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EFFECT OF MICROWAVE SOIL TREATMENT FOR WEED MANAGEMENT ON CEREAL PRODUCTIVITY

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Abstract

The evolution of herbicide-resistant weeds has encouraged the development of new non-herbicidal weed management technologies for sustainable food production. Agricultural pests are enormously reducing the sustainability of farmland and the traditional usage of chemicals for pest control has shifted the pests' adaptability to resist this widely adopted control measure. This adaptability of pest organisms (*i.e.*, *pesticide and herbicide resistance*) can impose a significant challenge to sustainable food production. In addition, chemicals are deteriorating the cropping environment and soil health, which is a significant threat to the environment and is one of the potential factors to climate change. Therefore, an environment-friendly non-chemical weed control approach is needed to ensure sustainability of agricultural systems and food security. In pursuit of this, numerous thermal weed management technologies have been proposed and developed. However, a novel and effective solution to herbicide-resistant weeds is still in question. Therefore, this thesis evaluated the effect of microwave (MW) soil treatment to minimize the regeneration capacity of the weed seedbanks as a novel tool for weed management in temperate rice and dryland wheat production systems in Australia. During the initial field trials at Dookie, Victoria, an open structure horn antenna assembled with a waveguide and a 750 W magnetron was employed to channel the MW energy for soil treatments. MW energy was assigned in a randomized complete block design with five replicates under field conditions. Soils exposed to 120 s of MW energy rapidly increased in temperature, with increases of up to 75 – 80 °C in top soil horizons of 0 – 8 cm. This temperature range significantly inhibited (40 – 60% reduction) the germination of weeds relative to untreated control soils for both temperate rice and dryland wheat crops. In addition to weed suppression, soil microwaving substantially increased the grain yield of both crops.

Subsequently, a 2-kW MW weed killer was tested for pre-emergence soil treatment to reduce seedbank viability in a rice crop under field conditions, in two agroecological zones of Australia. This transient soil heating resulted in an average of 50 – 60% reduction in weed establishment compared to untreated control plots; however, there was no statistically significant difference detected between MW and untreated control plots at both study locations. Irrespective of this pattern for weed control, the MW

soil treatment gave 20 – 52% increases in rice grain yields. Furthermore, under the MW soil treatment, the agronomic efficiency of applied nitrogen ($\text{kg grain kg}^{-1} \text{ N}$) was 10 times higher and irrigation water use efficiency (t ML^{-1}) was 50 – 100% higher compared with untreated control plots at both study locations because of lower weed-crop competition for resources and/or soil heating impact on the indigenous N supply for crop yields. In summary, investment in this non-chemical weed control technique can potentially reduce weed establishment and enhance rice productivity and thus this study clearly supports the practicability of this technology for sustainable weed management.

Furthermore, a series of pot trials were conducted to evaluate the impact of MW soil treatment on crop growth and nitrogen use efficiency in a wheat crop using an isotope dilution technique over two seasons. Pre-cropping soil heating at up to 75 – 80 °C did not significantly increase the N% in wheat dry matter and N derived from fertilizers (^{15}N atom% recovery) relative to untreated soils; however, despite this, there was a substantial increase in crop growth and development. Additionally, the total N accumulation in the wheat dry biomass significantly increased with soil heating intensities compared with untreated control soils. The increased growth effect in response to a once-off MW soil heating continued for up to two seasons (residual effect; 440 and 740 days after MW soil treatment), confirming the positive long-lasting impact of soil heating on soil fertility and productivity.

Finally, in order to fully understand the potential effects of the MW weed management treatment and its role in shaping the soil microbiota, the soil microcosms exposed to the MW weed disinfestation treatment at three MW durations and monitored the bacterial community composition (16S rRNA amplicon sequencing and 16S rRNA qPCR) as well as the total abundance of ammonia oxidising archaea (AOA's) and bacteria (AOB's). The microcosms were sampled immediately after treatment and 28 days later. Because the treatments were expected to be more extreme on the upper soil strata, the soil was sampled at 5 cm and 10 cm separately to evaluate the effectiveness of the MW treatment in regard to penetration depth for treatment efficacy. Therefore, these results demonstrate that MW soil heating (75 – 80 °C) intensities proposed for weed management had a strong rearrangement impact on bacterial community composition without significantly influencing the ammonia oxidisers abundance. These results also indicate that the survival of ammonia-oxidisers after MW soil heating may favor the fertilizer

nitrogen use efficiency in cropping systems and could potentially support the organic production of high-value crops. Additionally, the triggering of biologically important taxa following heat disturbance could cope with the yield increase behaviour of crops because of ample availability of decomposed organic carbon to release the numerous yield-limiting nutrients.

