RESEARCH ARTICLE

Differential impacts of brassicaceous and nonbrassicaceous oilseed meals on soil bacterial and fungal communities

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Received 7 August 2012; revised 21 September 2012; accepted 25 September 2012.
Final version published online 6 November 2012.

DOI: 10.1111/1574-6941.12020

Editor: Ian C. Anderson

Keywords
oilseed; seed meal; soil microbial community; pyrosequencing.

Abstract

Demand for alternative fuels has sparked renewed interest in the production of biodiesel from oil-rich seeds. Oilseed meals are a byproduct of this process, and given their relatively high nutrient content, land application represents a potential value-added use. In this microcosm-based study, soil microbial community responses to amendments of a glucosinolate-containing brassicaceous oilseed meal (*Brassica juncea*, mustard), a non-glucosinolate-containing, non-brassicaceous oilseed meal (*Linum usitatissimum*, flax), and a nonoilseed biomass (*Sorghum bicolor*) were characterized using a 28-day time series of replicated 16S rRNA gene and fungal ITS gene sequence libraries. We hypothesized that biomass type and glucosinolate content would alter community composition but that effects would diminish over time. Distinct separation occurred by amendment type, with mustard inducing large increases in the abundance of bacterial taxa associated with fungal disease suppression (e.g. *Bacillus*, *Pseudomonas*, and *Streptomyces* spp.). Dramatic shifts were seen among the fungi, too, with phylotype richness decreasing by > 60% following mustard addition. Changes in bacterial and fungal community composition were rapid, and distinct community types persisted throughout the study. Oilseed amendment, and mustard in particular, has the potential to alter soil microbial community structure substantially, and such changes are likely to be important in the context of ecosystem health.

Introduction

The push to develop alternative fuel sources has sparked renewed interest in the use of oil-rich seeds for the production of biodiesel. Oilseed meals are a byproduct created during the process of oil seed extraction, and it is expected that increased amounts of oilseed meal will be produced as the demand for biodiesel rises (Gasiorek & Wilk, 2011). Although some oilseed meals, such as those produced from soybean or canola, can be used to supplement poultry and livestock feed (Dale, 1996), other oilseeds currently being targeted for large-scale cultivation and use in biodiesel production are known to contain toxic or antinutritive compounds, which may limit their use in animal feeds (Bell, 1995; Katamoto *et al.*, 2001; Pekel *et al.*, 2009). Given that oilseed meals are relatively nutrient rich, land application as organic fertilizers or biofumigants represents potential routes for their use and disposal (Wang *et al.*, 2012).

Past studies have found that oilseed meals produced from glucosinolate-containing plants of the *Brassicaceae* family have the potential to serve as biofumigants (Kirkegaard *et al.*, 1996; Mazzola *et al.*, 2007). Isothiocyanates, the hydrolysis products of glucosinolate compounds, are often identified as the bioactive materials responsible for brassicaceous seed meals' ability to suppress weeds and plant pathogens (Brown & Morra, 1997; Rice *et al.*, 2007; Mazzola & Brown, 2010; Hu *et al.*, 2011). Although it has been shown that isothiocyanates degrade rapidly in the environment (i.e. > 99% of emissions occur within 24–72 h) (Mazzola *et al.*, 2007; Mazzola & Zhao, 2010), soils amended with brassicaceous plant materials often retain the ability to suppress weeds and patho-

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gens for much longer periods of time, up to several weeks postamendment (Mazzola *et al.*, 2007; Rice *et al.*, 2007).

It is believed that rather than being a direct chemical effect, this longer-term suppression may be due to shifts in the overall composition of the microbial community. In the handful of studies that have examined the effects of isothiocyanates on nontarget, nonpathogenic members of the soil microbial community, it has been reported that isothiocyanates and related compounds may alter bacterial and eukaryotic community structure (Rumberger & Marschner, 2003; Cohen *et al.*, 2005; Wang *et al.*, 2012) and inhibit nitrifying bacteria (Bending & Lincoln, 2000). Most of these studies, however, used relatively low-resolution techniques, such as denaturing gradient gel electrophoresis or fatty acid profiles, and their results provide limited taxonomic or phylogenetic information regarding these changes. Given the integral role that soil microbial communities play in C mineralization, nutrient cycling, and disease transmission and resistance, additional research is needed to specifically address the effects of oilseed meals on soil microbial communities.

In this study, soil bacterial and fungal community responses to amendments of a brassicaceous, glucosinolate-containing oilseed meal [Brown mustard (*Brassica juncea*)], a nonbrassicaceous, non-glucosinolate-containing oilseed meal [flax (*Linum usitatissimum*)], and nonoilseed biomass source [sorghum–sudangrass (*Sorghum bicolor*)] were characterized using community qPCR assays and replicated 16S rRNA gene and fungal ITS gene pyrotag sequence libraries at four time points over the course of a 28-day laboratory-scale incubation. This study represents the first use of high-throughput sequencing technology to characterize the impact of brassicaceous and nonbrassicaceous oilseed meals on soil bacterial and fungal communities.

Materials and methods

Soil and seed meal collection and characterization

The soil used in this study was a Weswood loam (fine-silty, mixed, superactive, thermic, Udifluventic Haplustept, collected from 0 to 15 cm depth, at the Texas AgriLife Research Farm (Burleson County, TX). Bulk soils were composited, homogenized, and passed through 2-mm sieve prior to use.

Mustard seed meal was obtained from the Brassica Breeding and Research Group at the University of Idaho. Flax was obtained commercially, and seed meal was created from it using a Komet oil press (Model CA59; IBG Monforts Oekotec, Mönchengladbach, Germany). The seed meals were ground with a mortar and pestle and passed through a 1-mm sieve prior to their use. Dried, chipped sorghum–sudangrass was obtained from the Sorghum Breeding and Genetics Program at Texas A&M University and ground using a Wiley Mill with a 20-mesh screen. Carbon and nitrogen content and mineralization rates, as well as additional chemical characterization of the Westwood loam, oilseed meals, and sorghum biomass, can be found in Wang *et al.* (2012).

