade. Additions of composted organic matter are considered beneficial for vegetable cropping systems because they contribute positively to soil quality and provide fertility. The recipe, curing process, and maturity have unique

CONTACT Deborah A. Neher ✉ deborah.neher@uvm.edu ▣ Department of Plant and Soil Science, University of Vermont, 63 Carrigan Drive, Burlington, VT 05405, USA.

impacts on the soil microbiology (Neher et al. 2013) and influence the ecological succession of soil microbial communities. These impacts on soil biology are 26% effective in suppressing plant pathogens (Bonanomi et al. 2007; Jack et al. 2011).

Rhizoctonia solani Donk (Kuhn [teleomorph *Thanatephorus cucumeris* (Frank)] is listed as one of the most important pathogens on crop plants, which provide the primary sources of human nutrition (Strange and Scott 2005). *R. solani* is an aggressive fungal pathogen with a wide host range, including Solanaceae, Fabaceae, Asteraceae, Brassicaceae, and has a global distribution. When alone with a host, it colonizes the root, monopolizing a resource to the point of competitive exclusion (Klein et al. 2013). It is a difficult disease to manage because it is a facultative saprophyte, which is able to survive as sclerotia under adverse environmental conditions (González García, Portal Onco, and Rubio Susan 2006). Few methods are available for managing *R. solani* aside from not planting too deeply, and removing weeds to ensure good air circulation. There is no genetic resistance to *R. solani* (Grosch et al. 2004) and a limited choice of seed varieties that meet the organic certification standards of seed production and pelleting.

Successful and reliable suppression of *R. solani* has been shown in greenhouse trials with vermicompost (Ersahin, Haktanir, and Yanar 2009), and thermophilic composts made from hardwood bark (Nelson and Hoitink 1983), organic household waste (Tuitert, Szczech, and Bollen 1998), viticulture and enological factory residues (Pane et al. 2011), cow manure (Pane et al. 2013), swine manure (Diab et al. 2003), municipal waste (Mathout 1987), and grape marc. However, grape marc compost also showed conduciveness towards *R. solani* (Santos et al. 2008), showing the inconsistency of compost use in disease suppression. Municipal waste compost stored near piles of composted hardwood bark suppressed *R. solani*, while those that were not stored near composted hardwood bark were not suppressive (Kuter, Hoitink, and Chen 1988). These conflicting findings stress the importance of a consistent feedstock chemistry, curing process, and maturity in *R. solani* suppression.

Fungi *Trichoderma harmatum* and *Gliocladium virens* are known antagonists of *Rhizoctonia* (Hoitink and Boehm 1999). Composts prepared from hardwood tree barks become colonized by *Trichoderma* spp. and *Gliocladium* spp. or other related Hypocreaceae (Hoitink and Boehm 1999; Neher et al. 2013). Wood-based carbon has higher lignin:cellulose ratios than hay or straw-carbon-based composts. Pathogens, such as *Rhizoctonia solani*, are favored in early stages of composting when a concentration of labile carbon is high (Chung, Hoitink, and Lipps 1988). Once labile carbons are depleted, the efficacy of biological control fungi such as *Trichoderma hamatum* increases (Chung, Hoitink, and Lipps 1988). *T. hamatum* hyperparasitizes mycelium of *Rhizoctonia solani* by producing cellulases, glucanases, and chitnases. Given the variety of composts shown to suppress *R. solani* suggests that managing carbon quality and compost maturity will alter relative competition between biocontrol microbes and the pathogen (Nelson and Hoitink 1983; Ersahin, Haktanir, and Yanar 2009).

The objectives of the study were: (1) determine whether process, maturity, or feedstock of commercial compost products is a consistent predictor of disease suppression, and (2) identify the most reliable indicators of disease suppression. Indicators evaluated included current industry standards (Thompson et al. 2001) as well as indicators proposed by research laboratories, including extracellular enzyme assays (Castaldi et al. 2008), nematode community indices (Neher 2010; Steel et al. 2010), and a compost extract plate competition assay (Alfano et al. 2011). The ultimate goal was to identify a quick and accurate test to predict suppressive potential of compost toward *R. solani*.

Materials and methods

Model pathogen system

Local isolates of *Rhizoctonia solani* Donk (Kuhn [teleomorph *Thanatephorus cucumeris*]) (Frank) were collected from Vermont grown potatoes and radishes. Infected pieces were excised, grown in water agar, and incubated at room temperature. We chose radish (*Raphanus sativus*), Ping Pong variety from Johnny's Seeds (Fairfield, ME, USA), for its quick growth and ease of detecting *R. solani* infection at the white root crown. Radish is recommended for germination tests because of its precision and accuracy among compost types compared to other vegetable seeds (Komilis and Tziouvaras 2009). The isolates most virulent on radish were kept in long-term storage on a minimal media of corn meal agar slants (at 5°C), and used to infest soils

for greenhouse bioassays. *R. solani* is less likely to lose virulence in culture than Oomycota or bacteria pathogens (personal observation).

Inoculum was cultured in a sterile mix of 96 g sandy soil, 4 g corn meal, and 20 ml of water. The culture medium was autoclaved for 1 h over three consecutive days to ensure that any endospores of *Bacillus* were killed. Plugs of *R. solani* growing on potato dextrose agar were transferred to the culture medium, covered with foil, and incubated at room temperature for 2–3 weeks until the culture medium was overgrown with mycelium.

Compost types

Composts from a variety of different commercial facilities were chosen based on maturity and pile type (Table 1). Production processes included: (1) aerated static piles (ASP) often followed by windrow curing, (2) windrows aerated by a bucket loader or excavator, (3) vermicomposts produced by a two-step process where materials were first composted past the thermophilic stage, then fed to compost worms (*Eisenia fetida*) for maturation. Immature and mature compost was just 0 and 3 to 6 months of curing after the thermophilic phase, respectively. Feedstocks were mixed food waste, poultry manure, food waste and poultry manure, dairy manure and silage only, or hardwood bark as the primary carbon substrate. We were not able to achieve a full factorial design, as we relied on what local producers had available in Vermont and New York.

