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Weed seed mortality in soils with contrasting agricultural management histories

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It has been proposed that cropping systems can be managed to promote the development of soil microbial communities that accelerate weed seed mortality. We examined soil fungal and bacterial communities, soil C:N ratio, soil particle size fractions, and weed seed mortality in soil from fields with over 10 yr of five contrasting management histories with the objective of determining if seed mortality could be explained by differences in soil properties. Seed mortality of giant foxtail and velvetleaf were greatest in soil from the conventionally managed systems and lowest in soil from a reduced input system. Principal-components analysis of soil microbial communities, as determined through denaturing gradient gel electrophoresis of polymerase chain reaction–amplified ribosomal RNA genes (PCR-DGGE), showed distinct differences in the composition of fungal and bacterial communities among the study soils. The first principal component of the 18S rDNA PCR-DGGE analysis of fungal community composition showed a strong negative correlation with both giant foxtail (-0.52, $P < 0.05$) and velvetleaf (-0.57, $P < 0.01$) seed mortality, as did ordination with nonmetric multidimensional scaling (NMS) [giant foxtail (-0.54, $P < 0.01$) and velvetleaf (-0.60, $P < 0.01$)], suggesting that seeds of the two species were affected similarly by changes in the soil fungal community. For giant foxtail, weed seed mortality was also positively correlated ($r = 0.48$, $P < 0.05$) with the first NMS axis of the bacterial 16S rDNA analysis. None of the other measured soil properties were significantly correlated with weed seed mortality. Thus, for the soils tested here, management history, microbial community composition, and weed seed mortality were linked. To extend these results to the field, more work is needed to identify components of the fungal and bacterial communities that are active in seed degradation, and to develop conservation biocontrol recommendations for these species.

Nomenclature: Giant foxtail, *Setaria faberi* Herrm., SETFA; velvetleaf, *Abutilon theophrasti* Medik., ABUTH.

Key words: Soil properties, PCR-DGGE, soil microbial community, fungi, bacteria, principal-components analysis, nonmetric multidimensional scaling, weed seedbank.

Developing effective methods for managing weed seedbanks is a difficult yet important problem for weed scientists to solve (Buhler et al. 1997). Because many weed species form persistent soil seedbanks (Burnside et al. 1996), replenishment of the seedbank by residual weed populations ensures an ongoing struggle to maintain acceptable control. At present, seedbanks are managed primarily by the control of seed production from emerged plants, or by the manipulation of seed germination by cultivation and herbicides (Buhler et al. 1997). An improvement in our ability to reduce weed seedbanks directly could be of considerable value in developing improved integrated weed-management strategies. Unfortunately, our understanding of the factors controlling the persistence of weed seeds in the soil seedbank is currently too limited for the development of such strategies.

Conservation biocontrol has been suggested as one method of reducing the persistence of weed seeds in the soil seedbank (Hallett 2005; Kremer 1993). Such an approach would enhance populations of natural enemies of weed seeds through agroecosystem-level activities. Populations of macrofaunal predators of weed seeds and their feeding activity are directly affected by agricultural management practices (Cromar et al. 1999; Harrison et al. 2003; Menalled et al. 2000, 2001; Westerman et al. 2003). Similarly, agricultural practices can have large impacts on soil microbial community composition (Bossio et al. 1998; Garbeva et al. 2004; Johnsen et al. 2001; Kennedy and Smith 1995; Wander et al. 1995). Therefore it may be reasonable to extend the conservation biocontrol approach by managing soils with the intent of increasing soil borne microbes (such as deleterious rhizobacteria and fungi) that accelerate seed decay, suppress seed germination or inhibit seedling development (Kremer 1993). Some soilborne fungi can have a direct impact upon weeds. For example, the incorporation of *Trichoderma virens*-colonized composted manure into the soil can suppress weed growth (Héraux et al. 2005a, b; Hutchinson 1999).

Gallandt et al. (1999) hypothesized that improvements in soil quality and soil biological activity from activities such as organic amendments may result in greater loss of weed seeds to decay and pathogen attack. Our objective in the present study was to look for gross differences in seed mortality between soils with contrasting agricultural management histories and then to determine whether this mortality could be explained by any of a number of soil properties. Decreasing weed seedbank persistence through management of soil properties would be of particular importance to pro-

ducers in low-external-input and organic systems, in which costs for managing weeds are high, as are potential crop yield losses due to weed interference (Liebman and Davis 2000).