Microcosm incubation and sample collection

Seed meals and sorghum–sudangrass biomass were incorporated into the microcosm soils at an application rate of 2.5% (w/w), or a field equivalent of approximately 45 Mg ha^{-1}. Each microcosm contained 400 g soil, maintained at 13% (w/w) water content (approximately 40% field capacity), and was incubated in a 1-L mason jar, under aerobic conditions, at 25 °C in a controlled environment incubator. Microcosms were set up in triplicate, with a series of unamended controls also included in the experimental design. A subsample from each microcosm was collected at days 3, 7, 14, and 28 to track potential changes in bacterial and fungal community composition. All samples were stored at −80 °C until DNA extraction.

DNA extraction and quantification

Community DNA was extracted from 0.5-g aliquots of each soil sample using a PowerSoil DNA extraction kit (Mo Bio Laboratories, Inc., Carlsbad, CA). Samples were purified with illustra MicroSpin S-400 HR columns (GE Healthcare Bio-Sciences Corp, Piscataway, NJ), and they were quantified using both a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and a Quant-iT PicoGreen dsDNA assay kit (Invitrogen Corp, Carlsbad, CA).

Bacterial and fungal qPCR

Community qPCR assays, based upon Fierer *et al.* (2005) and Boyle *et al.* (2008), were used to evaluate the relative abundances of bacteria and fungi in the microcosm communities. Assays were performed in triplicate, using a Rotor-Gene 6000 series thermal cycler (Qiagen, Valencia, CA). Each 10-μL reaction contained the following: 4.5 μL 2.5× RealMasterMix with 20× SYBR solution (5Prime, Inc., Gaithersburg, MD), 1.0 μL BSA (10 mg mL^{-1}), 0.5 μL of each primer (10 μM,), 2.5 μL molecular-grade water, and 1.0 μL template DNA (2.5 ng μL^{-1}). Thermocycling consisted of an initial denaturation at 95 °C for 15 min, followed by 40 cycles of 95 °C for 1 min and 53 °C for 30 s, and 72 °C for 1 min. Primer set Eub338/518 was used for bacteria (Fierer *et al.*, 2005), and primer set 5.8S and ITS1F were used for fungi (Boyle *et al.*, 2008).

Plasmid standards for the bacteria and fungi were generated from the genomic DNA of *Escherichia coli* DH10B (pUC19) (obtained from Carlos Gonzales, Texas A&M University) and *Neurospora crassa* 74-OR23-1VA (H.H. Wilkinson). Regions of interest were amplified from each organism using a FailSafe PCR kit (Epicentre Biotechnologies, Madison, WI) and their corresponding qPCR primer sets. The PCR products were confirmed for size on a 1.2% (w/v) low-melting point agarose gel with UV light, excised, and extracted using a Wizard SV Gel and PCR Clean Up kit (Promega). Then, they were cloned into a pGEM®-T Easy vector (Promega), and plasmids were isolated using a Wizard SV Miniprep kit (Promega). Plasmid DNA concentrations were quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies), and serial dilutions of 5.0×10^{-3} to 5.0×10^{-7} ng μL^{-1} DNA were amplified to generate standard curves. The rRNA gene copy numbers were estimated from the plasmid-based standard curves and were calculated as a function of DNA concentration, the average molecular weight of a base pair of DNA (660 g mol^{-1}), the size of our cloning vector, and the size of our DNA fragment of interest.

The standard curves generated from our bacterial and fungal controls displayed linear relationships between the log values of their gene copy numbers and calculated threshold cycles ($r^2 \geq 0.98$, y-intercept = 29.48, efficiency = 2.23 for bacteria, and $r^2 \geq 0.98$, y-intercept = 34.49, efficiency = 2.07 for fungi). Efficiency values were calculated as described by Pfaffl (2001) and fell within a range that has been reported by others (Fierer *et al.*, 2005 and references therein).

Bacterial and fungal tag-encoded amplicon pyrosequencing

Purified community DNA samples were submitted to the Research and Testing Laboratory (Lubbock, TX) for tag pyrosequencing using 454 GS FLX titanium technology (454 Life Sciences, Branford, CT). Bacterial 16S rRNA genes were amplified using primers 530F and 1100R and were sequenced in the forward direction as described by Acosta-Martínez *et al.* (2008). The fungal ITS region was sequenced in a similar manner, substituting fungal primers ITS1F and ITS4, as described by Amend *et al.* (2010), for the initial generation of the amplicons. The fungal amplicons were also sequenced in the forward direction, generating reads from ITS1F.

Bacterial and fungal sequence analysis

Initial sequence processing was carried out as described by Acosta-Martínez *et al.* (2008). In addition, any sequences shorter than 400 bp in length and/or containing ambiguous base pair reads were removed from the data set. The 16S rRNA gene sequence data were then imported into MOTHUR, v1.15 (Schloss *et al.*, 2009) and aligned using the package's internal alignment feature, and the chimera.slayer function was used to identify potentially chimeric sequences. All sequences that were flagged as potential chimeras were excluded from further analysis.

Substantial variation occurred with respect to library size across our 48 bacterial samples (4 amendments × 4 time points × 3 replicates of each). Given that a number of diversity and richness estimators tend to suffer from sample size bias (Magurran, 2004), we 're-sampled' our sequence libraries so that they contained similar numbers of sequences. The sub.sample function in MOTHUR was used to randomly select a subsample of sequences from each library, and these equally sized, reduced data sets were used in all subsequent analyses of our sequence libraries.