Plant bioassay

Field soil (Adams and Windsor loamy sands) was steam pasteurized at 70°C for 4 h to destroy native pathogens, then re-inoculated with its endemic microbial community by adding 4 L of 10-μm filtered-soil extract, and sat for 3 weeks to allow the microbial community to re-establish itself. Half of the soil was inoculated with mixed local isolates of *R. solani* (100 ml inoculum per liter of soil), and was allowed 1 week to equilibrate with the soil before compost was incorporated. ASP, windrow, and vermicompost were applied respectively at 10, 10, and 1.25% (v/v). These application rates exhibited the greatest disease suppression in preliminary trials (Fang 2015). A negative control of no compost in uninfested soil served as a reference. Treatments were replicated four times. Compost-soil mixtures were allowed to equilibrate for 1 week after which 25 radish seeds were planted into each pot using a customized dibble-stick to ensure a distance of 254 mm between each seed. Four replicate pots were ascribed to each treatment sample. Plant bioassays were performed in the greenhouse under natural day lengths and watered daily. Radish seedlings were allowed to grow for 2 weeks until the

Table 1. Recipe/primary feedstock, pile type, maturity level in factorial combinations with two stages of compost maturity and infestation by *R. solani* pathogen.

Recipe/primary feedstock	Facility	Pile type	Maturity
Hardwood bark[a]	Highfields Ctr for Composting	Windrow	Very mature
Commercial standard[b]	Highfields Ctr for Composting	ASP/windrow	Fresh [f]
Commercial standard	Highfields Ctr for Composting	ASP/windrow	Mature [g]
Commercial standard	Highfields Ctr for Composting	Vermicompost	Mature
Cow manure—silage	Worm Power	Vermicompost[WP]	Mature
Commercial standard[c]	Vermont Compost	Windrow	Fresh
Commercial standard	Vermont Compost	Windrow	Mature
Commercial standard[d]	Green Mountain Compost	ASP/windrow	Fresh
Commercial standard	Green Mountain Compost	ASP/windrow	Mature
Commercial standard[e]	Grow Compost	Windrow	Fresh
Commercial standard	Grow Compost	Windrow	Mature
On-farm[h]	Riverside Organic Farm	Windrow	Mature
Cow Manure/Silage[i]	Green Mountain Power	Anaerobic digestion	N/A

[a]Custom prepared with yellow birch as a carbon source mixed in a 5:5:3 ratio of manure/silage:hardwood bark:softwood shavings resulting in a C:N ratio of 34:1.
[b]Twenty percent food residuals, 10–15% 2.4-cm woody materials (e.g., hardwood bark and mixed wood chips), 10% hay, up to 5% shredded paper, up to 2% dry sawdust or shavings, and 50–60% mixed livestock manures (e.g., horse, cow, heifer, calf) mixed with various bedding materials (e.g., straw and hay).
[c]Variety of materials, including food waste, manure, especially chicken manure, and wood materials, no biodegradable plastic products.
[d]A municipal compost facility taking in food and yard waste and some manure, as well as biodegradable plastic products.
[e]Mixture of wood materials, food scraps, hay, manure, and yard waste, no biodegradable plastic products.
[f]Fresh, but post-thermophilic.
[g]Ready to sell.
[h]Poultry manure and softwood cedar shavings.
[i]Manure/silage fed to digester; remaining solids are separated and dehydrated and analyzed.

emergence of one true pair of leaves, after which each bioassay was harvested. Each seedling hypocotyl was examined under a stereoscope and rated for disease severity on a scale of 1 to 5. A percentage disease severity was ascribed for each rating based on the Horsfall-Barratt scale (Horsfall and Cowling 1978). The mean disease severity was computed from the midpoint percentages. Treatment results were expressed as a percent change in disease severity from the negative control.

Indicators of disease suppression

Each compost sample was homogenized and subsamples were analyzed for a variety of bioindicators: microbial carbon, CO_2 respiration, ratio of total carbon to total nitrogen (C:N), electrical conductivity (EC), pH, plate competition assay, extracellular enzyme activity (hereafter referred to as ecoenzymes), and nematode community indices (Table 2). Compost industry typically relies on measures of respiration, carbon, salinity, and pH as indicators of stability and phytotoxicity (Thompson et al. 2001). In addition, we evaluated three additional indicators, which are described briefly below.

Plate competition assay

A half gram of compost was added to 50 ml of sterile water and shaken overnight (adapted from Alfano et al. 2011). The next day, 1.5 g agar was added to 50 ml deionized water and autoclaved for 30 min. It was cooled to 55°C, mixed in with the compost water extract, swirled gently to mix, and poured into 100 mm × 15 mm plastic petri plates. The next day, plugs of *R. solani* growing on potato dextrose agar were transferred onto the compost water extract plates, and pure water agar plates were used as a control. Plates were incubated for 24 h at room temperature. The mycelium radius was then measured to the nearest 1 mm using a microscope. Three of the longest radii were recorded, and the mean was used as a representative measure to compare suppressive potential among different compost samples. This assay was completed five times in replicate per compost type. All measurements were standardized against the control of mean mycelium radial growth on water agar.

Ecoenzymes

Hydrolase, oxidase, amino-peptidase, and esterase activity was quantified as indicators of microbial functional activity and expressed as nmol h^{-1} g^{-1} of dry compost. Hydrolases (BG = β-glucosidase and NAG = β-1,4-N-acetylglucosaminidase) serve as an indicator for hydrolysis of plant and fungal cell walls, respectively. Oxidase (peroxidase and phenol oxidase), L-leucine aminopeptidase (LAP), and phosphatase (PP) activity are indicators for degradation of lignin, proteins, and phosphate, respectively (Moorhead et al. 2013). Sample suspensions were prepared by adding 0.5 g compost to 100 ml of 50 mM, pH 7.0, sodium bicarbonate buffer and homogenizing for 90 s with a Brinkman Polytron. The microplates were organized to assay three samples per plate, with two columns of eight wells each, for 16 replicates for each sample, along with controls (250 μl buffer alone, 200 μl buffer with 50 μl reference, and 200 μl buffer with 50 μl substrate). The reference standard was a 50-μM solution. Substrates were prepared as 200-μM solutions in nanopure (18.2 megohm) water. Microplates were covered and incubated at 20°C for 2 h. After incubation, they were quantified using a microplate fluorimeter (FLx800, Bio-Tek Instruments) with 360-nm excitation and 460-nm emission filters.

