Biological indicators and compost for managing plant disease

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Abstract.

Compost can limit disease through thermophilic exposure, release of toxic products and/or microbial antagonists that colonize compost during the cooling, curing stage. Mature compost contains microorganisms that can promote plant growth by mineralizing nutrients, producing plant hormone imitations, or secreting antibiotics to defend against other microorganisms. As indicators, soil communities both integrate soil chemical and physical properties, and reflect the status of ecological processes including disease suppression. General microbial activity or biomass measured as respiration or phospholipids are simple indicators but difficult to interpret. Instead, indicators that reflect ecological succession or compost maturity are better predictors of disease suppression. Emerging tools that measure competition, ecoenzymes, and functional diversity are relatively quick assays that provide ecological insights beyond general measures of activity or biomass. These tools reflect composition of soil communities and predict their potential to suppress soilborne pathogens. These indicators are quicker than plant bioassays and could be adopted as tools to certify commercial products.

Keywords

Competition plate, disease suppression, ecoenzymes, ecological succession, EcoPlates™, fungi to bacteria ratio, maturity index, PLFA

INTRODUCTION

Compost is the product of a controlled aerobic process that degrades organic waste to stable material with the resident microbial community mediating the biodegradation and conversion processes (Neher et al., 2013). The product has undergone mesophilic and thermophilic temperatures, which significantly reduces the viability of pathogens and weed seeds (Neher et al., 2015) and stabilizes the carbon such that it is beneficial to plant growth (Wichuk and McCartney, 2010). Composition of microbial communities starts similarly after the sanitation phase and the composition of the community changes as the temperature declines and the chemistry of the compost changes (Neher et al., 2013). The product resulting from proper composting techniques is a nutrient-rich substrate that promotes plant growth and supports a diversity of microbial life.

Companies want shelf-ready tools and products and the marketplace offers numerous packaged microbial products. Even when approved products are available commercially, consumers are skeptical whether they are effective or provide return on investment. Intensifying research on compost microbiology not only provides insights on basic biology of the mechanisms of disease suppression but also adds disclosure of product properties to consumers. Establishing standards for compost recipes, processing, and markets would provide metrics to increase confidence that products are safe in the food chain, protect the environment from unintentional contamination, and provide ecosystem services that protect plants and increase yields. Relative consistency in product and product grade is a win-win proposition by not only defining product uses and economic value but also provides composters a potential tool that confirms a 'quality seal' brand for their commercial product.

Need for consistent and defined products

Microorganisms are abundant and their communities diverse in compost. Beneficial microbes compete with and antagonize pathogens to keep them at bay. An extensive study of 120 bioassays involving 18 composts and seven pathosystems found positive disease suppression in 54 percent of the treatment combinations, a disease stimulating effect only rarely (3%), and no effect in 43 percent of the treatment combinations (Termorshuizen et al., 2006). Suppressive properties are unique to each pathosystem (Bonanomi et al., 2018) implying the need for "designer composts".

Generally, we have a poor understanding of the microbiological dynamics that occur during the composting process. There are also no current regulations or guidelines that define desirable microbiological properties of compost. Most studies have utilized culture-based methods that only capture a small portion of the microbial diversity found in environmental samples. My pioneering study that used high-throughput sequencing techniques showed that compost recipe, choice of post-thermophilic process, and duration of curing (maturation) of composts influences the assembly of bacterial and fungal communities (Neher et al., 2013).

Developing consistent products with disease suppressive properties demands a better understanding of the microbiology of composting. With a better understanding, we can learn the pivotal points where it can be managed to enhance disease suppressiveness. This knowledge will elucidate which recipe and post-thermophilic practices are best to develop compost for more reliable strategies to manage ubiquitous and difficult to manage root pathogens more sustainably than the biopesticide products on the market. Moreover, this information will inform how to test compost for suppressiveness. As examples, this review will focus on three fungal soilborne pathogens, globally distributed, and famous for their broad host range, *Rhizoctonia solani* (teleomorph *Thanatephora cucumeris*), *Phytophthora* spp., and *Pythium* spp.

Commercial composting guidelines require a high-temperature phase designed to facilitate the removal of human and plant pathogens. However, these requirements stop short of guidelines for compost curing (cooling) in the post-thermophilic phase. This curing phase offers a substrate and climate conducive for microbial recolonization that is expedited by inoculating post-thermophilic compost or preparing a palatable substrate that provides a competitive advantage for colonization by bacteria and fungi that offer biological control. Plant disease suppression is the result of the activity of antagonistic microorganisms that naturally recolonize the compost during the cooling phase of the process (Hadar and Papadopoulou, 2012).

