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BARD Project Number: US-4266-09R

Date of Submission of the report: 10/1/2013

Project Title: The effect of combined solarization and *in solum* compost decomposition on soil health

Investigators		Institutions
Position in Proposal	Name	Affiliated institutions
1) Principal Investigator (PI)	Jean VanderGheynst	UC Davis
2) co-PI	Michael Raviv	ARO
3) Collaborating	Jim Stapleton	Kearney Agricultural Center, Parlier
4) Collaborating	Dror Minz	ARO

microbial communities, soil disinfestation, soil fumigation alternative, organic soil amendment

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Abbreviations commonly used in the report, in alphabetical order:

Budget: IS: \$ 162,000

US: \$ 163,000

Total: \$ 325,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged		2		2
Submitted, in review, in preparation	5	3	4	12
Invited review papers				
Book chapters				
Books				
Master theses		1		1
Ph.D. theses			1	
Abstracts		4		4
Not refereed (proceedings, reports, etc.)		2		2

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

No postdoctoral scholars received more than 50% of their salary from this grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	0	2	0	2
Longer Visits (Sabbaticals)				

Description Cooperation:

Collaboration was accomplished through exchanges via e-mail, meetings on Skype, and in-person meetings.

Dr. Michael Raviv visited UC Davis in February, 2010 and presented his research in a departmental seminar in Biological and Agricultural Engineering. He also met with Dr. VanderGheynst and Dr. Stapleton to discuss the BARD project and experimental plans. Dr. Raviv was provided with compost that is being used in UC Davis for calorimetry analyses. Dr. Raviv visited UC Davis again in March, 2013 to discuss results and plans for joint manuscripts from the project. Please see the table on the following page. Discussions are still underway regarding manuscript preparation. Dr. VanderGheynst also plans to analyze pathogen inactivation data collected by Dr. Raviv's team.

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During our March 2013 meeting the Israeli and US teams discussed the preparation of possible manuscripts. A list of manuscripts discussed and their status appears in the table below.

Proposed submission order	Proposed (tentative) title	Group(s)	Current state	Possible journal
1	Managing compost stability and amendment to soil to enhance soil heating during soil solarization.	UCD	published	<i>Waste Management</i>
2	Characterization of bacterial communities in solarized soil amended with lignocellulosic organic matter.	UCD	published	<i>Applied Soil Ecology</i>
3	Predicting and validating thermal inactivation of <i>Brassica nigra</i> seeds in soil.*	UCD	In preparation	<i>Journal of Pest Management Science</i>
4	Survival of propagules of SBP as a function of soil temperature and compost maturity.* ¹	Both	In preparation	<i>Phytopathology, or Plant Pathology</i>
5	How the combination of compost and soil solarization affects soil microbial activity and communities? ¹	Both	In preparation	<i>Applied Soil Ecology</i>
6	Dynamics of soil temperature as affected by the properties of organic amendments.	Both	In preparation	<i>Compost Sci. & Util.</i>
7	The development of soil suppressiveness against SBD as affected by composts of different maturity levels and solarization.	ARO	In preparation	<i>Applied Soil Ecology</i>
8	Modeling pathogens survival as a function of temperature regime and its validation <i>in vivo</i> .	Both	In preparation	To be determined
9	Can moderate temperatures, achieved during solarization, affect antagonists of extremely resistant pathogens?	ARO	In preparation	To be determined
10	The effect of combined compost application and solarization on soil's microbial communities.	Both	In preparation	<i>Journal of Food and Ag. Chem.</i>
11	Organic acid profiles in solarized soil amended with varying levels of organic matter.	UCD	In preparation	To be determined
12	Contributions of biotoxic volatile compounds and elevated temperature to mortality of <i>Brassica nigra</i> L. seeds in soil amended with destabilized green waste compost	UCD	In preparation	To be determined

* After the above indicated papers are accepted for publication, we plan to work on a review, in order to generalize the process. Potential journal: Crop Protection.

¹ Housam Kanaan, a PhD student advised by Dr. Raviv, will be the first author of these joint papers

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Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted	0	0	0	0
Issued (allowed)	0	0	0	0
Licensed	0	0	0	0

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Submitted by the Israeli team:

Michael Raviv, Dror Minz, Housam Kana'an (Ph.D. student), Eddie Cytryn, Shlomit Medina and Arkady Krassnovsky (Research Engineers).

Objective 1: Develop and validate a heat generation model during solarization associated with organic matter decomposition at various degrees of stability

Compost production: The Israeli team used cattle manure-based composts produced in Newe Ya'ar Research Center, in a Rutgers-type, temperature- and moisture-controlled composting facility. This is the type of compost most widely used by organic growers, the group which is most likely to adopt first the results of this project.

In order to test the project hypothesis, composts of 3 maturity levels were produced: fully mature, semi-prepared and immature. In all cases the pile temperatures were at least 3 weeks above 50°C and turned at least once, to ensure eradication of zoonotic pathogens. In the third year the composts consisted of 75% cattle manure and 25% wheat straw (on a volumetric basis). On a weight basis the ratio was 85% manure and 15% straw. This year the composting processes were initiated on January 8, 2012 (fully mature), April 29, 2012 (semi-prepared) and June 6, 2012 (young) and lasted till July 2, 2012. Temperatures were controlled using forced aeration, operated whenever temperature reached 60°C, or for 1 minute every hour when temperatures were below the set-point in order to prevent the development of anaerobic pockets. Moisture content was maintained at 50-60% during the thermophilic stage and 35-45% afterwards. Temperature evolution and time of turnings are shown in [Figure 1](#) and the chemical characteristics of the composts appear in [Table 1](#).

Figure 1: Temperature evolution of the 3 composts. Triangles denote turnings.

Table 1: Some chemical and physical characteristics of the 3 composts at application time (July 2, 2012). Ions were measured in a 1:10 water extract.

MEASURED PARAMETER (%)	COMPOST 1	COMPOST 2	COMPOST 3
Moisture content (%)	36.0	36.1	48.8
Bulk density (gr cm ⁻³)	0.45	0.23	0.13
Ash (%)	52.7	39.1	19.5
N (%)	1.94	2.34	2.26
P (%)	0.80	0.64	0.52
K (%)	3.34	2.27	1.75
Ca (%)	9.0	6.6	3.5
Mg (%)	1.34	1.10	0.63
Na (%)	0.59	0.73	0.75
C/N	14.3	15.3	21.0
pH	6.2	7.5	7.6
EC (dS m ⁻¹)	11.48	6.76	5.56
N-NO ₃ ⁻ (ppm)	373.1	80.5	4.4
N-NH ₄ ⁺ (ppm)	29.1	30.8	29.7
N-NO ₃ /N-NH ₄	12.8	2.61	0.15
P-PO ₄ ⁻³ (ppm)	21.9	23.8	17.8
K ⁺ (ppm)	2835	1279	991

The results demonstrate the difference in maturity level among the composts: The maturity of compost 1 is reflected in its high N-NO₃/N-NH₄ ratio and higher levels of bulk density and ash content. Compost 1 characterizes normal, mature composts used by Israeli growers.

Respiration of the three composts was assessed using the method of [Bartha and Pramer \(1965\)](#). The results appear in [Figure 2](#).

Figure 2: Respiration rates (mg CO₂ g DW compost⁻¹)

As can be predicted, the three composts revealed very different respiration rates, following their maturity levels. Heat generation of these composts (for materials and methods see below) is shown in [Table 2](#).

Table 2: Height of the line above the baseline ($\mu\text{watt mg}^{-1}\text{dry weight}$) 12 hours after start, and heat generation during 12 hours ($\mu\text{Joule mg}^{-1}$), of the 3 composts produced during the third year. Different letters next to means within a column indicate significant differences (Tukey – Kramer HSD, $P < 0.05$).

Compost type	Heat generation during 12 hours	Height
Mature	31790 b	0.582 b
Partly mature	36908 b	0.678 b
Young	78716 a	1.440 a

The difference in heat generation between the mature compost and the other two is clearly demonstrated, as in the previous two years, although in the third year the young compost was not significantly different from the partly mature compost (unlike the case of respiration). This is in spite of the fact that chemical measures such as C/N and nitrate/ammonium ratios showed clear difference between the two.

Dehydrogenase activity of the three composts was measured according to [Casida et al., \(1964\)](#). The results appear in [Figure 3](#).

Figure 3: Dehydrogenase activity of the three composts.

As in the case of heat generation, the mature and partly mature composts are not significantly different from each other ($p < 0.05$), while young compost showed higher (~35%) activity.

It can be concluded (also based on analyses done in the previous two years) that the young compost may be able to contribute some heat energy during the solarization stage.

Estimating heat generation associated with combined soil solarization and compost amendment. Heat generation potential, associated with aerobic microbial activity during *in solum* compost decomposition, was estimated using microcalorimetric measurements according to [Laor et al. \(2004\)](#) and [Medina et al. \(2009\)](#). A CSC 4100 Multi-cell Differential Scanning Calorimeter, equipped with four 1.43 ml Hastelloy removable ampoules closed with Viton gaskets was used. The microcalorimeter was operated under isothermal mode at 25°C. Composts and compost-amended soils (200-300 mg DW, soil or 40-60 mg DW composts) were placed into three removable ampoules while the reference ampoule stayed empty and served as blank. Microcalorimetric curves were obtained by

continuous recording the emitted heat versus time for each sample ampoule and by calculating the area under the power line minus the baseline. Measurements were done in triplicates. Typically this measurement lasts 12 hours.

For both measures of heat generation, in order to determine the heat generation associated with the compost, the difference in the heat generation between the soil + compost and soil alone were calculated. A Tukey – Kramer HSD test was performed on the differences to determine if the compost and soil treatments (i.e. compost degree of stability and the solarization treatment) vary in their potential to heat the soil.

Soil heat generation was measured before starting this years' solarization and before amending the soil with composts. Results are shown in [Table 3](#). In this case samples were taken on 21.6.12, a week after the soil was irrigated in preparation for the solarization.