Materials and Methods

Site Description and Study Design

Soil-management effects on weed seed mortality were studied in controlled-environment bioassays with the use of soil collected from two long-term cropping systems experiments at the W. K. Kellogg Biological Station (KBS) in Hickory Corners, MI, with over 10 yr of contrasting agricultural management histories: the Living Field Lab (LFL) (Fortuna et al. 2003) and the Long Term Ecological Research (LTER) site (Davis et al. 2005a). The LTER and LFL have similar treatments, but the LTER features large (1 ha) plots with only one rotation entry point per treatment, whereas the LFL has all rotation entry points, additional cropping system treatments, and small plot sizes (< 100 m^2). The dominant soil series represented at the LTER and LFL sites was Kalamazoo silt loam (Typic Hapludalf; 43% sand, 40% silt, 17% clay, 1.1% OC and pH 6.7). Soil textural data were obtained from the KBS LTER online data archive (http://lter.kbs.msu.edu/Data/DataCatalog.html) as well as through direct measurements by the hydrometer method (Day 1965). There were no pH differences in study soils (mean pH was 6.7); therefore, this parameter was not included in the study. Soil and seed total C and total N (including both inorganic and organic forms of N) were determined on oven-dried soil samples with the use of a CHN combustion analyzer.[1]

Study soils were sampled from fields with five different management histories: (1) a corn–corn–soybean–wheat rotation in the LFL receiving recommended rates of fertilizer and herbicide (CONV-4); (2) a reduced-input corn–corn–soybean–wheat rotation in the LFL receiving nutrients from compost only and weed control through cultivation only (RI-4); (3) a conventional corn–soybean–wheat rotation in the LTER receiving recommended rates of fertilizer and herbicide (CONV-3); (4) an organic corn–soybean–wheat rotation in the LTER with nutrients from red clover overseeded in the wheat phase and weed control through cultivation only (ORG-3); and (5) an early successional treatment in the LTER that received no agricultural chemical inputs and was mowed or burned in alternate years (SUCC).

Plots were selected for sampling in each of the agricultural management treatments that were in the corn entry point of the rotation. In mid-March, 2003, 30 soil samples were collected in a randomly located "W" pattern within each plot, with 10 m between sampling points. A 5-cm-diameter soil probe was used to pull cores from the top 10 cm of the soil profile. Soil cores were bulked to form a composite sample within each replication of each treatment. This sampling date followed a field season in which vegetation in the SUCC treatment had been mowed. Samples were taken between crop rows from the previous field season. Soil samples were stored at 4 C for 1 wk before bioassay establishment.

Bioassays of Seed Mortality

Bioassays were conducted from March 2003 through June 2003, in glasshouses in East Lansing, MI. The experiments were performed with the use of an unbalanced completely randomized design that corresponded to the number of replications in the long-term field experiments: four replications for the CONV-4 and RI-4 treatments, which were located in the Living Field Laboratory, and five replications for the CONV-3, ORG-3, and SUCC treatments, which were located in the LTER site. Bioassays were repeated in a second run that started 2 wk after the first. Day and night temperatures in the glasshouses were 25 and 15 C, respectively. No supplemental light was used.

Experimental units consisted of 7.5-cm-diameter by 10-cm-deep opaque plastic pots containing 100 seeds of either giant foxtail or velvetleaf sown over a 5-cm-deep layer of field soil, and filled to the rim with additional field soil. Experimental soils were sieved through a 2-mm standard soil sieve, poured loosely into each pot with no additional tamping, and allowed to equilibrate at 20 C for 4 d before seeds were placed in the soil. Seeds of giant foxtail and velvetleaf were collected in East Lansing, MI, in October 2002, and light seeds and chaff were removed with a seed cleaner before storage in airtight containers at 4 C. Initial seed viability was estimated at the beginning of the bioassay with the use of tetrazolium seed testing procedures (Peters 2000). Newly emerged seedlings were counted and plucked weekly, and care was taken to ensure that the growing point was removed.

To remove confounding effects of soil-management history on soil matric potential in bioassays, we ran soil water content characteristic curves for each soil (Klute 1982) before bioassays were started. We then used this information to bring experimental units to a common matric potential of -30 kPa (near field capacity for these soils; A. Smucker, personal communication), which was between 18 and 21% gravimetric soil water content for the study soils. The starting weight of each experimental unit was recorded, and kept constant by weighing the pots and adding water daily, if required.