Key factors of suppressiveness

Disease suppression is mediated by microbial communities, both microbe-microbe and microbe-plant interactions. Microbes naturally produce chemical defenses to antagonize other microbes. Likewise, biocontrol fungi and bacteria can suppress disease-causing pathogens through competition, antagonism, or regulating plant growth. Competition (for nutrients, trace elements, or colonization sites) occurs when taxa have differential efficiencies in utilizing specific sources of nitrogen or carbon compounds in the same space and time. Production of siderophores, nutrient chelators, is a means of increasing competition for nutrients. One mechanism of antagonism is production of antibiotics (against bacteria or fungi) (Noble, 2011). Antibiotics secreted in low concentrations can mediate intercellular signaling (communication), and in high concentrations can inhibit pathogen growth (Neher and Hoitink, 2021).

Compost-mediated disease suppression ranges from specific to general. Specific suppression is provided by activities of a narrow spectrum of one or a few specific populations of beneficial microorganisms of which some do not colonize composts. General suppression results from the collective activity of many species of microorganisms in field or potting soils. Companies that formulate, produce and market specific antagonistic strains of microorganisms take advantage of specific suppression. However, no single species by itself is responsible for general suppression (Bonanomi et al., 2018). General suppression relies on the activity and interaction among bacterial and fungal communities (Postma and Shilder, 2015), and their chemical communication with the plant (van Dam and Bouwmeester 2016).

For example, an individual of a popular biocontrol fungus (i.e., *Trichoderma harzianum*) is barely detectable with high throughput sequencing but a multitude of many other species in the same order (Hypocreaceae) are abundant (Neher et al., 2013), collectively creating a consortium.

1. Initial feedstocks.

Different composted material supports distinctly different bacterial and fungal communities as characterized by high throughput sequencing in dairy manure composted with different quality of carbon (Neher et al., 2013). Dairy manure itself is the likely source of relatively high abundances of bacteria in phyla Proteobacteria and Bacteroidetes (Neher et al. 2013). Within Proteobacteria, the γ-proteobacteria are more abundant than α-proteobacteria, β-proteobacteria, and δ-proteobacteria. Bacteria in the phylum Bacteriodetes, classes Flavobacteriia (*Flavobacterium* spp.) and Spingobacteriales (*Fluviicola*, *Flavobacteriia*, and *Pedobacter*), are two to four times more abundant in soils amended with poultry litter compost than dairy manureost comp (Neher et al., 2020). Relative dominance of fungal phyla depends on the type of animal manure. Specifically, dairy manure and poultry litter composts contained relatively large abundance of fungi in the phyla Ascomycota and Zygomycota, respectively (Neher et al., 2020). Zygomycota are considered earlier successional species than Ascomycota.

There is empirical evidence that poultry litter composts are conducive to both *R. solani* and *E. coli* pathogens. These microbial community differences were apparent in thrice-repeated field experiments (Neher et al., 2020). Controlled laboratory experiments demonstrated that the presence of indigenous microbial communities in soil help keep population growth of a pathogen in check (Neher et al., 2019). Furthermore, greenhouse experiments, suggest poultry litter composts can decrease water infiltration of soil resulting in anerobic conditions (Readyhough, 2021). These experiments challenge the prevailing explanation that poultry composts perform differently than dairy composts simply because of their high ammonium content. Clearly, the situation is far more complex.

Altering sources or types of carbon, while holding the source of manure constant, also changes composition of bacterial and fungal communities. For example, hardwood carbon supports relatively abundant bacterial taxa within the Acidobacteria and Verrucomicrobia phyla, and hay carbon is favorable habitat for Actinobacteria and Gemmatimonadetes. In contrast, dairy bedding and hay contained greater relative abundances of Firmicutes than hardwood. Carbon originating from dairy bedding or hay contain relatively more of the fungal phyla Chytridomycota and Zygomycota (especially Mortierellales and Mucorales) than hardwood carbon (Neher et al., 2013). In contrast, tree bark colonizing fungi, e.g., Sordariomycetes and Agaricomycetes, are more common in compost containing hardwood than compost with softwood or hay.

