Assessing the Impact of Microwave Treatment on Soil Microbial Populations

Graham Brodie[1,*], Michelle Grixti[1], Eloise Hollins[1], Andrew Cooper[1], Tianyao Li[1] and Mary Cole[2]

[1]*Melbourne University, Dookie Campus, Nalinga Rd, Dookie 3647, Australia*

[2]*AgPath Pty Ltd, 105 Gunn Road, Vervale, 3814, Australia*

Abstract: Microwave soil treatment can kill weed plants and their seeds in the soil. It has also been demonstrated elsewhere that microwave soil treatment can kill nematodes in the soil; however few studies have considered the effect of microwave soil treatment on other key soil biota. This study explored the effect of microwave soil treatment on soil bacteria, fungi, and various protozoa. The research used a series of experiments using different techniques to verify the effect of varying degrees of microwave treatment on these soil biota. Microwave treatment reduces bacterial populations in the top layers of soil, but populations that are deeper in the soil are relatively unaffected. Bacterial populations increased significantly within a month of microwave treatment. *E. coli* populations experienced a 10^{-5} reduction in numbers in the top layer of soil by 500 J cm^{-2} of microwave energy; however other soil bacteria survived over 3000 J cm^{-2} of microwave energy applied to the soil surface, suggesting that some species are more susceptible to microwave treatment than others. No significant response of soil fungi, ciliates, amoeba and flagellates could be attributed to a microwave dose response.

Keywords: Microwave, soil, soil biota, bacteria, fungi, protozoa.

1. INTRODUCTION

Soil fumigants, such as Methyl bromide (bromomethane), have been used widely in agriculture since the 1940's. They can eradicate nematodes, plant pathogens, weeds and insects in the soil, largely due to: their wide spectrum of activity against soil biota; their ability to penetrate the fumigated zones; and their ease of application [1]. Soil fumigants are used for many commercial crops, including: strawberries; tomatoes; peppers; eggplants; tobacco; ornamentals; nursery stocks; vines; and turves [1]. Soil fumigants are hazardous to work with and some of these chemicals have been phased out in developed countries [2]. This has prompted a search for alternative methods of controlling weeds, insects, nematodes, and other plant pathogens.

Microwave treatment of soil has been shown to kill weeds and their seeds [3-6]. Experiments have demonstrated that raising the soil temperature above 80 °C will kill seeds of: wheat [7], ryegrass [8], rubber vine (*Cryptostegia grandiflora* R.Br.), parthenium (*Parthenium hysterophorous* L.), bellyache bush (*Jatropha gossypiifolia* L.) [9], Prickly Paddy Melon (*Cucumis myriocarpus*) [10], wild oats (*Avena fatua* L.) [11], white clover (*Trifolium repens*), and hemlock (*genus Tsuga*) [12].

Davis [4] showed that seed volume significantly correlates with susceptibility to microwave damage; however this may not be due to direct interaction of the seeds with microwave energy, but rather it may be due to better heat transfer from surrounding soil [13] because the radar cross section [14] of seeds is very small and therefore direct absorption of microwave energy will also be small. Imbibing of water significantly increases susceptibility of seeds to damage by microwave treatment [4, 8, 15] (Figure **1**).

Other studies have revealed that the amount of microwave energy required to kill emerged broad leafed weed plants is at least an order of magnitude less than the energy needed for seed inactivation in the top layers of soil [11]. Microwave soil treatment also kills nematodes [16, 17].

Studies of microwave effects on soil invertebrates are rare. It has been demonstrated that long exposure of earth worms (*Eisenia fetida*), in the absence of soil, to low intensity microwave fields (23 V m^{-1} for 2 hours at frequencies of 900 MHz and 1.8 GHz) induced measurable DNA damage; however, lower intensity fields (10 V m^{-1} for 2 hours at frequencies of 900 MHz and 1.8 GHz) had no measureable effect on DNA [18]. Note that this study was focused on the effect of long term exposure to low power electronic communication systems rather than microwave soil treatment.

Speir *et al*. [19] demonstrated that fungi are more susceptible to microwave soil treatment than bacteria.