Ambient densities of viable seeds in study soils were determined through elutriation and tetrazolium testing. After a 2-mo incubation period, giant foxtail and velvetleaf seeds were recovered by washing study soils in a mechanical elutriator (Wiles et al. 1996) for 30 min. Percent weed seed mortality (μ) in bioassays was calculated with the use of Equation 1:

$$\mu = \frac{(s_0 + a_0) - (s_1 + g)}{(s_0 + a_0)} \quad [1]$$

where s_0 = the number of viable seeds added at the start of the bioassay, a_0 = the ambient density of seeds present in the study soils at the start of the bioassay, s_1 = the number of viable seeds recovered at the end of the assay, and g = the number of emerged seedlings. We did not examine experimental units before elutriation for evidence of fatal germination (Fenner and Thompson 2005); therefore no attempt was made to distinguish between seed decay and fatal germination as causes of seed mortality.

Soil Microbial Community Analysis

Soil samples for microbial community analysis were taken from the same bulked soil samples that were put in bioassay pots. Soil microbial communities were characterized with

the use of denaturing gradient gel electrophoresis of polymerase chain reaction–amplified small subunit ribosomal RNA genes (PCR-DGGE) (Muyzer et al. 1996) with two laboratory replicates per sample. DNA was extracted from soil samples with the use of a bead-beating technique[2] with 0.5 g of sample per extraction. PCR reactions used a touchdown protocol where the annealing temperature was decreased by 1 C each cycle to maintain the highest level of stringency in the first few reactions (Don et al. 1991) and one primer was modified with a 40-bp GC clamp to arrest the migration of amplified rDNA fragments during electrophoresis (Sheffield et al. 1989).

Gradients were formed with an agitated CBS gradient former, and amplified DNA products were separated by electrophoresis on a CBS-2201 DGGE apparatus.[3] A small fragment (196 bp) of the V3 variable region of the bacterial 16S rDNA was amplified with the use of the universal bacterial primers of Muyzer et al. (1996). The PCR cycle was: 5 min at 95 C, then 11 cycles of 30 s at 93 C, 30 s at 65 C, 30 s at 72 C, then 19 cycles of 30 s at 93 C, 30 s at 55 C, 30 s at 72 C, with a final extension of 15 min at 72 C. Two different primer sets were used for the analysis of fungal community composition. A small fragment (390 bp) of the eukaryotic 18S rDNA was amplified with the use of the universal fungal primers of Vainio and Hantula (2000). The PCR cycle was: 8 min at 95 C, then 11 cycles of 30 s at 95 C, 45 s at 60 C, 2 min at 72 C, then 19 cycles of 30 s at 93 C, 45 s at 50 C, 2 min at 72 C, with a final extension of 10 min at 72 C. Additionally, the fungal ribosomal internal transcribed spacer (ITS) region (550 bp) was amplified with the primers of White et al (1990). The PCR cycle was: 5 min at 95 C, then 11 cycles of 30 s at 95 C, 45 s at 65 C, 2 min at 72 C, then 19 cycles of 30 s at 95 C, 45 s at 55 C, 2 min at 72 C, with a final extension of 5 min at 72 C. PCR products were separated on DGGE gels as follows: bacterial 16S: 10% T, 19:1 [5%C] acrylamide/bis-acrylamide, 160 V, 18 h, 60 C, 35–75% denaturant gradient; fungal 18S: 8% T, 37.5:1 [2.6%C] acrylamide/bis-acrylamide, 120 V, 18 h, 60 C, 40–70% denaturant gradient; fungal ITS: 8% T, 37.5:1 [2.6%C] acrylamide/bis-acrylamide, 140V, 18 h, 60 C, 30–65% denaturant gradient.

The advantage of the above approach was that it was the least complex, allowing comparison of samples. All comparisons were relative: the gel bands did not represent the entire catalog of species in the microbial communities. The data generated relied on the PCR reaction to find template DNA. If one template (i.e., organism) was more abundant, it would have been likely to compete successfully with a less-abundant template (i.e., organism). Consequently, certain organisms may not be represented on the gel. This would have been a problem if we had made absolute comparisons between communities, but was not a problem with the analyses and conclusions we have made, based on relative comparisons.

