

Relative effects of biological amendments and crop rotations on soil microbial communities and soilborne diseases of potato[☆]

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Abstract

Various biological amendments, including commercial biocontrol agents, microbial inoculants, mycorrhizae, and an aerobic compost tea (ACT), were evaluated, alone and in conjunction with different crop rotations, for their efficacy in introducing beneficial microorganisms, affecting soil microbial community characteristics (SMCC), and reducing soilborne diseases of potato in greenhouse and field trials in Maine. Most amendments successfully delivered microorganisms into the soil, altering microbial populations and activity in accordance with the particular organisms added, and significantly altering SMCC (as determined by FAME analysis) to various degrees from 2 to 24 weeks. Amendment effects were greatest early on (2 weeks after amendment), but effects associated with crop treatment became more dominant at subsequent assessments (10 and 24 weeks after amendment). In field trials, effects on microbial characteristics, soilborne diseases and tuber yield were variable, with some microbial inoculants and a biostimulant producing no significant effects, whereas arbuscular mycorrhizae, reduced stem canker and black scurf by 17–28%. When used in three different 2 yr crop rotations (barley/ryegrass, barley/clover, and potato, all followed by potato), biological amendments reduced soilborne disease and improved yield in some rotations, but not others. Soil-applied ACT and the combination of ACT with a mixture of beneficial microorganisms (Mix) reduced stem canker, black scurf, and common scab on tubers by 18–33% and increased yield 20–23% in the barley/ryegrass rotation, but not in the other rotations. Mix also reduced disease (20–32%) in the barley/clover rotation only. None of the amendments significantly reduced disease in continuous potato plots. Both crop rotation and amendment treatments significantly affected SMCC, but rotation effects were more dominant. These results indicate that certain rotations were better able to support the added beneficial organisms from amendments and enable more effective biological control, and also that favorable crop rotations may be more effective than amendments in manipulating or altering SMCC. Establishment and persistence of amendment effects may depend on many factors, but an effective and supportive crop rotation is apparently important.

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Keywords: Biological amendments; Crop rotation; Soil microbial communities; Potato; Soilborne disease; Compost tea; FAME

1. Introduction

Numerous soilborne diseases are persistent, recurrent problems in commercial production of potato (*Solanum tuberosum* L.), reducing plant vigor, tuber yield, and tuber quality (Stevenson et al., 2001). Among the most prevalent in the northeast US are stem canker and black scurf,

caused by *Rhizoctonia solani* Kühn, and common scab, caused by *Streptomyces scabiei* (Thaxter) Lambert and Loria. For these and other soilborne diseases, current control methods are not always practical or effective and integrated sustainable disease-control options are needed.

Active management of soil microbial communities is a promising approach as a means to develop natural suppression of soilborne diseases and improve crop productivity (Garbeva et al., 2004; Mazzola, 2004; Welbaum et al., 2004). The goal of this approach is to manipulate, alter, or augment the microbial characteristics of the soil through various management practices that increase soil microbial activity, diversity, populations of

[☆]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture

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plant-beneficial organisms, and antagonism towards pathogens, resulting in disease suppression. Unfortunately, relatively little is known regarding the specific populations, characteristics, interactions, and relationships among plants and soil microorganisms that confer stable disease suppressiveness. Although the mechanisms of microbial disease suppression and organisms responsible are known for some specific pathogen and disease-suppressive soils, such as take-all decline (Raaijmakers and Weller, 1998; Weller et al., 2002), in most cases, and particularly for nonspecific general suppression of multiple diseases, the microbial components necessary and the means to achieve adequate disease suppression in a particular field with a history of disease problems are just not known.

It is known that plants and plant products (organic amendments, crop residues, green manures) can dramatically affect soil microbial communities, and are primary drivers of soil microbial dynamics (Garbeva et al., 2004; O'Donnell et al., 2001; van Elsas et al., 2002), and thus may be important components in establishing and maintaining soil suppressiveness. Crop rotations and residue amendments have been shown to have major effects on soil microbial communities and can result in significant reductions in soilborne diseases (Abawi and Widmer, 2000; Bailey and Lazarovits, 2003; Davis et al., 1996). Previously, our research with crop rotations, cover crops, and green manures established that different crop rotations resulted in distinct differences in soil microbial community characteristics (SMCC) (Larkin, 2003) that were associated with suppression of soilborne potato diseases (Larkin and Honeycutt, 2006; Larkin et al., 2006a), and that *Brassica* rotations and green manures could reduce multiple soilborne diseases of potato (Larkin and Griffin, 2007).

However, addition of microorganisms, either to provide specific known beneficial or pathogen-antagonistic strains, or to supplement the indigenous microbial biodiversity, may enhance the development of microbial disease suppression (Cook, 1993). Biological amendments of various types, including recognized biological control agents with known activity against specific pathogens, and diversified microbial inoculants may effectively introduce, augment, or stimulate soil populations of plant-beneficial microorganisms, and may also interact to some degree with crop rotations. Previous research indicated that several established biocontrol agents, including *Bacillus subtilis*, *Trichoderma harzianum*, and *Trichoderma virens* can reduce soilborne potato diseases in the greenhouse and field (Brewer and Larkin, 2005; Larkin, 2004).

There has also been much interest and activity regarding various alternative and diversified biological amendments with potential to enhance, restore, or regenerate soil microbial communities and soil health. Among these, compost teas, produced by mixing compost with water and other ingredients in various ways and incubating (or “brewing”) for defined periods to extract or enhance the microorganisms, have had some success in suppressing disease and increasing crop growth (Scheuerell and

Mahaffee, 2002, 2004, 2006). In Asia, the use of a defined mixture of fermenting yeast, lactic acid bacteria, phototrophic bacteria, and other microorganisms, called effective microorganisms or EM, has been associated with improving productivity and disease control (Higa, 1991, 1994). Arbuscular mycorrhizae are well known to benefit plant growth, but supplemental additions may also help suppress disease (Jeffries et al., 2003; Harrier and Watson, 2004). In addition, amendments known as biostimulants (organic acids, enzymes, plant extracts, vitamins, growth regulators, etc.) are formulated to stimulate microbial activity in soil and on plant roots, theoretically resulting in improved crop growth and disease suppression.