Table 3: Height of the line above the baseline ($\mu\text{watt mg}^{-1}\text{dry weight}$) 12 hours after start, and heat generation during 12 hours ($\mu\text{Joule mg}^{-1}$), of the soil treatments of the second year, prior to solarization and soil amendment. No significant differences were found at $p \leq 0.05$.

Treatment	Solarized in the previous year	Heat generation during 12 hours	Height
Unamended soil	+	309	0.003
Soil + mature compost	+	505	0.009
Soil + partly mature compost	+	449	0.007
Soil + young compost	+	501	0.008
Unamended soil	-	360	0.006
Soil+ mature compost	-	551	0.012
Soil + partly mature compost	-	511	0.011
Soil + young compost	-	657	0.013

Heat generation of these samples was generally low, probably due to the absence of the typical burst of activity, characterizes dry soil immediately after wetting. Although no significant differences were found among the treatments (of the previous season) it is interesting to note that amended soil was in both cases (solarized and non-solarized) somewhat more energetic than the unamended control. It is also intriguing to see that even

11 months after the end of the previous solarization, the non-solarized treatments were somewhat more energetic than their corresponding solarized treatments.

Before starting the third solarization cycle, dry soil samples of the eight different treatments (control, 3 compost ages, $\pm$ solarization) of the second year were taken on 24.5.12. They were air dried, grinded, screened on a 2-mm mesh and amended (or not) with similar newly-produced composts at a rate equivalent to 4 Kg m² (based on a mixing depth of 20 cm). After mixing the composts with the soils, a similar test to the one described above was conducted and its results are shown in [Table 4](#). At the same time samples were inserted in nylon mesh bags within the soil, to be tested again 2 and 4 weeks after the end of solarization.

Table 4: Height of the line above the baseline (μ watt mg⁻¹ dry weight) 12 hours after start, and heat generation during 12 hours (μ Joule mg⁻¹), of the soil and the composts-containing soil (3rd year) before starting the solarization. Different letters next to means within a column indicate significant differences (Tukey – Kramer, $P < 0.05$).

Treatment	Solarized in the previous year	Heat generation during 12 hours	Height
Unamended soil	+	1587 d	0.03 e
Soil + mature compost	+	2048 d	0.04 de
Soil + partly mature compost	+	2805 cd	0.06 cde
Soil + young compost	+	4935 abc	0.09 abc
Unamended soil	-	3785 bcd	0.08 bcd
Soil+ mature compost	-	3665 bcd	0.07 bcde
Soil + partly mature compost	-	5189 ab	0.10 ab
Soil + young compost	-	6682 a	0.13 a

Unlike the background measurement ([Table 3](#)), the differences among the treatments after adding composts ([Table 4](#)) were statistically significant. Amending the soil with immature compost resulted with a clear increase in its heat-generating capacity. This allowed us to verify our initial hypothesis, namely that immature compost added to soil, releases a significant amount of energy which eventually can be expressed in higher soil temperatures. Another interesting observation is that previously solarized soil was able to generate less heat than the control, as was also shown in [Table 3](#).

After the end of the solarization period, heat generation of the soils was tested again (Table 5).

Table 5: Height of the line above the baseline ($\mu\text{watt mg}^{-1}$ dry weight) 12 hours after start, and heat generation during 12 hours ($\mu\text{Joule mg}^{-1}$), of the soil and the composts-containing soil (3rd year) after the end of solarization. Different letters next to means within a column indicate significant differences (Tukey – Kramer , $P<0.05$).

Treatment	Solarized in the previous year	Heat generation during 12 hours	Height
Unamended soil	+	410 d	0.008 d
Soil + mature compost	+	541 cd	0.010 cd
Soil + partly mature compost	+	610 cd	0.012 cd
Soil + young compost	+	913 bc	0.019 bc
Unamended soil	-	674 cd	0.013 cd
Soil+ mature compost	-	1272 b	0.026 b
Soil + partly mature compost	-	1883 a	0.038 a
Soil + young compost	-	1882 a	0.038 a

The summer period caused (as previously found) a clear decline in microbial activity in both solarized and non-solarized treatments. In spite of the additional 4-week *in solum* maturation period, and as in the previous case, “young” compost significantly increased heat generation in both solarized and non-solarized treatments. Similarly, solarization caused a significant decline in heat generation in both amended and non-amended treatments.

Other tested measures of soil’s microbial activity were respiration, dehydrogenase and FDA. All these were measured along the solarization period + the 4-week recovery period. Due to space considerations only the respiration results are shown (Figure 4).

Figure 4: Soil respiration as affected by solarization and amendment type.

+ covered, - uncovered, M – mature, PM – partly mature, Y – young, C – unamended control.

Although the young and partly manure composts caused an increased respiration rate in the uncovered soil, their effect was lower once covered. Similar results were obtained for the tested enzymatic activities.

Objective 2: Characterize the combined effect of solarization and compost application on microbial community dynamics during and after solarization.

Soil samples from the 2nd year of the field experiment were collected and DNA was extracted. DNA was PCR amplified using DGGE primers specific to 16S rRNA gene fragment. PCR products were analyzed by DGGE and the resulting patterns were documented, analyzed using Fingerprinting II software (Bio-Rad) and compared using PCA. The results are shown in [Figure 5](#).

Figure 5: PCA analysis of bacterial community DGGE fingerprints in soils.

The most obvious observation is that regardless of the stage of the experiment (weeks 0, 4 and 8), the biggest effect is that of the solarization - all solarized samples clustered together and separated from all control samples. Within each treatment (control and solarized), samples separated according to the amended compost type. In addition, it seems that the effect of solarization on communities in compost-amended soils was less robust, as in weeks 4 and 8 the communities of compost-amended soils were closer in solarized and control soils than the communities of the non-amended soils.

The bacterial community composition in these soils is currently being analyzed by sequencing of the 16S rRNA gene fragments to identify bacterial groups of importance.

Objective 5: Conduct solarization experiments in the field to confirm soil temperature and inactivation models and to study the microbial community and plant pathogen and weed dynamics during recolonization.

A field experiment was carried out at Newe Ya'ar, an agricultural research center located in the Jezre'el Valley, Israel. The soil is a Chromic Haploxerert (fine-clayey, montmorillonitic, thermic). The climate is typical to the Mediterranean region: cool winter

(average minimum of 8-9°C), warm summer (average maximum of 28-29°C). The average annual precipitation is about 530 mm, concentrated mostly between November and March.

Field plots were irrigated to 80% field capacity to a depth of 30 cm and then covered with polyethylene-solarization film (UVA Arava, 0.1 mm, Ginegar Plastics, Israel) or left uncovered (control). In order to prevent spatial gradients in soil temperature from affecting results, each replicate occupied a flat (non-bedded) 3 m x 6 m space, but only the inner 1 m x 4 m was used for measurements and for inserting inoculated soil sample (Grinstein et al. 1995; Mahrer and Katan 1981). Nylon mesh bags were filled with soil or soil + compost inoculated with 2×10^4 microsclerotia per gram of *V. dahliae*. Similarly, different nylon mesh bags were inoculated with of 6×10^5 sclerotia per gram soil of *Macrophomina phaseolina*. The bags were randomly distributed in the field plot at 10 cm and 20 cm below the soil surface. At 16 locations in each plot, temperature were continuously monitored using thermistors (TMC20 – HD) and a data logger (H08-008-04, HOBO, Onset Co., Bourne, MA, USA) at depths of 10 and 20 cm continuously throughout the experiment. Weather data from a standard weather station that is located in the vicinity of the test field were collected. In addition to bags containing soils infested with propagules, additional bags were inserted containing soil naturally infested with both fungi. These samples were later excavated, inserted in pots, to which test plants were planted.

Solarization started on July 2, 2012 and lasted 4 weeks. After the polyethylene film has been removed, some nylon bags were left in the soil for additional 4 weeks in order to study potential soil recolonization. Sampling was done once a week throughout solarization and twice during recolonization. At each sampling time, one nylon bag was removed from each plot, each depth and for each pathogen and analyzed for pathogens survival. In addition, soil samples were collected for DGGE, q-PCR, respiration, and other chemical analyses.

Temperatures were clearly affected by the PE cover, as can be seen in [Figure 6 a and b](#).

Figure 6: Temperature increase by the PE cover, during 4 weeks of solarization. Each data point represents the average reading of two sensors, covered minus uncovered.

a – 10 cm, b – 20 cm.

a.

b.

The solarization itself caused a temperature increase of about 10 and 18°C during the night and day, respectively, over the bare soil at the 10 cm depth. In absolute terms, the daily temperature average was about 45°C (data not shown). Depth moderated the night-day difference and it was 8 and 14 °C, respectively at the 20 cm depth. In absolute terms, the daily temperature average was about 41°C at the 20 cm depth (data not shown). At the upper layer the specific contribution of the young compost was quite significant at 5-7°C above the unamended soil. As can be predicted by the lower heat generation potential of the compost in the deeper layer (see [Table 5](#)), this difference was much more limited at 20 cm depth. Overall, our hypothesis that young compost can increase soil temperature during was verified.

In order to characterize the relative contribution of both factors (i.e. solarization and compost age) to soil heating, the number of degree-hours above 35°C was calculated at the end of the solarization period. The results are shown in [Table 6](#).

Table 6: Accumulated number of degree-hours above 35°C during the solarization period, as affected by cover, compost type and depth. Each data point represents the average reading of two sensors. Negative values means temperatures were mostly below 35°C.