Overall, MW soil heating appeared to be effective for reducing the viability of soil weed seedbanks and increasing crop yields. In addition, the heating intensities proposed for deactivation of the soil weed seedbanks also modified soil bacterial communities - activating important dormant taxa - and enhancing soil health and crop productivity. Therefore, MW technology can be adopted as an environmentally-sound weed control technique. Further it can be integrated into the existing weed control approaches to improve the sustainability of farming systems.

Declaration

This is certified that;

- i. The thesis comprises only my original work towards the PhD,
- ii. Due acknowledgment has been made in the text to all other material used,
- iii. The thesis is less than 100, 000 words in length, exclusive of tables, maps, bibliographies and appendices.

Portions of this thesis have already been published in the papers listed below;

Papers in Peer-reviewed archived Journals

Khan, M.J., Jurburg, S., He, J., Brodie, G., and Gupta, D. (2019). Impact of Microwave Disinfestation Treatments on the Bacterial Communities of No-till Agricultural Soils. *European Journal of Soil Science*, 1 – 12.

Khan, M.J., Brodie, G., and Gupta, D. (2019). Potential of Microwave Soil Heating for Weed Management and Yield Improvement in Rice Cropping. *Crop and Pasture Science* 70, 211-217.

Khan, M.J., Brodie, G.I., Gupta, D., and He, J. (2019). Microwave Soil Treatment Increases Soil Nitrogen Supply for Sustained Wheat Productivity. *Transactions of the ASABE* 62, 355-362.

Khan, M.J., Brodie, G.I., Gupta, D., and Foletta, S. (2018). Microwave Soil Treatment Improves Weed Management in Australian Dryland Wheat. *Transactions of the ASABE* 61, 671-680.

Brodie, G., Khan, M.J., Gupta, D., and Foletta, S. (2018). Microwave Weed and Soil Treatment in Agricultural Systems. *Global Journal of Agricultural Innovation, Research & Development*, 5, 1-14.

Khan, M.J., Brodie, G. and Gupta, D. 2017. The effect of Microwave Soil Treatment on Rice Production under Field Conditions. *Transactions of the ASABE*, 60(2); 517 – 525.

Khan, M.J., Brodie, G., and Gupta, D. (2016). Effect of Microwave (2.45 GHz) Treatment of Soil on Yield Components of Wheat (*Triticum aestivum* L.). *Journal of Microwave Power and Electromagnetic Energy*, 50(3); 191-200.

Other Publications

- Khan, M.J., Brodie, G., Cheng, L., Liu, W. And Jhajj, R. (2019). Impact of Microwave Soil Heating on the Yield and Nutritive Value of Rice Crop. *Agriculture* 9.
- Khan, M.J., and Brodie, G. (2018). "Microwave Weed and Soil Treatment in Rice Production," in *Rice Crop - Current Developments*. Intech Open: London, 99 - 127.
- Khan, M.J. (2018). The effect of microwave soil treatment on subsequent crop growth and development, in the book "Microwave Based Weed Control and Soil Treatment". Publisher: De Gruyter online.
- Brodie, G., Khan, M.J. and Foletta, S. (2016). Trialling microwave-based weed management for rice. *IREC Farmer's Newsletter*. 26-28.
- Brodie, G., Khan, M.J., Gupta, D., Foletta, S., and Bootes, N. (2017). Microwave Weed and Soil Treatment in Agricultural Systems. *AMPERE Newsletter*, Issue 93. Association for Microwave Power in Europe for Research and Education.