All of these amendments provide different types and assemblages of microorganisms, and potentially may have different effects on and interactions with existing soil microbial communities, and presumably different potential benefits and limitations. The role and extent of effects of such biological amendments on soil microbial communities, and vice versa, and as affected by crop rotations and related factors, remains a critical area of investigation and increased understanding before development of biological control and natural disease suppression can be implemented to its fullest (Handelsman and Stabb, 1996; Milner et al., 1997). The objectives of this research were to evaluate a variety of different biological amendments in conjunction with different crop rotations for their effects on SMCC, soilborne diseases, and tuber yield in greenhouse and field trials.

2. Materials and methods

2.1. Biological amendments

Amendments representative of a variety of different types of biological amendments were evaluated, including three commercially available biological control agents with known activity against *R. solani* and other soilborne pathogens, four different microbial inoculants containing distinctly different classes and types of microorganisms, and a biostimulant. The biological control agents used (followed by abbreviations to be used in parentheses) were: *Bacillus subtilis* GB03 (*Bsub*) (Kodiak; Bayer CropScience LP, Research Triangle Park, NC), *Trichoderma virens* GI-21 (*Tvir*) (SoilGard 12G; CertisUSA, Inc., Columbia, MD), and *Trichoderma harzianum* strain T-22 (*Tharz*) (RootShield; BioWorks, Inc., Geneva, NY). Bacterial and fungal cell counts in the commercial formulations were determined using standard serial dilution plating on 0.1% tryptic soy agar (TSA) for bacteria and potato dextrose agar (PDA) with 50 mg chlortetracycline and 1 ml/L tergitol for fungi (Larkin, 2003). Bacterial counts of $\sim 1 \times 10^9$ CFU/g formulation for *Bsub* and fungal counts of $6.5\text{--}7 \times 10^6$ CFU/g for *Tvir* and *Tharz* were observed.

EM (EM-1; EMRO USA, Tucson, AZ, USA), a mixture of fermenting yeast, lactic acid bacteria, phototrophic bacteria, and other microorganisms, was obtained as a

concentrate, and activated before use. EM was activated by adding EM-1, molasses, and water in a 1:1:20 (volume) ratio, followed by mixing and fermenting in a tightly sealed (anaerobic) container for 3 to 7 days. The mixture was used after the pH dropped below 3.9. The activated mixture had bacterial and fungal plate counts of 8×10^7 and 4×10^4 CFU/ml, respectively. An aerated compost tea (ACT) was aerobically brewed for 24 hours using a commercial 83.3 L Earth Tea Brewer (Sustainable Agriculture Technologies, Inc., Cottage Grove, OR) from vermicompost and nutrient additives obtained from and according to manufacturer recommendations. The source material for the vermicompost consisted of culled produce wastes, coffee grounds, composted horse manure, paper, and straw, and the nutrient additives contained clay, blue-green algae, sugar, yeast, and kelp (Sustainable Agricultural Technologies, Inc.). Plate counts of different batches of ACT averaged $0.2\text{--}1.0 \times 10^9$ and $2\text{--}8 \times 10^4$ CFU/ml for bacteria and fungi, respectively. The arbuscular mycorrhizal inoculant (AMF) (endomycorrhizal inoculant; Bio-Organics, Inc., La Pine, OR) used contains a mixture of endomycorrhizae, including *Glomus aggregatum*, *G. clarum*, *G. deserticola*, *G. interadices*, *G. monosporus*, *G. mosseae*, *Gigaspora margarita*, and *Paraglomus brazilianum*. The commercial inoculant mixture of potentially beneficial microorganisms (Mix) used (Compete Plus; Plant Health Care, Inc., Pittsburgh, PA) contained six species of *Bacillus*, *Streptomyces griseoviridis*, *Trichoderma harzianum*, and various organic nutrients (humic acids, sea kelp, and yeast extracts). Plate counts of the formulation were 5×10^8 , 2.5×10^6 , and 5×10^4 CFU/g for bacteria, actinomycetes, and fungi, respectively. The biostimulant vitazyme (vita) (Vital Earth Resources, Gladewater, TX) contains a proprietary mixture of vitamins, enzymes, organic acids, growth regulators, and plant extracts, and is expected to increase plant growth and yield through stimulation of soil and root microbial activity.

2.2. Greenhouse trials

The ability of biological amendments to deliver microorganisms to a natural field soil and effects on SMCC as affected by crop plants were evaluated in greenhouse pot trials. Each biological amendment was added as a suspension (*Bsub*, 1.0 g; AMF, 2.0 g; *Tvir*, *Tharz*, Mix, 2.5 g; EM, 5 ml) in 50 ml deionized water to each of three replicate pots (10 cm diameter) containing 500 g of field soil for each of three different crop treatments. A control treatment of 50 ml water was also included and ACT was added without dilution (50 ml/pot). The soil was a Bangor silt loam collected from the research farm at Newport, ME (see field trial section). The biostimulant (vitazyme) was not included in these trials. However, a preliminary trial indicated that vitazyme did not significantly affect soil microbial parameters. The crop treatments included no crop (control), ryegrass (*Lolium multiflorum*-‘Lemtal’), and rapeseed (*Brassica napa*-‘Dwarf Essex’). Approximately, 30

seeds/pot were planted 2 days after addition of biological amendments. Pots were incubated at ambient greenhouse temperatures (18–25 °C) and watered as needed to maintain moist soil. All pots were fertilized once during the trial, at 4 weeks after planting, using a 20–20–20 water-soluble fertilizer (J. R. Peters, Inc., Allentown, PA) at the recommended rate. A 50 g soil sample was carefully taken (to minimize root disturbance and destruction) from each pot at 2, 10, and 24 weeks after planting for determination of soil microbial parameters as described below. The experimental design was a randomized complete block (RCB) factorial with eight amendments, three crops, and three replicate pots for each treatment combination, and the experiment was conducted twice.