Treatment	10 cm	20 cm
Unamended soil	4266	3769
Soil + mature compost	4357	3849
Soil + partly mature compost	4767	4003
Soil + young compost	4462	3792
Unamended soil	-2823	-3337
Soil+ mature compost	-3402	-3251
Soil + partly mature compost	-2902	-2651
Soil + young compost	-1964	-2237

The results presented in [Table 6](#) suggest that, unlike the results of the previous two years, the heat contributed by the young compost under cover was marginal. Some heat contribution of the young compost was apparent in the uncovered soil: at the depth of 20 cm the young compost contributed, on the average, 1.8°C, and at the depth of 10 cm – 1.4°C. At this point we have no solid explanation for this inconsistency. One explanation can be that oxygen deficiency under the cover delayed organic matter decomposition. At any rate, as can be seen in the following, this low temperature increase cannot significantly affect survival of *Macrophomina phaseolina*, while even lower temperatures were sufficient to eradicate *Verticillium dahliae*.

Objective 3: Characterize the combined effect of solarization and soil compost amendment on thermal inactivation of model plant pathogens.

Lethal temperatures are the main driving force of pathogen inactivation during solarization. Although several researchers have examined the effect of constant temperature and exposure time on inactivation of *V. dahliae*, a thermal death kinetic model has not been validated or applied to predict inactivation under non-isothermal temperatures.

***Verticillium dahliae* inoculum production and detection of viable propagules**

To produce microsclerotia inoculum, *V. dahliae* that was grown on a medium composed of 20 g l⁻¹ potato dextrose agar and 10 g l⁻¹ of agar, and 100 mg l⁻¹ of streptomycin were used to inoculate autoclaved wheat straw. *V. dahliae* was allowed to completely colonize the straw and then the mixture was air dried in order to maintain microsclerotia but eliminate short-lived conidia, and enable grinding. The inoculated straw was ground and the powder was used as inoculum. The presence of microsclerotia was verified under binocular. To quantify *V. dahliae* microsclerotia from the straw powder or from sieved soil, a sample was air-dried, mixed using vortex, diluted, and then dispersed with Drigalski spatula onto a semi-selective medium, containing ethanol and streptomycin. For each sample, 2X10⁻³ g subsamples were dispersed onto each of four Petri plates.

***Macrophomina phaseolina* inoculum production and detection of viable propagules**

Sorghum seeds were soaked in deionized water overnight and autoclaved. Discs of PDA agar inoculated with 4-days old *M. phaseolina* mycelium were placed on the sterile wet sorghum. Incubation was conducted at 30°C for 2 weeks, during which the seeds were aerated and mixed every 3-4 days, until all sorghum seeds turned black due to sclerotia formation. After drying at 37°C for additional 6 days, the seeds were ground and sieved at 425 µm. The inoculum was kept at 4°C. Soil bags from the field were dried at 37°C for 15 days, and kept at 4°C until use.

Inoculated soil samples were shaken in distilled water for 30 minutes, diluted, and then dispersed with Drigalski spatula onto a modified SSM medium (Ndiaye et al., 2010). In short, the SSM medium was prepared using 1.5 ml of 0.525% sodium hypochlorite, 1 ml of 0.5% chloramphenicol dissolved in 95% alcohol, 10 ml of 2.25% quitozene (PCNB). All the above were added to 100 ml of a potato dextrose agar (PDA) with 10 mg streptomycin, after being cooled to 55°C. 2X10⁻³ g subsamples were dispersed onto each of four Petri plates. Colonies were counted after 7 day incubation at 32°C.

***Survival of Verticillium dahliae* – 3rd year**

Survival results for *Verticillium dahliae* are presented in Figure 7 a, b, c and d.

Figure 7: Survival rate of *Verticillium dahliae* propagules, as affected by soil solarization, compost types and the relevant controls, at 10 and 20 cm depth.

a: +solarization, 10 cm; b: -solarization, 10 cm; c: +solarization, 20 cm; d: -solarization, 20 cm.

After one week of solarization, no propagules of *V. dahliae* survived, irrespective of the compost treatment or depth. Without solarization, a reduction of ~2 orders of magnitude occurred, followed by partial slow recovery after the end of the solarization period. Compost showed no distinct suppressive capacity against the *V. dahliae*.

***Survival of Macrophomina phaseolina* – 3rd year**

Survival of *M. phaseolina* propagules during the solarization process, and 4 week later are shown in Figure 8.

Figure 8: Survival rate of *M. phaseolina* propagules, as affected by soil solarization, compost types and the relevant controls, at 10 and 20 cm depth.

a: +solarization, 10 cm; b: -solarization, 10 cm; c: +solarization, 20 cm; d: -solarization, 20 cm.

Unlike in the previous year, a sharp decline in infestation with *M. phaseolina* was noticed after 2 weeks, in both solarized and bare soils. However, in all treatments and depths some (and in the case of solarization, 10 cm significant) recovery was observed during the following weeks. No clear effect of the various composts could be observed in any of these cases.

Objective 6: Measure the relative contribution of mature vs. immature composts to soil suppressiveness against soil-borne pathogens with and without solarization.

One of our original hypotheses was that mature compost confers to the soil some degree of suppressiveness against soil-borne diseases. We further assumed that after *in solum* young compost maturation, it will also result with some degree of suppressiveness. In order to test these hypotheses, soil samples were collected at time 0, after the end of the solarization process, for weeks and 6 months later from the tested field plots (described in Objective 5).

In this case we used the pathosystem melon/fusarium as previously described (Yogev *et al.*, 2006) to characterize soil suppressiveness. Inoculated vs. uninoculated

Melon plants (CV. Ofir, *Fusarium oxysporum* F. Sp. *melonis*) were planted into pots filled with the tested soil samples and their disease-related symptoms were monitored during 21 days. Seeds of melons were planted in sand and irrigated with tap water. Six days after sowing the seedlings were removed; their roots were washed, cut to 2 cm and dipped for 2 minutes in the conidial suspension that contained 3×10^5 conidia ml^{-1} . The seedlings were then transplanted into the tested soils or white fine peat (Tref, Kildare, Ireland) that was used as a conducive medium, to ascertain the aggressiveness of the propagules. As an example, the results of the last sampling date (6 months from the start of solarization) are shown in Figure 9.

Figure 9: Fusarium wilt development in melon plants, planted in solarized vs. non-solarized soils amended or not amended with composts of different maturity levels, as compared to peat. Different letters indicate significant difference between AUDPC values ($p \leq 0.05$).

All non-inoculated plants showed no disease symptoms (data not shown). In general, composts increased soil suppressiveness of the non-solarized soils, but had lower effect on the solarized soils. Young compost fully matured in the non-solarized soil, and showed complete suppressiveness. When applied to solarized soil, where biological activity was more moderate (as shown in Figure 4 and Table 5), young compost did not result with a

suppressive soil while mature and partly mature composts showed significant suppressiveness.

Concluding remarks

Although we were able to measure a significant heat generation capacity of young composts, our main hypothesis, namely, that young compost will increase soil temperature during soil solarization proved incorrect. As a result, no improved *M. phaseolina* eradication was achieved. In this respect it is interesting to note that young compost did, in fact, warm up non-solarized soil by 1-2°C.

Some of our other initial hypotheses could not be validated, as well. For example, young compost did not undergo full *in solum* maturation, and did not turn the soil suppressive.

An important observation is that both compost application and soil solarization have distinct residual effect. As mentioned in the previous report due to technical problem we could not conduct the experiments in the same plot for three consecutive years. However, even two years (year 2 and 3) were sufficient to demonstrate this effect in terms of microbial activity.

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Final Report

Submitted by the US team

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Soil solarization is a nonchemical alternative to chemical fumigation for soil disinfection prior to planting. Moist soil is covered with clear plastic, resulting in an increase in soil temperature and inactivation of soil-borne weed and pathogen propagules. However, soil solarization treatments must be applied for a longer duration than is feasible for many growers, and are also subject to climatic restrictions. The addition of compost to soil prior to solarization could improve the efficacy of solarization by generating higher soil temperatures and/or by inducing soil suppressiveness over time.

This report describes progress made determining heat generation and cumulative biological activity in soil amended with compost and organic matter under a diurnal temperature cycle, determining microbial community composition in soil amended with varying levels of compost and organic matter, modeling weed inactivation in sand at constant temperatures, and initiation of a field trial to measure the effects of solarization and compost amendment on weed inactivation, soil respiration, and microbial community composition.

Objective 1: Model of heat generation during soil solarization

Accurate modeling of the soil solarization process will be important in bringing solarization technology to its full potential. With mounting environmental concern and pressure on growers to transition to more sustainable agricultural practices, the need for reliable models also increases. These models would allow growers to predict outcomes, plan for them, and therefore more easily adopt the new practice by minimizing the impact of transition. The model developed for stable soil (Marshall, 2012) is one such tool but it does not take into account heat generated from the breakdown of organic matter that may be incorporated into the soil prior to solarization. In order to accurately predict amended soil temperatures, the development of a more general solarization model is needed, ideally accounting for the quantity and type of amendment used. A model was developed for a soil amendment source term to predict the heat generated during solarization. This was incorporated into the source term in the solarization model resulting in a general soil solarization model. The model was validated using year 2 field trial data.

For the purposes of developing an amended soil solarization model, Simmons et al. (2013) conducted field trials during the summer of 2011 in Parlier, CA at the Kearney Agricultural Center (KAC) under the support of BARD funding. These trials compared the solarization temperature profiles of non-amended, stable soil (SS), and soil amended with undigested organic matter (AS). AS plots showed a substantial increased temperature profile for all time points relative to the SS plots. This was attributed to the heat generation associated with the decomposition of amended organic matter.

Heat generation from the degradation of soil organic matter (SOM) is the result of microbial action and is mechanistically linked to the rate at which unstable carbon-based compounds (i.e., starch, cellulose, hemi-cellulose, lignin, etc.) are converted to stable carbon molecules such as CO₂. Therefore, mathematical modeling of energy generation associated with microbial activity can be done indirectly by modeling this carbon mineralization rate, specifically the rate at which CO₂ is evolved from the soil. For the case of carbon mineralization

in compost-amended soil, a first-order model showed as good or better fit to validation data than other models including second order and Monod kinetic models ¹. High-solids aerobic microbial degradation processes, such as compost stabilization, have been estimated to produce 9500 kJ per kg of O₂ consumed during decomposition ². Since for every mole O₂ consumed one mole of CO₂ is evolved, the estimated heat yield becomes 6909 kJ/kg CO₂ evolved.

