

**Final Report to the CERES Trust  
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**Toward effective microbial weed management: Effects of manure application and cover crop use on native soil microbial communities and weed seed demise in the soil seedbank**

**Summary**

- Seed of common Midwestern agricultural weeds was buried in 47 fields on 15 certified organic farms in Minnesota.
- Long-term cropping history was collected from organic certification records.
- Weed seed was excavated following a year of burial and tested for viability. Surrounding soil was sampled and analyzed for physicochemical composition, and bacterial and fungal DNA was extracted and sequenced.
- Higher sand content in soil, and greater frequency of winters under living vegetative cover, were associated with greater bacterial community diversity. However, increased bacterial diversity was not associated with higher rates of weed seed death.
- Weed seed death was not associated with management history or bacterial family abundances, but did vary with soil composition. Specifically, higher silt content was correlated with increased death of *Setaria viridis* and *Chenopodium album* seed.
- In a controlled plot experiment, one year of cover crop planting did not produce detectable differences in soil bacterial communities.

**Introduction**

Weed control is one of the central challenges of organic agricultural production. Mechanical cultivation is the main tool available for removal of weeds in organic fields; however, frequent cultivation is both labor-intensive and disruptive to soil structure, leading organic farmers to look to long-term ecological strategies for managing soil weed seedbanks. Limiting input to seedbanks, by reducing the number of weeds maturing in the field and promoting aboveground seed predation, has been the principal approach. However, fewer strategies are available for reducing the viability of weed seed once it has entered the soil seedbank. The goal of this project is to build a foundation for the development of techniques for cultivating weed-suppressive soil microbial communities, offering farmers a range of

ecological strategies encompassing the entire weed life cycle to reduce the negative impacts of both weeds and unwanted tillage

Current understanding of weed seedbanks and cropping systems gives us reason to expect that rates of weed seed decay will differ in soils with differing microbial communities, and that these communities can be affected by agricultural management choices. Microbial decay can play a significant role in the rate of demise of seeds (Kremer, 1993; Chee-Sanford and Fu., 2010; Wagner and Mitschunas, 2008). Microbial colonization can also affect seed dormancy or can play a protective role, limiting the ability of pathogens to attack dormant seeds (Dalling et al., 2011; Long et al, 2014).

Organic management practices can affect the microbial conditions that weed seeds will encounter in the soil. Both manure application and cover crop use are well documented to produce increases in soil organic matter and microbial biomass (Peacock et al., 2001; Snapp et al., 2005). Weed seedbank density is inversely correlated with microbial biomass and soil organic carbon (De Cauwer et al., 2011). These correlations may be due to the effects of soil organic matter on moisture conditions, as higher organic matter is associated with more water-stable aggregates, which in turn was associated with higher prevalence of weed-suppressive bacterial isolates (Kremer and Li, 2003).

Past research has yielded mixed results on whether organic and diversified cropping practices, on balance, tend to increase or decrease weed seed decay. Liebman et al. (2008) and Davis et al. (2006) found that weed seed decay was higher in two-crop rotations and conventional management than in longer rotations and lower-input systems, respectively. In contrast, Gomez et al. (2014) and Liebman et al. (2014) found that weed seed decay was higher in diversified rotations. Ullrich et al. (2011) found that the association between soil microbial biomass and weed seed demise was not consistent across fields and years.

Although several “biological herbicides” have been commercialized (Stubbs and Kennedy, 2012), the effectiveness of bioherbicide products is limited because the ability of cultured inoculant strains to naturalize in soil environments is dependent on soil conditions and the complex microbial communities present (Wagner and Mitschunas, 2008), and because the prevalence and activity of relevant strains under field conditions is governed by many factors other than the simple availability of inoculum. Several groups have been active in research on microbial community effects on weed seedbanks, including the effects of organic management (Ullrich et al., 2011; Chee-Sanford et al., 2006; Gomez et al., 2014), and their findings have confirmed that weed seed persistence and decay are governed by complex interactions of many microbial community members and abiotic factors, and are not easily attributable to the presence or absence of any single microbe. Weed reducing inoculant products may be possible, but identifying appropriate strains and predicting the conditions under which they may be effective will require much more understanding of how the members of naturally occurring

microbial communities interact to influence seed germination, protection, dormancy, and decay (Muller-Stover et al., 2016).