Following chimera detection, and the re-sampling of the larger sequence libraries, the RDP Classifier function was used to assign identities to the bacterial pyrotag sequence data (Wang *et al.*, 2007). MOTHUR was used to align the re-sampled data set and create an all-sample distance matrix, as well as assign sequences to operational taxonomic units (OTU = 97% similarity, using the hcluster function), calculate diversity indices and richness estimates, and determine the degree of overlap shared among the soil communities. Overlap was calculated using the Yue-Clayton similarity estimator (θ_{YC}), a metric that is scored on a scale of 0–1, where 0 represents complete dissimilarity and 1 represents identity (Yue & Clayton, 2005; Schloss *et al.*, 2009). When comparing any given set of communities, θ_{YC} considers the distribution of OTUs between the communities, as well as their relative abundances.

Chimeric sequences were identified from the ITS sequence libraries using the Fungal Metagenomics Pipeline chimera tool (http://www.borealfungi.uaf.edu) provided by the University of Alaska Fairbanks. All potentially chimeric reads were flagged and excluded from downstream analysis. Substantial variation in library size was also noted among our 48 fungal samples. As such, the quality-checked fungal libraries were subject to the re-sampling procedure described previously. Sequences from all of the reduced-sized fungal libraries were combined into a common file and were clustered into OTUs (97% similarity) using CD-HIT-EST (Li & Godzik, 2006). Identities were assigned to the OTUs using the UNITE database's 454 pipeline (Tedersoo *et al.*, 2010). Hits with BLAST scores ≤ 200 were considered to represent unknown or unclassified fungi. Diversity indices and similarity metrics, based upon the OTU data, were calculated using MOTHUR.

Statistical analysis

Variation in soil community qPCR values was assessed with respect to amendment type and time using both one-way and repeated measures ANOVA in R (version 2.10.1). Values were log-transformed prior to analysis, and *post hoc* testing was carried out using Bonferroni-adjusted *P*-values. Variation in community richness and diversity metrics was also evaluated with respect to amendment type and time using repeated measures ANOVA in R.

Nonmetric multidimensional scaling of the bacterial and fungal communities, based upon OTU composition, was carried out using the Bray–Curtis similarity metric in the PAST software package, version 2.03 (Hammer *et al.*, 2001). PAST was also used to conduct two-way analysis of similarity (ANOSIM) with respect to the effects of seed meal type and incubation time on bacterial and fungal community OTU composition. Heatmaps displaying the replicate-level relative abundances of bacterial phyla and fungal genera for each amendment type and time point were created using the heatmap.2 function in the gplots package for R. Samples were clustered with one another using Euclidian distance-based hierarchical agglomerative clustering, and the abundance of each taxonomic group was scaled relative to the mean across all samples.

All tag pyrosequence data generated from this study were deposited in the NCBI Sequence Read Archive under project accession numbers SRP007815 (16S reads) and SRP007824 (ITS reads).

Results

Community qPCR profiles

Bacteria tended to be numerically dominant within the microcosm communities (Fig. 1), and significant differences were observed among the bacterial and fungal communities with respect to soil amendment type, time, and the interaction of these two variables. Among all treatments, the flax-amended soils tended to yield the greatest numbers of bacterial 16S rRNA gene copies. The mustard- and sorghum–sudangrass-amended soils tended to harbor similar or reduced 16S rRNA gene copy numbers relative to both the flax-amended soils and the unamended controls. Fungal copy numbers from biomass-amended soils were significantly greater than those observed in the unamended controls throughout the entire 4-week incubation, and accordingly, the bacterial-to-fungal ratios were also significantly lower than those observed among the unamended controls.