*Address correspondence to this author at the Melbourne University, Dookie Campus, Nalinga Rd, Dookie 3647, Australia; Tel: +61 3 5833 9273; Fax: +61 3 5833 9201; E-mail: grahamb@unimelb.edu.au

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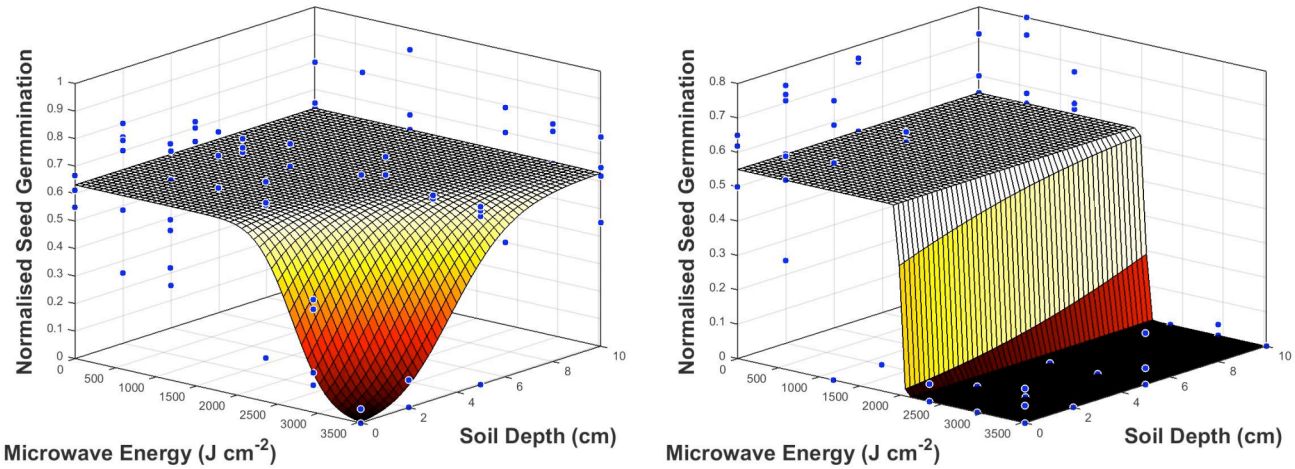

Figure 1: Effect of microwave treatment on annual ryegrass (Lolium rigidum) seeds as a function of applied microwave energy and soil depth (left = dry seeds in dry soil; right = moist seeds in moist soil).

This has been verified by others [20-22]; and a microwave induced "heat shock" activation of bacterial and fungal spores has also been observed [21]. Vela *et al*. [21] also demonstrated that soil bacteria, bacterial spores, actinobacteria, fungi, nitrogen-fixing bacteria, and nitrifying bacteria were all resistant to over 40,000 J cm^{-2} of microwave energy applied to the soil surface; therefore the literature is not clear about the effect of microwave soil treatment on various soil biota and there is scope for further experimental studies. Therefore the objective of this paper is to present the results of a recent study into the effect of microwave soil treatment on populations of soil bacteria, soil fungi, and soil protozoa.

2. METHOD

As with any systematic study, it is critical to verify important experimental results using a number of experimental procedures which employ slightly different methodologies. Therefore this study involved a series of linked experiments with slightly different methodologies to verify the impact of microwave treatment on soil bacteria, soil fungi, and soil protozoa. One experiment investigated the effect of microwave treatment on a number of soil phyla, while the remaining two experiments applied different experimental techniques to verify specifically the effect of microwave treatment on soil bacteria.

2.1. Experiment 1 – Assessment of Microbial, Fungal, and Protozoa Levels in Microwave Treated Soil

On the 19th of August, 2014, twenty soil profile samples were sampled randomly from a paddock at Dookie Campus of the University of Melbourne dominated by the Caniambo Loam soil type. A larger than needed volume of soil was removed carefully from the ground using a shovel so that the soil profile in the sample experienced minimal disturbance. Samples were then cut to fit into a 150 mm diameter pot using a knife and the soil was carefully placed into the pot to maintain the existing soil profile. If the profile was disturbed in this process, samples were discarded. The pots were placed into the Dookie campus glass house and watered.