Previous studies have focused on culturable microbial isolates or older microbial community profiling methods such as phospholipid fatty acid (PLFA) analysis that do not produce taxonomic identifications of microbial community members. Several authors have called for more detailed investigation into the characteristics of native soil microbial communities as relevant to seed survival or demise (Long et al., 2014; Chee-Sanford and Fu, 2010; Stubbs and Kennedy, 2012; Muller-Stover et al., 2016). Our project responds to this call by using cutting-edge next-generation DNA sequencing to generate much more comprehensive and detailed inventories of soil bacteria and fungi, classified to the genus level where possible. This level of detail and taxonomic identification allows us to associate management practices and weed seed demise with specific groups of organisms, individually or in combination.

This project provided the opportunity to observe the long-term effects of cropping system choices in situ and in practice, rather than in a controlled plot environment, which may not reflect the many ways in which management decisions and their effects interact in a real-world farming situation. The project has two objectives that will contribute to the development of effective techniques for the development of weed-suppressive soil communities: 1) to identify bacterial and fungal community structural features associated with reduced weed seed viability, and 2) to determine the effects of long-term organic cropping practices, particularly winter cover, tillage, and rotational diversity, on rates of weed seed demise and the structure of weed seed-associated microbial communities.

## **Methods and Materials**

### *Field Phase 1*

Cover crop treatment plots were established between August 26-September 4 2015 at three locations with at least 10 years of certified organic cropping history: the Elwell Farm at the Southwest Research and Outreach Center (SWROC) in Lamberton, MN, Carmen Fernholz's A-Frame Farm in Madison, MN, and Scott Johnson's Spruce Valley Organics in Farmington, MN. Treatments used were pennycress, rye, an oat/berseem mix, and a no-cover control. Plots were 20' x 20' with 15' alleys between plots to prevent dragging of soil or crop residues. Experimental design at each location was a randomized complete block with four replicates.

Weed seed was collected in summer and fall 2016 from volunteer populations found on the UMN St. Paul campus's Student Organic Farm and at the Rosemount Research and Outreach Center. Weed seed burial took place in November 2016. Each burial consisted of five common Midwestern agricultural weed species: common lamb's quarters (*Chenopodium album*), redroot pigweed (*Amaranthus retroflexus*), velvetleaf (*Abutilon theophrasti*), giant

ragweed (*Ambrosia trifida*), and yellow foxtail (*Setaria viridis*). Pre-burial seed viability was tested by the Wisconsin Crop Improvement Association using a tetrazolium assay. Seed of each species was enclosed in heat-sealed nylon mesh packets. Each packet was attached to an aluminum tag. One packet of each species was buried in each plot to a depth of two inches such that the seed packet was fully underground, with the aluminum tag serving as an aboveground marker.

During the winter of 2015-16, weed seed packets were brought to the surface by frost heave and exposed to rodent predation. We were therefore unable to obtain post-burial weed seed viability data from this phase of the experiment. This prompted us to revise our seed burial procedure for the second phase, as described below.

Soil samples were collected in May 2016 immediately following cover crop termination and biomass incorporation. Ten cores were taken to a depth of 10 cm from each plot. All cores from a plot were pooled and homogenized for physicochemical analysis and DNA extraction (described below).