One generalization is that the most reliably disease-suppressive composts are those made with woody materials, like tree bark, wood chips and woody yard trimmings. For example, in a field experiment, natural epidemics of early blight (caused by fungal pathogen *Alternaria brassicinae*) were more severe in brassica crops when planted in hay than hardwood mixtures (Neher et al., 2015). Tree bark and other woody materials consist mostly of lignin, cellulose, tannins and waxes, a mixture that resists decomposition. After composting, the disease suppressive effects of composted barks last for several years in soil, depending on the tree species from which the bark was removed and how much compost was added to the soil. Decomposition of wood waste releases nutrients very slowly and produces humic acids (large molecular weight organic acids that are very complex and difficult to degrade). In contrast, food and feed wastes, animal manures and biosolids mostly consist of readily decomposable compounds and nutrients and produce fulvic acids (low molecular weight organic acids). Both fulvic and humic acids grab and bind (chelate) essential micro-nutrients and keep them available for uptake by plants. Chelates can strongly mediate the severity of diseases caused by soilborne plant pathogens. However, these beneficial effects usually do not last more than one or two years in soils.

2. **Maturity.**

Managing carbon quality and compost maturity are the tools that one has to manage the colonization of compost with microorganisms that will favor disease suppression. Biological control organisms can grow effectively on both immature and mature products but shift to become relatively more or less competitive against pathogens depending on the particular substrates and the disease. A classic example is for pathogens like *Rhizoctonia solani* (teleomorph *Thanatephorus cucumeris*) that are favored in early stages of composting when concentrations of water-soluble carbon compounds are high (Chung et al., 1988). The efficacy of biological control fungi such as *Trichoderma hamatum* increases as compost matures and the ratio of cellulose to lignin decreases. Wood-based carbon has lower ratios of cellulose to lignin than hay or straw-carbon based composts.

With the exception of *Pythium* and *Phytophthora* diseases, different compost recipes and maturity affect pathogen(s) and host crop(s) differently. Compost naturally suppresses oomycota pathogens, *Pythium* and *Phytophthora* (Chung et al., 1988) but only 20 percent naturally suppresses *Rhizoctonia* (Voland and Epstein, 1997). Diseases with saprophytic phases such as *Rhizoctonia* are more difficult to control. However, disease caused by *R. solani* are more likely suppressed by mature (Scheuerell et al., 2005; Coventry et al., 2006) and immature compost is conducive to disease development (Kuter et al., 1988; Hoitink et al., 1996). Mature composts have greater C:N and lignin: cellulose ratios, and slow-release of nutrients than immature composts.

At the other end of the spectrum, composts that are excessively stable or fully decomposed after months or years of decomposition in soil no longer have the ability to support populations of beneficial organisms. As beneficial organism populations decline, plant pathogens increase in numbers and activity and diseases increase in severity in the now "worn out" soil, even though humic acids produced from the compost are still present in the soil. For this reason, highly stabilized organic matter, such as peat, is not effective in controlling plant pathogens unless new sources of stable organic matter are added.

3. **Curing.**

When carbon quality is held constant, the post-thermophilic compost curing method also alters the bacterial and fungal community composition (Neher et al., 2013). This conclusion is based on an experiment with a common recipe and process of composting through the recommended thermophilic stage after which compost was cured by three methods: windrow, aerated static pile, and vermicompost. The bacterial and fungal community composition changed uniquely by curing method (Neher et al., 2013).

Experiments conducted by two independent research groups demonstrated that thermophilic compost cured by vermicompost holds unique properties that foster disease suppression of *R. solani* on radish (Neher et al., 2017) and *Pythium aphanidermatum* on cucumber (Jack et al., 2011; Jack, 2012). The specific vermicompost tested was prepared from a consistent source of dairy manure bedding and was produced by a two-step process where materials were first composted past the thermophilic stage, then fed to compost worms (*Eisenia fetid*a) for maturation. A mature vermicompost has the greatest potential for suppressing *R. solani* on radish (Figure 1; Neher et al. 2017).

A mature vermicompost has substantially different bacterial and fungal communities when compared to those from a common recipe produced by windrow, and has much greater bacterial diversity, which may support its ability to outcompete pathogens (Neher et al., 2013). Earthworms (*Eisenia fetida*) promote the growth of bacteria Verrucomicrobia and γ-Proteobacteria, and *Arthrobotrys* and *Mortierella* fungi (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 electrical conductivity (salinity) 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-microbe interactions (Zaller, 2007; Jack et al., 2011). These organisms may manipulate plant growth by excreting

exogenous microbial plant hormone analogs (Robert-Seilaniantz et al., 2011; Pangesti et al., 2013), and may further be responsible for disease suppression.