2.3. Soil microbial assessments

Soil microbial populations and communities were evaluated by soil dilution plating, substrate utilization (SU) profiles, and fatty acid methyl ester (FAME) profiles. General populations of culturable bacteria and fungi were determined by plating on 0.1% TSA and PDA with 50 mg chlortetracycline and 1 ml/L tergitol added, respectively (Larkin, 2003). For each 50 g soil sample, two 10 g subsamples were assayed, and added separately to 90 ml sterile 0.2% water agar, stirred for 5 min, and serially diluted and plated. Bacterial plates were incubated at 28 °C for 3 days, and fungal plates at 25 °C for 7 days prior to enumeration of viable colonies. Colonies of *Trichoderma* spp. were identified on fungal plates by their distinctive colony morphology and enumerated separately.

Sole carbon source SU of soil microbial communities was assessed using BIOLOG GN2 plates (BIOLOG Inc., Hayward, CA) by a procedure adapted from Garland and Mills (1991) as described by Larkin (2003). One GN2 plate was prepared for each of two soil subsamples (10 g soil serially diluted as for microbial plate counts), with 150 μ l aliquots of a final dilution of 1:5000 added to each of the 96 wells per plate. The plates were incubated at 22 °C and optical density read on a plate reader (model: Emax; Molecular Devices, Inc., Sunnyvale, CA) at 590 and 760 nm after 72 and 96 h of incubation. Optical density readings were corrected for the control (blank) wells on each plate before data analyses. Average well color development (AWCD), calculated as the average optical density across all wells per plate, was used as an indicator of general microbial activity (Larkin, 2003).

Soil community fatty acid profiles were constructed from whole soil extractions of FAMES according to a modification of the microbial identification system (MIS; MIDI, Inc., Newark, DE) standard protocol as described by Larkin (2003). Extractions were conducted on each of three 4 g soil subsamples per pot or plot. Each sample was saponified, mixed, heated, methylated, mixed, cooled, extracted, and washed as previously described (Larkin, 2003). The organic phase was then transferred to a vial for subsequent analysis by gas chromatography by an

automated procedure (MIDI, Inc.) using an HP 6890 gas chromatograph (Hewlett-Packard, Wilmington, DE) with an HP Ultra-2 capillary column and flame-ionization detector. The fatty acids were identified according to the Eukary method and naming table software developed for the MIS (MIDI, Inc.). Only fatty acids that accounted for at least 0.25% of the total fatty acid content over all observations from any given sampling date were included in the analyses. This prevented fatty acids that were only sporadically detected or unreliably quantified from influencing the analyses (Bossio and Scow, 1998; Larkin, 2003). In addition, dicarboxylic acids and fatty acids with a chain length of > 20 carbons were not included in the analyses because these are generally not of microbial origin (Zelles et al., 1995). With these criteria, analyses consisted of 45 unique fatty acids.

2.4. Biological amendment field trials

Selected biological amendments were evaluated in field trials conducted at the USDA research farm in Newport, ME. The farm is located in central Maine and the soil type is a Bangor silt loam (coarse loamy, mixed, frigid, Typic Haplorthod). The average rainfall (25-yr average) for the months May to September is 46 cm and the average daily temperature over that period is 17.1 °C. Plots consisted of two, 6.1-m rows planted with potato variety 'Shepody' on 0.9-m centers, with 35 cm spacing between plants, arranged in a RCB design with four replicate blocks. Each biological amendment was applied as an in-furrow liquid (500 ml/plot) at the time of planting. Treatments contained 10 g formulated product/L for AMF, 10 ml concentrate for vitazyme, and 10 ml activated product for EM. Additional applications of EM (broadcast 2 L/plot) were applied at 2, 4, and 10 weeks after planting, and a supplemental application of Vita was applied 5 weeks after planting. Plots were fertilized with the equivalent of 169 kg N/ha using a 10–10–10 fertilizer applied immediately prior to planting, and plots were also sprayed regularly with alternating applications of mancozeb and chlorothalonil at recommended rates for the control of late blight throughout the growing season. Plants were monitored for symptoms of disease throughout the season, and in late August, four potato plants per plot (each containing multiple roots and stems) were destructively sampled to assess stem and stolon canker and other root diseases as described by Larkin and Honeycutt (2006). Tubers were harvested and rated for incidence and severity (% surface coverage) of disease (black scurf, common scab, and others). Yield was evaluated as the total and marketable (>4.8 cm diameter) weight of potatoes per 6.1 m row and converted to the equivalent value expressed as Mg/ha. Soil samples consisting of eight cores (2.5 cm × 15 cm) were collected from each plot at the beginning (prior to planting) and end (after harvest) of each field season. Each composite sample was mixed and sieved (3 mm mesh) prior to processing for soil microbial analyses. The

experiment was conducted in the same field over two consecutive field seasons (2 yr), and since results were similar in both seasons, data are presented as the combined results from both years.

2.5. Crop rotation field trial

Three 2-yr crop rotations, consisting of barley underseeded with ryegrass (bar/rye), barley underseeded with red clover (bar/clo), and a potato nonrotation control (P/P) followed by potato in the second year of all rotations, were established in research plots in Newport ME in 2001 as a RCB design with four replicate plots (18 m × 3.6 m). Seeding rates were 110, 28, and 17 kg/ha for the barley, ryegrass, and clover rotation crops, respectively, and fertilization of 84 kg N/ha was applied at planting. Once rotation crops were planted, they were not tilled (incorporated) until the following spring. In the 2004 potato year (second rotation cycle), main plots were split into four subplots (4.5 m × 3.6 m) for testing biological amendments. Biological amendment treatments included ACT, Mix, a combination of ACT and Mix, and a nontreated control. Mix was applied as an in-furrow liquid at planting (1 L/plot of a 10 g/L suspension). ACT was applied to soil as a broadcast spray 3 L/plot 1 day prior to planting, in-furrow at planting, and as supplemental sprays at 4 and 8 weeks after planting. Cut seedpieces of potato variety 'Shepody' were planted by hand in each plot (four rows, 0.9 m centers, with a 35 cm spacing between plants), with fertilization, crop management, and disease and yield parameters as described for the previous biological amendment trials.

2.6. Statistical analyses

Disease data, microbial population counts, and most other data were analyzed using standard analysis of variance (ANOVA) with factorial treatment structure and interactions. FAME data were analyzed by principal components analysis (PCA) using the covariance matrix followed by multivariate ANOVA (Glimm et al., 1997) and by canonical variates analysis (CVA), which serves to maximize differences among treatment groups (Buyer et al., 1999). Significance was evaluated at $P < 0.05$ for all tests. Mean separation was accomplished by Fisher's protected least significant difference test. All analyses were conducted using the statistical analysis systems ver. 7 (SAS Institute, Cary, NC).