### *Field Phase 2*

Weed seed was buried in 47 fields on 15 farms across Minnesota. All sites were certified organic with at least eight years of cropping history under organic practices and a history of grain and/or canning crop production. Sites were selected to include a wide range of soil types and cropping histories, particularly with regard to cover crop use and/or perennial (pasture or hay) production. Site locations are given in Table 1. To preserve farmer confidentiality, farms have been de-identified. Field history was collected from organic certification records, and was classified into summary variables describing crop diversity and over-winter ground cover (Table 2).

**Table 1. Weed seed burial farm sites and fields on 15 Minnesota farms**

<b>Farm code</b>	<b>Number of fields</b>	<b>County</b>
AB	4	Sibley
AM	3	Clay
BL	3	Redwood
BP	2	Otter Tail Lac Qui
FM	5	Parle
GC	4	Houston
HL	3	Redwood
JF	4	Dakota
KC	3	Otter Tail
LH	2	Freeborn

ME	3	Pipestone
RK	4	Swift
SC	4	Polk
TK	1	Goodhue
TM	2	Clay

**Table 2: Variables used to quantify management history in 47 study fields, 2009-2016**

<b>Management variable</b>	<b>Max</b>	<b>Min</b>	<b>Criteria</b>
Living cover winters			Winters under living cover, including cover, perennial, and winter annual crops.
Stubble or residue winters			Winters under non-tilled crop stubble or winter-killed residue
Disturbed winters			Winters with soil disturbance after growing season; moldboard, chisel, or disc tillage applied
Legume seasons			Seasons (summers and/or winters) with legume species present
Grass seasons			Seasons (summers and/or winters) with grass species present
Brassica seasons			Seasons (summers and/or winters) with brassica species present
Crop species			Total number of crop species (including in mixtures) grown since 2009

Weed seed was collected and enclosed in mesh packets as described above. One packet of each species was placed in a 3"x4" nylon mesh drawstring bag. The remainder of the bag was filled with soil from the burial place. The drawstring bag was placed in a cage of rodent-proof stainless steel mesh, and the remainder of the cage was also filled with soil. The cage was buried to a depth of 4" and marked with aboveground flags.

Soil samples were taken at the time of weed seed burial by collecting ten cores to a depth of 10 cm at random within a 5' radius of the buried cage. Cores were pooled and the soil was homogenized by passing through a #8 sieve. Aliquots of approximately 10 g were stored at -20 C for DNA extraction. The remainder of the sample was refrigerated for physicochemical analysis. Soil for a potted seed burial was collected in November 2016 within a 5' radius of the buried cage.

Approximately 3 cubic ft of soil was placed in a tub and transported to St. Paul, where soils were homogenized and used to fill 8" greenhouse pots. 8" pots were placed inside 7" pots, in order to provide a gap below the bottom of the soil pot to allow drainage and prevent movement of organisms between the collected soils and the surrounding soil in the common pot-burial field. A nylon drawstring bag of weed seed, constructed as described above, was buried to a depth of 4" in each pot. Pots were dug into a common field of a well-drained

Waukegan silt loam soil to a depth such that the top of the soil in the pot was level with the surrounding field soil. The purpose of the potted soils was to provide a common environment in which soil effects on weed seed could be observed without confounding with differences in temperature and precipitation across sites. However, this data was compromised by ongoing drainage problems, which resulted in anaerobic conditions in many of the pots.

Burial sites were observed in April 2017 and a 10-core soil sample was collected as described above. Weed seed cages were excavated in October 2017. Four of 47 cages had been disturbed by field operations and were excluded from analysis. Drawstring bags of weed seed were removed from cages and refrigerated until they were opened for submission for viability testing. Soil from within the drawstring bags was collected and classified as Seed-Adjacent Soil (SAS). An additional 10-core soil sample was collected when boxes were excavated. All soil samples were homogenized as described above.