Table 2. Indicators of compost maturity or stability tested for their ability to predict disease suppression of *Rhizoctonia solani*.

Assay	Function	Units	Reference
Chloroform fumigation extraction	Microbial biomass carbon (C$_{mic}$)	μg C/g dry weight compost	Allison (2008)
Sodium hydroxide titration	Microbial activity via CO_2 respiration	mg CO_2/h/g dry weight compost	Oviedo-Ocaña et al. (2015)
Solvita™	Stability measured by respiration and N mineralization rate	8-Point color scale	Solvita (2009); Brinton et al. (1995)
CHN analysis	Limiting nutrient	Ratio	Goyal, Dhull, and Kapoor (2005)
pH and electrical conductivity (EC)	Salt concentration	EC measured in mS/cm	Smith and Doran (1996)
Ecoenzyme analysis	Decomposition activity; nutrient availability	nmol/h/g dry weight compost or μmol/h/g dry weight compost; ecoenzymatic C:N:P acquisition	Saiya-Cork et al. (2002)
Plate competition assay	Competition against pathogen	Millimeters (mm) mycelium/day	Alfano et al. (2011)
Nematode community	Nematode ecological successional index	MI, F:B	Steel et al. (2010); Neher (2010)

Oxidative enzyme substrates consisted of 50 mM L-DOPA for the phenol oxidase assay and 50 mM L-DOPA with 0.3% hydrogen peroxide for the peroxidase assay. The plates were covered and incubated for 1.5 h at 20°C. Absorbance was read on a microplate spectrophotometer (Bio-Tek μQuant microplate reader) with a 520-nm filter. Actual oxidative activity is the sum of phenol oxidase and peroxidase.

Nematode indicators

Nematode communities were extracted from compost using a mist extraction method and collected in a water-filled tube. The total number of nematodes were counted per sample and a 10% subsample was identified to family (Bongers 1987) and placed into a trophic group (Yeates et al. 1993). A maturity index (MI) was calculated as a weighted mean (Bongers 1990). The ratio of fungivorous to bacterivorous nematode abundance was calculated as F/B = fungivores/(fungivores + bacterivores).

Statistical analyses

Analysis of covariance was employed to analyze effects of compost process, maturity, and feedstock as independent variables on disease severity, ecoenzyme activity, and nematode ecology as dependent variables. The source facility of the compost was held as a random effect, and the covariables were microbial biomass carbon, respiration, C:N, pH, EC, and the compost extract plate assay. Tukey post-hoc comparisons were performed to compare among levels of independent variables. Multiple stepwise regression (forward selection) was performed with disease severity as the dependent variable. Independent variables were microbial carbon, respiration, C:N, pH, EC, compost extract plate assay, ecoenzyme activity, and nematode indices. Normality, analysis of covariance, and multiple stepwise regression were performed using the UNIVARIATE, MIXED, and REG procedures, respectively, in SAS version 9.3. All variables were assessed for normality using the UNIVARIATE procedure. Only C:N and EC required transformation, each with a natural log transformation $(x + 1)$. All statistically analyses were performed with transformed variables.

Results

Process, maturity and feedstock

Disease severity was affected consistently by compost process and maturity (Figure 1, Table 3). Vermicompost and anaerobic digestate were more suppressive than windrow or ASP composts. Mature composts were more suppressive than immature composts. Feedstock containing dairy manure and/or hardwood bark tended to
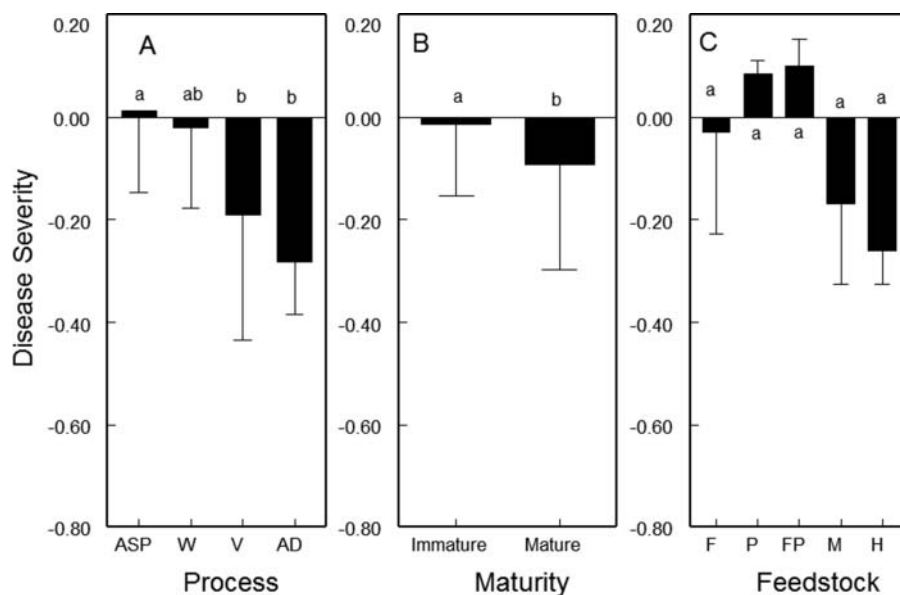

Figure 1. Disease severity affected by compost: (A) process (F = 8.82, P = 0.0003, ASP: aerated static pile, W = windrow, V = vermicompost, AD = anaerobic digestate; (B) maturity (F = 4.59, P = 0.041); and (C) feedstock (F = 0.59, 0.674, F = food waste, P = poultry manure, M = dairy manure, FP = food waste and poultry manure, H = hardwood bark). Both controls and treatment comparisons were inoculated with virulent *Rhizoctonia solani*. Illustrated are means ± 1 standard error of percent change from non-compost control.

Table 3. Analysis of covariance.