3. Results

3.1. Greenhouse trials

Biological amendments added to field soil in pots significantly affected soil microbial communities after incubation in the greenhouse for 2 weeks. All biological amendments, except AMF and *Tharz*, increased populations of culturable bacteria ($P < 0.001$) relative to the

nontreated control. Populations observed for *Bsub*, Mix, and *Tvir* treatments were higher than all other treatments, averaging $1.2\text{--}2.5 \times 10^8$ CFU/g soil compared to $3.5\text{--}4.7 \times 10^7$ CFU/g for AMF, *Tharz*, and the nontreated control. Bacterial populations for ACT and EM treatments were intermediate ($6.4\text{--}6.6 \times 10^7$ CFU/g soil). Based on formulation plate counts and treatment doses, bacterial inoculum levels applied to pots averaged $\sim 8 \times 10^5$, 2×10^6 , 5×10^6 , and 1×10^8 CFU/g soil, for EM, Mix, *Bsub*, and ACT, respectively. Total fungal populations were not substantially different among treatments ($1.6\text{--}3.2 \times 10^5$ CFU/g soil), but *Tharz* and *Tvir* treatments significantly increased ($P < 0.001$) the counts of *Trichoderma* spp. observed ($2\text{--}4 \times 10^4$ /g soil) compared to all other treatments ($0.3\text{--}0.8 \times 10^4$). Fungal inoculum levels added in the various treatments averaged $2\text{--}8 \times 10^3$ CFU/g soil for EM, Mix, and ACT, whereas *Tvir* and *Tharz* added $\sim 3 \times 10^4$ *Trichoderma* CFU/g soil. All biological amendments significantly increased soil microbial activity ($P < 0.001$), as indicated by average utilization across all substrates (AWCD) in Biolog plates, relative to the nontreated control. Highest activity was measured in *Bsub*, Mix, and *Tvir* treatments, averaging optical densities of 0.58–0.80 after 96 h readings compared to 0.39 for the nontreated control. Crop effects were relatively minor at the 2-week assessment, although culturable bacteria and *Trichoderma* populations were significantly higher with cropping to rapeseed than no crop ($P < 0.02$ and 0.001, respectively), and soil from the rapeseed and ryegrass cropping treatments averaged higher microbial activity than the noncropped control (0.59–0.61 vs. 0.53) ($P < 0.001$).

Characteristics of the soil microbial communities, as determined by CVA of FAME profiles, were quite distinct among the different biological amendment treatments at 2 weeks after planting (Fig. 1A), with only FAME profiles from AMF and *Tharz* treatments not significantly different from the nontreated control ($P < 0.001$). Addition of *Tvir*, *Bsub*, and Mix treatments resulted in the most divergent effects, with *Tvir* increasing levels of fatty acids associated with selected fungal groups (high CV 1, low CV 2 values), *Bsub* increasing bacterial group (particularly gram-positive) fatty acid biomarkers (high CV 2 values), and Mix increasing levels of both groups. Amendment effects dominated the profile variability at this assessment, with no significant crop effects (average values across all crop treatments indicated).

At 10 weeks after planting, crop treatment effects were more obvious than amendment effects on soil microbial parameters, although both were still significant factors. Populations of culturable bacteria and fungi, and microbial activity, were significantly greater in both crop treatments (rapeseed and ryegrass) than the no-crop control, and microbial activity was significantly greater for rapeseed than for ryegrass (optical densities of 0.71, 0.50, and 0.17 for rapeseed, ryegrass, and no crop control, respectively). Amendment treatments *Bsub*, ACT, *Tvir*, and EM averaged higher bacterial populations across all crop

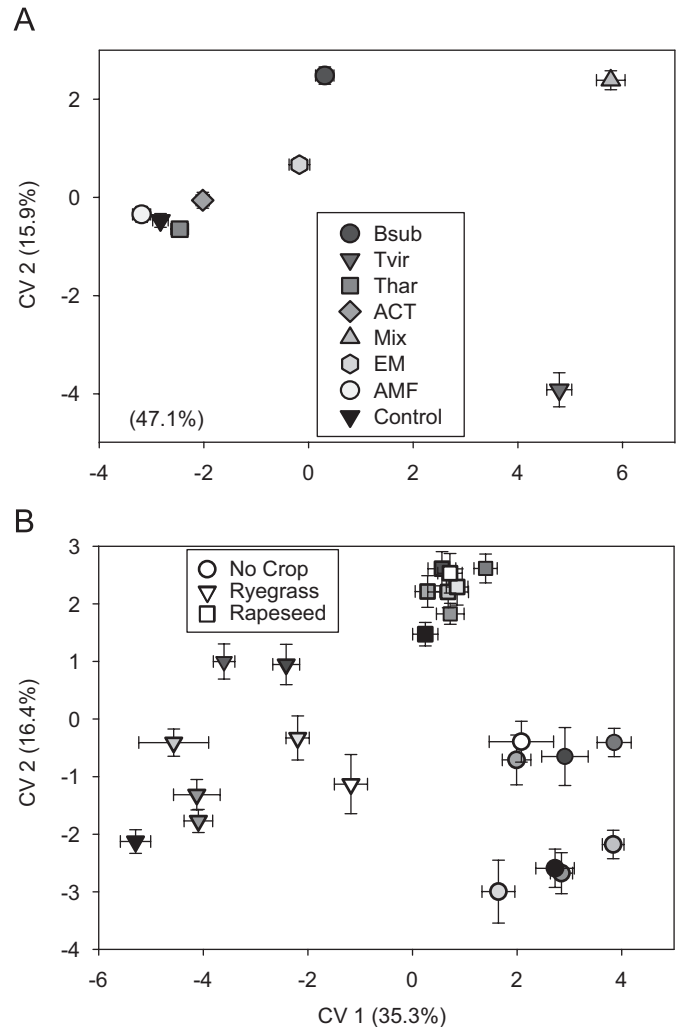


Fig. 1. Effect of biological amendments and cropping treatments on soil FAME profiles represented by CVs 1 and 2 from canonical variates analysis (CVA) measured at: (A) 2 weeks (values averaged over all crop treatments, since crop effects were minor) and (B) 24 weeks after amendment and crop planting in greenhouse pot trials. Error bars represent the standard error of the mean for each CV.

treatments ($1.6\text{--}2.1 \times 10^8$ CFU/g soil) than the nontreated control (0.9×10^8 CFU/g soil), and *Tharz* treatment maintained higher *Trichoderma* populations than all other treatments. FAME profile results also indicated greater crop effects, with both amendment and crop treatments resulting in significant effects, and some significant amendment by crop interactions (data not shown).