### *Soil analysis*

Physicochemical analysis was performed by the University of MN Research Analytical Laboratory using standard procedures as described at <http://ral.cfans.umn.edu/tests-analysis/soil-analysis>. Parameters analyzed included macro- and micronutrients, soil texture, organic matter (OM), moisture, and pH. Weed seed viability was tested at the Wisconsin Crop Improvement Association using a tetrazolium assay. Post-burial weed seed was classified as live, empty, or dead. For the purpose of statistical analysis, empty seedcoats were considered to be live seed, on the assumption that the seed coats were a residual of a germinated seed.

DNA was extracted from 0.35 g of soil using the DNeasy PowerSoil kit according to manufacturer guidance on a QiaCube robotic workstation (Qiagen, Venlo, Netherlands). Extracted DNA was submitted to the University of MN Genomics Center for library preparation and sequencing. The V5-V6 region of the bacterial 16S rRNA gene and the ITS2 region of the eukaryotic (fungal) 18S rRNA gene were selectively amplified and tagged with Nextera dual-indexing primers. Sequence libraries were normalized and pooled for paired-end sequencing on the HiSeq platform (Illumina, San Diego, CA). The 16S and ITS2 regions were used for sequencing because they have a slow rate of evolution and are therefore highly (but not perfectly) conserved across related organisms, which allows them to be used to identify organisms against a database of known sequences.

16S sequence data was processed using mothur v. 1.33.3 (Schloss 2009). Sequences were trimmed to 160 bp and paired-end joined. Sequences were screened for quality and removed if they had a quality score < 35 over a window of 50 nt, had >2 mismatches to a primer sequence, had homopolymers > 8 nt, or had an ambiguous base (N). Sequences were aligned to the SILVA database (Quast et al. 2013). Sequences were pre-clustered, and sequences that were singletons or were identified as chimeras by UCHIME (Edgar et al. 2011) were removed.

Sequence reads were classified to the RDP database v. 14 (MI State University, East Lansing, MI), and reads corresponding to chloroplast lineages were removed. Remaining sequence reads were clustered into Operational Taxonomic Units (OTUs) at 97% similarity using the average-neighbor algorithm. OTUs were classified to the RDP database. Further data analysis was done in R v. 3.3.2 (R Core Team, 2016) using the *vegan* package (Oksanen et al., 2017). We tested the relationships among soil test values, weed seed mortality results, cropping history variables, and relative abundances of bacterial community members using redundancy analysis, a multivariate ordination method that can identify when differences in one group of variables co-occur with differences in another group of variables.

## Results

### *Phase 1*

Soils from these farms sites were home to extremely diverse soil bacterial communities. An average of 2331 operational taxonomic units, or OTUs (genetically distinct bacterial types, distinguished at approximately a genus level) were detected in each sample, representing 43 phyla (high-level subdivisions of the bacterial kingdom). *Proteobacteria* and *Actinobacteria* were the dominant phyla, with their members comprising 24% and 22% of the total sequence reads, respectively. The four cover crop treatments showed similar bacterial communities, but significant differences in community composition were detected between the sites (PERMANOVA  $p < 0.0001$ ), with the sandier Farmington site emerging as most distinct from the heavier soils at Lamberton and Madison (Figure 1). This is consistent with our previous work, as well as others', which have indicated that, while short-term crop effects on bacterial community composition are sometimes detectable, they are much smaller than the differences associated with different underlying soil types.

Because the seed packets were damaged during the winter, we were not able to associate the observed differences in soil microbial community with differences in weed seed survival as we had initially planned. However, we were able to make those associations in the second phase of the experiment, as described below.