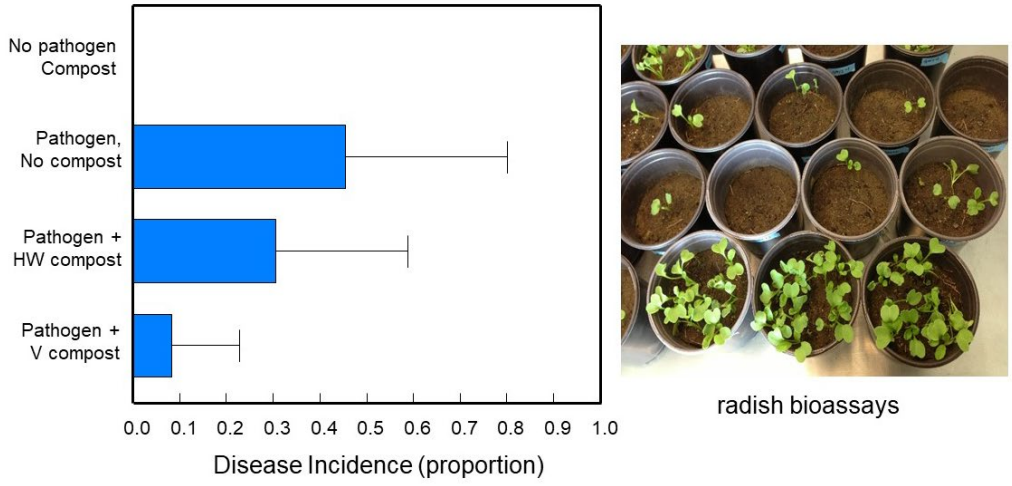

Figure 1. Incidence of damping-off caused by fungal pathogen, *Rhizoctonia solani*, in radish bioassay (right). Treatments were compost without pathogen, pathogen without compost, pathogen with hardwood (HW) compost applied at 22.4 metric tons ha-1 (10 tons acre-1) and thermophilic compost cured by vermicompost (V) applied at 2.24 metric ton ha-1 (1 ton acre-1).

Promoting plant growth

Plants regulate microbial communities by altering the quantity and quality of root exudates (Garbeva et al., 2004; Raaijmakers et al., 2009; Lakshmanan, 2015; van Dam and Bouwmeester, 2016). Root exudates account for a stunning 20-40% of plant carbon metabolism (Bais et al., 2006). The most abundant root exudates are sugars, but also include a wide variety of low molecular weight metabolites, ranging from the highly water-soluble amino acids, organic acids, and glycosides to more lipophilic phenolics, terpenoids, and alkaloids. A direct attack by pathogens stimulates the plant to release stress chemical signals consisting of phenolic compounds (e.g., coumaric, cinnamic, salicylic acids) or saponins (glycosides with triterpene or steroid backbones) into the rhizosphere (Chapelle et al., 2015). Mechanistically, it has been proposed that *R. solani* is an invading pathogenic fungus that induces, directly or via the plant, stress responses in the rhizosphere bacterial community (Chapelle et al., 2015). As a result, the microbiome composition shifts and activates antagonistic traits that restrict the pathogen infection and, ultimately, protect the roots from infection. Root associated bacteria can distinguish among their neighbors and fine-tune the biosynthesis of antimicrobial metabolites. These types of natural suppression are highly coordinated events influenced by the plant host and soil (Bais et al., 2006; Chapelle et al., 2015). It is only recently that scientists have the tools to solve the mysteries of how these highly co-evolved relationships work (van Dam and Bouwmeester, 2016) so we can incorporate those insights into management practices.

Phenolic compounds stimulate germination of fungal conidia in low concentrations and inhibit fungal growth in high concentrations. Mal-timed chemical signals can trick fungal pathogens into germination in unfavorable conditions disarming them from a successful infection. Indirectly, root exudates stimulate microorganisms to produce small water-soluble molecules, called volatile organic compounds (VOCs). The type and temporal dynamics of VOC production are extremely species-specific, at least for *Trichoderma* that produces a diversity of sesquiterpene emission patterns (Guo et al., 2020). VOCs evaporate easily at room temperature and distribute into the surrounding air, enabling them to act as communication

signals within and among organisms. When the density of these molecules exceeds a certain threshold (measured as parts per trillion with modern instrumentation), it triggers a coordinated community response (quorum sensing) that activate various plant defense related genes that either suppress disease symptoms or stimulate plant growth. Microbial species promote plant growth by production of antibiotics to combat pathogens, manufacturing plant growth mimics, and/or induced systemic resistance (ISR) that protects non-infected tissues throughout the plant. ISR in plants by compost occurs in cucumber Pythium root rot (i.e., *Pythium ultimum* and *Pythium aphanidermatum*). Traditionally, the role of VOCs was overlooked partly due to analytical limitations. With modern tools, we are likely to gain knowledge on they operate ecologically and are modified by soil type and irrigation events (Leff and Fierer, 2008; Rossabi et al., 2018) and how the can be harnessed as a useful tool to enhance crop defense and production (Brilli et al., 2019).