On the 20th of August, the pots were subjected to five treatments using a 2 kW trailer mounted microwave prototype operating at 2.54 GHz feeding into a horn antenna with aperture dimensions of 110 mm by 55 mm. The pots were set up with the soil surface at a range of 100 mm from the horn antenna's aperture. The treatments were: A control; a light treatment of 30 seconds exposure; a moderate treatment of 60 seconds exposure; and two treatments of 120 seconds of exposure. Four of the eight pots exposed to 120 seconds of microwave treatment received an application of compost tea at an equivalent rate of 200 litres ha^{-1}, after the pots had cooled to ambient temperature. The compost tea treatment was to determine whether re-inoculation of the soil may help improve microbial recovery.

On the 21st of August, access points were made in the sides of the pots with a scalpel. These access points were at the surface of the soil, at 5 cm below the soil surface and at 10 cm below the soil surface. Soil samples were removed from the pots at these locations using an apple corer. The soil samples were individually wrapped in a paper towel and placed into a labelled zip-lock plastic bag. Each pot was planted with three wheat seeds, which were watered regularly and

allowed to grow in the glasshouse until a second set of soil samples was taken from the same access points on the 22nd of September, 2014.

Soil biology assays were carried out at Agpath Laboratories. To measure protozoa, 1 g of each soil sample underwent serial dilutions to 10^{-6}. Each serial dilution was plated onto soil agar in 4-times replicated wells and incubated for 4 days at 22 °C. Microscopic analysis was carried out by experienced staff to derive counts of ciliates, amoeba and flagellates at each dilution.

Active bacteria, active fungi, and total fungal assessments for the soil samples were carried out on 10^{-1} dilutions by applying fluorescent dye to a known volume of sample, mounting the sample in agar and viewing under an appropriate wavelength of light to facilitate fluorescence in the cells. Total bacteria were assessed on 10^{-2} dilution by a similar method using a different fluorescent stain.

Fluorescence microscopy is a rapidly expanding technique that is used in both medical and biological sciences. The technique has made it possible to identify cells and cellular components with a high degree of specificity [23]. The technique is used to study specimens, which can be made to fluoresce, usually by the addition of a fluorescing chemical that binds to the target cells of interest. The fluorescence microscope is based on the phenomenon that certain materials emit energy detectable as visible light when irradiated with the light of a specific wavelength; usually in wavelengths shorter than the visible range.

Fluorescence can be induced in cells by addition of various chemicals: fluorescein diacetate for living cells and fluorescein isothiocyanate for non-living cells [23]. With adequate training, this technique can be used to determine the portions of living and dead specimens of bacteria and fungi extracted from the soil.

The resulting data from the soil samples taken immediately after microwave treatment and one month later were analysed using a multi-factor analysis of variance, after determining that the data was normally distributed.

3. SOIL BACTERIA

The impact of microwave treatment on soil bacteria was clarified experimentally by: culturing indigenous soil bacteria; and assessing the impact of microwave treatment on a suitable test species.

3.1. Experiment 2 – Culturing of Indigenous Soil Bacteria

Twenty soil samples were excavated randomly from a paddock at the Dookie campus of the University of Melbourne that was predominantly “Dookie Clay” and carefully layered into wooden boxes with pre-drilled soil sampling points at various locations down the side of the box (0, 2.5, 5, 10, 20 and 40 cm depths). These boxes were treated using five levels of microwave treatment times (0, 2, 4, 8, and 16 minutes) using a 1 kW microwave prototype with a 180 mm by 90 mm horn antenna mounted 50 mm above the soil surface (Figure **2**). Soil samples were harvested from the six different depths in the soil profile after the soil had cooled to ambient temperature.

After microwave treatment, extracted soil samples were placed in a 160 mL dilution bottle that contained 100 mL of Phosphate-buffered saline. Four glass

Figure 2: Microwave prototype system based on a modified microwave oven, including wooden box used for soil bacteria study [Source: 9].

beads were placed into the dilution bottle and the lid replaced. The dilution bottle containing the soil suspension was shaken on a mechanical shaker for 10 minutes with the bottles in a horizontal position.

A useful technique for culturing indigenous bacteria is the pour plate method, which requires the use of 1, 0.1, 0.01, or 0.001 mL samples [24]. The difficulty of measuring and working with the two smaller volumes, 0.01 and 0.001 mL required the use of sample dilutions. These solutions were prepared by adding 1 mL of undiluted sample, using a pipette, into 99 mL of Phosphate-buffered saline diluent. Diluting the sample allows 1 mL of diluted sample to be used instead of 0.01 mL of undiluted sample, and 0.1 mL of diluted sample instead of 0.001 mL of undiluted sample.