Formulation of first-order heat generation source term

The rate equation for mineralizable carbon in compost can be modeled as a first order degradation:

$$\frac{dC}{dt} = -k_1 C \quad (1)$$

where C is the concentration of mineralizable carbon in grams CO₂-C/gram dry soil and k₁ (s⁻¹) is the first order mineralization rate constant. Assuming k₁ is constant over time, integration gives an expression for C_r(t), the mineralizable carbon remaining in the soil at any time:

$$C_r(t) = C_0 e^{-k_1 t} \quad (2)$$

where C₀ is initial mineralizable carbon content (g CO₂-C/g dw). The first order rate constant, k (s⁻¹) is defined by equation 3:

$$k_1 = A_1 \exp \left[-\frac{E_a}{RT} \right] \quad (3)$$

where A₁ is an experimentally determined pre-exponential (time⁻¹), E_a is the activation energy (J · mole⁻¹), R is the universal gas constant (J · mole⁻¹ · K⁻¹) and T is the temperature (K) ³. The parameter k₁ is clearly temperature dependent and so, in fact, would not be constant over time, especially near the surface where the temperature fluctuations are the largest. Equation 2 only holds if k₁ is constant with respect to time and therefore an estimated average temperature is used in equation 3.

Differentiating equation 2 yields the degradation rate in terms of initial concentration:

$$\frac{d}{dt} C_r(t) = -k_1 C_0 e^{-k_1 t} \quad (4)$$

Multiplying this result by the heat yield, Q_{CO₂} (J/g-CO₂ evolved), gives the rate of heat generation per unit mass of dry soil, q_{gen} ($\frac{W}{g_{dw}}$):

$$q_{gen} = Q_{CO_2} k_1 C_0 e^{-k_1 t} \quad (5)$$

Multiplying by the dry-weight bulk density and letting B = ρ_{dw} Q_{CO₂} k₁ C₀ ($\frac{W}{m^3}$) gives the volumetric heat rate, q̇ ($\frac{W}{m^3}$), represented in equation 6:

$$\dot{q} = \rho_b q_{gen} = B e^{-k_1 t} \quad (6)$$

The variables required for the calculation of B and k₁ were either obtained directly from a reference or calculated based on a referenced value as summarized in table 2.4. The amount of heat generated in an aerobic degradation process is coupled to the oxygen uptake, and VanderGheynst et al. (1997) estimated this value to be Q_{O₂} = 9500 J/g of O₂ consumed. Simmons (2013), on the other hand, measured mineralizable carbon content based on CO₂ released from amended soil reactors. Aerobic degradation of organic matter releases approximately one CO₂ molecule for every O₂ consumed, so the carbon dioxide-linked heat yield

was calculated as follows:

$$Q_{CO_2} = Q_{O_2} \left(\frac{m_{O_2}}{m_{CO_2}} \right) = \left(9500 \frac{J}{g} \right) \left(\frac{32 \text{ g/mol}}{44 \text{ g/mol}} \right) = \mathbf{6909 \text{ J/g } CO_2 \text{ released}} \quad (7)$$

Table 1.1: First-order source term parameters and variables

Symbol	Parameter	Value	Units
A_1	First orde pre-exponential	$\ln(A_1) = 15.5$	$days^{-1}$
E_a	Activation energy	45400	J/mol
R	Gas Constant	8.314	$J/mol \cdot K$
C_0	Total Mineralizable Carbon	16.9	$\frac{mg}{g_{dw}}$
Q_{O_2}	Heat Generation (O_2 – linked)	9500	$\frac{J}{g \text{ } O_2 \text{ consumed}}$
Q_{CO_2}	Heat Generation (CO_2 – linked)	6909	$\frac{J}{g \text{ } CO_2 \text{ evolved}}$
ρ_{dry}	Soil Bulk Density	$1.7 * 10^6$	g/m^3

Model Validation

The 1-D amended-soil heat transfer model is described by equation 7:

$$k \frac{\partial^2 T_s}{\partial x^2} + \dot{q} = C_s \frac{\partial T_s}{\partial t} \quad (8)$$

Note that if the source term, $\dot{q} \left(\frac{W}{m^3} \right)$, is set to zero, equation 8 becomes equivalent to that described by Marshall et al. (2013) for stable soils. Validation of the amended soil model consisted of comparison with two independent field data sets ⁴:

1. actual measured field temperature profiles
2. measurements of organic matter mineralization potential

The simulated field temperature profiles with the first-order source term ($\dot{q} = B e^{-k_1 t}$) were validated against field trial data ⁴. These results were also compared to zero source term ($\dot{q} = 0$) and constant source term ($\dot{q} = \text{constant}$) simulation results. The validation cases are summarized below (table 1.2).

Table 1.2: Amended soil temperature profile simulation cases

Case	Source term	Description
Case 1 (base case)	$\dot{q} = 0$	Amended soil profile using the stable-soil model
Case 2	$\dot{q} = B e^{-k_1 t}$	Amended soil profile using first order source term
Case 3	$\dot{q} = \text{constant}$	Amended soil profile using constant source term

As a secondary validation method, the simulated heat generation over the solarization period (i.e., $Q_s = \int \dot{q} dt$) was compared to the experimentally determined mineralization potential from amended soils collected from field site ⁴. During the soil stabilization process via microbial respiration, shown below, soil microbes convert mineralizable organic matter to carbon dioxide and heat, Q_m , is released in the process:

Using CO_2 emission data, Q_m can be calculated to validate the modeled heat generation.

Table 1.3: Site dependent model parameter values.

Input Variables		
experimental variables	Soil Only	Amended
maximum time (min)	31440	31440
latitude of field site (°)	36.6	36.6
longitude of field site (°W)	119.5	119.5
longitude, local standard meridian (°W)	120	120
soil parameters		
mass fraction, water	0.1248	0.22
mass fraction, om	0.0049	0.1034
mass fraction, sand	0.71	0.71
mass fraction, silt	0.23	0.23
mass fraction, clay	0.06	0.06
bulk density (dry g/cm ³)	1.60	1.39
soil type	2	2
soil color value, moist	4	4
soil color value, dry	6	6
mass fraction, water, field capacity	0.15	0.22
mass fraction, water, air dry	0.006	0.006
plastic parameters		
plastic thickness (μm)	17.8	17.8
air gap height (cm)	0.45	0.45
plastic dimension, wind, L (m)	8.5344	8.5344
plastic dimension, W (m)	1.8288	1.8288
condensation thickness (mm)	0.5	0.5
max. fraction of tarp area with condensation	0	0
emittance, long-wave	0.15	0.15
transmittance, long-wave	0.75	0.75

In order to evaluate the performance of the first order amended soil model, three distinct source term simulation cases were examined:

Case 1: Zero source term $\rightarrow \dot{q} = 0$

The model was run assuming that there was no heat generation.

Case 2: First-order source term (eq. 2.28) $\rightarrow \dot{q}_{gen} = B e^{-k_1 t}$

The values for B and k_1 were determined as described above and summarized in table 1.1. The calculated value of $Q_{CO_2} = 6909 \text{ J/g (CO}_2\text{)}$ was used for the initial simulations and further simulations were conducted to find a value of Q_{CO_2} that optimized the temperature profile prediction results.

Case 3: Constant source term $\rightarrow \dot{q} = \text{constant}$

Various constant values were used for the heat generation term to serve as a comparison for the first-order source model. An attempt was made to optimize model performance for the first, most crucial week of solarization.

Standard Error Metrics: To assess overall model accuracy, predicted temperatures generated by model simulation (T_s) were compared to measured field temperatures (T_m) by calculating the following error values (eq. 9-11):

- root mean squared (RMSE) – descriptor of average model performance;
- mean bias error (MBE) – indicator of long term over or under-prediction;
- mean absolute error (MAE) – additional descriptor of average model performance based on potential RMSE ambiguities.

$$RMSE = \left(\frac{1}{n} \sum_{i=1}^n (T_{s,i} - T_{m,i})^2 \right)^{\frac{1}{2}} \quad (9)$$

$$MBE = \frac{1}{n} \sum_{i=1}^n (T_{s,i} - T_{m,i}) \quad (10)$$

$$MAE = \frac{1}{n} \sum_{i=1}^n |T_{s,i} - T_{m,i}| \quad (11)$$

Simulated total heat generation, $Q_{sim} (J/cm^3)$, is obtained by numerically integrating the source term over the period of solarization (eq. 12):

$$Q_{sim} = \int_0^t \dot{q} dt \quad (12)$$

The volumetric heat generated, $Q_{gen} (J/cm^3)$, from mineralization of organic soil amendment was calculated from respiration data using equation 13,

$$Q_{gen} = U \cdot C_0 \cdot \rho_{dry} \cdot Q'_{CO_2} \quad (13)$$

where U is utilization, C_0 is the theoretical maximum amount of CO_2 that can be evolved ($g CO_2/g dry soil$), ρ_{dry} is the density of dry soil, and Q'_{CO_2} is the apparent CO_2 -linked heat yield. The dry basis density of soil was calculated according to equation 14,

$$\rho_{dry} = \rho_{wet}(1 - w) \quad (14)$$

Case 1: Zero source term $\rightarrow \dot{q} = 0$

The stable soil model showed decreased correlation with field data than when modeling stable soil (figure 1.1 and table 1.4).

Figure 1.1: Kearney field trial predicted (case 1) and measured temperatures for amended plots

Table 1.4: Summary of results for case 1 (source term, $\dot{q} = 0$)

	Days 2-8	Days 9-22	Days 2-22
RMSE (°C)	1.4	1.5	1.4
MAE (°C)	1.2	1.2	1.2
MBE (°C)	-1.0	1.1	0.4
Number of points (N)	1007	1993	3000
Avg. Max T_m (°C)	44.1		
Avg. Max T_s (°C)	43.1		
Avg. ΔT_{max} (°C)	-1.0		

RMSE = root mean squared error, MBE = mean bias error, MAE = mean absolute error

Though the RMSE, MAE, and MBE show small errors in on aggregate and suggest a slight over-prediction trend, the average maximum temperature difference during the first week of solarization reflects an approximately 2.4% under-prediction. One of the principal aims of the current research is to minimize the error in maximum temperature prediction.