By 24 weeks after planting, crop effects were more dominant than amendment effects and significant amendment by crop interactions were evident. The effect of crop treatment on soil microbial characteristics was evident in the FAME profiles after 24 weeks (Fig. 1B), with each crop treatment shaping the microbial characteristics and amendment effects modifying these characteristics. Amendment effects were smallest in conjunction with the rapeseed planting and most substantial (divergent) in the ryegrass planting. Overall bacterial and fungal populations were similar to those after 10 weeks ($0.9\text{--}2.1 \times 10^8$ and

$6.7\text{--}11.3 \times 10^5$ CFU/g soil, respectively), but soil microbial activity showed substantial differences among amendments depending on crop treatments (Fig. 2). Different amendments accounted for the highest levels of activity in the three different crop treatments, although activity was significantly lower for the nontreated control than most other treatments under all crop treatments.

3.2. Biological amendment field trials

The biological amendments AMF and EM, and the biostimulant vitazyme, were assessed over two consecutive field seasons. Both years produced similar and consistent results, with comparable disease levels and yields observed. Significant differences among treatments were noted in both seasons, and the combined data for both years provided the best representation of the overall results (Table 1). In disease and yield assays, AMF significantly reduced stem canker and black scurf severity (17–28%) and slightly increased yield (4–7%) over the two field seasons, but EM and vitazyme treatments did not (Table 1). No treatments significantly reduced common scab ($P = 0.06$). AMF and EM significantly affected soil microbial parameters measured at the end of the field season (October), with EM showing higher bacterial populations, and EM and AMF resulting in higher microbial activity and significantly different FAME profiles (represented by the value of CVI from CVA) than the nontreated control (Table 2), whereas microbial characteristics for the biostimulant vitazyme treatment were not significantly different from the nontreated control for any parameter.

3.3. Crop rotation field trial

Soil-applied ACT, Mix, and a combination of ACT and Mix were evaluated in three different crop rotations. In disease and yield assays, there was a significant amendment

Table 1

Effect of selected biological amendments on the severity of stem canker, black scurf, and common scab, and total and marketable yield of potato tubers in the field

Biological amendment ^b	Disease severity ^a			Yield (Mg/ha)	
	Stem canker (0–5)	Black scurf (%)	Common scab (%)	Total	Marketable
AMF	1.58 b ^c	0.78 b	4.07 a	23.15 a	20.33 a
EM	2.16 a	1.11 a	4.21 a	22.15 a	19.71 a
Vitazyme	1.76 ab	0.91 ab	3.34 a	22.22 a	18.40 a
Control	1.90 ab	1.09 a	4.05 a	22.20 a	18.97 a

Data represent average values over 2 yr.

^aStem canker severity based on 0–5 rating scale: 0 = no symptoms; 1 = discoloration, slight lesion; 2 = substantial lesion, necrosis covering <50% stem diameter; 3 = lesion >50% stem diameter; 4 = lesion girdling stem; 5 = stem girdled, plant dead. Black scurf and common scab severity ratings represent the percentage of the tuber surface covered by scurf or scab.

^bBiological amendments consisted of arbuscular mycorrhizae (AMF), effective microorganisms (EM), and the biostimulant, vitazyme (vita) (see Section 2).

^cMeans within columns followed by the same letter are not significantly different according to Fisher's protected LSD test at $P = 0.05$.

Table 2

Effect of selected biological amendments on soil microbial parameters, including total populations of culturable bacteria and fungi, soil microbial activity as represented by AWCD from substrate utilization assays, and the parameter CVI from canonical variates analysis (CVA) of soil FAME profiles, measured at the end of the field season (October)

Biological amendment ^c	Microbial populations ^a (CFU/g soil)		SU profiles ^b	FAME profiles ^b
	Bacteria ($\times 10^7$)	Fungi ($\times 10^4$)	Activity (AWCD)	CVI (CVA)
AMF	6.1 b ^d	25.8 a	0.384 b	–8.93 c
EM	9.0 a	32.1 a	0.543 a	6.16 a
Vitazyme	7.1 ab	35.0 a	0.345 bc	0.59 b
Control	6.6 b	30.5 a	0.302 c	2.38 b

^aMicrobial populations based on dilution plating on 0.1% TSA (bacteria) and PDA (fungi).

^bSU profiles represent the average well color development (AWCD) across all substrates (as optical density) in Biolog plates and indicates general microbial activity. FAME profiles represent results from CVA and indicate differences in soil fatty acid profiles among treatments.

^cBiological amendments consisted of arbuscular mycorrhizae (AMF), effective microorganisms (EM), and the biostimulant, vitazyme (vita) (see Section 2).

^dMeans within columns followed by the same letter are not significantly different according to Fisher's protected LSD test at $P = 0.05$.

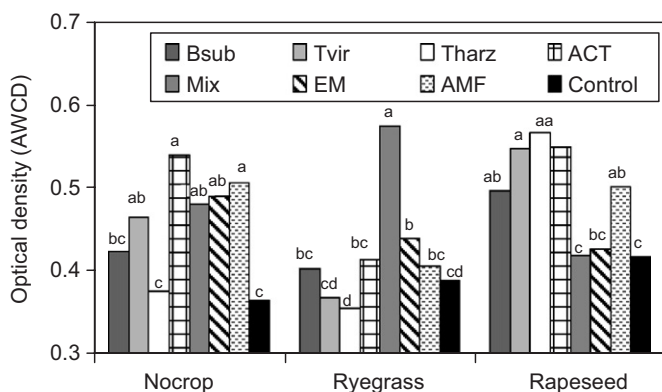


Fig. 2. Effects of biological amendments and crop treatments on soil microbial activity in greenhouse pot trials as indicated by average substrate utilization of a wide variety of carbon sources (in Biolog plates) after 24-week incubation. Bars within crop treatments topped by the same letter are not significantly different according to Fisher's protected LSD test ($P < 0.05$).