An agar medium was created and poured into a 1 litre glass container. This was treated in an autoclave for sterilization. Following autoclaving, the liquid agar was placed in a water bath set at a temperature of 45°C, until used.

The samples were diluted twice and 1 mL of the diluted sample was placed into a sterile Petri dish using a pipette, while in a laminar flow cabinet. Fifteen millilitres of sterilised, liquefied plate count agar was placed into the Petri dish. The melted medium was thoroughly mixed with the sample in the Petri dish by rotating the dish in opposite directions (10 times in each direction). The plates were placed on a level surface to solidify. The plates were inverted and placed in a sealed plastic bag. The bags were incubated for 48 hours at 35°C. All colonies on the plates were counted with the aid of a Quebec Colony Counter. The resulting data was analysed by regression analysis using MatLab® to determine a dose response equation. It needs to be acknowledged that agar plate culturing grows only approximately 0.3 of 1% of possible species present in a gram of soil.

3.2. Experiment 3 – Treatment and Culturing of a Known Test Species

In this strategy twelve soil samples from a paddock at the Dookie campus of the University of Melbourne that was predominantly "Currawa Loam" were treated in an autoclave at 121°C at 15 psi for 20 minutes to sterilise the soil. Previously cultured *Escherichia coli* (*E. coli*) bacteria were inoculated into sterilised soil sub-samples. *E coli* is a gram-negative, easily cultured bacterium and therefore would be a suitable representative of a number of bacterial species.

One gram samples of inoculated soil were placed inside small paper envelopes. Sterilised soil was used to fill twelve pots to a depth of 20 cm. Envelopes of inoculated soil were placed at various depths in the sterilised soil (suggested: 0, 2.5, 5, 10, and 20 cm). Each pot was placed under the microwave antenna of the trailer mounted 2 kW system, at a range of 100 mm from the horn antenna, for treatment (treatment times: 0, 10, 30, and 120 seconds). Pots were allowed to return to ambient temperature. Each layer of inoculated soil was carefully removed from the pots and mixed with 9 ml of nutrient broth. Each mixture underwent a serial dilution to 10^{-6}. From this, 1.0 mL aliquots underwent a similar Petri dish assessment as described in the previous experiment. The resulting data was analysed by regression analysis using MatLab® to determine a dose response equation.

4. RESULTS

4.1. Experiment 1 – Assessment of Microbial, Fungal, and Protozoa Levels in Microwave Treated Soil

Analyses of the soil biota data revealed that microwave treatment significantly reduced the number of soil bacteria (Table **1**) but did not sterilise the soil profile; however their numbers significantly increased

Table 1: Soil Bacterial Numbers Shortly after Microwave Treatment (Entries in the Table with Different Superscripts are Significantly Different to One Another)

Soil Depth (cm)	Estimated Microwave Treatment (J cm^{-2})			
	0	150	300	600
0	6.20^{a}	5.57^{a}	4.73^{ab}	1.78^{c}
5	3.78^{abc}	4.71^{ab}	4.23^{ab}	1.18^{c}
10	4.06^{ab}	2.93^{bc}	3.87^{abc}	1.74^{c}
LSD (P = 0.05)				2.60

Table 2: Soil Bacterial Numbers as a Function of Microwave Treatment, Soil Depth and Recovery time after Treatment (Entries in the Table with Different Superscripts are Significantly Different to One Another)

Soil Depth (cm)	Time from Microwave Treatment (Days)	Estimated Microwave Treatment (J cm^{-2})			
		0	150	300	600
0	1	6.20^{d}	5.57^{d}	4.73^{d}	1.78^{d}
	31	18.90^{c}	38.48^{a}	38.25^{a}	19.67^{c}
5	1	3.78^{d}	4.71^{d}	4.23^{d}	1.18^{d}
	31	18.73^{c}	24.28^{bc}	29.95^{b}	28.22^{b}
10	1	4.06^{d}	2.93^{d}	3.87^{d}	1.74^{d}
	31	16.93^{c}	26.13^{bc}	28.90^{b}	18.00^{c}
LSD (P = 0.05)					7.30

after a month (Table **2**) and ended significantly higher than at the start of the experiment. Bacterial cells form the most concentrated C; N ratio of soil biota. Killing the cells through the microwave treatment provides extra nutrients for the remaining bacteria leading to an increase in the populations during the period following the treatment. The other soil biota experienced no statistically significant effect that could be attributed to microwave treatment dose response (Tables **3-6**), even though there were some significant results in the data. Although every effort was made to randomise the soil sampling procedure, it is likely that significant differences in these data were due to natural spatial variability of soil biota captured in the soil sampling process.