DNA in gels was stained with SYBR Green and photographed over an ultraviolet transilluminator. Band pixel intensity in photographs was normalized to total lane intensity and analyzed with the aid of a band recognition program,[4] and a composite profile was created for each experimental unit representing the bands found in any lane. Bands were then scored as having an intensity of 0, 1, 2, 3, or 4, where a score of zero represents a band that was absent from the sample, but present in the composite. A score of 4 was given to the brightest band on the gel.

Data Analysis

Seed mortality data for velvetleaf and giant foxtail were subjected to analysis of variance with the GLM subroutine of SYSTAT 11.0.[5] Models contained terms for experimental run, replication, and soil management. Tests for normality and constant error variance (Neter et al. 1996) indicated that the data met the requirements for analysis of variance; therefore data were not transformed before analysis. There was no significant effect of experimental run; therefore data for experimental runs were combined. Protected, Bonferroni-corrected multiple comparisons (Neter et al. 1996) were used to separate mean seed mortality for different soil-management treatments. Seed mortality data were correlated to soil properties with the use of Pearson correlations with Bonferroni-corrected P values.

The PCR-DGGE band scores for each sample were analyzed by two different ordination techniques as a means of confirming that observed trends were not due to artifacts of the ordination method (Rees et al. 2004). Data were subjected to principal-components analysis with the use of the PRINCOMP procedure in SAS.[6] The same data were also analyzed with the use of nonmetric multidimensional scaling (NMS) to ordinate Bray-Curtis similarity coefficients (McCune and Mefford 1999) in PC-ORD.[7] All ordinations were performed on untransformed data.

Results and Discussion

Bioassays of Seed Mortality

There was a strong main effect ($P < 0.001$ for both species) of soil-management history on seed mortality of velvetleaf and giant foxtail in bioassays. Velvetleaf seed mortality was greatest in the CONV-4, CONV-3, and ORG-3 soil-management treatments, and lowest in the RI-4 and SUCC treatments (Figure 1a). Seed mortality was more than 25% lower in the RI-4 and SUCC treatments compared to the other treatments. Giant foxtail seed mortality was greatest in the CONV-4, CONV-3, and SUCC soil-management treatments, intermediate in the ORG-3 treatment, and lowest in the RI-4 treatment (Figure 1b). Giant foxtail seed mortality in the RI-4 treatment was 44% lower than in the CONV-4, CONV-3, and SUCC treatments.

The general relationship between seed mortality and soil management was somewhat similar for the two weed species: seed mortality was high in both management treatments receiving no organic amendments (CONV-4 and CONV-3) and low in the management treatment receiving compost amendments plus a red clover green manure preceding corn (RI-4). This does not support the hypothesis that soil-quality improvements associated with amendment with organic residues should produce elevated levels of weed seed mortality (Gallandt et al. 1999). Rather, they suggest that soil organic amendments have an inhibitory effect upon weed seed mortality. Seed mortality results were variable in soil from the SUCC treatment that received no fertilizer inputs. In this case, giant foxtail seed mortality was high, whereas velvetleaf seed mortality was low.

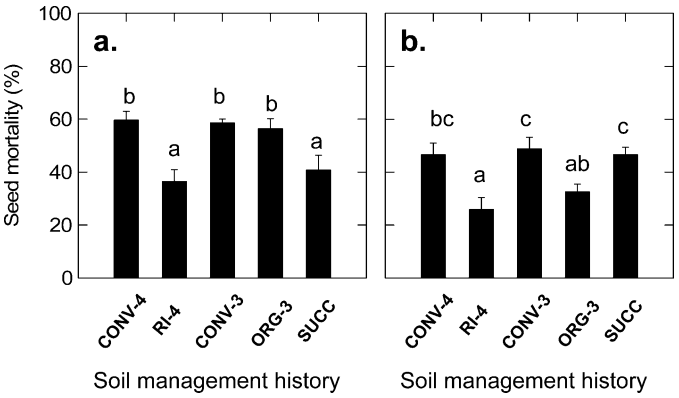

FIGURE 1. Seed mortality of (a) velvetleaf and (b) giant foxtail in controlled environment bioassays in field soil. Field soil-management histories are as follows: CONV-4 = a corn–corn–soybean–wheat rotation receiving recommended rates of fertilizer and herbicide; RI-4 = a reduced input corn–corn–soybean–wheat rotation receiving nutrients from compost only and weed control through cultivation only; CONV-3 = a conventional corn–soybean–wheat rotation receiving recommended rates of fertilizer and herbicide; ORG-3 = an organic corn–soybean–wheat rotation with nutrients from red clover overseeded in the wheat phase and weed control through cultivation only; and SUCC = an early successional treatment that received no agricultural chemical inputs and was mowed or burned in alternate years. Bars represent mean + SE, and are averaged over four replications for soils A and B, and five replications for soils C through E. Bars with different lower-case letters indicate means that are significantly different at the $P < 0.05$ level, as determined by protected Bonferroni-corrected multiple comparison tests.