by crop interaction, with black scurf and common scab reduced significantly (by 18–33%) with ACT and ACT/Mix treatments in the bar/rye rotation, but not in the P/P rotation (Fig. 3A,B). In addition, Mix reduced scurf and ACT reduced scab in the bar/clo rotation only. Stem

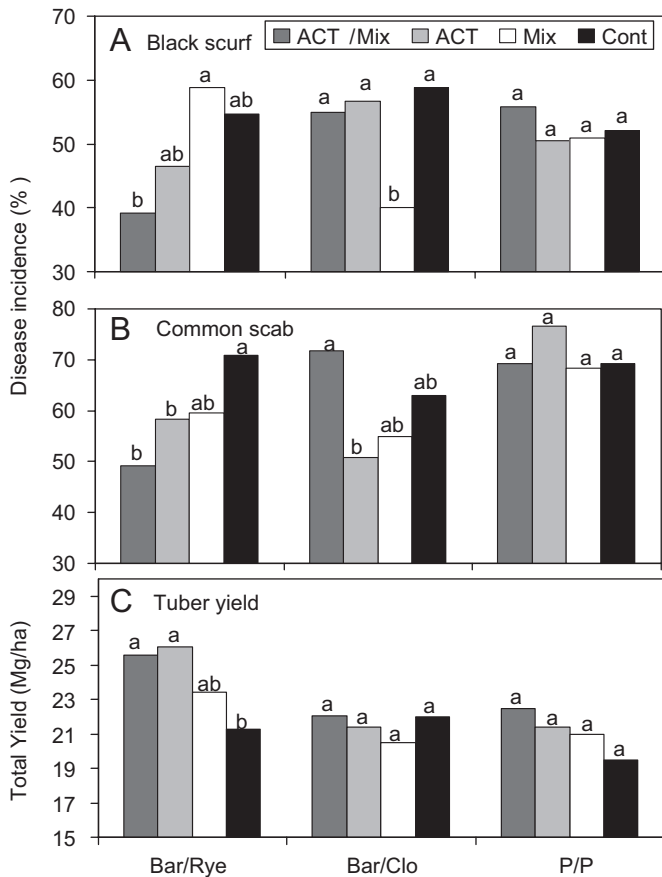


Fig. 3. Effects of biological amendments (aerated compost tea (ACT), microbial mixture (Mix), combination (ACT/Mix), and nontreated control (Con)) on incidence of: (A) black scurf, (B) common scab on potato tubers, and (C) tuber yield in three different crop rotations (barley/ryegrass [bar/rye], barley/clover [bar/clo], and potato [P/P], all followed by potato). Bars within crop treatments for each disease topped by the same letter are not significantly different according to Fisher's protected LSD test ($P < 0.05$).

canker was also significantly reduced ($P = 0.03$) in the ACT and ACT/Mix treatments in the bar/rye rotation, but not in the other rotations (data not shown). Amendments also affected yield differently in the rotations, with ACT and ACT/Mix resulting in significantly greater tuber yield (20–23% increase) only in the bar/rye rotation (Fig. 3C). Bacterial population and microbial activity data also indicated significant interaction between amendment and crop factors, but there were some overall consistent trends. Bacterial populations were significantly higher ($P = 0.01$) in bar/clo than the other rotations overall (1.0×10^8 vs. 4.6 – 5.9×10^7 CFU/g soil) and also tended to be higher ($P < 0.001$) with the ACT/Mix combination treatment than with the other amendments (8.5×10^7 vs. 5.4 – 6.0×10^7 CFU/g soil) when measured at mid-summer (August). Soil microbial activity as indicated by average substrate utilization AWCD also showed the highest activity in soil from the bar/clo rotation and the combination ACT/Mix amendment (average optical densities of 0.64 vs. 0.55–0.56 and 0.61 vs. 0.56–0.58 for the rotation and amendment

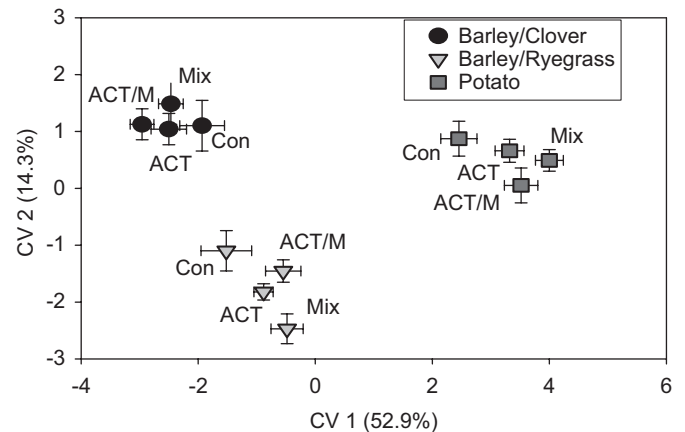


Fig. 4. Effects of biological amendments (aerated compost tea (ACT), microbial mixture (Mix), combination (ACT/M), and nontreated control (Con)) on soil FAME profiles, represented by CVs 1 and 2 from canonical variates analysis, in three different crop rotations (barley/ryegrass, barley/clover, and potato, all followed by potato). Error bars represent the standard error of the mean for each CV.

comparisons, respectively), although values varied among amendment/rotation combinations.

Analysis of SMCC by FAME profiles in the fall of 2004 indicated significant microbial effects due to both rotation and biological amendments (Fig. 4). Each crop rotation resulted in microbial communities with distinct characteristics that were different from one another regardless of amendment treatments. Within each rotation, however, amendment treatments did significantly affect microbial communities. Microbial characteristics were most closely defined by crop rotation, and modified somewhat by biological amendments. Thus, relative changes in SMCC associated with crop rotation were much larger than those associated with biological amendments, as estimated by FAME profiles.