Dependent variable[†]	Independent variables												Covariables					
	Process				Maturity				Feedstock				C_{mic}	Rs	pH	EC	C:N	Rhiz
	F	P	N	D	F	P	N	D	F	P	N	D	P	P	P	P	P	P
Disease severity	8.82	0.0003	3	28	4.59	0.0410	1	28	0.59	0.6740	4	28	n.s.	*	n.s.	n.s.	n.s.	n.s.
Plate competition assay	2.1	0.1217	3	29	0.02	0.8993	1	29	4.19	0.0085	4	29	*	n.s.	n.s.	n.s.	n.s.	—
BG	2.79	0.0606	3	26	0.14	0.7144	1	26	6.54	0.0009	4	26	n.s.	*	n.s.	n.s.	**	*
NAG	453.6	<0.0001	3	26	1.32	0.2620	1	26	1.21	0.3302	4	26	n.s.	*	n.s.	n.s.	***	**
OX	1.12	0.3613	3	25	0.98	0.3314	1	25	1.34	0.2843	4	25	n.s.	n.s.	***	***	n.s.	n.s.
LAP	42.54	<0.0001	3	26	6.44	0.0175	1	26	2.45	0.0714	4	26	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PP	18.98	<0.0001	3	26	0.17	0.6880	1	26	8.91	0.0001	4	26	n.s.	***	n.s.	n.s.	**	**
ESI	0.64	0.5370	2	20	2.54	0.1270	1	20	12.19	<0.0001	4	20	n.s.	n.s.	*	n.s.	***	***
F/B	1.67	0.2130	2	20	10.0	0.0050	1	20	2.22	0.1031	4	20	**	**	*	n.s.	**	n.s.

Note. Effects of facility as random effect, process, maturity, and feedstock on disease severity, compost extract plate assay, ecoenzymes, and nematode indices, with microbial biomass carbon (C_{mic}), respiration (Rs), pH, electrical conductivity, C:N ratio, and plate competition assay (Rhiz) as covariables. Degrees of freedom for numerator (N) and denominator (D), F-values (F) and levels of significance (P) are illustrated.

[†]PP: phosphatase; BG: β-glucosidase (cellulose); LAP: L-leucine aminopeptidase; NAG: β-1,4-N-acetylglucosaminidase (chitinase); OX: actual oxidative activity = peroxidase + phenol oxidase; ESI: ecological succession of nematode index; F/B: fungivorous nematodes/(fungivorous + bacterivorous nematodes).

n.s.: $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; —: not included.

have significant disease suppression. In contrast, poultry manure-based compost promoted disease. Growth of *R. solani* mycelium on compost extract was affected by process and feedstock but not maturity. Growth was reduced most by vermicompost and windrow processes and feedstock containing hardwood bark (Figure 2).

Effects of compost process, maturity, and feedstock depended on ecoenzyme type. For example, NAG activity was greater in vermicompost than ASP and windrow. In contrast, PP and LAP activity was greater in windrow than ASP or vermicompost (Figure 3A).

LAP activity was greater in mature than immature compost (Figure 3B), but activity of the other ecoenzymes was similar among maturity classes. PP and BG activity were greater in dairy manure than poultry, food waste, or hardwood bark feedstocks (Figure 3C). Effects on oxidative activity were similar across process, maturity, and feedstock.

Nematode indices differentiated levels of compost maturity and feedstock but not process (Figure 4). Specifically, F/B were greater in immature than mature composts ($P < 0.005$).
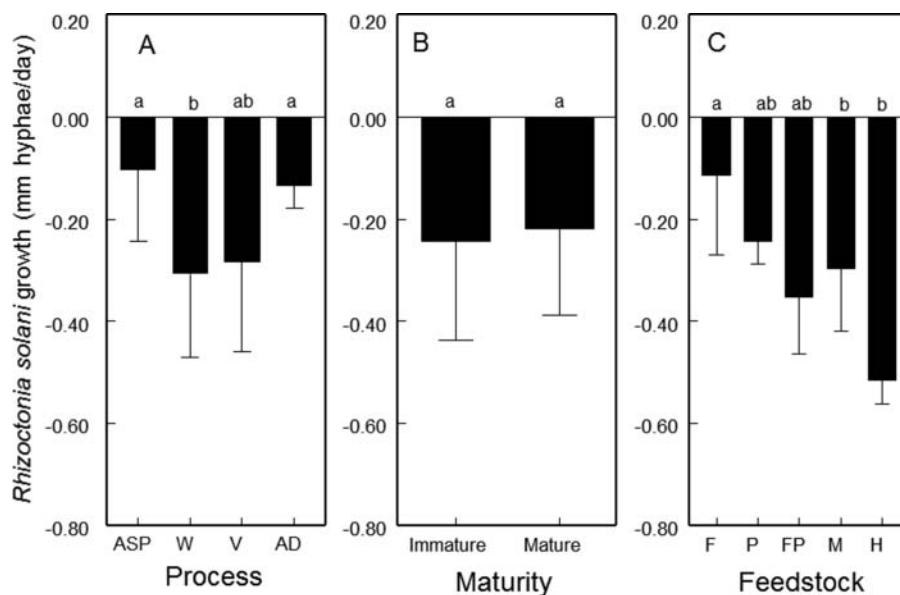