Case 2: First order source term $\rightarrow \dot{q} = Be^{-k_1 t}$

When the model was run using parameter values as described in table 1.1, the model did not converge on a solution temperature profile. Since the model did converge for the zero source

term (i.e., when $B = 0$), the first order equation was rewritten as,

$$\dot{q} = B e_1^{-kt} = Q'_{CO_2} D e^{-k_1 t} \quad (15)$$

where Q'_{CO_2} is the apparent CO_2 -linked heat yield (J/g- CO_2) and D comprises the remaining pre-exponential constants (i.e., $D = \rho_{dw} k_1 C_0$) with units of $\left(\frac{g-CO_2}{m^3 s}\right)$. An optimal value between 0 and 6909 J/g- CO_2 was sought. It seemed reasonable to vary the value of Q'_{CO_2} since this value was determined for purely aerobic systems with forced aeration² where the field soil system described by our model is likely not purely aerobic. The model was run using values of heat yield values, at regular intervals up to 6909 J/g- CO_2 , and plotted against each corresponding RMSE value as a measure of model performance over days 2-8. An optimal value of approximately 300 J/g- CO_2 emerged (figure 1.2).

Figure 1.2: Optimization curve for the apparent CO_2 -linked heat yield

The resulting temperature profile for this optimal Q'_{CO_2} (figure 1.3) shows a very tight correlation for the first week (RMSE = 0.97 °C), a slightly decreased correlation for the final 2 weeks (RMSE = 1.9 °C), and an overall RMSE of 1.65 °C. There was a very slight general over-prediction for the first week (MBE = 0.02) however inspection of the high points of figure 3.2 shows a slight under-prediction by the model. The average measured maximum daily temperature, T_m , was 44.13 °C where the corresponding average predicted maximum, T_s , was 44.12 °C. This indicates that during the critical first week of solarization, the predicted daily maximum temperatures given by the model with a first order source term showed near perfect

correlation to measured daily maximum temperatures. Table 1.5 provides a summary of these results.

Figure 1.3: Case 2 amended soil temperature profile (Predicted vs. Measured)

Table 1.5: Summary of results for case 2 (1° source term with $Q'_{CO_2} = 300 \text{ J/g-CO}_2$)

	Days 2-8	Days 9-22	Days 2-22
RMSE ($^\circ\text{C}$)	1.0	1.9	1.7
MAE ($^\circ\text{C}$)	0.9	1.7	1.4
MBE ($^\circ\text{C}$)	> 0.1	1.7	1.1
Number of points (N)	1007	1993	3000
Avg. Max T_m ($^\circ\text{C}$)	44.1		
Avg. Max T_s ($^\circ\text{C}$)	44.1		
Avg. $\Delta T_{\max}$ ($^\circ\text{C}$)	> 0.1		

RMSE = root mean squared error, MBE = mean bias error, MAE = mean absolute error

Case 3: Constant source term $\rightarrow \dot{q} = \text{constant}$

The objective of this case study was to determine if a constant heat generation model could be optimized to outperform the first-order model over days 2-8 of the solarization period. As with case 2, an optimal value was sought by implementing several constant source terms into the

model and plotting the resulting RMSE values (figure 1.4); these data suggest an optimal value of $\dot{q} = 10 \text{ W/m}^3$.

Figure 1.4: RMSE for solarization days 2-8 vs. constant source term, $\dot{q}$

Figure 1.5: Case 3 amended soil temperature profile (Predicted vs. Measured)

The resulting temperature profile for this optimal constant source term (figure 1.5) shows a slightly weaker correlation for the first week (RMSE = 1.11 °C), with a marked increase in error for the final 2 weeks (RMSE = 2.96 °C), and an overall RMSE of 2.50 °C (table 1.6). The relative error in predicted maximum daily temperatures is within 1%.

Table 1.6: Summary of results for case 3 (Constant source term, $\dot{q} = 10 \text{ W/m}^3$)

	Days 2-8	Days 9-22	Days 23-44
RMSE (°C)	1.1	3.0	2.5
MAE (°C)	0.9	2.8	2.2
MBE (°C)	-0.2	2.8	1.8
Number of points (N)	1007	1993	3000
Avg. Max T_m (°C)	44.1		
Avg. Max T_s (°C)	43.7		
Avg. $\Delta T_{\max}$ (°C)	-0.4		

RMSE = root mean squared error, MBE = mean bias error, MAE = mean absolute error

The model results for case 2 (i.e., for $\dot{q} = Q'_{CO_2} De^{-k_1 t}$ where $Q'_{CO_2} = 300 \text{ J/g-CO}_2$) were analyzed to determine the total volumetric heat generated. Equation 12 was integrated numerically using the trapezoid method and resulted in a net heat generation of $Q_{sim} = 6.0 \text{ J/cm}^3$. Equation 313 was then evaluated using the parameter values shown below (table 1.7) with a resulting heat generation, $Q_{gen} = 6.5 \text{ J/cm}^3$.

Table 1.7: Mineralization Parameters

Symbol	Parameter	Value	Units	Source
U	Utilization	0.85	--	5
ρ_{wet}	Wet basis density	1.7	g/cm^3	5
w	Water content	18.2%	g/g	5
ρ_{dry}	Dry basis density	1.39	g/cm^3	Calculated from ρ_{wet}
C_0	Max. mineralization	16.9×10^{-3}	$\text{g-CO}_2/\text{g}_{dw}$	5
Q_{CO_2}	CO_2 -linked heat yield	300	J/g-CO_2	Optimized via simulation

These results (as summarized in table 1.8) confirm that a first order source term can be used to model soil heating associated with organic matter stabilization via microbial decomposition of organic amendment during the solarization process. Based on RMSE (and a point by point comparison), case 2 appears to give the best overall fit for the initial solarization period (days 2-8) with RMSE = 1.0; however, all other RMSE values are within one standard deviation. More indicative of the first order model performance over the initial period is the prediction of the daily high temperatures. The Marshall model (stable-soil) ⁶ performed well for soil-only ($\Delta T_{\max} = 0.1^\circ\text{C}$) but not as well for amended soil ($\Delta T_{\max} = 1.1^\circ\text{C}$). Implementation of the first order source term resulted in tight correlation of daily maximum temperatures ($\Delta T_{\max} > 0.1^\circ\text{C}$), as did a constant source term ($\Delta T_{\max} = 0.4^\circ\text{C}$).

The difference between the first order and constant source terms can be clearly seen in the overall performance as presented in table 1.9. Case 3, though comparable to case 2 over the short term, clearly shows poor average performance during the final 2 weeks (RMSE = 2.5). Moreover, the constant source over-estimates the total source heat generated relative the calculated field value ($Q_{\text{sim}} = 19 \text{ J/cm}^3$ vs. $Q_{\text{gen}} = 6.5 \text{ J/cm}^3$). The first order model showed only a slight overall under-estimate of generated heat ($Q_{\text{sim}} = 6.0 \text{ J/cm}^3$).

Table 1.8: Summary of Results for days 2-8

	RMSE (°C)	Avg. ΔT_{max} (°C)	%Error ΔT_{max}
Case 0	1.3	0.1	0.33%
Case 1	1.4	1.1	2.4%
Case 2	1.0	< 0.1	< 0.2%
Case 3	1.1	0.4	1.0%

Table 1.9: Summary of Results for entire solarization period

	RMSE (°C)	Q_{gen} (J/cm ³)	Q_{sim} (J/cm ³)	% Error Q_{sim}
Case 0	1.6	--	--	--
Case 1	1.4	--	--	--
Case 2	1.7	6.5	6.0	-7.7%
Case 3	2.5	6.5	19	190%

It should be noted that, in all cases, there is a slight phase shift of the simulated temperature profile relative to the measured profile. This means that the maximum and minimum temperatures of the overlaid data sets do not correspond to the same time measurement. This shift has a slight effect on the values of RMSE, MAE, and MBE. Furthermore these phase-shifts are not uniform from case to case so, without a phase correction, small differences in these values should be taken with a grain of salt. The daily maximum temperature comparison (table 1.8) takes into account this phase shift and may provide a more informative measure of model's ability to predict solarization effectiveness.

The initial estimate for the heat yield (i.e., the expected heat released due to aerobic digestion of mineralizable biomass) of 6909 J/g-CO₂ released had to be reduced in order for the model to converge on a solution. The optimized value of 300 J/g-CO₂ (i.e. a 23-fold reduction in expected heat yield) indicates that the decomposition is not completely aerobic and is likely due in part to much less exothermic anaerobic decomposition processes.

Another possible contributor to such a large reduction in effective heat yield may lie in *vertical* discontinuity of amendment level. The model assumes that the soil amendment is continuous and consistent between the boundaries (i.e., $0 < x \leq 2 \text{ m}$) whereas the actual amended depths in these field trials did not exceed 17.4 cm (i.e., the depth of the soil microcosm). The effect, if any, of the *horizontal* discontinuity of soil amendment (due to the use of microcosms as opposed to evenly amended plots) is also unknown and would need to be considered in deployment of the model.

Objective 2: Microbial community dynamics during and after solarization

Microbial respiration in soils, especially those with increasing levels of organic matter, can augment solarization to produce soil temperatures higher than those achieved by solar heating alone. Currently, it is unclear how solarization affects microbial community structure in soils amended with organic matter to promote microbial activity. In this study, a field trial was conducted in the San Joaquin Valley of California to solarize an irrigated, agricultural field soil which was either amended with mature green waste compost destabilized with wheat bran, or not amended (Simmons et al. 2014). Following 22 days of treatment during July-August 2011, soil from three depth increments (0-5.8, 5.8-11.6, and 11.6-17.4 cm) was subjected to 16S ribosomal RNA gene sequencing to characterize microbial communities.