Seed mortality levels were quite high for both giant foxtail (27–50%) and velvetleaf (38–60%), especially given the short duration of the experiment. Giant foxtail is known to have a transient seedbank, whereas the velvetleaf forms persistent seedbanks (Buhler and Hartzler 2001; Kremer 1986). Values at the low end of the range of seed mortality rates observed in this study are similar to those observed for field burial of the same species from October through April in a regional seed biology study (Davis et al. 2005b), but the high end of the range of seed mortality rates is nearly double those observed in the field study.

Given the uniform environmental conditions in these bioassays, and the relatively close field position of soils forming the different samples (all samples were collected within a 45-ha area at Kellogg Biological Station), the large differences observed in weed seed decay were most likely due to differences in soil microbial communities. We offer additional evidence for this argument in the following section.

Soil Microbial Community Analysis

Principal-components analysis (PCA) was used in three separate multivariate ordinations of DGGE profiles generated from PCR-amplified ribosomal DNA fragments from bacteria and fungi. The first two principal components represented 57, 61, and 51% of the variability in the data for the fungal 18S rDNA, fungal ITS region, and bacterial 16S rDNA analyses, respectively. The first four principal components represented 73, 74, and 74% of the variability in the data for the fungal 18S rDNA, fungal ITS region, and bacterial 16S rDNA analyses, respectively. Nonmetric multidimensional scaling (NMS) of the DGGE profiles also explained sample variation well, with stress scores of 0.19, 0.12, and 0.15 for the fungal 18S rDNA, fungal ITS region, and bacterial 16S rDNA analyses, respectively (data not shown). Stress scores in NMS analyses less than 0.20 indicate useful structure in the ordination, and a stress score of 0.10 indicates an ideal ordination, with all variation explained (Clark 1993). In the PCA analysis of the DGGE profiles of fungal 18S rDNA, soils with CONV-4, CONV-3, and ORG-3 management histories formed tight clusters, with looser associations between data points for soils with RI-4 and SUCC management histories (Figure 2a). In the PCA analysis of the DGGE profiles from both the fungal ITS region (Figure 2b) and bacterial 16s rDNA (Figure 2c), all soils formed distinct associations. Additional levels of organization were also evident in the fungal ITS region and bacterial 16S rDNA ordinations. First, the soils collected from the LFL site (open and closed circles in Figures 2b and 2c) segregated from the soils collected from the LTER site (squares, diamonds, and triangles in Figures 2b and 2c). For the fungal ITS region analysis, the LFL soils all had PC1 values between 2 and 6, whereas the LTER soils all had PC1 values between 0 and − 6.

A second level of organization apparent in these ordinations was the distinction between active agricultural soils and old field (early successional) soils within the LTER site. For the bacterial 16S rDNA and fungal ITS region analyses, the CONV-3 and ORG-3 soils clustered tightly together and were well separated from the data points for the SUCC soils (Figure 2b). The distinct differences among study soils reflect both the heterogeneity of soils at the research location, which differed in clay content (Table 1) and differences in soil properties, such as soil C:N ratio, resulting from long-term agricultural management practices (Fortuna et al. 2003; Robertson et al. 1997). Although the differences in microbial communities between the study sites were at least as great as the differences in communities between soils with varying management histories, a post-hoc 1 df contrast showed that the seed decay rates for the two experimental locations were not significantly different for either of the weed species ($P = 0.12$). We were able to detect significant differences in seed decay due to contrasting management histories, however (Figures 1a and 1b). This suggests that soil differences due to agricultural management history were more important to seed decay than soil differences due to underlying heterogeneity in W. K. Kellogg Biological Station soils.

We note that study soils were sampled only once, in late March, and that the composition of soil microbial communities are likely to change through time. Sampling at multiple times may have broadened our understanding of the dynamics of soil microbial communities through the season. However, we chose to sample field soils in late March on the rationale that differences in the microbial communities of study soils at this date, before crops were planted, would be most representative of the cumulative effects of agricultural management history rather than effects of the current plant community.