Figure 2. Plate competition assay measuring hyphal growth of *Rhizoctonia solani* on compost water extract agar affected by compost: (A) process (F = 2.1, P = 0.1217, ASP: aerated static pile, W = windrow, V = vermicompost, AD = anaerobic digestate; (B) maturity (F = 0.02, P = 0.8993); and (C) feedstock (F = 4.19, P = 0.0085, F = food waste, P = poultry manure, FP = food waste and poultry manure, M = dairy manure, H = hardwood bark). Illustrated are means ± 1 standard error of the change from non-compost control. Both controls and treatment comparisons were inoculated with virulent *Rhizoctonia solani*.
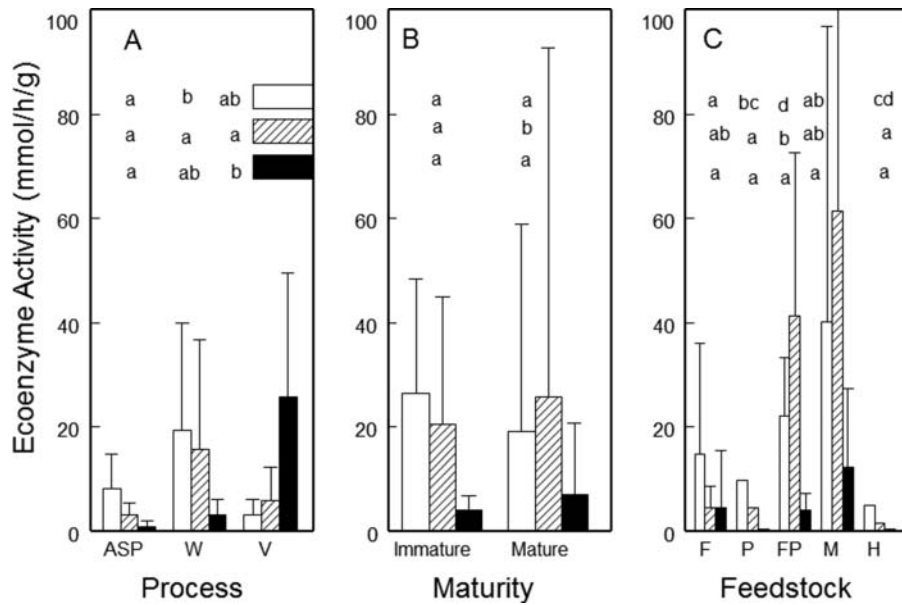
Figure 3. Ecoenzyme activity by: (*A*) process (ASP: aerated static pile, W = windrow, V = vermicompost); (*B*) maturity; (*C*) feedstock (F = food waste, P = poultry manure, FP = food waste and poultry manure, M = dairy manure, H = hardwood bark). Fill patterns represent ecoenzyme activities where white = phosphatase; slash = L-leucine aminopeptidase; and black: β-1,4-N-acetylglucosaminidase (chitinase). Illustrated are means $\pm$ 1 standard error of percent change from non-compost control. Both controls and treatment comparisons were inoculated with virulent *Rhizoctonia solani*. Post-hoc mean comparisons are represented by contrasting letters ($P < 0.05$) to the left of the fill pattern key within the first panel.

Successional maturity was greater in hardwood bark than other feedstocks (Figure 4C). Relatively few fungivorous nematodes in three families (Aphelenchidae, Paraphelenchidae, Qudsianematidae) were present.

Best predictors of disease suppression

Disease severity was better predicted by the indicators not currently used by the compost industry. Nematode MI, compost extract plate assay, and three
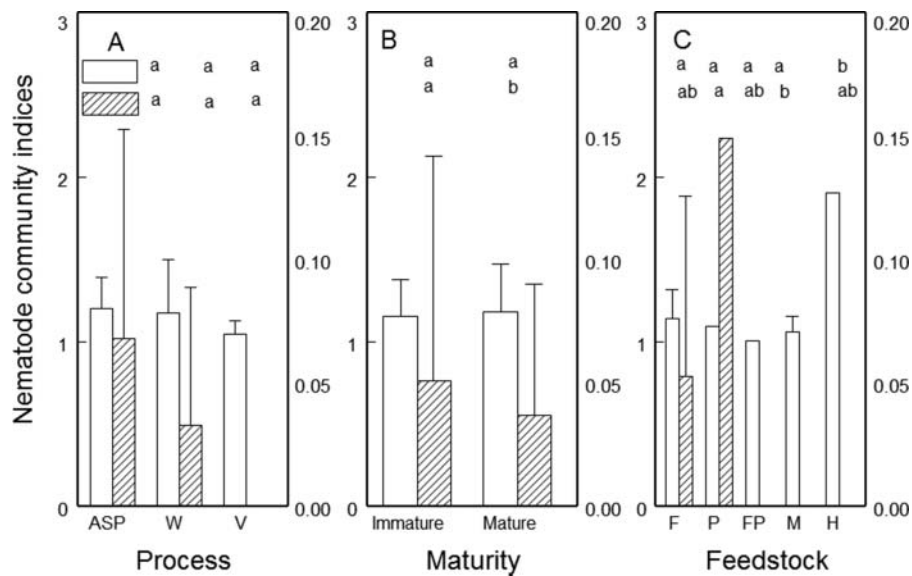

Figure 4. Nematode indices affected by compost: (*A*) process (ASP: aerated static pile, W = windrow, V = vermicompost); (*B*) maturity (M = mature; I = immature); (*C*) feedstock (F = food waste, P = poultry manure, FP = food waste and poultry manure, M = dairy manure, H = hardwood bark). Fill patterns represent community indices where open = maturity index (left *y*-axis), slash = fungivorous to bacterivorous (right *y*-axis) nematode abundance. Illustrated are means $\pm$ 1 standard error. Post-hoc mean comparisons are represented by contrasting letters ($P < 0.05$) to the right of the fill pattern key within the first panel.

Table 4. Multiple stepwise regression ($R^2 = 0.6861$) to identify best predictors of disease severity of *Rhizoctonia solani* on radish seedlings.

Variable	Parameter estimate	Partial R-square	F-value	P-value
Intercept	0.47874		33.62	<0.0001
Ecological successional index (ESI)	−0.31887	0.1741	24.97	<0.0001
Plate competition assay	0.37964	0.0358	14.78	0.0007
Phosphatase (PP) activity	0.00918	0.0493	14.68	0.0007
β-Glucosidase (BG) activity	0.06435	0.0439	7.95	0.0089
β-1,4-N-Acetylglucosaminidase (NAG) activity	−0.13379	0.0808	21.3	<0.0001

ecoenzymes (phosphatase, BG, and NAG) were the best predictors of disease suppressiveness (Table 4). In contrast, microbial carbon, CO_2 respiration, C:N, EC, pH, nematode F/B, oxidative and leucine amino peptidase ecoenzymes were less satisfactory predictors of disease suppression.

Discussion

This study was designed to identify a biological indicator(s) that could be used to routinely test compost for general or specific disease suppression properties. A mature vermicompost containing hardwood bark as a carbon source has the greatest potential for suppressing *Rhizoctonia solani* on radish. This result is sufficiently robust to span a representative sample of commercial products that have all met thermophilic requirements to kill plant pathogens and weed seeds established by the National Organic Standards Board (www.ams.usda.gov/nop). Achievement of these temperatures requires feedstock blends that average 25:1 to 30:1 C:N ratio, have 55–60% moisture, and a plentiful supply of oxygen, whether it be by turning piles or forcing air through a pile.