Soil temperature varied during field solarization depending on soil depth and amendment (Figure 2.1). Temperature differences between the amended and non-amended treatments were most apparent during the first 7 days of solarization. As such, only temperature data from this period is presented here. For both soil treatments, soil closer to the surface experienced a wider range of temperature variation, compared to greater depths. Daily temperature differences during the first three days of solarization spanned approximately 21 °C at a depth of 5.1 cm and 13 °C at a depth of 12.7 cm. Furthermore, soil nearer to the surface achieved more extreme minimum and maximum temperatures, relative to soil further from the surface. Amendment of soil with compost and wheat bran increased soil temperature during solarization, compared to non-amended field soil alone. Soil at 5.1 cm depth achieved average maximum temperatures of 48.6 and 50.5 °C for non-amended and amended soil, respectively, while average maximum temperatures of 43.9 and 44.7 °C were observed at 12.7 cm depth for non-amended and amended soil, respectively.

Figure 2.1. Mean soil temperature during solarization for non-amended and amended field soil. Data for the first 7 days of solarization are presented for two soil depths. Values on the upper horizontal axis represent the time of day. $n = 4$ for measurements at 12.7 cm. $n = 2$ for measurements at 5.1 cm. Elements of this figure were adapted or reproduced from prior work performed by Simmons et al. (2013).

NMDS revealed differences in community composition between amended and non-amended solarized soil, as well as community changes with soil depth (Figure 2.2). Non-solarized soil and mature green waste compost had differing initial community structures, which is reflected in the combined community structure of amended soil. Both soil treatments exhibited changes in community composition following solarization, relative to their initial states. While both treatments were affected by solarization, overall community dissimilarity between the solarized amended and non-amended soil treatments reflected dissimilarity between the initial communities. This is evidenced by the fact that communities in solarized non-amended soil were most similar to those in non-solarized, non-amended soil and, likewise, solarized amended soil communities were most similar to those in non-solarized amended soil.

Figure 2.2. Non-metric multidimensional scaling of microbial communities from non-amended and amended soil prior to and following solarization at different soil depths. S = non-amended soil, C = compost only, SCW = soil amended with compost and wheat bran. Controls are non-solarized samples.

Both solarized non-amended and solarized amended soil showed changes in community structure with soil depth. For amended soil, the community in the uppermost soil layer (0-5.8 cm) was most similar to the initial community in non-solarized amended soil. Community

structure in solarized amended soil diverged more from the initial state with increasing soil depth. Alternately, for solarized non-amended soil, the community in the deepest layer (11.7-17.4 cm) was most similar to that of non-solarized non-amended soil and dissimilarity from the initial state increased with proximity to the surface.

Calculation of diversity indices revealed additional information regarding how the microbial communities differed (Figure 2.3). Non-amended field soil contained a highly diverse microbial community prior to solarization. The mature green waste compost and the compost + wheat bran-amended soil contained significantly less diverse communities ($p<0.0001$ and $p=0.0008$, respectively). Community diversity in both non-amended and amended soil decreased following solarization. However, the decreases were only statistically significant in the uppermost layer of soil (0-5.8 cm) ($p=0.009$ for non-amended soil and $p=0.01$ for amended soil). Diversity increased significantly with soil depth in solarized, non-amended soil ($p=0.02$), with indices for the lowest layer being similar to those for non-solarized soil. Alternately, there was no evidence of diversity varying significantly with soil depth for solarized, amended soil ($p=0.36$). There were no significant differences in species richness among all treatments and soil depths ($\alpha=0.05$, data not shown). Thus, significant differences in diversity indices stemmed primarily from changes in species evenness (data not shown).

Figure 2.3. Shannon diversity indices for microbial communities at varying soil depths in non-amended and amended soil prior to and following solarization. Error bars represent one standard deviation. Horizontal lines indicate mean diversity values for freshly mixed, non-solarized soil and compost preparations. S = non-amended soil, C = compost only, SCW = soil amended with compost and wheat bran. $n=5$ for all samples except SCW, 0-5.8 cm for which $n=4$.

Several changes in phylum abundance were shared between amended and non-amended soil following solarization (Figure 2.4). Regardless of amendment treatment, there was a significant decrease in the relative abundance of Actinobacteria ($p<0.0001$) at all depths examined. Amended soil uniquely exhibited enrichment of Bacteroidetes following solarization ($p<0.05$) for all depths tested while non-amended soil did not. Abundance of several phyla differed with soil depth for each treatment following solarization (Figure 2.4B and 4C). For amended soil, relative abundance of Proteobacteria increased significantly with soil depth ($p<0.001$) while relative abundance levels of Acidobacteria, Actinobacteria, and Chloroflexi dropped significantly with increasing soil depth ($p=0.03$, 0.03 , and <0.001 , respectively). In contrast, relative abundance of Proteobacteria did not significantly vary with depth in solarized,

non-amended soil, whereas Acidobacteria levels increased significantly with depth ($p < 0.0001$). Solarized, non-amended soil also exhibited decreased abundance of Actinobacteria and Firmicutes with soil depth ($p < 0.001$ for both).

SIMPER analysis revealed that a diverse array of OTUs was responsible for community structure differences between certain treatments. In particular, comparison of the top layers of solarized non-amended and amended field soils (where greatest soil heating occurred) showed that 36 OTUs were responsible for approximately 50% of Bray-Curtis dissimilarity between the two communities (Table 2.1). The OTU contributing the most to dissimilarity accounted for only 6.9% of the overall dissimilarity between the two communities. The five largest contributors to community dissimilarity (accounting for approximately 20% of total dissimilarity) in solarized non-amended and amended field soil were a bacterium that could not be binned at the phylum level (enriched in amended soil), *Brevibacillus levickii* (enriched in non-amended soil), bacteria from the Pseudomonadaceae family (enriched in amended soil), *Luteimonas mephitis* (enriched in non-amended soil), and *Bacillus fumarioli* (enriched in non-amended soil). Comparison of the upper (0-5.8 cm) and lower (11.7-17.4 cm) layers of solarized amended soil showed that 21 OTUs contributed approximately 50% of the dissimilarity between the two communities (Table 2.2). *Azotobacter beijerinckii*, which was a major constituent of the community present in the lower soil layer but was undetectable in the upper layer, was the largest contributor to dissimilarity with a contribution of 10.7%. Comparison of the community in the uppermost layer of solarized amended soil to that present pre-solarization indicated that the two OTUs responsible for approximately 11% of total dissimilarity were bacteria that could not be binned to a phylum (Table 2.3). Among the subsequent OTUs accountable for approximately 20% of total community dissimilarity, a decrease in *Streptomyces radiopugnans* and increases in several Proteobacteria, Bacteroidetes, and Firmicutes OTUs following solarization were observed.

Figure 2.4. Phylum composition of microbial communities from non-amended and amended soil following solarization. (A) Relative abundance of dominant phyla in communities. Controls represent freshly mixed, non-solarized soil and compost preparations. (B) Relative abundance of phyla by soil depth in solarized, non-amended soil. (C) Relative abundance of phyla by soil depth in solarized amended soil. Dotted lines represent the line of best fit for each phylum. P-values indicate the probability of falsely concluding that a slope value is non-zero for a particular fitted line. Relative abundance values are the mean of 5 replicates for all samples except SCW, 0-5.8 cm for which $n = 4$. S = non-amended soil, C = compost only, SCW = soil amended with compost and wheat bran.

Table 2.1. SIMPER analysis of OTUs responsible for >50% of the Bray-Curtis dissimilarity between microbial communities in solarized non-amended and amended soils at 0-5.8 cm depth after 22 days of solarization.

Relative abundance (%)			
Amended soil (0-5.8 cm)	Non-amended soil (0-5.8 cm)	% contribution	Taxonomy ^a
12.5	0.0	6.9	k-Bacteria
0.0	9.4	5.2	<i>Brevibacillus levickii</i> (Firmicutes)
6.4	0.0	3.5	f-Pseudomonadaceae (Proteobacteria)
0.0	4.9	2.7	<i>Luteimonas mephitis</i> (Proteobacteria)
2.0	5.9	2.2	<i>Bacillus fumarioli</i> (Firmicutes)
3.9	0.0	2.2	o-Sphingobacteriales (Bacteroidetes)
4.1	0.2	2.1	<i>Bacillus nealsonii</i> (Firmicutes)
0.0	3.6	2.0	<i>Sphingomonas wittichii</i> (Proteobacteria)
3.6	0.0	2.0	<i>Flexibacter flexilis</i> (Bacteroidetes)
3.8	0.9	1.6	c-Gammaproteobacteria (Proteobacteria)
2.6	0.0	1.5	k-Bacteria
2.6	0.0	1.4	(Chloroflexi)
2.1	0.0	1.2	o-Rhizobiales (Proteobacteria)
0.0	2.1	1.2	<i>Alicyclobacillus pohliae</i> (Firmicutes)
2.0	0.1	1.0	f-Streptosporangiaceae (Actinobacteria)
1.8	0.0	1.0	k-Bacteria
1.7	0.0	0.9	<i>Bacillus thermoamylovorans</i> (Firmicutes)
1.6	0.0	0.9	<i>Oscillochloris trichoides</i> (Chloroflexi)
0.0	1.6	0.9	k-Bacteria
1.8	0.3	0.8	k-Bacteria
1.4	0.0	0.8	k-Bacteria
1.4	0.0	0.8	<i>Oscillochloris trichoides</i> (Chloroflexi)
0.2	1.5	0.8	<i>Bacillus flexus</i> (Firmicutes)
0.0	1.3	0.7	o-Sphingobacteriales (Bacteroidetes)
0.1	1.2	0.6	<i>Rhodocista pekingensis</i> (Proteobacteria)
1.1	0.0	0.6	(Proteobacteria)
0.0	1.0	0.6	k-Bacteria
0.0	1.0	0.6	f-Sulfobacillaceae (Firmicutes)
1.0	0.0	0.5	<i>Salinibacter ruber</i> (Bacteroidetes)
0.0	0.9	0.5	<i>Sphingomonas wittichii</i> (Proteobacteria)
0.0	0.9	0.5	f-Comamonadaceae (Proteobacteria)
0.1	1.0	0.4	c-Betaproteobacteria (Proteobacteria)
0.1	0.8	0.4	<i>Methylobacterium podarium</i> (Proteobacteria)
0.7	0.0	0.4	<i>Flexibacter flexilis</i> (Bacteroidetes)
0.7	0.0	0.4	<i>Chondromyces robustus</i> (Proteobacteria)
0.0	0.7	0.4	<i>Aeromicrobium marinum</i> (Actinobacteria)

^aTaxonomy is given as the species name with phylum in parentheses. Repeat species in the table represent different strains. For OTUs that could not be binned at the species level, the best taxonomic resolution is specified (k, kingdom; o, order; c, class; f, family; g, genus).