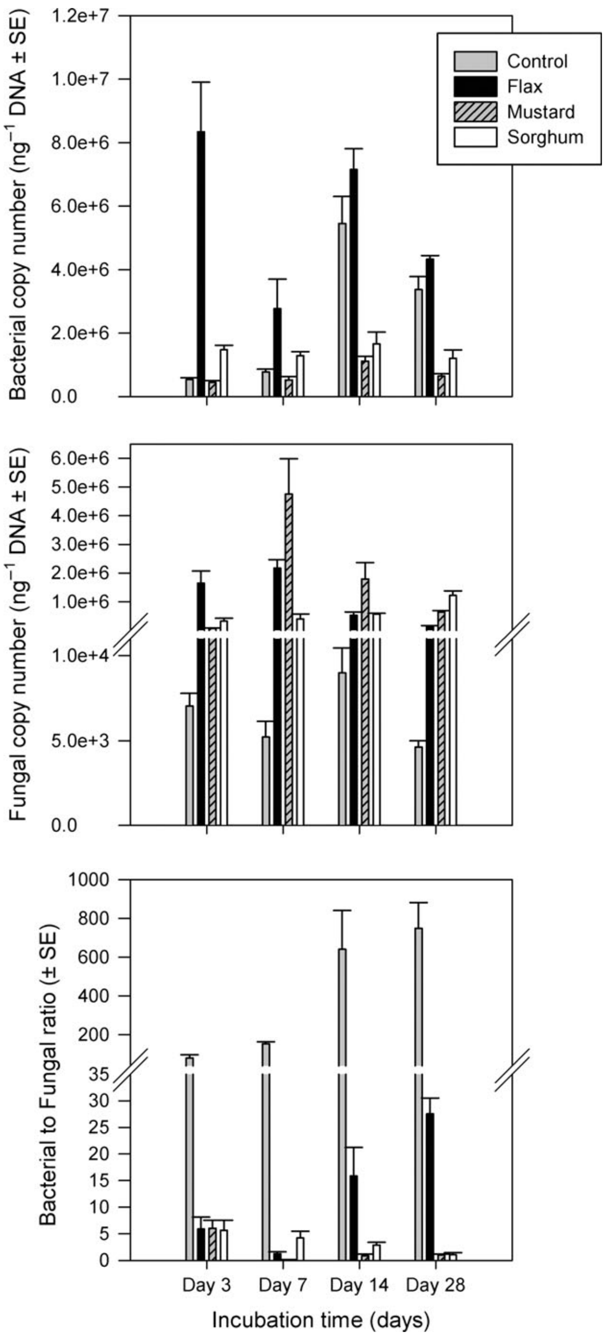

Fig. 1. Community qPCR gene copy numbers of bacteria (top) and fungi (middle), as well bacterial to fungal ratios (bottom) among biomass amendment types and over the course of a 4-week, laboratory-scale incubation. Error bars indicate the standard error of the mean of the biological replicates that were analyzed for each amendment type and time point combination.

Community composition and between-community comparisons

Following the removal of short, ambiguous, or low-quality reads, and normalizing sequence library size across all samples, each bacterial library contained 2169 sequence reads, and each fungal library contained 2008 sequence reads. Our final data set consisted of 104 112 bacterial and 96 384 fungal reads and displayed substantial variation with respect to average OTU content, diversity (Shannon H'), and estimated richness (Chao I) values (Table 1). Among the bacteria, both OTU richness and Shannon diversity values differed significantly by amendment type, time, and the interaction of amendment type and time with one another. Estimated richness values (Chao I) showed significant differences with respect to amendment type only. Both mustard and flax amendment demonstrate these differences in Table 1, where each resulted in reduced numbers of OTUs and estimated richness among the bacterial communities, with impacts lessening over time. Among the fungi, OTU richness, estimated richness (Choa I), and Shannon diversity all showed significant differences with respect to amendment type and amendment-by-time interactions. Mustard amendment reduced fungal OTUs, diversity, and estimated richness by > 60%, relative to each of the other microcosms, including the nonamended control. These reductions persisted throughout the course of the incubation. Interaction plots illustrating the effects of amendment type, time, and their interaction with one another on community richness and diversity metrics are included in Supporting Information, Fig. S1. Accompanying ANOVA results are provided in Table S1.

The dominant phyla associated with each of the bacterial communities varied along amendment type lines (Fig. 2a). The nonamended control was enriched in Acidobacteria, Gemmatimonadetes, Chloroflexi, and Verrucomicrobia and contained a large number of unclassified/uncharacterized bacteria. Bacteroidetes were highly enriched within the flax- and sorghum-amended soils, while increased levels of Proteobacteria (particularly *Pseudomonas* sp. and *Sinorhizobium*/*Ensifer* sp.) and Actinobacteria (especially *Streptomyces* sp.) were found among the mustard-amended soils. Additional details regarding the relative abundances of the bacterial and fungal communities at the genus level can be found in Tables S2 and S3.

Fungal community composition also varied along amendment type lines. The nonamended control was characterized by the relative enrichment of fungi associated with the genera *Spizellomyces*, *Alternaria*, *Mortierella*, and a variety of other, unclassified fungi. Flax-amended soils were enriched in members of the genera *Fusarium* and *Microdochium*. The sorghum-amended soils were enriched with members of the genera *Schizothecium*, *Chaetomium*, *Humicola*, *Ascobolus*, and *Paecilomyces*, and mustard amendment resulted in a distinct and sustained enrichment of *Retroconis* sp., a member of the Ascomycota.

Analysis of similarity (ANOSIM) found that both bacterial and fungal community composition varied significantly with respect to amendment type and time (Table 2). As depicted in Fig. 3a, distinct shifts in soil bacterial community composition were detected within the first 3 days postbiomass amendment. Mustard, flax, and sorghum amendment all produced significant shifts away from the

Table 1. Summary of soil bacterial and fungal community diversity attributes based upon OTUs (97% similarity) and their relative abundances

Sample		Community characteristics (mean ± SE)					
		Bacterial communities			Fungal communities		
Amendment	Day	Number of OTUs	Shannon (H')	Chao I richness estimate	Number of OTUs	Shannon (H')	Chao I richness estimate
Unamended	3	1339 ± 19	6.95 ± 0.02	3409 ± 169	182 ± 12	3.85 ± 0.08	283 ± 24
Unamended	7	1319 ± 9	6.94 ± 0.01	3296 ± 81	182 ± 17	3.73 ± 0.20	271 ± 36
Unamended	14	1328 ± 10	6.93 ± 0.02	3293 ± 73	173 ± 12	3.78 ± 0.04	293 ± 54
Unamended	28	1327 ± 36	6.94 ± 0.04	3411 ± 306	168 ± 6	3.82 ± 0.06	252 ± 30
Mustard	3	410 ± 22	4.70 ± 0.07	814 ± 68	60 ± 7	0.94 ± 0.10	114 ± 22
Mustard	7	583 ± 23	5.39 ± 0.05	1306 ± 72	32 ± 3	0.48 ± 0.04	56 ± 8
Mustard	14	591 ± 29	5.46 ± 0.09	1298 ± 76	36 ± 3	0.47 ± 0.05	82 ± 19
Mustard	28	642 ± 16	5.42 ± 0.09	1419 ± 17	29 ± 2	0.33 ± 0.02	48 ± 14
Flax	3	725 ± 18	5.68 ± 0.04	1894 ± 171	150 ± 8	2.53 ± 0.10	262 ± 19
Flax	7	738 ± 12	5.72 ± 0.02	1752 ± 76	152 ± 1	2.48 ± 0.07	391 ± 55
Flax	14	773 ± 16	5.84 ± 0.04	1859 ± 97	153 ± 12	2.75 ± 0.03	369 ± 74
Flax	28	812 ± 29	5.91 ± 0.07	1748 ± 122	192 ± 15	2.89 ± 0.14	398 ± 9
Sorghum	3	899 ± 21	5.89 ± 0.03	2628 ± 145	162 ± 12	2.95 ± 0.07	327 ± 10
Sorghum	7	1081 ± 24	6.43 ± 0.06	2948 ± 259	186 ± 2	3.36 ± 0.04	390 ± 37
Sorghum	14	1126 ± 12	6.60 ± 0.02	2749 ± 65	221 ± 7	3.56 ± 0.08	530 ± 52
Sorghum	28	1177 ± 27	6.69 ± 0.04	2995 ± 84	193 ± 6	3.07 ± 0.03	442 ± 21