Process

Regardless of feedstock mixture, this study supports other reports that thermophilic compost cured by vermicompost holds unique properties that foster more disease suppression than thermophilic composts cured by windrow or ASP. This has been shown not only for *R. solani* on radish (this study), but also *Pythium aphanidermatum* on cucumber (Jack 2012; Jack et al. 2011). Vermicompost has substantially different bacterial and fungal communities when compared to those from a common recipe produced by windrow, and also has much greater bacterial diversity, which

may support its ability to outcompete pathogens (Neher et al. 2013). Earthworms promote the growth of bacteria including Bacteriodetes, Verrucomicrobia, Firmicutes, and Proteobacteria. There is also a trend for greater diversity of fungi in vermicompost and a relatively high abundance of fungi including *Mortierella* and *Arthrobotrys* (Neher et al. 2013). Application rates of <5% promoted disease suppression and rates exceeding 25% promoted phytotoxicity. Vermicompost typically contains much nitrate but may have high EC that can result in germination problems and phytotoxicity in some plants (Pathma and Sakthivel 2012). In other cases, the benefits of vermicompost do not correlate with dosage, but affect plant growth indirectly possibly through plant microbial interactions (Jack et al. 2011; Zaller 2007). These organisms may manipulate plant growth by excreting exogenous microbial plant hormone analogs (Pangesti et al. 2013; Robert-Seilaniantz et al. 2011), and may further be responsible for disease suppression.

Maturity

Specific communities of saprophytic fungi and bacteria can be found at different successional stages of composting (Neher et al. 2013). Maturity or curing time is an indicator of substrate composition and microbial community, not necessarily stability (Oviedo-Ocaña et al. 2015). This study suggests that mature composts offer more disease suppression of *R. solani* than immature composts. This finding affirms other studies of *Rhizoctonia* diseases that report immature compost was conducive to disease development (Hoitink et al. 1996). Compost that had been cured for 5 months showed better suppression than compost that had been cured for 3 or 7 months (Tuitert, Szczech, and Bollen 1998). Mature composts with high chitin content contain abundant rhizosphere bacteria producing chitin and β-glucosinase that damage cell walls of fungal pathogens (Kavroulakis et al. 2010). Chitin amendments increase populations of Actinobacteria and γ-Proteobacteria (Postma and Schilder 2015).

Feedstock

Use of hardwood bark compost suppressed hyphal growth of *R. solani* in the compost extract plate assay. Pathogens, such as *R. solani*, are favored in early stages of composting when concentrations of labile carbon

are high (Chung, Hoitink, and Lipps 1988). Once labile carbons are depleted, the efficacy of biological control fungi such as *Trichoderma hamatum* increases (Chung, Hoitink, and Lipps 1988). *T. hamatum* hyperparasitizes mycelium of *R. solani* by producing cellulases, glucanases, and chitinases. Contrary to other studies, we did not find *Trichoderma* (Hypocreaceae) in our composts. However, other fungi in the Hypocreales were common at the end of the curing process, e.g., *Acremonium* (Neher et al. 2013). This supports the hypothesis that specific biocontrol organisms may not be as important as microbial consortia (Postma and Schilder 2015).

Best predictors of disease suppression

Similar to the conclusions and recommendations of Alfano et al. (2011), the plate competition assay is a quick preliminary assessment of disease suppression, but not reliable as a standalone assay. Confirmation by a greenhouse bioassay makes a more robust assessment. There is more complexity in the soil and compost ecosystem than could be mimicked entirely by a laboratory assay. Microbial communities play a significant role, as does the presence of a plant. Two other in situ measures contributed to predicting disease severity related to ecoenzyme activity and nematode communities in the soil food web.

Among all of the indicators assessed, ecoenzymes were the best potential indicator of disease suppressive compost. Ecoenzymes integrate information about environmental substrate composition, microbial nutrient acquisition, and microbial community metabolic function (Allison et al. 2007). Ecoenzymes capture the current state of microbial community metabolism, and serve as indicators of which substrates and decomposition functions are most abundant, or which nutrients are most limited. The most studied case of ecoenzymatic stoichiometry is the generally inverse relationship between phosphatase activity and environmental P availability (Chróst and Overbeck 1987).

Greater values of either nematode index (F/B, MI) reflect less disturbance or a later stage of succession (Neher 2010). In this study, F/B was responsive to compost maturity and MI affected by feedstock, but not vice versa. MI predicted disease severity of *R. solani* on radish seedlings but not F/B. Fungivorous nematodes were expected to be abundant in relatively mature composts (Steel et al. 2010; Termorshuizen et al. 2006), but few

were found in any compost samples of this study. Although Aphelenchideae were present, they were not associated with suppressing Rhizocotonia damping off on radish as reported on cauliflower (Lagerlöf et al. 2011). MI values require family identification rather than simply trophic group, perhaps increasing its sensitivity as an indicator of disease suppression. This supports the hypothesis that suppressive ability depends on a specific ecological environment (Hadar and Papadopoulou 2012; Termorshuizen et al. 2006).

Oxidative activity, or lignin degradation activity, was expected to be significant in disease suppression, representing an ecological condition favoring biological control agents over a pathogen. However, we were unable to identify significant differences among process, maturity, or feedstock. These results contrast another study suggesting the incorporation of lignin into soil reduced the viability of *R. solani* sclerotia (van Beneden et al. 2010).

Conclusions

Our study provides empirical data to support adoption of a compost extract competition assay, nematode MI, and ecoenzymes active on cellulose and chitin as indicators of disease suppression by *R. solani*. General measures of microbial respiration may measure compost stability but not necessarily disease suppression of a facultative saprophyte like *R. solani* (Bonanomi et al. 2010; Scheuerell, Sullivan, and Mahafee 2005). These indicators are quicker than plant bioassays and could be adopted as tools to certify commercial products.

References

Alfano, G., G. Lustrato, G. Lima, D. Vitullo, and G. Ranalli. 2011. Characterization of composted olive mill wastes to

predict potential plant disease suppressiveness. *Biological Control* 3:199–207.