4.2. Experiment 2 – Culturing of Indigenous Soil Bacteria

Only a very small portion of the total number of bacteria species present in soil can be cultured using the technique described in the method for Experiment 2 and it is not clear which species may have survived to create colonies in the agar; however several outcomes

Table 3: Total Fungal Numbers as a Function of Microwave Treatment, Soil Depth and Recovery Time after Treatment (Entries in the Table with Different Superscripts are Significantly Different to One Another)

Soil Depth (cm)	Time from Microwave Treatment (Days)	Estimated Microwave Treatment (J cm^{-2})			
		0	150	300	600
0	1	79.03^{a}	850.73^{a}	220.98^{a}	240.18^{a}
	31	209.48^{a}	146.13^{a}	191.00^{a}	253.88^{a}
5	1	77.30^{a}	4443.50^{b}	185.65^{a}	108.43^{a}
	31	146.90^{a}	142.45^{a}	171.10^{a}	223.86^{a}
10	1	36.83^{a}	380.95^{a}	106.48^{a}	114.66^{a}
	31	106.33^{a}	76.30^{a}	150.55^{a}	133.08^{a}
LSD (P = 0.05)					1956.60

Table 4: Flagilate Numbers as a Function of Microwave Treatment, Soil Depth and Recovery Time after Treatment (Entries in the Table with Different Superscripts are Significantly Different to One Another)

Soil Depth (cm)	Time from Microwave Treatment (Days)	Estimated Microwave Treatment (J cm^{-2})			
		0	150	300	600
0	1	4311.00^{a}	2931.50^{b}	2167.00^{b}	1855.38^{b}
	31	1208.50^{b}	4000.75^{a}	397.33^{b}	1536.88^{b}
5	1	2567.50^{b}	3343.25^{b}	2303.50^{b}	2672.50^{b}
	31	1386.75^{b}	1414.50^{b}	1068.33^{b}	499.75^{b}
10	1	1902.25^{b}	310.75^{b}	469.00^{b}	1901.13^{b}
	35	965.00^{b}	1282.25^{b}	246.75^{b}	184.75^{b}
LSD (P = 0.05)					2654.23

Table 5: Amoeba Numbers as a Function of Microwave Treatment, Soil Depth and Recovery Time after Treatment (Entries in the Table with Different Superscripts are Significantly Different to One Another)

Soil Depth (cm)	Time from Microwave Treatment (Days)	Estimated Microwave Treatment (J cm^{-2})			
		0	150	300	600
0	1	2859.50^{a}	29406.25^{b}	1889.00^{a}	7563.75^{a}
	31	2299.75^{a}	5722.75^{a}	2626.67^{a}	2458.50^{a}
5	1	941.50^{a}	6411.00^{a}	1303.50^{a}	10862.63^{a}
	31	2076.25^{a}	3785.25^{a}	1809.33^{a}	2280.63^{a}
10	1	926.50^{a}	4956.25^{a}	1037.50^{a}	4431.25^{a}
	31	735.25^{a}	2191.75^{a}	287.75^{a}	1179.25^{a}
LSD (P = 0.05)					10653.90

Table 6: Ciliate Numbers as a Function of Microwave Treatment, Soil Depth and Recovery Time after Treatment (Entries in the Table with Different Superscripts are Significantly Different to One Another)

Soil Depth (cm)	Time from Microwave Treatment (Days)	Estimated Microwave Treatment (J cm^{-2})			
		0	150	300	600
0	1	650.00^{a}	1015.25^{a}	119.75^{a}	196.38^{a}
	31	1747.25^{a}	505.25^{a}	115.67^{a}	88.13^{a}
5	1	45.25^{a}	1908.50^{b}	50.25^{a}	573.13^{a}
	31	91.50^{a}	127.00^{a}	46.33^{a}	241.75^{a}
10	1	403.75^{a}	37.50^{a}	41.25^{a}	53.62^{a}
	31	127.00^{a}	109.25^{a}	123.25^{a}	83.13^{a}
LSD (P = 0.05)					1132.89

are evident: there is considerable variability in bacterial numbers in the untreated soil; extended microwave treatment significantly reduces bacterial numbers, but does not sterilise the soil; and the surface soil is most affected by microwave treatment.