Mature, thermophilic composts cured by vermicompost (VC) prompt a greater growth response in tomato seedlings than dairy manure compost when incorporated into growing media and induces longer lasting change in the soil microbial community after transplant (Jack et al., 2011). Although VC appears to be a promising amendment to promote healthy vegetable starts and suppress disease, we still have a limited understanding of the microbial composition of manure-derived fertilizers and how these communities affect plant growth directly or indirectly through alteration of the rhizosphere microbiome. Not until we understand these biological dynamics will we be able to establish guidelines that define desirable microbiological properties of compost amendments.

Indicators of stability

Disease suppression is best tested by plant bioassays (Wichuk and McCartney, 2010) such as germination, phytotoxicity (response to abiotic factors, maturity) and disease suppression. 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. Besides, these assays do not consider the bacterial and fungal communities altered by compost amendments. Ideally, methods should reflect a composite of species and mechanisms and not require a specialist and expensive analytical equipment. Promising emerging tools include 1) competition plate assays (Pane et al., 2013; Neher and Weicht, 2018), 2) ecoenzymes (Neher et al., 2017), and/or 3) physiological profiles using Biolog EcoPlates™ that screen for utilization of 31 carbon types and define functional species (Scotti et al., 2020; Wright, 2020).

1. Microbial biomass and activity.

Simple measures of microbial activity or biomass predict *Pythium ultimum* and *Pythium irregulare* but not *R. solani* (Scheuerell et al., 2005). Compost analytical labs have a variety of measures to reflect microbial activity by respiration (CO_2 evolution) by dehydrogenase, Solvita™ test, and/or hydrolysis of fluorescein diacetate (FDA) (Green et al., 2006). Advantages of these methods are their simplicity and rapid response. However, the methods are criticized for imprecision and weak associations with populations of known biological control agents such as fluorescent *Pseudomonas* spp. and *Trichoderma* spp. (Pane et al., 2013; Scotti et al., 2020). FDA has been a tool that works to predict suppression of Oomycota pathogens (e.g., *Pythium* and *Phytophthora*) but not necessarily fungal soilborne pathogens (Hadar and Papadopoulou, 2012). Simple activity or biomass is too simple and difficult to interpret.

2. Competition plate assays.

Antibiosis activity on plate assays are effective tests for *R. solani* (Neher and Weicht, 2018) and *Fusarium* (Borrero et al., 2006). Suppressive colonies create a visible zone of inhibition around the pathogen colony. These assays consistently differentiate among curing process, maturity, and feedstock material as a function of disease severity of a seedling bioassay (Neher et al., 2017). The plate competition assay is a quick preliminary assessment of disease

suppression, but not reliable as a standalone assay (Alfano et al., 2011; Neher et al., 2017). 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.

3. Microbial ecoenzymes.

Compost microbial communities are saprophytes, decomposing organic compounds for their own energy and nutrients. These microbes are osmotrophs, which means they exude ecoenzymes outside their cell, where digestion takes place, and dissolved food is absorbed (Sinsabaugh and Follstad Shah, 2012). Each species of fungus and bacteria produces a unique suite of ecoenzymes, and each ecoenzyme has built-in precision to cleave compounds at specific types and locations of chemical bonds. 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 phosphorus availability (Chróst and Overbeck, 1987). Ecoenzymes integrate information about environmental substrate composition, microbial nutrient acquisition, and microbial community metabolic function (Allison et al., 2007).

Ecoenzymes active on chitin and cellulose are better predictors of disease suppressiveness by fungal pathogens than microbial respiration (Neher et al., 2017). Mature composts with high chitin content contain abundant rhizosphere bacteria producing chitin and β-glucosinase (Postma and Schilder, 2015) that damage cell walls of fungal pathogens such as *Rhizoctonia*, *Fusarium*, and *Verticillium* (Kavroulakis et al., 2010). However, Oomycota *Phytophthora* and *Pythium* have cellulose in their cell walls instead of chitin. Ecoenzymes track the temporal change bacterial and fungal communities through the composting process (Neher et al., 2015) and are a relatively promising indicator of disease suppressive compost (Neher et al., 2017).