Soil Microbiology and Weed Seed Decay

Simple correlations were run between weed seed mortality in bioassays and the first two principal components and first two nonmetric multidimensional scaling (NMS) axes for the fungal 18S rDNA, fungal ITS region, and bacterial 16S rDNA analyses, as well as soil C:N ratio, % sand and %

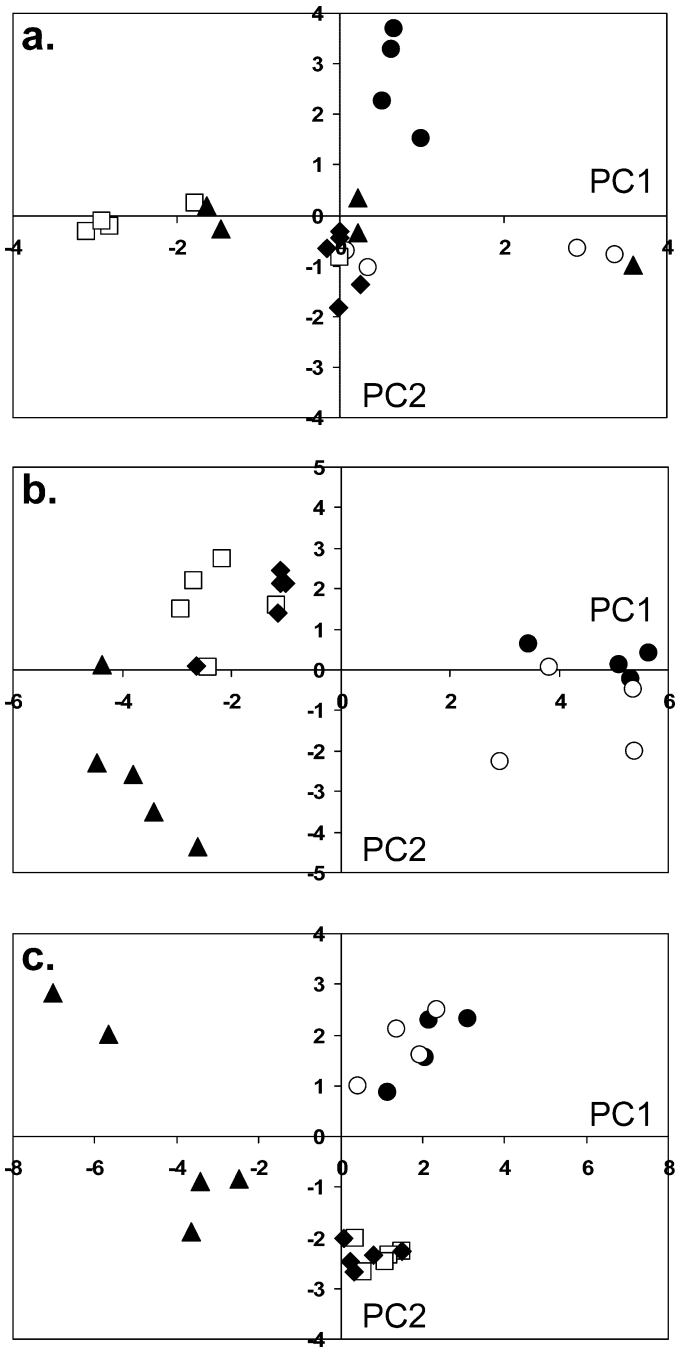

FIGURE 2. First and second principal components of polymerase chain reaction–amplified ribosomal RNA genes (PCR-DGGE) band scores for primers specific for the (a) fungi, and amplifying a variable region of the 18S rRNA gene; (b) fungi, and amplifying the ITS region of the ribosomal operon; and (c) bacteria, and amplifying a variable region (V3) of the 16S rRNA gene. Symbols represent different soil-management treatments: black circles = CONV-4; open circles = RI-4; open squares = CONV-3; black diamonds = ORG-3; black triangles = SUCC. See text for further explanation of abbreviations.

silt (Table 2). Interestingly, the microbial community measure (fungal 18S rDNA) that was least effective at distinguishing among the different soil-management histories in the PCA ordinations (Figure 2a) and NMS ordinations (data not shown) had the clearest relationship to soil-management–related variation in weed seed mortality (Table 2). Giant foxtail seed mortality was negatively correlated with the

TABLE 1. Soil particle size fractions and C:N ratio of study soils.