### *Phase 2*

An average of 2565 OTUs were detected in the bulk field soil at each site in November of 2017. These included members of 47 phyla. Phylum composition of the bacterial community at each site is shown in Figure 2. We measured the richness of the bacterial communities, or estimated number of distinct bacterial types present in the soil sample, as well as the diversity, which encompasses the number of types as well as how evenly they are represented in the

community (for example, a sample with 10 OTUs where 90% of the sequences were from a single OTU would be less diverse than a sample with 10 OTUs, each making up 10% of the sequences). The level of bacterial diversity that we detected in these soils was strongly associated with the texture of the underlying mineral soil (Figure 3). Soils with higher sand content were higher in both richness ( $r=0.40$ ,  $p=0.007$ ) and diversity ( $r=0.49$ ,  $p<0.001$ ) of bacterial OTUs, while silt and clay content were negatively correlated with OTU richness and diversity. Neither number of crop species nor frequency of undisturbed winters (which included winters under stubble or winter-killed residue) were correlated with OTU richness or diversity; however, winters under living cover were positively associated ( $r=0.33$ ,  $p=0.026$ ) with OTU richness. This effect was not simply due to farmers with sandy soils using more winter cover; living cover winters were not more frequently observed on sandier soils ( $p=0.379$ ).

We did redundancy analysis to test the relationship between the rates of death of the five weed species and three other sets of variables: soil composition (sand, silt, clay, OM, and moisture); management history (living cover winters, disturbed winters, legume seasons, grass seasons, brassica seasons, livestock presence, and total crop species); and relative abundances of bacterial families. Weed seed death was not associated with management history or bacterial family abundances ( $p=0.98$  and  $p=0.94$ , respectively), but was associated ( $p=0.048$ ) with soil composition (Figure 4). Specifically, abundance of silt in the soil was correlated with increased death of seed of yellow foxtail and common lamb's quarters ( $p=0.049$  and  $p=0.012$ , respectively).

Neither bacterial OTU diversity nor OTU richness in the bulk field soil was associated with any difference in the rate of demise of any weed species over the one-year burial period. This finding fits into a broader pattern that we have observed in our own work and in the published literature: while there is a general consensus that more diversity in the microbial community is "good," in soil environments that are host to massively diverse communities (nearly 50 phyla in this case), the functional significance of a further increase in diversity from an already-high baseline is unclear.

Analysis of this data is ongoing. These results have focused on bulk field soil; bacterial community profiles from seed-adjacent soil are also being analyzed and explored. We are in the process of identifying individual bacterial taxa associated with specific management practices, and with weed seed survival rates. Fungal sequence data have also been obtained, and will be incorporated into our analysis of the microbial community profiles.

## Outreach

Ongoing work and interim findings on this project were discussed with farmers as a part of the Organic Field Day at the Southwest Research and Outreach Center in Lamberton, MN, and in presentations on soil health and microbial communities at two Transitioning to Organic workshops in December of 2017. Approximately 95 farmers and agricultural professionals attended these workshops. We have also provided information and participated in an interview with the *Agri-News* newspaper, which resulted in an article featuring this project. Currently, an article is in preparation that uses the lessons and findings of this project, as well as our previous CERES-funded research on the effects of cover crops and organic fertilizers on soil bacterial communities and nutrient cycling, to provide a summary of the current state of scientific understanding of the roles of soil microbes in farm soils, how they are affected by cropping practices, and how to interpret news and advertising promoting products or systems to “improve” soil microbial function. This article will be written for a general/farming audience and will be published on our UMN organic research website.

We strongly believe that among the most important “big picture” implications of our results is the fact that the scientific research is still in the early stages of developing an understanding of the myriad microbial groups that can be detected using sequencing-based community profiling techniques, and how these groups interact to determine crucial soil functions. This means that claims of benefits from any commercial microbial inoculant product (except *rhizobium* inoculants) should be treated with extreme caution.

Scientific publications detailing our findings are under development and will include a paper on soil sampling methods for overcoming spatial heterogeneity to obtain replicable and representative samples of soil communities, which we anticipate will generate strong interest and be of particular value to future research at the UMN and elsewhere.