Allison, S. 2008. Chloroform fumigation direct extraction (CFDE) protocol for microbial biomass carbon and nitrogen. University of California, Irvine. http://allison.bio.uci.edu/protocols/microbiabiomasscn.pdf (accessed October 7, 2014).

Allison, S. D., T. Gartner, K. Holland, M. Weintraub, and R. L. Sinsabaugh. 2007. Soil enzymes: Linking proteomics and ecological process. In *Manual of environmental microbiology*, ed. by C. Hurst, R. Crawford, J. Garland, D. Lipson, A. Mills, and L. Stetzenbach, 704–11. Washington, DC: American Society of Microbiology Press.

Bonanomi, G., V. Antignani, M. Capodilupo, and F. Scala. 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biology and Biochemistry* 42:136–44.

Bonanomi, G., V. Antignani, C. Pane, and E. Scala. 2007. Suppression of soilborne fungal diseases with organic amendments. *Journal of Plant Pathology* 89:311–24.

Bongers, T. 1987. *De Nematoden van Nederland*. Schoorl, Netherlands: Pirola.

Bongers, T. 1990. The maturity index: An ecological measure of environmental disturbance based on nematode species composition. *Oecologia* 83:14–19.

Brinton, W. F. J., E. Evans, M. L. Droffner, and R. B. Brinton. 1995. Standardized test for evaluation of compost self-heating. *Biocycle* 36:64–68.

Castaldi, P., G. Garau, and P. Melis. 2008. Maturity assessment of compost from municipal solid waste through the study of enzyme activities and water-soluble fractions. *Waste Management* 28:534–40.

Chan, S., B. Caldwell, B. Rickard, and C. Mohler. 2011. Economic performance of organic cropping systems for vegetables in the northeast. *Journal of Agribusiness* 29:59–82.

Chung, Y. R., H. A. J. Hoitink, and P. E. Lipps. 1988. Interactions between organic-matter decomposition level and soilborne disease severity. *Agriculture Ecosystems and Environment* 24:183–93.

Chróst, R. J., and J. Overbeck. 1987. Kinetics of alkaline phosphatase and phosphorus availability for phytoplankton and bacterioplankton in lake plussee. *Microbiology Ecology* 13:229–48.

Diab, H. G., S. Hu, amd D. M. Benson. 2003. Suppression of Rhizoctonia solani on Impatiens by enhanced microbial activity in composted swine waste-amended potting mixes. *Phytopathology* 93:1115–23.

Ersahin, Y. S., K. Haktanir, and Y. Yanar. 2009. Vermicompost suppresses *Rhizoctonia solani* Kühn in cucumber seedlings. *Journal of Plant Disease Protection* 116:182–88.

Fang, L. 2015. Biological indicators of compost-mediated disease suppression against the soilborne plant pathogen. *Rhizoctonia solani*. MS thesis, University of Vermont.

Gómez, R. B., F. V. Lima, and A. S. Ferrer. 2006. The use of respiration indices in the composting process: A review. *Waste Management Research* 24:37–47.

González García, V., M. A. Portal Onco, and V. Rubio Susan. 2006. Review. biology and systematics of the form genus Rhizoctonia. *Spanish Journal of Agricultural Research* 4:55–79.

Goyal, S., S. K. Dhull, and K. K. Kapoor. 2005. Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. *Bioresource Technology* 96:1584–91.

Grosch, R., T. Koch, and A. Kofoet. 2004. Control of bottom rot on lettuce caused by Rhizoctonia solani with commercial biocontrol agents and a novel fungicide. *Journal of Plant Diseases and Protection* 111:572–82.

Hadar, Y., and K. Papadopoulou. 2012. Suppressive composts: Microbial ecology links between abiotic environments and healthy plants. *Annual Review of Phytopathology* 50:133–53.

Hoitink, H. A. J., and M. J. Boehm. 1999. Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon. *Annual Review of Phytopathology* 37:427–46.

Hoitink, H. A. J., A. G. Stone, and M. E. Grebus. 1996. Suppression of plant diseases by compost. In *The science of composting*, ed. by M. de Bertoldi, P. Sequi, B. Lemmes, T. Papi, 373–81. Dordrecht, The Netherlands: Springer.

Horsfall, J. G., and E. B. Cowling. 1978. Pathometry: The measurement of plant diseases. In *Plant Disease: An Advanced Treatise. Vol. II: How Disease Develops in Populations*, ed. J. G. Horsfall and E. B. Cowling, 119–36. New York, NY: Academic Press.

Jack, A. 2012. Vermicompost suppression of *Pythium aphanidermatum* seedling disease: Practical applications and an exploration of the mechanisms of disease suppression. PhD diss., Cornell University.

Jack, A. L. H., A. Rangarajan, S. W. Culman, T. Sooksa-Nguan, and J. E. Thies. 2011. Choice of organic amendments in tomato transplants has lasting effect on bacterial rhizosphere communities and crop performance in the field. *Applied Soil Ecology* 48:94–101.

Kavroulakis, N., S. Ntougias, M. I. Besi, P. Katsou, A. Damaskinou, C. Ehaliotis, G. I. Zervakis, and K. K. Papadopoulou. 2010. Antagonistic bacteria of composted agro-industrial residues exhibit antibiosis against soil-borne fungal plant pathogens and protection of tomato plants from *Fusarium oxysporum* f.sp. *radicis-lycopersici*. *Plant and Soil* 333:233–47.

Klein, E., M. Ofek, J. Katan, D. Minz, and A. Gamliel. 2013. Soil suppressiness to Fusarium disease: Shifts in root microbiome associated with reduction of pathogen root colonization. *Phytopathology* 103:23–33.

Komilis, D. P., and I. S. Tziouvaras. 2009. A statistical analysis to assess the maturity and stability of six composts. *Waste Management* 29:1504–13.

Kuter, G. A., H. A. J. Hoitink, and W. Chen. 1988. Effects of municipal sludge compost curing time on suppression of *Pythium* and *Rhizoctonia* diseases of ornamental plants. *Plant Disease* 72:751–56.