Diversity and richness estimates are based upon the reduced sized sequence libraries, each of which contained 2169 or 2008 sequences in the bacterial and fungal libraries, respectively.

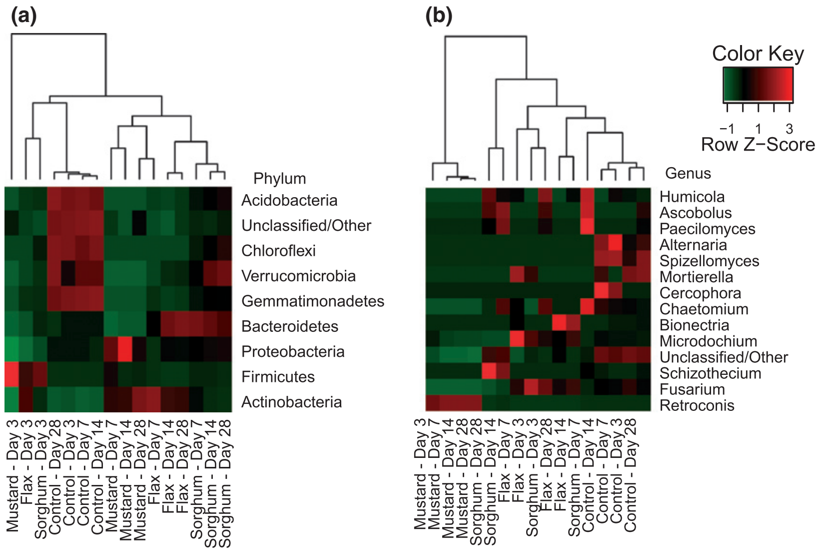

Fig. 2. Heatmaps depictinH:\journals\W3G \FEMSEC\12020\femsec_12020.3dg replicate-level relative abundances of (a) bacterial phyla and (b) fungal genera. Abundances for each taxonomic group (i.e. rows) were scaled relative to the mean across all samples. The dendrogram depicts the hierarchical clustering of samples relative to one another. Red indicates relative increases in abundance, and green indicates relative decreases in abundance.

Table 2. Analysis of similarity evaluating OTU-based community composition with respect to seed meal amendment and time

Microbial community	Treatment	ANOSIM metric *R* score	*P* value
Bacteria	Seed meal	0.9722	< 0.0001
	Time	0.7357	< 0.0001
Fungi	Seed meal	0.9738	< 0.0001
	Time	0.6335	< 0.0001

R values range from 0 to 1 with values approaching 0 representing strong similarities and values approaching 1 representing strong dissimilarities among the communities being compared.

unamended control, but mustard amendment elicited the most extreme response. These differences persisted throughout the course of the 28-day incubation; however, they were not static, as community composition continued to change through day 14. Following this, the bacterial communities began to stabilize, with only minor differences detected between the 14- and 28-day samples for a given biomass type.

In contrast, the fungal communities separated primarily along mustard vs. nonmustard amendment type lines (Fig. 3b). The mustard treatment altered soil fungal communities dramatically, and its effects were longer-lived than those imparted upon the bacterial portion of the soil community. Following their initial shift away from the control state, minimal change was observed with respect to community composition of the mustard-amended samples during the remainder of the 28-day incubation. Temporal shifts in community structure were observed among the other fungal communities, but these shifts

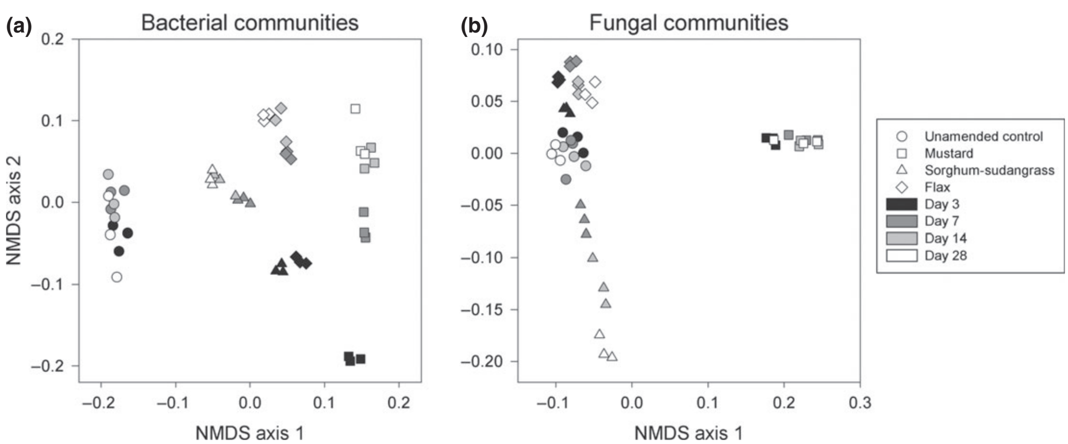

Fig. 3. Nonmetric multidimensional scaling of the (a) bacterial and (b) fungal communities based upon OTU composition. Separation of communities by both amendment type (shape) and time (color) occurred over the course of the experiment. OTUs were defined as sequences sharing $\geq$ 97% similarity with one another.

tended to be less dramatic than those observed among the bacteria.