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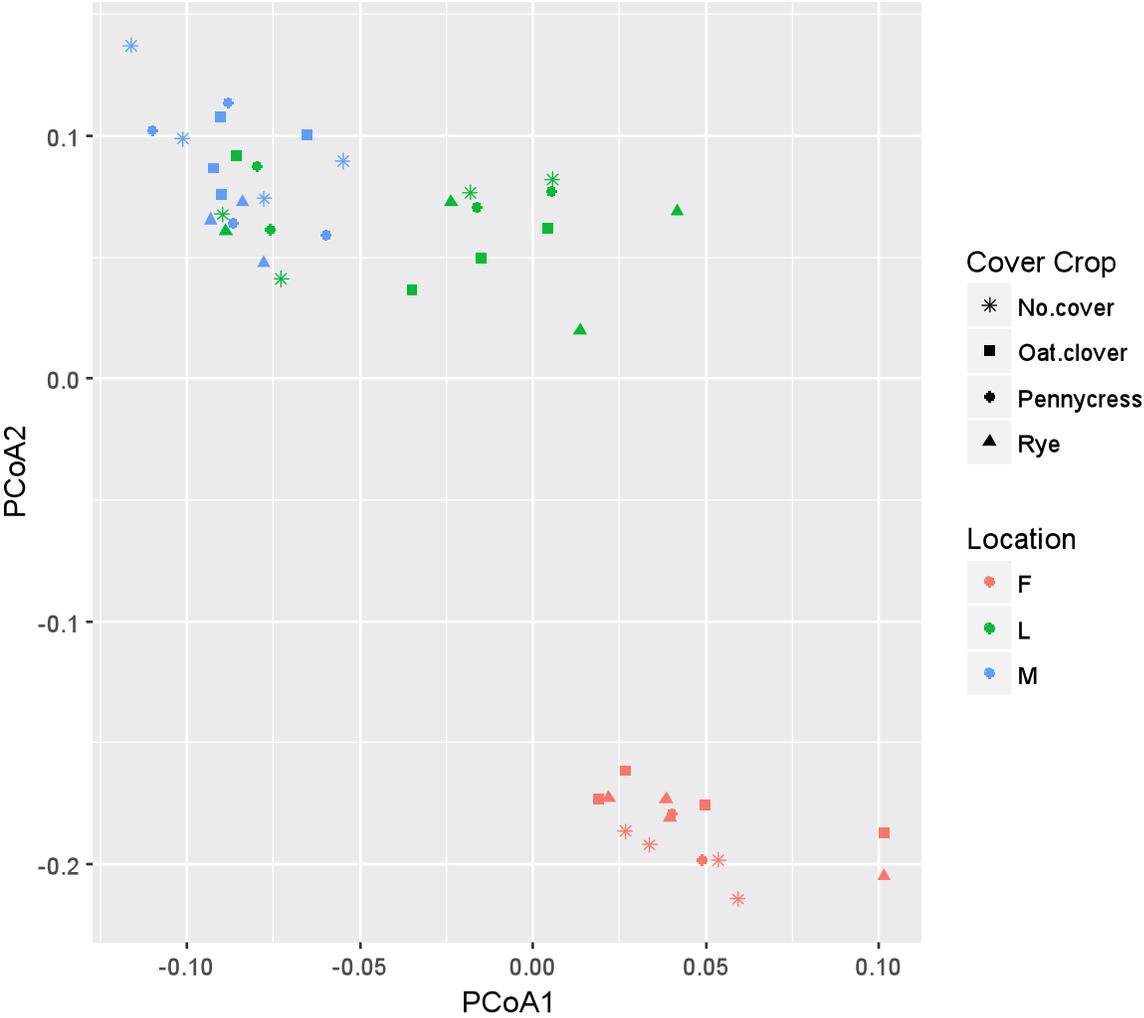
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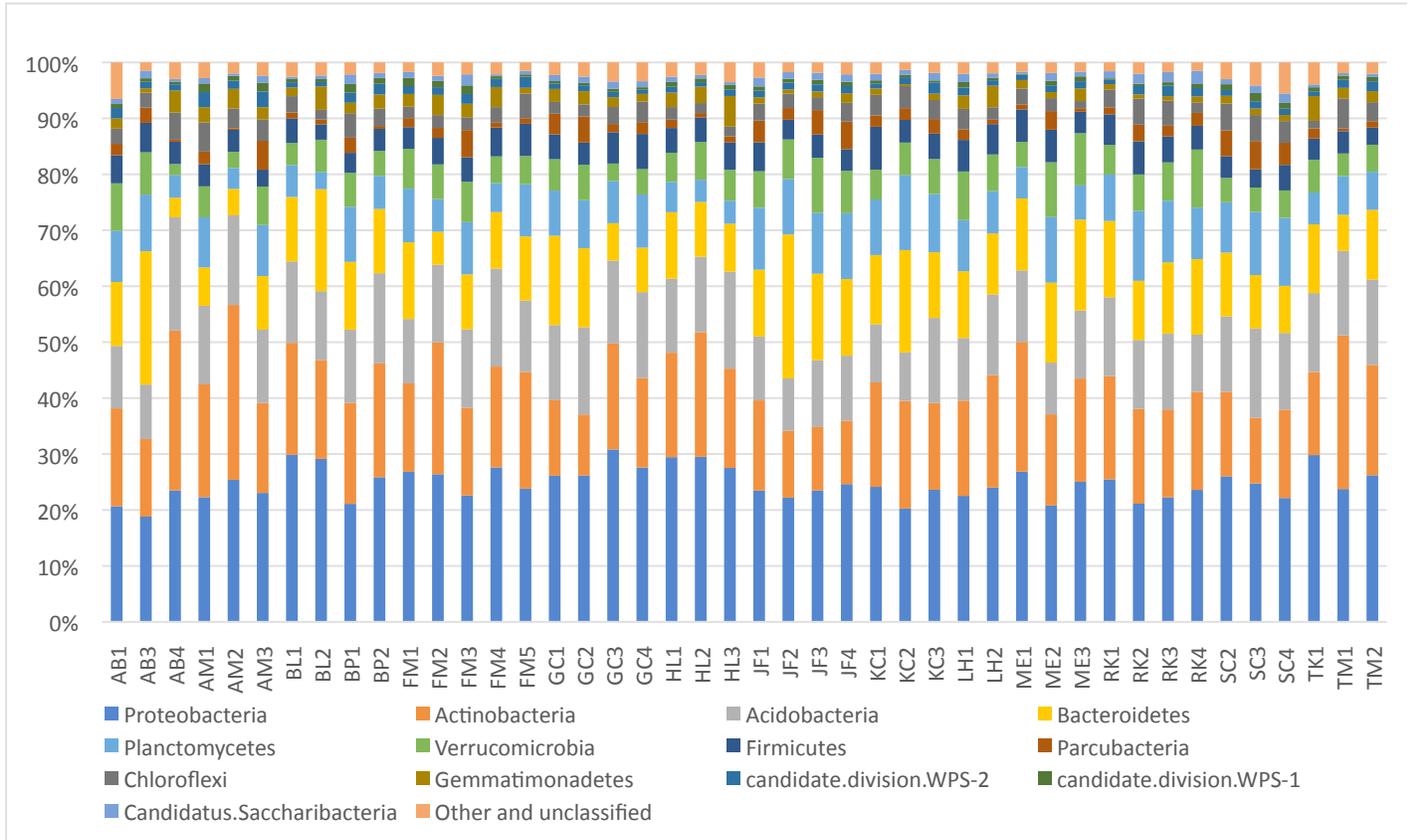
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**Figure 1. Principal coordinate analysis of bacterial community composition at three sites following cover crop termination in May 2016. Site and underlying soil type were stronger drivers of community composition than short-term cover crop treatment.**