Treatment ID[b]	Study soil properties[a]		
	Sand	Clay	C:N ratio
	%	%	
CONV-3	37.9 a	17.8 b	8.4 a
ORG-3	42.9 ab	16.6 b	9.5 a
SUCC	44.6 b	16.3 b	10.1 ab
CONV-4	43.1 ab	6.7 a	12.0 b
RI-4	40.4 ab	5.3 a	11.9 b
SE	1.7	0.40	0.26

[a] Means followed by different lower-case letters were different according to a protected Bonferroni-corrected multiple comparison test.
[b] See text for further explanation of treatments.

first and second principal components and first NMS axis of the fungal 18S rDNA analysis, and the second NMS axis of the fungal ITS region analysis. Velvetleaf seed mortality was negatively correlated with the first principal component and first NMS axis for the fungal 18S rDNA analysis, and positively correlated with the second NMS axis for the bacterial 16S rDNA analysis (Table 2). The strength and direction of the correlation between seed mortality and PC1 of the fungal 18S rDNA analysis was similar for both giant foxtail ($r = -0.52$, $P < 0.05$) and velvetleaf ($r = -0.57$; $P < 0.01$), as was the correlation between the first NMS axis and giant foxtail ($r = -0.54$, $P < 0.01$) and velvetleaf ($r = -0.60$, $P < 0.01$) seed mortality. There is no direct biological meaning for the first principal component or first NMS axis of the fungal 18S rDNA analysis; they simply are the result of reducing patterns of variation in the fungal

TABLE 2. Correlation between weed seed mortality in bioassays and selected soil properties.

Soil parameter[a]	Giant foxtail	Velvetleaf
	Pearson correlation (r)	
Fungal 18S PC1	−0.52*[b]	−0.57**
Fungal 18S PC2	0.49*	0.27
Fungal ITS PC1	−0.29	−0.05
Fungal ITS PC2	−0.03	0.36
Bacterial 16S PC1	−0.28	0.30
Bacterial 16S PC2	0.01	−0.37
Fungal 18S NMS1	−0.54**	−0.60**
Fungal 18S NMS2	−0.08	0.01
Fungal ITS NMS1	0.07	−0.15
Fungal ITS NMS2	−0.35†	0.11
Bacterial 16S NMS1	0.16	−0.17
Bacterial 16S NMS2	−0.15	0.48*
Soil C:N ratio	−0.21	−0.25
% sand	−0.22	−0.18
% clay	0.33	0.13

[a] Explanation of soil parameter abbreviations: fungal 18S PC1, PC2 (NMS1, NMS2), principal components 1 and 2 (nonmetric multidimensional scaling axes 1 and 2) using primers specific for the fungi, and amplifying a variable region of the 18S ribosomal subunit; fungal ITS PC1, PC2 (NMS1, NMS2), principal components 1 and 2 (nonmetric multidimensional scaling axes 1 and 2) using primers specific for the fungi, and amplifying the ITS region of the ribosome; and bacterial 16S PC1, 2 (NMS1, NMS2), principal components 1 and 2 (nonmetric multidimensional scaling axes 1 and 2) using primers specific for the bacteria, and amplifying a variable region (V3) of the 16S ribosomal subunit.
[b] The symbols †, *, and ** denote significant Pearson correlations between seed mortality and soil properties at $P < 0.10$, $P < 0.05$, and $P < 0.01$, respectively.

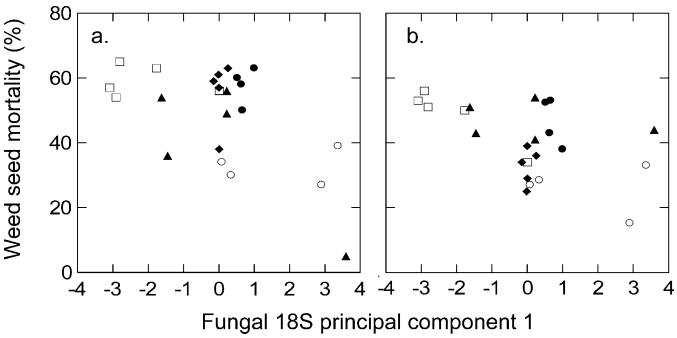

FIGURE 3. Scatter plot of seed mortality for (a) velvetleaf and (b) giant foxtail in relation to the first principal component of polymerase chain reaction–amplified ribosomal RNA genes (PCR-DGGE) band scores for primers specific for the fungi, and amplifying a variable region of the 18S ribosomal subunit. Symbols represent different soil-management treatments: black circles = CONV-4; open circles = RI-4; open squares = CONV-3; black diamonds = ORG-3; black triangles = SUCC. See text for further explanation of abbreviations.