Conference Proceedings

- Khan, M.J., Brodie, G. and Gupta, D. 2018. Microwave: A Novel Approach to Tackle Herbicide Resistance in No-till Farming Systems. *Proc. 21st Australian Weed Conference*. Sydney, Australia.
- Khan, M.J., Brodie, G. and Gupta, D. 2016. Effect of Microwave (2.45 GHz) on Agronomic Efficiency of Nitrogen in Wheat. *Proc. 50th International Microwave Power Institute Symposium*. 91-93. Orlando, Florida, USA.
- Khan, M.J., Brodie, G. and Gupta, D. 2016. Influence of Microwave Soil Treatment on Nitrogen Use Efficiency of Wheat. *Proc. Microwave and Radio Frequency Applications Symposium*. 31-36. Melbourne, Australia.
- Khan, M.J., Brodie, G., Gupta, D. and He, J. 2017. Residual Effect of Microwave Soil Treatment on Growth and Development of Wheat. *Proc. 51st International Microwave Power Institute Symposium*. 32-34. Miami, Florida, USA.
- Khan, M.J., Brodie, G., and Gupta, D. (2017). Microwave: A Novel Non-Chemical Weed Management Approach in Different Cropping Systems. *Proc. The 26th Asian-Pacific Weed Science Society Conference*, Kyoto, Japan; pp: 314.
- Khan, M.J., Brodie, G., Gupta, D. & He, J. (2016). Microwave (2.45 GHz) Soil Treatment for Weed Management in Rice (*Oryza sativa* L.). *Proc. 7th International Temperate Rice Conference*, New South Wales, Australia.

Preface

Sustainable weed management demands the implementation of new technology to outcompete the herbicides resistance problem in agriculture systems. One of the innovative technologies, MW weed management, has been investigated in this PhD thesis. This thesis comprises 9 chapters. Chapter 1 introduces the problem statements and the reasons why we conducted this research. Chapter 2 provides diverse literature on the use of MW technology in the agriculture systems. Most of information presented in this chapter has been published in a book chapter - *Rice Crop - Current Developments* on the 5th September 2018. Chapter 3 represents the effectiveness of MW soil treatment for weed seedbank depletion in dryland wheat production systems of the Australia. This paper has been published in the Transaction of the ASABE on the 18th January 2018. Furthermore, to understand the effectiveness of MW soil treatment for weed management in different cropping systems. We chose a rice crop and evaluated the similar microwave heating intensities for weed management; therefore, this suppressed the weed effectively under field conditions. This paper has been published in the Transaction of the ASABE on the 31st January 2017. Chapter 5 validated the implementation of MW technology for weed management in different regions. Irrespective of soil management practices and seedbank size, the MW heating intensities has potential to retard weed establishment (paper published in Crop and Pasture Science). Chapters 6 (published in Journal of Microwave Power and Electromagnetic Energy) and 7 (published in Transaction of ASABE) explored the impact of MW soil treatment on crop productivity and nitrogen use efficiency. Chapter 8 rigorously explored the impact of MW soil heating, which was proposed for weed management under field conditions, on the soil bacterial communities' responses based on the 16S rRNA amplicon sequencing (this has been accepted for publication in the European Journal of Soil Science). Overall, Chapter 9 concludes the effectiveness of MW soil treatment in the agriculture industry for weed management and provides the directions for future research work.

In all the presented papers, I 'Muhammad Jamal KHAN' performed all the research under the generous guidance of supervisors and prepared all the original drafts. Among all co-authors, Dr. Graham Brodie helped in methodology validation and conceptualization of field and glasshouse trials. He also provided statistical support for

data analysis and reviewed all the drafts. Prof Jizheng He provided guidance on how to proceed with the soil biology work (formal analysis and investigation) and its relevance. Dr. Dorin Gupta helped in experimental approaches, reviewed and provided suggestions for all papers. Dr. Stephanie Jurburg helped in analysing and interpretation of soil biology data. She provided valuable comments and suggestions for paper improvement.

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Also, I was lucky enough to get conceptual writing support from the **Professor Timothy Reeves**, Former Director General of CIMMYT: International Maize and Wheat Improvement Center. He helped me to understand the importance of sustainable weed management for the implementation of sustainable intensification.

I would like to thank **Dr. Stephanie D. Jurburg**, German Center for Integrative Biodiversity Research (iDiv), for her help in understanding the soil biology data and in preparing the manuscript presented in chapter 8.