**Figure 2. Phylum composition of bacterial communities at weed seed burial sites in November 2017. Members of 47 bacterial phyla were detected, with all sites showing highly diverse communities.**



**Figure 3. Correlation between percent sand in soil and bacterial community OTU richness and diversity. Soils with higher sand content showed higher OTU richness and diversity.**

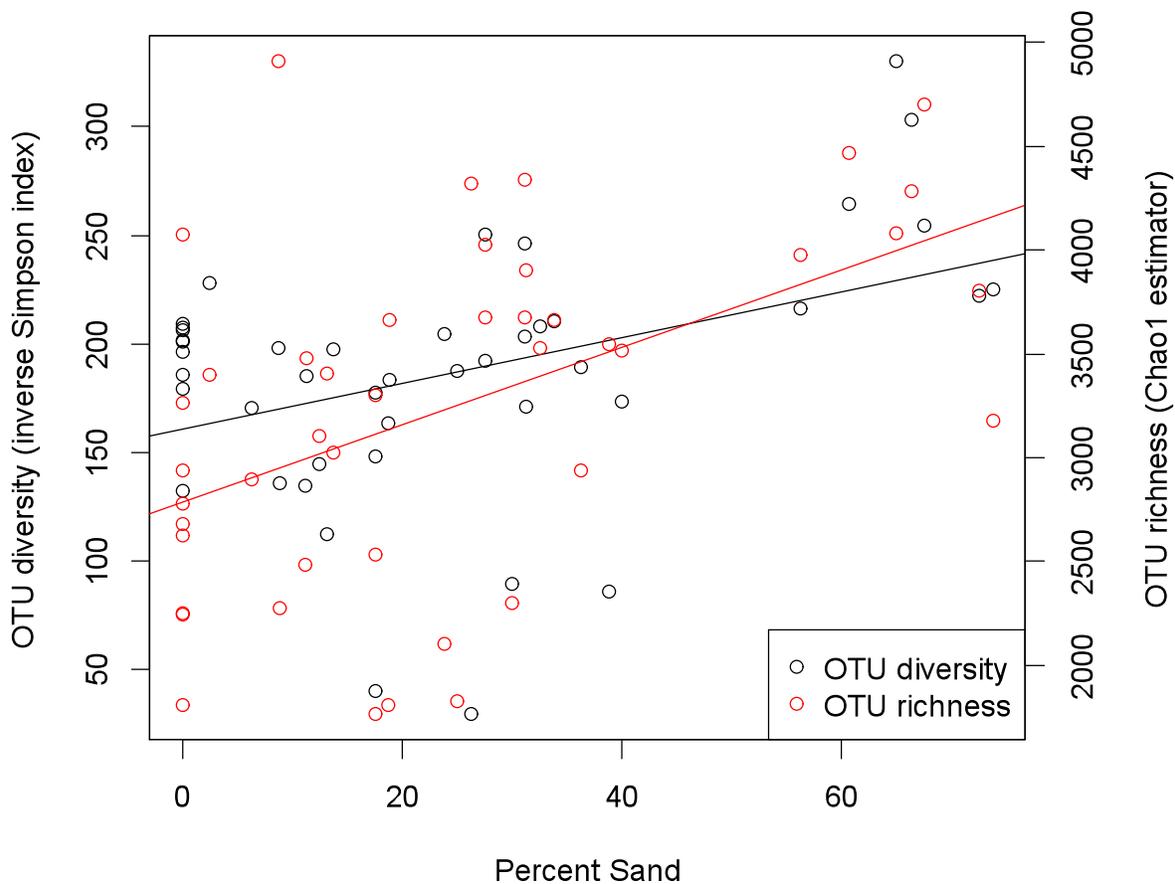


Figure 4. Redundancy analysis showing associations between weed seed death and soil composition. Letters in red represent mortality rates of weed species: GR, giant ragweed; LQ, common lamb's quarters; RP, redroot pigweed; VL, velvetleaf; YF, yellow foxtail. Mortality of lamb's quarters and yellow foxtail was highest in high-silt soils.