Discussion

Given the potential for oilseed residues to be used as soil amendments, it is important to understand their impacts on soil ecosystems. The addition of plant biomass to soils, whether as oilseed meals, standing crop residues, plant litter, or roots exudates, has the potential to stimulate microbial community activity, influence soil nutrient fluxes and storage potential, and alter microbial community composition (Cleveland *et al.*, 2007; Köhl *et al.*, 2007; Broeckling *et al.*, 2008; Bressan *et al.*, 2009; Wang *et al.*, 2012). Changes to microbial community composition following the addition of plant biomass to the soil may be of particular importance in agronomic settings should those changes alter the abundance of plant pathogens.

Biomass addition has been shown to have variable effects on pathogen loads in soil. In some instances, biomass incorporation may increase pathogen loads. For example, Bateman *et al.* (1998) found increased numbers of *Fusarium* colony-forming units following the incorporation of wheat straw into agricultural soils. However, in other cases, biomass amendments have been shown to have the opposite effect, particularly in instances where isothiocyanate- or glucosinolate-containing biomass has been added to soil systems (Kirkegaard *et al.*, 1996; Brown & Morra, 1997; Cohen & Mazzola, 2006; Aires *et al.*, 2009). In our study, we found that the addition of oilseed residues and sorghum altered bacterial and fungal community structure, including the relative abundance of organisms that share a high degree of sequence similarity with known pathogens. These responses varied by residue type and time and are likely to have important implications for crop and pathogen management.

Although both bacteria and fungi were affected by biomass amendment in our study, the greatest effects of biomass addition occurred within the fungal portion of our soil communities. Fungal ITS gene abundances, a rough proxy for biomass, increased significantly following biomass amendment. Concurrent with this, changes in community composition were also detected. Sorghum amendment resulted in the enrichment of fungi resembling *Humicola* sp., *Ascobolus* sp., and *Paecilomyces* sp. Members of these genera are saprobes that are generally associated with compost, soil, or animal waste and are recognized for the ability to produce cellulases, xylanases, and other biomass-degrading enzymes (de Almeida *et al.*, 1995 and references therein, Alexopoulos *et al.*, 1996; Yang *et al.*, 2006), such as those that would be needed to degrade the sorghum biomass. Flax amendment resulted in the enrichment of fungi sharing a high degree of similarity with *Fusarium* sp. and *Microdochium* sp., many of which are known plant pathogens (Köhl *et al.*, 2007). In contrast, the mustard amendment, containing relatively high levels of glucosinolate (158 ± 15 μmol g^{-1}; Wang *et al.*, 2012), resulted in the suppression of all fungi except *Retroconis*. *Retroconis* is a genus of plant- and soil-associated fungi (de Hoog & Batenburg-van der Vegte, 1989; Munguía-Pérez *et al.*, 2011) that is likely to be saprophytic but lacks thorough ecological characterization.

The major bioactive compounds associated with mustard and other brassicaceous plants are isothiocyanates, the hydrolysis products of glucosinolates (Gimsing & Kirkegaard, 2006). Past work has shown that majority of isothiocyanate emissions, including allyl isothiocyanate, occurs within 24–72 h following addition to the soil system (Mazzola *et al.*, 2007; Mazzola & Zhao, 2010). Isothiocyanates are thought to be effective in suppressing fungal pathogens, such as *Rhizoctonia solani*, during this relatively short window of time; however, soils amended with mustard seed meal still harbor the ability to suppress *Rhizoctonia* after 4 weeks of incubation (Mazzola *et al.*, 2007). It is believed that rather than being a direct chemical effect, this longer-term suppression may be a result of altered bacterial community structure as suggested by results from a recent study that found similar inhibition of *Pythium ultimum* following addition of *B. juncea* and *Brassica napus* seed meals to soil, despite the lower glucosinolate content of the *B. napus* seed meal (Handiseni *et al.* 2012).

Culture-based studies have consistently identified increases in *Streptomyces* spp. abundances following the addition of brassica oilseed meal to soil (Cohen *et al.*, 2005; Cohen & Mazzola, 2006; Mazzola *et al.*, 2007). *Streptomyces* spp. are known antibiotic-producing bacteria and, more importantly, they have been shown to suppress the growth of the plant pathogen *Rhizoctonia* (Cohen *et al.*, 2005 and references therein). Supporting the culture-based evidence for the brassica-bacterial community model soil fungal pathogen suppression, our 16S rRNA gene libraries found that mustard seed meal-amendment resulted in the enrichment of bacteria resembling *Streptomyces* spp. and *Pseudomonas* spp. Like *Streptomyces*, many *Pseudomonas* spp. produce antifungal compounds capable of repressing soil-borne pathogens, such as *Fusarium* (Haas & Défago, 2005). The paired enrichment of *Pseudomonas* spp. and *Streptomyces* spp. may have contributed to the extreme loss of fungal diversity in our mustard-amended soils (Table 1), including the repression of *Fusarium* relative to our other biomass amendments (Fig. 2).

In addition to the effects of biomass amendment type, time also played an important role in determining the effects of each amendment on soil community structure. As the duration of the soil incubations increased and the biomass added to each microcosm degraded (Wang *et al.*, 2012), shifts in community structure were detected. These tended to be strongest among the bacterial communities (Fig. 2a) but were also detected among the flax- and sorghum-amended fungal communities (Fig. 2b). Interestingly, the communities tended not to shift back toward the nonamended, control state. Rather, each progressed toward an alternative assemblage.