Oxidative activity, or lignin degradation activity, is expected to be significant in disease suppression, representing an ecological condition favoring biological control agents over a pathogen. However, we are unable to identify significant differences in oxidase activity in composts that vary among process, maturity, or feedstock (Neher et al., 2017). In contrast, another study suggests the incorporation of lignin into soil reduces the viability of *R. solani* sclerotia (van Beneden et al., 2010).

4. EcoPlates™.

Many biological control mechanisms exist among members of Actinobacteria including antibiotics, volatile organic compounds, and competition mechanisms that suppress the growth of pathogens (Palaniyandi et al., 2013). The practice of anaerobic soil disinfestation (ASD) as a means of disease suppression significantly increased the abundance and altered the composition of actinobacterial communities (Figure 2). 'Functional species' of actinobacteria were classified by which and how many of 31 carbon substrates of the Biolog EcoPlates™ were metabolized. These metabolic profiles are potentially useful in screening differences among compost recipes and communities of relative suppressiveness.

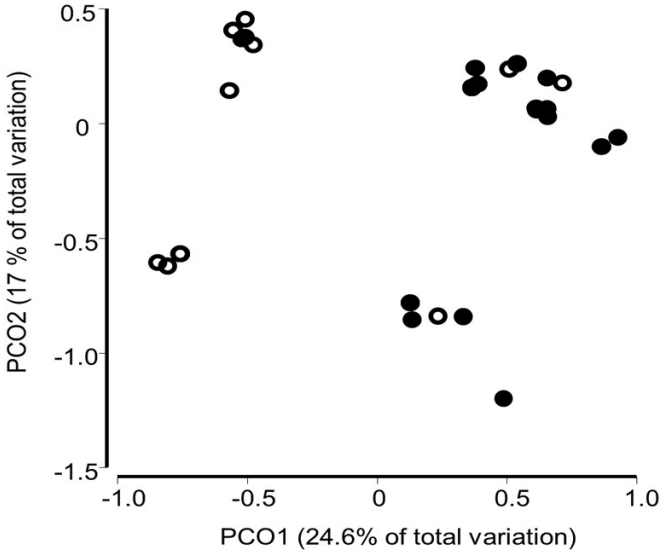

Figure 2. Principal Coordinates Analysis biplot of the Actinobacteria from soil treated with Anaerobic Soil Disinfestation (ASD; open) and unamended soil (Soil; closed) into 29 functional species based on relative similarity in utilization of 31 carbon sources from Biolog EcoPlates™ plates. Distance between samples is expressed as Euclidian (*Pseudo-F* = 7.64, *P(perm)* = 0.001). Source: Wright (2020).

5. Ecological succession.

Diversity indices are popular because they are easy to calculate. However, significant semantic, conceptual, and technical problems limit their usefulness as indicators of soil condition. Alternatively, a measure of ecological succession is more informative. This approach is based on the principle that different taxa have contrasting sensitivities to stress or disruption of the successional sequence because of their life-history characteristics. Measures of ecological succession are measures of compost maturity.

Immature compost corresponds with early stages in succession that favor microbial species that are most competitive when simple carbohydrates are abundant, earning the ecological title, copiotrophs. *Pythium* and *Phytophthora* species are examples of pathogens that thrive in high nutrient conditions and easily degradable simple carbohydrates (e.g., sugars). Mature compost corresponds with later succession that favor species that are most competitive with complex carbohydrates (e.g., lignin, tannins) earning the ecological title, oligotrophs. The ratio of oligotrophic to copiotrophic organisms increases through maturation. *R. solani* is an example because it can also metabolize starches and cellulose, both of which are abundant in compost (Scotti et al., 2020). However, the biological control *Trichoderma harzianum* is more competitive than *R. solani* on these complex substrates (Chung et al., 1988).

Indicators of ecological succession can be at the microbial or microbial grazer position in soil food webs. Values of fungi to bacteria (or fungivorous to bacterivorous nematodes) ratios and maturity indices of nematode communities are measures of ecological succession (Neher and Darby, 2006). Soil fauna have advantages over soil microbes as bioindicators. First, by being one or two steps higher in the food chain, they serve as integrators of physical, chemical, and biological properties related with their food resources. Second, their generation time (days to years) is longer than metabolically active microbes (hours to days), making them more stable temporally and not simply fluctuating with ephemeral nutrient flushes (Neher, 2001). Both types of indices are useful to monitor compost maturity. Values of both types of indices are significantly greater during curing then during the thermophilic and mesophilic phases (Steel et al., 2010; Neher et al., 2017).