Table 2.2. SIMPER analysis of OTUs responsible for >50% of the Bray-Curtis dissimilarity between microbial communities in solarized amended soil at 0-5.8 cm and 11.7-17.4 cm depths after 22 days of solarization.

Relative abundance (%)		% contribution	Taxonomy ^a
Amended soil (0-5.8 cm)	Amended soil (11.7-17.4 cm)		
0.0	10.5	10.7	<i>Azotobacter beijerinckii</i> (Proteobacteria)
12.5	2.4	10.3	k-Bacteria
6.4	9.5	3.2	f-Pseudomonadaceae (Proteobacteria)
3.6	0.5	3.2	<i>Flexibacter flexilis</i> (Bacteroidetes)
3.8	6.1	2.3	c-Gammaproteobacteria (Proteobacteria)
2.6	0.7	2.0	(Chloroflexi)
1.8	0.1	1.7	k-Bacteria
0.2	1.9	1.7	<i>Clostridium butyricum</i> (Firmicutes)
2.6	1.0	1.7	k-Bacteria
2.0	0.4	1.6	f-Streptosporangiaceae (Actinobacteria)
0.8	2.4	1.6	<i>Bacillus selenatarsenatis</i> (Firmicutes)
1.8	0.3	1.5	k-Bacteria
2.1	0.9	1.3	o-Rhizobiales (Proteobacteria)
1.6	0.4	1.3	<i>Oscillochloris trichoides</i> (Chloroflexi)
1.7	0.6	1.2	<i>Bacillus thermoamylovorans</i> (Firmicutes)
0.7	1.8	1.1	<i>Flexibacter flexilis</i> (Bacteroidetes)
1.1	0.1	1.0	(Proteobacteria)
1.4	0.5	0.9	<i>Oscillochloris trichoides</i> (Chloroflexi)
1.4	0.6	0.9	k-Bacteria
0.2	0.9	0.7	<i>Flexibacter flexilis</i> (Bacteroidetes)
1.0	0.3	0.7	<i>Salinibacter ruber</i> (Bacteroidetes)

^aTaxonomy is given as the species name with phylum in parentheses. Repeat species in the table represent different strains. For OTUs that could not be binned at the species level, the best taxonomic resolution is specified (k, kingdom; o, order; c, class; f, family; g, genus).

Table 2.3. SIMPER analysis of OTUs responsible for >50% of the Bray-Curtis dissimilarity between microbial communities in amended soil (0-5.8 cm depth) prior to and following solarization.

Relative abundance (%)		% contribution	Taxonomy ^a
Pre-solarization	Post-solarization (0-5.8 cm)		
11.1	2.6	5.8	k-Bacteria
4.5	12.5	5.5	k-Bacteria
8.4	0.7	5.3	<i>Streptomyces radiopugnans</i> (Actinobacteria)
0.1	6.4	4.3	f-Pseudomonadaceae (Proteobacteria)
0.1	4.1	2.8	<i>Bacillus nealsonii</i> (Firmicutes)
0.0	3.9	2.7	o-Sphingobacteriales (Bacteroidetes)
0.0	3.6	2.4	<i>Flexibacter flexilis</i> (Bacteroidetes)
0.7	3.8	2.1	c-Gammaproteobacteria (Proteobacteria)
2.8	0.0	1.9	o-Actinomycetales (Actinobacteria)
0.0	2.6	1.8	(Chloroflexi)
2.4	0.0	1.7	o-Actinomycetales (Actinobacteria)
0.1	2.0	1.3	f-Streptosporangiaceae (Actinobacteria)
0.0	1.7	1.2	<i>Bacillus thermoamylovorans</i> (Firmicutes)
0.2	1.8	1.1	k-Bacteria
0.2	1.8	1.1	k-Bacteria
0.2	1.4	0.9	<i>Oscillochloris trichoides</i> (Chloroflexi)
1.1	0.0	0.8	k-Bacteria
1.5	0.5	0.7	g- <i>Rubrobacter</i> (Actinobacteria)
0.0	1.0	0.7	<i>Salinibacter ruber</i> (Bacteroidetes)
1.2	0.2	0.6	k-Bacteria
1.0	0.1	0.6	<i>Pseudonocardia zijingensis</i> (Actinobacteria)
0.0	0.8	0.5	<i>Nonomuraea kuesteri</i> (Actinobacteria)
0.7	0.0	0.5	<i>Aeromicrobium marinum</i> (Actinobacteria)
0.0	0.7	0.5	<i>Chondromyces robustus</i> (Proteobacteria)
1.5	2.1	0.5	o-Rhizobiales (Proteobacteria)
0.6	0.0	0.4	<i>Algoriphagus antarcticus</i> (Bacteroidetes)
0.6	0.0	0.4	<i>Tetrathiobacter kashmirensis</i> (Proteobacteria)
0.6	0.0	0.4	<i>Flavobacterium gelidilacus</i> (Bacteroidetes)
0.5	1.1	0.4	(Proteobacteria)
0.0	0.6	0.4	g- <i>Geobacter</i> (Proteobacteria)
0.5	0.0	0.4	<i>Cellvibrio japonicus</i> (Proteobacteria)
0.0	0.5	0.4	<i>Nonomuraea roseoviolacea</i> (Actinobacteria)
0.3	0.8	0.3	<i>Bacillus selenatarsenatis</i> (Firmicutes)

^aTaxonomy is given as the species name with phylum in parentheses. For OTUs that could not be binned at the species level, the best taxonomic resolution is specified (k, kingdom; o, order; c, class; f, family; g, genus).

Objective 3: Thermal inactivation of a model plant pathogen/weed propagule Modeling weed inactivation in sand

- Times and temperatures for inactivation in semi-sterile sand
Temperatures: 42, 46, 50, and 54 °C. Fitting of Weibull and logistic models
- Validation of Weibull model with nonisothermal heating in incubator, in sand only.
Compared old and new seed lots, but this will not be included in the manuscript. 4 trials total.
Max temp: 44-51 °C, min temp 16-24 °C; trials 4, 7, 9 and 14 days long
- With new, validation data included, the revised manuscript will be submitted to *Journal of Pest Management Science* before 30 September 2013:

2014 Dahlquist, R.M., Marshall, M.N., Betts, S., Tuell-Todd, C., VanderGheynst, J.S., and Stapleton, J.J. Predicting and validating thermal inactivation of *Brassica nigra* seeds in soil
Journal of Pest Management Science: in preparation

Summary: To develop guidelines for weed seedbank control strategies using soil heating, treatment duration required for mortality of imbibed seeds of *Brassica nigra* (black mustard) in moist, silica sand was determined in laboratory microcosms at constant temperatures of 42, 46, 50, and 54 °C. Logistic and Weibull inactivation models were developed from the data and compared for accuracy in estimating seed mortality as a function of heat treatment duration and temperature. Nonlinear regression was performed to determine parameter estimates and 95% confidence intervals (CI) for the estimates. Four validation trials were conducted using different, diurnally fluctuating temperature regimens, to evaluate whether the Weibull model developed from constant temperature data would accurately predict mortality of *B. nigra* seeds.

Seed samples reached complete mortality by 3 hours at 54 °C, 16 hours at 50 °C, 72 hours at 46 °C, and 240 hours at 42 °C. Results were based on both germination and embryo vital staining with triphenyl tetrazolium chloride. At 42, 46 and 50 °C, the logistic and Weibull models predicted similar trends in mortality with respect to time. Differences in model predictions were most apparent near time zero. Under the experimental conditions and across all temperatures, the Weibull shape parameter estimate was 0.904 at 95% CI. This value, since it approximated one, indicated that a first order model may be sufficient to describe inactivation of *B. nigra* seeds heated in a sandy soil or substrate matrix with relatively low organic content and biological activity. The Weibull model accurately predicted seed mortality under fluctuating diurnal temperature regimens, varying ($P < 0.05$) at only three of nineteen sampling times, across the four trials.

CONCLUSIONS: The inactivation data, coupled with the parameter estimates developed in this study, provide a basis for predicting mortality of *B. nigra* seeds during heat-based control treatments (e.g., solarization, low-temperature steam and hot water) in the field. The Weibull model, constructed from seed mortality data, can also be used to predict seed mortality in the

range of diurnally-fluctuating soil temperatures commonly occurring during field solarization. This information can be used for decision support of integrated weed management programs by end users, and for subsequent, thermal inactivation studies on other weed species.

Comparison of weed seed mortality in sand, soil, and compost

A. Comparison of sand, soil, and compost (buried seeds only): Temperatures: 42 and 46 C

B. Comparison of sand, soil, and compost with buried seeds and suspended (headspace) seeds:
Temperatures: 39, 42, and 46 C

Introduction

This study evaluated mortality of black mustard (*Brassica nigra*) seeds buried in three different substrates: field soil amended with green waste compost and wheat bran, non-amended field soil, and silica sand. Our objective was to evaluate whether amendment with compost increases mortality of weed seeds compared to substrates with lower biological activity.