Dynamics such as these have been observed in terrestrial ecosystems, seeding the development of the alternative stable state hypothesis and 'state and transition' models (May, 1977; Westoby *et al.*, 1989). Likewise, similar transitions have been observed in gut microbial communities following the introduction of antibiotics. In some cases, gut communities recover quickly, resembling their pre-antibiotic treatment state within days to weeks following treatment (Young & Schmidt, 2004; De La Cochetiere *et al.*, 2005). In others, however, alternative states have persisted for up to 2 years post-treatment (Jernberg *et al.*, 2007).

The degree to which the perturbation caused by the addition of biomass to each of our microcosms may have set each community on a trajectory toward long-term alternative stable states is somewhat difficult to evaluate. It is possible that the failure of our communities to return to their pre-amendment state may be a function of incubation length, and if given enough time, they might have eventually converged upon one another. Given the trajectory of our communities over the first 4 weeks of our experiment, however, this scenario seems rather unlikely, as most of our communities moved farther away from their control and its 'baseline' state. Alternatively, their failure to converge may be an artifact of using laboratory microcosms for this study, rather than conducting this experiment under field conditions that might allow for the repopulation of the 'baseline' community from the surrounding soils. A field trial incorporating mustard green tissues (but not seed meals) reported differences in bacterial community structure up to 8 months post-treatment, but suppression of fungal pathogens was not maintained long term (Friberg *et al.*, 2009). The results of this study and others suggest that shifts in soil microbial community composition, in addition to direct effects of biocidal chemicals such as isothiocyanates, likely contribute to disease suppression by brassicaceous oilseed meals. However, longer-term, field-scale studies evaluating the impacts of these oilseed meals on soil microbial communities and pathogen suppression are needed to confirm this.

Acknowledgements

This work was supported by a grant from Chevron Technology Ventures, a division of Chevron, U.S.A. Inc. The authors would like to thank Heidi Mjelde and Steven Bookout for their assistance with this study. Grateful acknowledgement is also extended to the Texas A&M University Supercomputing center for providing access to computing resources.

References

Acosta-Martínez V, Dowd S, Sun Y & Allen V (2008) Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. *Soil Biol Biochem* **40**: 2762–2770.

Aires A, Mota VR, Saavedra MJ, Monteiro AA, Simoes M, Rosa EAS & Bennett RN (2009) Initial *in vitro* evaluations of the antibacterial activities of glucosinolate enzymatic hydrolysis products against plant pathogenic bacteria. *J Appl Microbiol* **106**: 2096–2105.

Alexopoulos CJ, Mims CW & Blackwell M (1996) *Introductory Mycology*. Wiley, New York.

Amend AS, Seifert KA, Samson R & Bruns TD (2010) Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. *P Natl Acad Sci USA* **107**: 13748–13753.

Bateman GL, Murray G, Gutteridge RJ & CoŞLkun H (1998) Effects of method of straw disposal and depth of cultivation on populations of *Fusarium* spp. in soil and on brown foot rot in continuous winter wheat. *Ann Appl Biol* **132**: 35–47.

Bell JM (1995) Meal and by-product utilization in animal nutrition. *Brassica Oilseeds* (Kimber DS & McGregor DI, eds), pp. 301–337. CAB International, Wallingford.

Bending GD & Lincoln SD (2000) Inhibition of soil nitrifying bacteria communities and their activities by glucosinolate hydrolysis products. *Soil Biol Biochem* **32**: 1261–1269.

Boyle SA, Yarwood RR, Bottomley PJ & Myrold DD (2008) Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. *Soil Biol Biochem* **40**: 443–451.

Bressan M, Roncato MA, Bellvert F, Comte G, Haichar FE, Achouak W & Berge O (2009) Exogenous glucosinolate produced by *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. *ISME J* **3**: 1243–1257.

Broeckling CD, Broz AK, Bergelson J, Manter DK & Vivanco JM (2008) Root exudates regulate soil fungal community composition and diversity. *Appl Environ Microbiol* **74**: 738–744.

Brown PD & Morra MJ (1997) Control of soil-borne plant pests using glucosinolate-containing plants. *Adv Agron*, Vol. 61. Academic Press Inc, San Diego, pp. 167–231.

Cleveland CC, Nemergut DR, Schmidt SK & Townsend AR (2007) Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry* **82**: 229–240.

Cohen MF & Mazzola M (2006) Resident bacteria, nitric oxide emission and particle size modulate the effect of *Brassica napus* seed meal on disease incited by *Rhizoctonia solani* and *Pythium* spp. *Plant Soil* **286**: 75–86.

Cohen MF, Yamasaki H & Mazzola M (2005) *Brassica napus* seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of *Rhizoctonia* root rot. *Soil Biol Biochem* **37**: 1215–1227.

Dale N (1996) Variation in feed ingredient quality: oilseed meals. *Anim Feed Sci Technol* **59**: 129–135.

de Almeida EM, Polizeli MDTM, Terenzi HF & Jorge JA (1995) Purification and biochemical characterization of β-xylosidase from *Humicola grisea* var *thermoidea*. *FEMS Microbiol Lett* **130**: 171–175.

de Hoog GS & Batenburg-van der Vegte WH (1989) *Retroconis*, a new genus of ascomycetous *Hyphomycetes*. *Stud Mycol* **31**: 99–105.

De La Cochetiere MF, Durand T, Lepage P, Bourreille A, Galmiche JP & Dore J (2005) Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge. *J Clin Microbiol* **43**: 5588–5592.

Fierer N, Jackson JA, Vilgalys R & Jackson RB (2005) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl Environ Microbiol* **71**: 4117–4120.