Phospholipid fatty acid (PLFA) is a cost-effective way to obtain data for a ratio of fungi to bacteria and has become one of the most popular methods for measuring microbial biomass and broad community structure (Frostegård et al., 2011). There is some debate over the taxonomic groupings and the method is not very sensitive to compost types (Figure 3). This method is available as a service by commercial labs, making this approach available to non-specialists. In contrast, nematode community indices are more sensitive and effective in differentiating compost quality but require a specialist capable of identifying nematodes to genus. A nematode maturity index predicted disease severity of *R. solani* on radish seedlings better than a ratio of fungivorous to bacterivorous nematodes (Neher et al., 2017). To my knowledge, only one commercial laboratory performs nematode community assays (Earthfort, www.earthfort.com/).

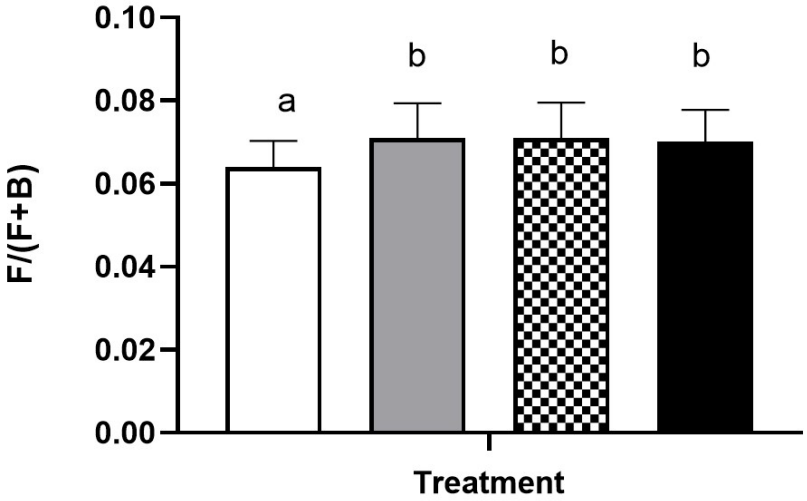

Figure 3. Ratio of fungal to bacterial membrane fatty acids. The fungi numerator is the sum of fatty acids associated with arbuscular mycorrhizae and other fungi. The denominator is the sum of the fungal numerator, and fatty acids associated with gram negative bacteria, gram positive bacteria, anaerobes, and actinobacteria. Soils were unamended (white, n = 35) or amended with dairy-windrow (gray, n = 35), dairy-vermicompost (stippled, n = 36), or poultry litter (black, n = 34) compost. Means and standard deviations of two fields across five time points. Adapted from Cutler (2016).

Finding the right consortium

By intentionally designing recipes and curing methods, compost can become a tool to manipulate or deliver a natural consortium of microorganisms in soil, onto seeds, and planting materials. Rather than single species, consortia are communities that mimic general disease suppression in soil. The advantage of assembling microorganisms with complementary or synergistic traits is to provide a more effective and consistent effect. For example, a consortium containing both *Flavobacterium* and *Chitinophaga* conferred significant and more consistent protection against fungal root infection than individual consortium members (Scotti et al., 2020).

The next scientific challenge is to find or select the right players of a consortium. Scientists are starting to reveal taxonomy and functional diversity yet know little about the ecological function of these microbes (Lugtenberg and Kamilova, 2009) and the operative mechanism(s) of how compost microbes suppress plant pathogens and disease. Amplicon sequencing is a useful tool to define community composition but identifying the mechanisms requires metagenomic analysis of functional genes. Unfortunately, it is neither practical nor affordable for practitioners to run DNA tests to look at specific consortia of bacterial and fungal species.

However, given more intensive research to identify key groups of taxa, kits could be developed and marketed that make these methods available to a non-specialist.