The Normalised dose response surface shown in Figure **3** is of the form:

$$S = 0.28 \cdot erfc\left[0.00018 \cdot \left(E \cdot e^{-0.22D} - 0.001\right)\right] + 0.72 \cdot erfc\left[0.001 \cdot \left(E + 432.1\right)\right] \quad (1)$$

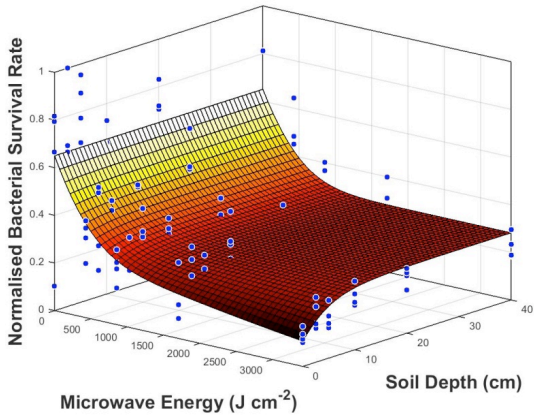

Figure 3: Normalised soil bacterial counts as a function of microwave energy and soil depth.

where E is the applied microwave energy (J cm^{-2}) and D is the soil depth (cm). However the goodness of fit is moderate (R^2 = 0.64).

Note: $erfc(z) = \frac{1}{\sqrt{2\pi}} \int_z^{\infty} e^{-\frac{t^2}{2}} \cdot dt$ - is the complementary Gaussian error function and assumes that the susceptibility of bacteria to microwave treatment is normally distributed. Including a term with the form $e^{-\alpha D}$, accounts for the natural attenuation of the microwave energy with depth in the soil.

4.3. Experiment 3 – Treatment and Culturing of a Known Test Species

As in the previous experiment, there is considerable variability in bacterial numbers in the untreated soil. Extended microwave treatment caused a 10^{-5} reduction in *E. coli* numbers in the top layer of soil; however populations at greater depth were not significantly affected by microwave treatment.

The Normalised dose response curve shown in Figure **4** is of the form:

$$S = 0.58 \cdot erfc\left(0.009\left(E \cdot e^{-0.33D} - 9.6 \times 10^{-9}\right)\right) \quad (2)$$

However the goodness of fit is moderate (R^2 = 0.47).

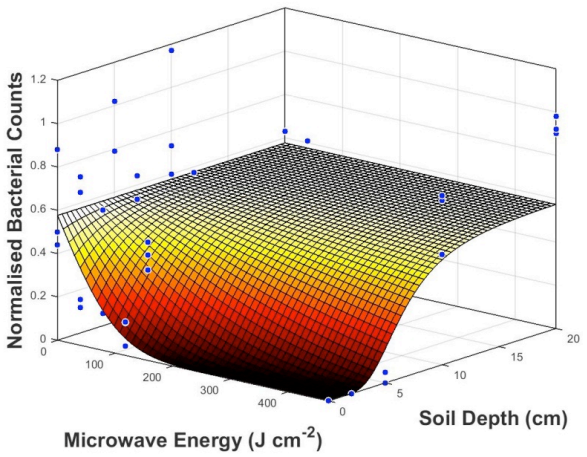

Figure 4: Normalised E. Coli counts as a function of microwave energy and soil depth.

5. CONCLUSIONS

Based on the results of these linked experiments it is clear that, unlike chemical soil fumigation techniques that have been used in agriculture for many decades, microwave soil treatment does not sterilise soil. Microwave treatment reduces bacterial populations in the top layers of soil, but populations that are deeper in the soil are relatively unaffected. Bacterial populations increased significantly within a month of microwave treatment. *E. coli* populations experienced a 10^{-5} reduction in numbers in the top layer of soil when 500 J cm^{-2} of microwave energy was applied to the surface; however other soil bacteria survived over 3000 J cm^{-2} of microwave energy applied to the soil surface, suggesting that some species are more susceptible to microwave treatment than others. No significant response of soil fungi, ciliates, amoeba and flagellates could be attributed to a microwave dose response.

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