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Table of Contents

Abstract	ii
Declaration	v
Chapter 1 – Introduction	1
1.1. Importance of Weed Management.....	1
1.2. Use of Herbicides for Weed Management.....	3
1.3. Microwave Weed Control.....	4
1.4. Impact of Microwave Soil Treatment on Soil Properties and Biota.....	6
1.5. Research Aims	7
1.6. Hypothesis.....	8
1.7. Thesis Overview	8
Chapter 2 – Literature Review.....	10
2.1. Weeds: An Important Yield Limiting Factor.....	10
2.2. Herbicide Resistant Problem.....	10
2.3. Impact of Thermal Technologies for Herbicides Resistant Weed Management.....	13
2.3.1. Flaming	14
2.3.2. Steam Treatment.....	14
2.3.3. Solarisation.....	15
2.3.4. Electrocuting	15
2.3.5. Infrared Radiation	16
2.3.6. Ultraviolet Radiation.....	16
2.4. Microwave and it's Interactive Mechanisms	17
2.4.1. Microwave Weed Management.....	18
2.4.2. Microwave Toxicity to Weed SeedBank	22
2.4.3. Effect of Microwave Heating on Soil Nutrient Dynamics	24

2.4.4. Impact of Microwave Soil Heating on Soil Organic Matter	26
2.4.5. Response of Soil Microorganisms to Microwave Heating.....	27
2.5. Summary	32
Chapter 3 – Microwave Soil Treatment Improves Weed Management in Dryland Wheat of Australia	34
3.1. Abstract.....	34
3.2. Introduction.....	35
3.3. Materials and Methods.....	37
3.4. Results.....	40
3.5. Discussions	41
3.5.1. Effect of Microwave Soil Treatment on Weed Establishment	41
3.5.2. Influence of Microwave Soil Irradiation on Wheat Productivity	46
3.5.3. Economic Impact of Microwave Soil Irradiation on Profitability	48
3.6. Conclusions and Future Work	50
3.7. Appendix: Simulating Power Density from a Horn Antenna for Soil Irradiation.....	50
Chapter 4 – The Effect of Microwave Soil Treatment on Rice Production Under Field Conditions.....	53
4.1. Abstract.....	53
4.2. Introduction.....	53
4.3. Materials and Methods.....	56
4.4. Results and Discussion	59
4.4.1. Inhibitory Effect of Microwave on Weeds Establishment.....	59
4.4.2. Influence of Microwave Irradiation of Soil on Rice Growth	61
4.4.3. Energy Analysis for Rice Field Trial at Dookie	65
4.5. Conculsion	67

Chapter 5 – Potential of Microwave Soil Heating for Weed Management and Yield Improvement in Rice Cropping	68
5.1. Abstract.....	68
5.2. Introduction.....	69
5.3. Materials and Methods.....	70
5.4. Results and Discussion	75
5.4.1. Spatial Distribution of Temperature into Soil	75
5.4.2. Inhibitory Effect of Heating on Weed	75
5.4.3. Rice Productivity in Response to Microwave Soil Heating.....	79
5.5. Conclusions.....	82
Chapter 6 – Effect of Microwave (2.45 GHz) Treatment of Soil on Yield Components of Wheat (<i>Triticum aestivum</i> L.).....	83
6.1. Abstract.....	83
6.2. Introduction.....	83
6.3. Materials and Methods.....	85
6.4. Results.....	86
6.4.1. Number of Tillers per Pot.....	86
6.4.2. Dry Biomass Yield (g pot ⁻¹).....	87
6.4.3. Grain Yield (g pot ⁻¹).....	87
6.5. Discussion.....	89
6.6. Conclusions.....	92
Chapter 7 – Microwave Soil Treatment Increases Soil Nitrogen Supply for Wheat Productivity	93
7.1. Abstract.....	93
7.2. Introduction.....	94
7.3. Materials and Methods.....	96

7.3.1. Soil Collection.....	96
7.3.2. Microwave Soil Treatment.....	97
7.3.3. ¹⁵ N Soil Labelling.....	97
7.3.4. Measurement of Nitrogen in Plant Biomass	98
7.3.5. Residual Experiments.....	99
7.3.6. Nucleic Acid Extraction and qPCR.....	99
7.3.7. Statistical Analysis	100
7.4. Results.....	100
7.4.1. Impact of Microwave Soil Treatment on Wheat Yields	100
7.4.2. Nitrogen Use Efficiency.....	101
7.4.1. Residual Impact of Microwave soil treatment on Wheat Yields	105
7.4.2. Functional Gene Abundance	105
7.5. Discussion	107
7.5.1. Crop Productivity and Nitrogen Accumulation in response to Microwave Soil Heating	107
7.5.2. Effect of Soil Heating on the Ammonia Oxidizers.....	111
7.6. Conclusions.....	112
7.7. Supplementary Material.....	113