Materials and methods: Comparison at 39 C

All substrates were wetted and drained to field capacity. Compost-amended soil contained 9% compost and 1% wheat bran to activate the microbial community. Twenty seeds and 15 ml of the appropriate substrate were placed in an organdy bag to provide direct contact (Fig. 1). Each substrate was tested in ten 0.47-l canning jars, with the seed bags buried in the substrate. Five jars were placed at room temperature (~20 °C) as controls, and five were placed in a water bath at 39 °C. Seed bags were removed from jars after 13, 18, 24, 36, 48, and 55 hours. Seeds were removed and placed in Petri dishes with moistened filter paper, and incubated in a growth chamber (Fig. 2). Percent germination was determined for seeds in each petri dish after 14 days. Percent germination for each seed bag was divided by the average percent germination of the controls, and percent mortality calculated as (1-germination %) (Dahlquist et al. 2007). Seeds that were not visibly germinated were confirmed as nonviable with tetrazolium staining. Data were analyzed using analysis of variance with two factors (temperature and substrate). Pairwise contrasts were performed to determine differences between means at each sampling interval.

Results and Discussion

Both heat treatment and substrate type had a significant effect on seed mortality, with higher mortality for heat-treated seeds (Fig. 3). Mortality of seeds buried in compost-amended soil was significantly higher than in sand and field soil, for all sampling intervals and temperatures ($P < 0.05$), except heated seeds at 13 hours ($P=0.09$ in soil; $P=0.17$ in sand) and unheated seeds at 24 hours ($P=0.78$ in soil; $P=0.08$ in sand). There were no significant differences in mortality between sand and field soil due to temperature or sampling interval.

Activated green waste compost added to the field soil without heat treatment resulted in a higher seed mortality rate than non-amended soil and sand. Seed mortality was relatively high in the compost-amended control soil, and was even higher when combined with heat treatment. At 48 and 55 hours of treatment, mortality of heat-treated seeds in compost-amended soil approached 100%, while mortality of heat-treated seeds in soil alone was 60-70%. This indicated that soil amendment with activated green waste compost could increase the efficacy of solarization in the field by shortening the duration of heat treatment required to achieve complete mortality of weed seeds.

C. GC-MS for headspace samples, 9% compost

Headspace volatile compounds emanating from green waste-amended soil were assayed by GC-MS in microcosms, to compare with mortality tests of buried *Brassica nigra* seeds and those suspended in microcosm headspace. There were two trials, each, at 46 C (0-24 hours exposure), and at 39 C (0-54 hours exposure). Data are currently under analysis – results expected by mid-December 2013, followed by manuscript preparation. A preliminary chromatogram is shown below:

D. Comparison of 3% and 9% compost

Five microcosm trials were conducted to compare effects on *Brassica nigra* seed mortality: 20 and 24 hours at 39 C, 24 hours at 39 C, and 5 and 7 hours at 46 C. Representative, preliminary results are shown on the following page:

E. Nonisothermal validation of model in soil and compost – Preliminary experiments were conducted as follows: 45 C max: 1, 3, and 5 days; 42 C max: 1, 3, and 5 days. This study will continue beyond the period of BARD grant funding.

Field Trial 2012

Mortality assays of *Brassica nigra* seeds were done in a field trial during 2012, repeating the study of the 2011 field trial. However, in addition to testing mortality in amended and nonamended soil, two ages of seed lots were tested. Preliminary results are shown below:

	Mortality			
	OLD SEED LOT		NEW SEED LOT	
Days	Soil	Compost	Soil	Compost
2	0.1640	1.0000	0.0061	1.0000
5	0.2173	1.0000	0.0094	1.0000
8	0.2877	1.0000	0.0634	1.0000
15	0.4776	1.0000	0.0754	1.0000

Objectives 4 and 5: Organic matter amendment effect on soil temperatures and solarization field experiments

Our first solarization experiment was completed in 2011. Results from this experiment are presented in the year-2 report and in two publications resulting from this project^{4,7}. Our second solarization field experiment was completed during July of 2012. Like the trial in 2011, these studies were performed on experimental soil contained within microcosms buried at a field site at the University of California Kearney Agricultural Research and Extension Center, Parlier, CA (KARE) in Parlier, CA (36.6 °N; 119.5 °W; elevation 96.9 m), in order to finely control conditions relating to soil amendment, temperature measurement and soil collection by depth. Soil for microcosms was collected from the field site prior to preparation. Mature green waste compost (Zamora Compost, Yolo County, CA) and autoclaved wheat bran (Giusto's Vita Grain, South San Francisco, CA) were used as soil amendments. Autoclaved wheat bran was added to increase the organic matter level and microbial activity in the soil + compost mixture.

Microcosms were prepared to assess the affect of compost and wheat bran level on heat generation (heat generation microcosms) and the effect of wheat bran and compost on weed inactivation (weed inactivation microcosm). The plot design is presented in Figure 5.1. Heat generation microcosms each contained 2% (wt/wt) compost with wheat bran varying from 0% to 5%. The weed activation microcosms contained 2% compost and 2% wheat bran. Temperature sensors were positioned at a depth of 12.7 cm in select microcosms to measure temperature during solarization. The field site is shown in figure 5.1.

After 15 days of solarization, heat generation microcosms were exhumed from field plots and stored at 1 °C overnight. Microcosms were stored at ambient conditions for approximately 3 hours during transport from the field site to the laboratory. Upon arrival, microcosms were cut into 5.8 cm sections to isolate soil samples from various depths. Soil sections were sealed in plastic bags and stored at -20 °C until analysis of residual respiration and extraction of DNA for microbial community analysis.

Respiration measurements were performed on 100 g samples (dry weight basis) of each soil section and mixtures of non-solarized soil + compost. Samples were placed into 250-ml bioreactors as previously described (May and VanderGheynst, 2001). Reactors were supplied with air at a rate of 20 ml/min and incubated at 35°C. Distilled water was added to reactors to maintain initial moisture contents. Carbon dioxide concentrations in reactor influents and effluents were measured using an infrared CO₂ sensor (Vaisala, Suffolk, UK) and mass flow rate through reactors was measured using a mass flow meter (Aalborg, Orangeburg, NY).

CO₂ evolution rate (CER) was calculated for each sample based on mass balances of CO₂ at each time point:

$$CER = F(CO_{2,out} - CO_{2,in}) \quad (5.1)$$

where F is the mass flow rate of gas through the reactor (mg/day/g dry weight), and $CO_{2,out}$ and $CO_{2,in}$ are the concentrations of CO₂ (%) in the reactor effluent and influent, respectively. Cumulative CO₂ evolution (cCER) was determined by integrating CER over time. A saturation model was fitted to cCER versus time data using KaleidaGraph v. 4.1.0 (Synergy Software, Reading, PA)⁸:

$$cCER = C_T t(c + t)^{-1} \quad (5.2)$$

where C_T is the theoretical maximum amount of CO_2 that can be evolved ($\text{mg CO}_2/\text{g dry weight}$), c is a constant describing the length of time needed to achieve half of C_T (days), and t is time (days).

Figure 5.1. Field trial treatments and plot layout.

Temperature data from heat generation microcosms are presented in figure 5.2. The temperature at 12.7 cm varied between 30°C and 48°C. For the first 10 days of solarization, temperatures in treatments containing wheat bran and compost were consistently warmer than treatments containing only compost (figure 5.3): the peak temperature differences for microcosms with wheat bran were, on average, 1.5°C greater than those without wheat bran. After 10 days of solarization the peak temperature in microcosms containing 5% wheat bran began to increase while those containing 2% wheat bran decreased. The results suggest an exhaustion of readily degradable organic matter in the 2% wheat bran microcosms.

Figure 5.2. Mean soil temperature during solarization for field soil amended with varying levels of wheat bran. 2C2W = 2% compost + 2 % wheat bran, 2C5W = 2% compost + 5% wheat bran, and 2C0W = 2% compost.

Figure 5.3. Mean soil temperature difference during solarization for field soil amended with varying levels of wheat bran. Temperature difference was calculated by subtracting the average temperature of treatments containing wheat bran from treatments containing only compost. 2C2W = 2% compost + 2 % wheat bran, and 2C5W = 2% compost + 5% wheat bran.

Figure 5.4. Daily peak soil temperature difference during solarization for field soil amended with varying levels of wheat bran. 2C2W = 2% compost + 2 % wheat bran, and 2C5W = 2% compost + 5% wheat bran.

Figure 5.5. Average residual respiration for soil mixtures after 15 days of solarization. 2C2W = 2% compost + 2 % wheat bran, and 2C5W = 2% compost + 5% wheat bran.

Figure 5.5 shows cumulative respiration for freshly prepared soil + compost + wheat bran mixtures and for samples collected from solarized microcosms. The soil containing only compost contained very little readily degradable organic matter and resulted in the least amount of respiration. As expected, soil amended with wheat bran yielded higher respiration that increased with increasing wheat bran amendment.

Exhaustion of the degradable organic matter amended with the wheat bran varied with treatment and soil depth. For treatments containing 2% wheat bran, about 55% of the respiration activity in the 0-5.8 cm layer was exhausted while only 25% was exhausted from the 11.6-17.4 cm layer during the fifteen days of solarization. In treatments containing 5% wheat bran about 60% of the respiration activity in the 0-5.8 cm layer was exhausted while only 25-30% was exhausted from the 11.6-17.4 cm layer. The higher levels of organic matter remaining in the 5% treatments could explain the higher temperatures observed throughout the solarization experiment.

Two critical questions remain regarding this study:

1. Would soils containing higher levels of residual organic matter be phytotoxic? Additional analyses are being completed on soil samples to determine organic acid levels. These will provide an indication of the potential for phytotoxicity.
2. How is the microbial community structure affected by the amount of organic matter amended and residual organic matter in the soil? We have extracted DNA from all microcosms originating from the 2012 field trial and have submitted these samples for 16S rDNA sequencing. We expect to learn more about differences in the soil microbial communities upon analysis of these data.

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