4. Discussion

In this research, a variety of biological amendments were assessed for their ability to deliver microorganisms to the soil, affect SMCC, and ultimately their effect on soilborne diseases of potato and potato yield. The results indicate that, in general, biological amendments can effectively deliver microorganisms to natural soil, resulting in a wide variety of effects on soil microbial communities depending on the particular types, numbers, and formulations of organisms added. All amendments significantly increased estimates of microbial activity and affected SMCC. After incubation for 2 weeks, each amendment affected soil microbial parameters in ways consistent with the organisms added. For example, the two treatments that specifically added high numbers of gram-positive bacteria, *Bsub* and Mix, resulted in the highest populations of bacteria on agar plates, and showed high levels of fatty acids associated with gram-positive bacteria in the FAME profiles. Both of the

Trichoderma amendments, *Tvir*, and *Tharz* also showed increased levels of *Trichoderma* in plate counts, which was comparable to the inoculum levels introduced in the amendments ($2\text{--}4 \times 10^4$ CFU/g soil).

The observed changes in microbial populations at the first assessment were not always directly attributable to the initial amendment inoculum inputs, however. For the *Bsub* and Mix treatments, the bacterial population increases observed were greater than that provided by the inoculum added in the treatments, since increases were on the order of $\sim 10^8$ CFU/g soil and additions accounted for $\sim 2\text{--}5 \times 10^6$ CFU/g soil. Thus, bacterial populations apparently multiplied in the soil after the amendment inputs. The *Tvir* amendment did not contain bacteria, yet significantly, higher bacterial populations were observed for this treatment than for most other treatments, indicating some stimulation of bacterial activity with this amendment. Thus, amendment effects extended beyond an increase in numbers of the added organisms, as has been observed with other biological amendments (Gilbert et al., 1993). The ACT amendment (because not diluted) actually supplied the greatest numbers of bacteria ($\sim 10^8$ CFU/g soil) to the soil, yet did not result in the highest populations at the first assessment (6.5×10^7 CFU/g soil). Thus, with this treatment there was a reduction from the introduced inoculum levels within the first 2 weeks after amendment, although bacterial populations were still greater than in the nontreated control. The ACT amendment contained a wide variety of microorganisms that were present in the vermicompost, compared to the single or few isolates of *Bacillus* spp. contained in the *Bsub* and Mix treatments. The greater relative success of the *Bsub* and Mix treatments in quickly establishing high populations may be due to the fact that the *Bacillus* isolates in these amendments are known to be good root colonizers, persistent, and capable of establishing in soil, whereas, presumably, a substantial portion of the organisms in ACT may not have been adapted to survive, persist, or become well established in this soil. Nonetheless, bacterial populations in the ACT-treated soils did increase at subsequent assessments such that populations were comparable to those in the *Bsub* and Mix treatments at the 24-week assessment, and perhaps the greater diversity of isolates added in the ACT treatments contributed to its ability to maintain higher bacterial populations than the nontreated control throughout the study.

FAME profiles showed widely divergent effects on soil microbial communities for different amendments at the 2-week assessment. Two amendments, AMF and *Tharz*, did not affect FAME profiles at this stage. However, AMF are plant symbionts and are not active outside plant roots, and since the crop treatments and their root systems were still not yet well established, AMF would not be expected to be very active in the soil. As for *Tharz*, the strain of *T. harzianum* used here is a rhizosphere-colonizing strain (Harman, 2000), and again, since the crops were not yet established, perhaps *Tharz* was also not very active at this

time. The plate counts showed that the organism (or at least viable conidia) was present. At later incubation times, all amendments continued to show significant effects on soil microbial parameters, indicating that effects of biological amendments can persist for extended periods in natural soil. Other previous research also demonstrated that biocontrol agents *Bsub*, *Tvir*, and *Tharz* could persist in soil and affect microbial communities throughout the field season (Larkin, 2004; Larkin, unpublished). By 10 weeks after planting, crop effects on soil microbial communities were evident, with effects due to crop greater than those associated with amendments in FAME profiles. This dominant crop effect on soil microbial communities was even more pronounced at week 24, with microbial community profile characteristics defined by the crop and then modified somewhat by the biological amendments.

This strong effect of crop rotation on SMCC, particularly in FAME profiles, was also observed in the field study, which indicates an important role for crop and plant characteristics in shaping the soil microbial communities, and potentially in the development of disease-suppressive soils. Different plant species and, in some cases even different cultivars, are known to greatly influence soil microbial communities (Garbeva et al., 2004; Mazzola, 2004), yet there is little information available on the extent and specific nature of these relationships. In some research studies, plant effects on soil microorganisms are considered secondary to other factors, such as soil type (Buyer et al., 1999; Girvan et al., 2003). However, these studies often are somewhat artificially constructed, by comparing extremely diverse soil types. It would appear that the dominant factor in influencing soil microbiology probably depends on the specific situation. That is, soil-type effects will be most critical when comparing highly divergent soil types and conditions (extreme differences in texture, parent materials, climatic conditions, etc.). Whereas, in cases where soil type is at least somewhat similar among samples, as is the case in a particular field, farm, area, or region (i.e., most practical examples), plant effects are undoubtedly most important. Numerous studies have documented the role and importance of plant species in determining SMCC (Grayston et al., 1998; Marschner et al., 2004; Wieland et al., 2001).

Plant effects on soil microbial communities, as well as different effects among plant species, are presumed to result primarily from the release of qualitatively and quantitatively different nutrients and organic compounds through roots and the breakdown of plant organic matter and residues (Curl and Truelove, 1986; Grayston et al., 1998). The rhizosphere, in particular, is where many of these effects occur. Besides the amendment inoculum and root exudate effects, another factor that could have influenced microbial characteristics in the greenhouse pot experiments was the presence of root material in the soil samples. Although soil samples were carefully extracted to minimize root damage and exclude root material, it is inevitable that some root organic matter was probably

included in samples, particularly at the later sampling times when root systems were larger and more complex. Such root material may have accentuated some differences between the crop treatments and the no-crop control. However, we believe these effects to be minimal and attribute most of the changes in microbial communities observed to be the result of root exudates and the presence of the crop plants in soil.