A case study investigation of *R. solani* is underway with the aim of identifying OTUs of fungi and/or bacteria that have suppressive function. However, these types of experiments need to be replicated for different diseases and soil types. Briefly, simple greenhouse experiments use pearl radish (*Raphanus sativus*) as a plant bioassay for disease suppression (Neher et al., 2017). Radishes were chosen for their short generation time and clear *R. solani* disease symptoms. Mineral soil was unamended or amended with 10% vermicompost. Vermicompost was chosen because its promise to suppress better than hardwood (Neher et al., 2017, Figure 1). Populations of radish were planted into factorial combinations of soil (unamended, amended with vermicompost) and *R. solani* inoculum (with or without). Radish seedlings were harvested after three weeks, and severity of damping-off disease was rated on a scale from 0 to 4. Roots and rhizosphere compartments were separated and treated with the photoreactive DNA-intercalating dye propidium monoazide (PMA) directly after collection to eliminate relic DNA (Carini et al., 2016). The bacterial and fungal microbiome were assessed independently for each additive component (soil, vermicompost, plant, pathogen), and plants were subsampled into root and rhizosphere subsamples. Community composition was determined by amplicon sequencing for both bacteria (16S, 515F/806R primers targeted for the V4 region) and fungi (ITS1/ITS2). Healthy roots were compositionally different from diseased roots. Composition of the fungal community composition was more sensitive to the successive addition of vermicompost, plant and pathogen (Figure 4) than composition of the bacterial community. The soil itself was dominated by fungi in the Sordariomycetes and Eurotiomycetes. Thermophilic compost cured by vermicompost contained relative abundant fungi in the Mortierellomycota (*Mortierella*) which are common in finished but immature compost. Adding the pathogen *R. solani* resulted in increased abundance of Agaricomycete (Basidiomycota) fungi. A combination of the plant and vermicompost supported Bacteroidia, which was related inversely to Bacilli bacteria. When the pathogen was added, the α-proteobacteria increased in the rhizosphere, and Oxyphotobacteria in the root. These results support those of Mendes et al. (2011) who noted relative abundance of γ- and β-Proteobacteria, Firmicutes and Actinobacteria in suppressive soils against *R. solani* (Mendes et al., 2011).

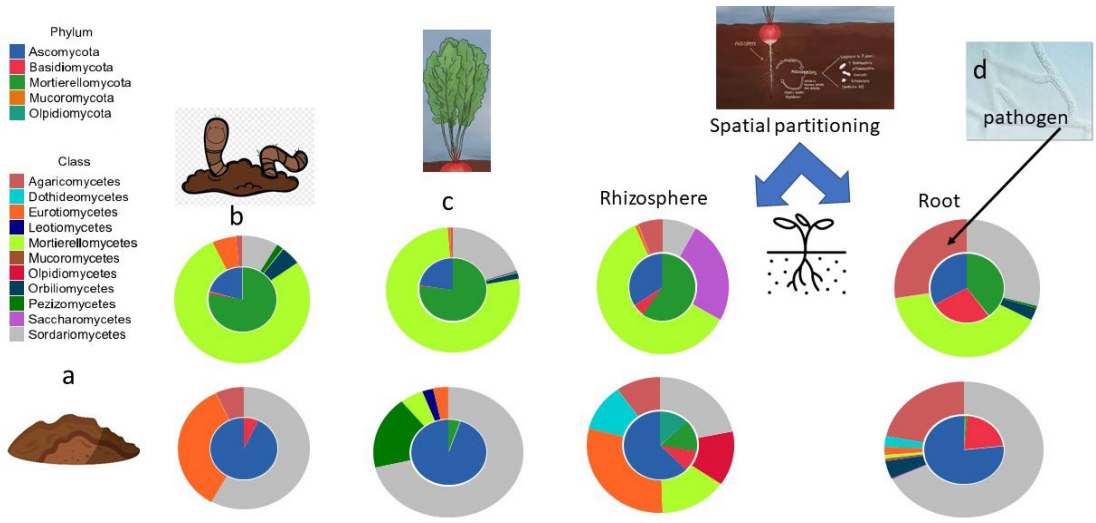

Figure 4. Community assembly of fungi by ITS amplicon sequencing for soil (a), soil plus thermophilic compost cured by vermicompost (b), addition of radish plant (c) and introduction of fungal pathogen, *Rhizoctonia solani* (d). Fungal community composition is illustrated by taxonomic phyla (inner pie) and class (outer pie).

CONCLUSIONS

Disease suppression can be promoted by compost amendments that indirectly regulate the microbe-to-microbe interactions or microbe-to-plant interactions in soil. Plant-competition assays and ecoenzymes are indicators that are quicker than plant bioassays and could be adopted as tools to certify commercial products (Neher et al., 2017; Neher and Weicht, 2018). Different pathogens on different crops require a different combination of microorganisms and/or mechanisms suggesting the need for "designer composts". As the use of high throughput DNA sequencing increases, some generalizable patterns are emerging. For example, compost with wood as an ingredient and/or passed through the gut of an earthworm provides a healthy microbiome that provides ecosystem functions including promoting plant growth (Neher et al., 2013) and enhance microbial-mediated release growth limiting nutrients to plants (Bernard et al., 2012). There is a need for a better understanding of the ecology and the niches within the soil microbiome such as the rhizosphere, rhizoplane, and seeds, rather than just the bulk soil (Hadar and Papadopoulou, 2012). Future research on compost microbiology can focus on the mechanisms of suppression and how that suppression can be transferred to other soils.

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