Friberg H, Edel-Hermann V, Faivre C, Gautheron N, Fayolle L, Faloya V, Montfort F & Steinberg C (2009) Cause and duration of mustard incorporation effects on soil-borne plant pathogenic fungi. *Soil Biol Biochem* **41**: 2075–2084.

Gasiorek E & Wilk M (2011) Possibilities of utilizing the solid by-products of biodiesel production – A review. *Pol J Chem Technol* **13**: 58–62.

Gimsing AL & Kirkegaard JA (2006) Glucosinolate and isothiocyanate concentration in soil following incorporation of Brassica biofumigants. *Soil Biol Biochem* **38**: 2255–2264.

Haas D & Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* **3**: 307–319.

Hammer O, Harper DAT & Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. *Palaeontol Electronica* **4**: 9.

Handiseni M, Brown J, Zemetra R & Mazzola M (2012) Use of brassicaceous seed meals to improve seedling emergence of tomato and pepper in Pythium ultimum infested soils. *Arch Phytopathology Plant Protect* **45**: 1204–1209.

Hu P, Wang AS, Engledow AS *et al.* (2011) Inhibition of the germination and growth of *Phymatotrichopsis omnivora* (cotton root rot) by oilseed meals and isothiocyanates. *Appl Soil Ecol* **49**: 68–75.

Jernberg C, Lofmark S, Edlund C & Jansson JK (2007) Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *ISME J* **1**: 56–66.

Katamoto H, Nishiguchi S, Harada K, Ueyama I, Fujita T & Watanabe O (2001) Suspected Oriental mustard (*Brassica juncea*) intoxication in cattle. *Vet Rec* **149**: 215–216.

Kirkegaard JA, Wong PTW & Desmarchelier JM (1996) *In vitro* suppression of fungal root pathogens of cereals by Brassica tissues. *Plant Pathol* **45**: 593–603.

Köhl J, de Haas BH, Kastelein P, Burgers SLGE & Waalwijk C (2007) Population dynamics of *Fusarium* spp. and *Microdochium nivale* in crops and crop residues of winter wheat. *Phytopathology* **97**: 971–978.

Li W & Godzik A (2006) Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22**: 1658–1659.

Magurran AE (2004) *Measuring Biological Diversity*. Blackwell Publishing, Maldan, MA.

May RM (1977) Thresholds and breakpoints in ecosystems with a multiplicity of stable states. *Nature* **269**: 471–477.

Mazzola M & Brown J (2010) Efficacy of brassicaceous seed meal formulations for the control of apple replant disease in conventional and organic production systems. *Plant Dis* **94**: 835–842.

Mazzola M & Zhao X (2010) *Brassica juncea* seed meal particle size influences chemistry but not soil biology-based suppression of individual agents inciting apple replant disease. *Plant Soil* **337**: 313–324.

Mazzola M, Brown J, Izzo AD & Cohen MF (2007) Mechanism of action and efficacy of seed meal-induced pathogen suppression differ in a Brassicaceae species and time-dependent manner. *Phytopathology* **97**: 454–460.

Munguía-Pérez R, Díaz-Cabrera E, Martínez-Montiel N, Muñoz-Rojas J & Martínez-Contreras R (2011) Fungal diversity in soils samples from a Mexican region with endemic dermatomycoses. *Micol Aplicada Int* **23**: 11–19.

Pekel AY, Patterson PH, Hulet RM, Acar N, Cravener TL, Dowler DB & Hunter JM (2009) Dietary camelina meal versus flaxseed with and without supplemental copper for broiler chickens: live performance and processing yield. *Poult Sci* **88**: 2392–2398.

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**: 2002–2007.

Rice AR, Johnson-Maynard JL, Thill DC & Morra MJ (2007) Vegetable crop emergence and weed control following amendment with different Brassicaceae seed meals. *Renew Agr Food Syst* **22**: 204–212.

Rumberger A & Marschner P (2003) 2-Phenylethylisothiocyanate concentration and microbial community composition in the rhizosphere of canola. *Soil Biol Biochem* **35**: 445–452.

Schloss PD, Westcott SL, Ryabin T *et al.* (2009) Introducing Mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537–7541.

Tedersoo L, Nilsson RH, Abarenkov K *et al.* (2010) 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytol* **188**: 291–301.

Wang Q, Garrity GM, Tiedje JM & Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73**: 5261–5267.

Wang AS, Hu P, Hollister EB *et al.* (2012) Impact of Indian mustard (*Brassica juncea*) and flax (*Linum usitatissimum*) seed meal applications on soil carbon, nitrogen, and microbial dynamics. *Appl Environ Soil Sci*, **2012**, 351609.

Westoby M, Walker B & Noymeir I (1989) Opportunistic management for rangelands not at equilibrium. *J Range Manage* **42**: 266–274.

Yang SQ, Yan QJ, Jiang ZQ, Li LT, Tian HM & Wang YZ (2006) High-level of xylanase production by the thermophilic *Paecilomyces themophila* J18 on wheat straw in solid-state fermentation. *Bioresour Technol* **97**: 1794–1800.

Young VB & Schmidt TM (2004) Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. *J Clin Microbiol* **42**: 1203–1206.

Yue JC & Clayton MK (2005) A similarity measure based on species proportions. *Commun Stat Theory Methods* **34**: 2123–2131.

Supporting Information

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

Fig. S1. Interaction plots depicting mean ± standard deviation of OTU richness, Chao I estimated richness, and Shannon diversity (H') with respect to amendment type and time in (A) bacteria and (B) fungi.

Table S1. Significance of the effects (as indicated by *P*-values) of amendment type, time, and their interaction with one another on OTU richness, Chao I estimated richness and Shannon (H') diversity values.

Table S2. Mean relative abundances of bacterial genera across amendment types and time points.

Table S3. Mean relative abundances of fungal genera across amendment types and time points.

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