community in a high-dimensional space into a low-dimensional space that explains most of the variation. Seed mortality of both giant foxtail and velvetleaf responded to variation in the fungal community in similar ways (Figures 3a and 3b), with negative slopes and similar R^2 values (0.27 and 0.29, respectively) in regressions against PC1 of the 18S rDNA analysis. However, only giant foxtail seed mortality was linked to changes in bacterial community composition.

In addition to being negatively correlated with seed mortality, the first principal component and first NMS axis of the fungal 18S rDNA analysis were also positively correlated with seed germination ($r = 0.58$, $P < 0.001$, and $r = 0.59$, $P < 0.0001$, for PC1 and NMS1, respectively). The second NMS axis of the bacterial 16S rDNA analysis was positively correlated with velvetleaf seed mortality and negatively correlated with seed germination of both species ($r = -0.42$, $P < 0.05$). Seed germination, in turn, was strongly negatively correlated with seed mortality ($r = -0.75$, $P < 0.001$). There are a number of possible interpretations for this suite of relationships. Differences in the composition of the fungal and bacterial communities in the various soil-management treatments may have caused different levels of germination, fatal germination, or decay of weed seeds by soil microbes. Alternatively, the decay or germination of weeds may have caused changes in the composition of microbial communities. Further experiments will be required to unravel cause and effect in these soils, but here we report that soil-management history, microbial community composition, and weed seed mortality in the experiments performed here were linked. More work is needed, particularly under field conditions, so that it can be determined if this relationship between soil microbial communities and weed seed decay may be applied more broadly.

The C:N ratios of soil samples used in the bioassay ranged between 8 and 12 (Table 1), giving little reason to suspect that soil C:N ratio was directly limiting organic matter decomposition (Brady and Weil 1996). Seed C:N ratios did not differ between velvetleaf (15.7 ± 0.88) and giant foxtail (14.2 ± 0.80), and were also not in a range that would suggest direct limitation of soil microbial decomposition of organic matter. Soil C:N ratio was not directly related to seed mortality, but there was a strong positive correlation between soil C:N ratio and the first principal component of the fungal 18S rDNA analysis ($r = 0.57$, $P < 0.001$) and second NMS axis of the fungal ITS region analysis ($r = 0.54$, $P < 0.01$). This relationship, considered along with the nonsignificant negative correlation between soil C:N ratio and seed mortality of both giant foxtail and velvetleaf, offers some additional support for the inverse relationship between weed seed mortality and soil C:N ratio observed elsewhere (Shem-Tov et al. 2005). Such a relationship could contribute to explaining the reduction in weed seed mortality observed in the soil-management treatments amended with organic residues compared to those only receiving synthetic fertilizers. We speculate that adding large amounts of carbon to the soil as organic amendments may limit microbial decay of weed seeds by immobilizing the nitrogen necessary for microbial proliferation.

The large differences in weed seed decay in soils with contrasting agricultural management histories, coupled with our results showing a strong correlation between weed seed decay and soil microbial community but not soil C:N ratio or texture, indicate that it may be possible to manipulate soil microbial communities to enhance weed seed decay in agroecosystems. Future studies will be necessary to replicate these results under field conditions, and to fully characterize the trade-offs between improving soils through organic amendments and reducing weed seedbank persistence.

Sources of Materials

[1] CE440 CHN combustion analyzer, Exeter Analytical, Inc., 7 Doris Drive, Unit No. 6A, North Chelmsford, MA 01863.

[2] FastDNA SPIN kit, QBiogene, 15 Morga, Irvine, CA 92618.

[3] CBS-2201 DGGE apparatus, CBS Scientific, P.O. Box 856, Del Mar, CA 92014.

[4] Quantity One band recognition program; Bio-Rad, 1000 Alfred Nobel Drive, Hercules, CA 94547.

[5] SYSTAT Software, 501 Canal Boulevard, Suite C, Richmond, CA 94804.

[6] SAS Institute, Inc., SAS Campus Drive, Cary, NC 27513-2414.

[7] PC-ORD, MJM Software Design, P.O. Box 129, Gleneden Beach, OR 97388.

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