In the amendment field trial, both AMF and EM significantly affected soil microbial parameters to different degrees, but only AMF reduced the severity of stem canker and black scurf (by 17–28%). Arbuscular mycorrhizae are well-known for their plant growth promotion capabilities, and their role in enhancing plant health and disease suppression has also been well documented and recently reviewed (Jeffries et al., 2003; Harrier and Watson, 2004; Whipps, 2004). However, there is little information available on the potential for AMF to control soilborne potato diseases (Whipps, 2004). Yao et al. (2002) demonstrated that AMF could reduce disease due to *R. solani* in potato plantlets, but effects varied somewhat by cultivar. In the present study, EM treatment resulted in increased bacterial populations and microbial activity, and substantially different FAME characteristics, both in relation to the nontreated control and AMF, but EM did not suppress disease. Thus, disease reduction was not necessarily associated only with higher bacterial populations or microbial activity, but most likely to the specific kinds of changes produced within the microbial communities. The organisms in EM are quite different from those in any of the other treatments, consisting primarily of fermenting bacteria and yeasts (dominated by *Lactobacillus* spp. and *Candida* spp.). Although these organisms have been associated with disease reduction in some cases, they are more known for enhancing decomposition and improving overall soil health and productivity (Xu et al., 2000). The biostimulant vitazyme, on the other hand, did not consistently show effects on soil microbial communities in the field, and did not reduce disease or increase yield in these tests. We also did not observe any measurable stimulation of plant or microbial activity.

Although the biological control agent amendments (*Bsub*, *Tvir*, and *Tharz*) were not evaluated in the field as part of this research, these amendments were previously evaluated in another series of experiments conducted in these same fields and under similar conditions (Larkin, 2004; Larkin, unpublished), as well as in different crop rotations (Larkin and Brewer, 2005). In that research, *Bsub*, *Tvir*, and *Tharz* all significantly reduced the incidence and severity of stem canker 37–75%, and *Bsub* and *Tvir* reduced black scurf 11–20%, and increased total and marketable yield 11–15% relative to the pathogen control over two field seasons. The amendments also significantly affected soil microbial parameters (plate counts, SU profiles) throughout the field season, and effects were still evident (in FAME profiles) 1 yr later. In experiments in conjunction with the same crop rotations observed in the

present research, interactions with *Tvir* amendment and rotation were also observed, with *Tvir* significantly reducing black scurf in the bar/clo rotation, but not in continuous potato or bar/rye (Larkin and Brewer, 2005).

When evaluated in different crop rotations, the biological amendments ACT and a diverse mixture of beneficial microorganisms (Mix), significantly affected soil microbial communities, reduced soilborne diseases, and improved yield under some crop rotations, but not others. ACT and ACT/Mix reduced disease in the bar/rye rotation only, and Mix reduced disease in the bar/clo rotation only. Neither amendment was effective in continuous potato. Thus, biological amendments may have different effects on soil microbial communities and development of disease suppression depending on the crops and rotations present. In previous research, bar/rye was observed to be a better rotation than bar/clo regarding soilborne diseases, with bar/rye resulting in a 20% reduction in black scurf and common scab over multiple years (Larkin et al., 2006a). The barley/ryegrass rotation includes a good cover crop with a complex root system and abundant residue biomass that does not lead to problems with *R. solani*, as clover can. Thus, bar/rye rotation alone can help reduce these soilborne diseases, but addition of the biological amendments ACT and ACT/Mix substantially enhanced the suppression of disease in this rotation. This efficacy within certain rotations and lack of efficacy in continuous potato suggests that the biological amendments require a minimum level of support from the soil microbial environment in order to be effective and successful. If soils are depleted or do not provide the support needed, biological amendments will not properly establish or be active and will not produce the intended effect. This finding has important implications for biocontrol research and the development of disease suppression. But does this imply that biological amendments are not effective? On the contrary, this research provides information to make better use of biological amendments and to potentially make them more effective.

Other factors that may have contributed to the observed effects on microbial communities in the crop rotation trial include soil fertility, chemical residues, and soil organic matter. Based on previous nutrient determinations of these rotations at this site (T.S. Griffin, unpublished), the bar/clo and bar/rye rotations result in biomass residues of approximately 3000 and 2500 kg/ha, respectively, and N contributions of approximately 80–100 and 50–70 kg N/ha, respectively. Whereas, potato (as a ‘rotation’ crop), contributes no more than ~20 kg N/ha in residues. Thus, combined with the 84 kg N/ha of fertilizer added to the grain crops at planting and the 169 kg N/ha added to the potato crop, total N inputs for the rotation year were fairly comparable between the bar/clo and potato rotations, averaging 160–190 kg N/ha, and slightly lower for the bar/rye rotation, at ~130–150 kg N/ha. However, the form of these inputs are very different, with the grain rotation inputs coming largely as organic matter residues that are

actually incorporated at the start of the potato crop year. Thus, the grain rotations result in higher soil organic matter and C and N inputs as crop residues that are absent from the continuous potato soil. The presence of these cover crop residues themselves results in substantial effects on soil microbial communities, and significant differences in effects have been observed between the clover and ryegrass covers (Larkin et al., 2006a). In addition, the continuous potato treatment is also subjected to other crop management practices, such as fungicide and herbicide treatments used in typical potato production, and multiple tillage operations throughout the season. Thus, there are numerous ways that the rotation crop treatments are different and may affect soil microbial characteristics, but differences in N inputs, per se, are probably not an important factor. Also, previous research with manure amendments indicated that the amounts of C inputs were more important than N inputs in their effects on SMCC (Larkin et al., 2006b).

In conclusion, this research indicated that biological amendments such as arbuscular mycorrhizae, ACT, and mixtures of beneficial microorganisms could be used to alter SMCC and significantly reduce soilborne diseases. However, the establishment and persistence of biological amendment effects on soil microbial communities may depend on many factors, with a supportive crop rotation apparently important. Relative treatment effects due to crop rotation were greater than those of biological amendments, suggesting rotations may have a greater influence in shaping microbial communities and may be more influential in the manipulation of those communities to achieve disease suppression. This research also indicated that a single management approach or practice, such as a biological amendment or crop rotation, alone will probably not be effective in establishing disease suppression, but multiple approaches, such as combinations of rotations, cover crops, organic, and biological amendments, need to be optimized and coordinated together as part of an integrated soil management program. Active management of soil microbial communities for disease suppression through the use of effective crop rotations and biological amendments has much potential, but more research is needed to determine effects and interactions among microorganisms, the most effective crop and amendment combinations, and practical implementation.

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