Lagerlöf, J., V. Insunza, B. Lundegårdh, and B. Rämert. 2011. Interaction between a fungal plant disease, fungivorous nematodes and compost suppressiveness. *Acta Agriculturae Scandinavica: Section B, Soil and Plant Science* 61:372–77.

Mathout, P. 1987. Emploi de composts dans la lutte contre Rhizoctonia sp. Meded. *Fac. Land Wetensch. Rijks. Gent* 52:1127–32.

Moorhead, D. L., Z. L. Rinkes, R. L. Sinsabaugh, and M. N. Weintraub. 2013. Dynamic relationships between microbial biomass, respiration, inorganic nutrients, and enzyme activities: Informing enzyme-based decomposition models. *Frontiers in Microbiology* 4:1–12.

Neher, D. A. 2010. Ecology of plant and free-living nematodes in natural and agricultural soil. *Annual Review of Phytopathology* 48:371–94.

Neher, D. A., T. R. Weicht, S. T. Bates, J. W. Leff, and N. Fierer. 2013. Changes in bacterial and fungal communities across compost recipes, preparation methods, and composting times. *PLOS One* 8:e79512.

Nelson, E. B., and H. A. J. Hoitink. 1983. The role of microorganisms in the suppression of Rhizoctonia solani in container media amended with composted hardwood bark. *Phytopathology* 73:274–8.

Oviedo-Ocaňa, E. R., P. Torres-Lozada, L. F. Marmoloejo-Rebellon, L. V. Hoyos, S. Gonzales, R. Barrena, D. Komilis, and A. Sanchez. 2015. stability and maturity of biowaste composts derived by small municipalities: Correlation among physical, chemical and biological indices. *Waste Management* 44:63–71.

Pane, C., A. Piccolo, R. Spaccini, G. Celano, D. Villecco, and M. Zaccardelli. 2013. Agricultural waste-based composts exhibiting suppressivity to diseases caused by the phytopathogenic soil-borne fungi *Rhizoctonia solani* and Sclerotinia minor. *Applied Soil Ecology* 65:43–51.

Pane, C., R. Spaccini, A. Piccolo, F. Scala, and G. Bonanomi. 2011. Compost amendments enhance peat suppressiveness to *Pythium ultimum, Rhizoctonia solani*, and *Sclerotinia minor. Biological Control* 56:115–24.

Pangesti, N., A. Pineda, C. M. J. Pieterse, M. Dicke, and J. J. A. van Loon. 2013. Two-way plant-mediated interactions between root-associated microbes and insects: From ecology to mechanisms. *Frontiers in Plant Science* 4:414. doi: 10.3389/fpls.2013.00414

Pathma, J., and N. Sakthivel. 2012. Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. *SpringerPlus* 1:26.

Postma, J., and M. T. Schilder. 2015. Enhancement of soil suppressiveness against *Rhizoctonia solani* in sugar beet by organic amendments. *Applied Soil Ecology* 94:72–79.

Robert-Seilianiantz, A., M. Grant, and J. D. G. Jones. 2011. Hormone crosstalk in plant disease and defense: More than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology* 49:317–43.

Saiya-Cork, K. R., R. L. Sinsabaugh, and D. R. Zak. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology and Biochemistry* 34:1309–15.

Santos, M., F. Diánez, M. González del Valle, and J. C. Tello. 2008. Grape marc compost: Microbial studies and suppression of soil-borne mycosis in vegetable seedlings. *World Journal of Microbiology and Biotechnology* 24:1493–505.

Scheuerell, S. L., D. M. Sullivan, and W. F. Mahafee. 2005. Suppression of seedling damping-off caused by *Pythium ultimum, P. irregulare*, and *Rhizoctonia solani* in container media amended with a diverse range of pacific northwest compost sources. *Phytopathology* 95:306–15.

Smith, J. L., and J. W. Doran. 1996. Measurement and use of pH and electrical conductivity for soil quality analysis. In *Methods for Assessing Soil Quality*, ed. J. W. Doran and A. J. Jones, 169–85. Madison, Wisconsin: Soil Science Society of America Special Publication 49.

Steel, H., E. de la Peña, P. Fonderie, K. Willekens, G. Borgonie, and W. Bert. 2010. Nematode succession during composting and the potential of the nematode community as an indicator of compost maturity. *Pedobiologia* 53:181–90.

Strange, R. N., and P. R. Scott. 2005. Plant disease: A threat to global food security. *Annual Review of Phytopathology* 43:83–116.

Termorshuizen, A. J., E. van Rijn, D. J. van der Gaag, C. Alabouvette, Y. Chen, J. Logerlöf, A. A. Malandrakis, et al. 2006. Suppressiveness of 18 composts against 7 pathosystems: Variability in pathogen response. *Soil Biology and Biochemistry* 38:2461–77.

Thompson, W. H., P. B. Leege, P. D. Millner, and M. E. Wilson. 2001. *Test methods for the examination of composting and compost.* Holbrook, NY: United States Composting Council Research and Education Foundation.

Tuitert, G., M. Szczech, and G. J. Bollen. 1998. Suppression of *Rhizoctonia solani* in potting mixtures amended with compost made from organic household waste. *Phytopathology* 88:764–73.

USDA-ERS. 2016. Overview—Vegetables and pulses. http://www.ers.usda.gov/topics/crops/vegetables-pulses.aspx (accessed October 13, 2016).

van Beneden, S., D. Roobroeck , S. C. Franca, S. De Neve, P. Boeckx, and M. Hofte. 2010. Microbial populations involved in the suppression of Rhizoctonia solani AG1-1B by lignin incorporation in soil. *Soil Biology and Biochemistry* 42:1268–74.

Wichuk, K. M., and D. McCartney. 2010. Compost stability and maturity evaluation—A literature review. *Canadian Journal of Civil Engineering* 37:1505–23.

Yeates, G. W., T. Bongers, R. G. M. de Goede, D. W. Freckman, and S. S. Georgieva. 1993. Feeding habits in soil nematode families and genera—An outline for soil ecologists. *Journal of Nematology* 25:315–31.

Zaller, J. G. 2007. Vermicompost in seedling potting media can affect germination, biomass allocation, yields and fruit quality of three tomato varieties. *European Journal of Soil Biology* 43:S332–36.