Chapter 8 – Impact of Microwave Disinfestation Treatments on Bacterial Communities of No-till Agricultural Soils 116

8.1. Abstract.....	116
8.2. Introduction.....	117
8.3. Materials and Methods.....	119
8.3.1. Soil Collection and Microwave Heating:	119
8.3.2. DNA Extraction and qPCR	121
8.3.3. 16S rRNA Sequencing	122
8.3.4. Statistical Analysis	122

8.4. Results.....	123
8.4.1. Temperature Distribution in Soil.....	123
8.4.2. Bacterial Community Composition.....	123
8.4.3. Bacterial Community Richness.....	125
8.4.4. Bacterial Responses	127
8.4.5. Taxonomic and Functional Gene Abundances	129
8.5. Discussion	129
8.6. Conclusions.....	136
8.7. Supplementary Material.....	138
Chapter 9 – General Discussion and Conclusions.....	142
9.1. Microwave Soil Heating for Weed Seedbank Control	142
9.2. Crop Productivity.....	144
9.3. Soil Bacterial Community Responses to Heat Disturbance.....	146
9.4. Energy and Economic Analysis for Soil Treatment	148
9.5. Risk and Safety Issues with Microwave Weed Control Technology ...	149
9.6. Industrial Applications of this Research	151
9.7. Future Research Work	151
References.....	153

List of Figures

Figure 2.1: The overall number of herbicide-resistant species by crops. Source: Heap (2016).	11
Figure 2.2: Confirmed cases of glyphosate resistance in summer weeds of Australia. Source: Australian glyphosate sustainability working group (https://www.glyphosateresistance.org.au/).	12
Figure 2.3: Confirmed cases of glyphosate resistance in winter weeds of the Australia. Source: Australian glyphosate sustainability working group (https://www.glyphosateresistance.org.au/).	12
Figure 2.4: The earlier design of the infrared flame weeder for pre- and post-emergence weed management under field conditions; adapted from Parish (1990).	17
Figure 2.5: The Zapper III (60 kW; microwave weed killer) developed by Phytoc Corporation to zap weeds and soil treatment under field conditions.	20
Figure 2.6: Impact of various doses of soil temperature increase following microwave soil treatment on the germination capacity of ryegrass, wheat and oat seeds, revealing the importance of soil-seed moisture of microwave efficacy. Source: Brodie et al. (2009).	23
Figure 2.7: Influence of microwave soil treatment on the (A) total bacterial abundance and (B) potentially active bacterial abundance. Source: Nunes et al. (2018).	29
Figure 2.8: The hypothetical depiction of soil bacterial communities' responses to any disturbance regimes and their recovery trajectory according to time following disturbance. Source: Griffiths and Philippot (2013).	31
Figure 3.1: Schematic diagram of free-standing microwave prototype for soil irradiation used in this study under field conditions.	38
Figure 3.2: Thermal image captured with an infrared camera (C2; FLIR Systems Inc; Wilsonville, Oregon, USA) and post-processed in MATLAB® R2015b (The Mathworks Inc., Natick Massachusetts, USA), after 120 s of microwave irradiation of soil under the horn antenna (60.5 cm ²), evidenced the average temperature increase of 75 ± 5°C.	42

Figure 3.3: Power intensity in the top 0.5 cm of soil exposed to microwave radiation through a horn antenna (5.5×11 cm) for 120 s using a 600 W microwave sources. Note: Orientation of the Horn is such that the long dimension is parallel to the vertical axis.....	42
Figure 3.4: Visual comparison of weeds emergence in a microwave treated plot (A) and an untreated control plot (B). The dryland wheat crop rows (25 cm apart) were clean of weeds (MW treated plots) and highly invaded by weeds (untreated control plot).....	45
Figure 3.5: Relationship between the number of wheat tillers and dry weed biomass (left) at early crop growth stage and wheat dry biomass production as a function of dry weed biomass (right).	46
Figure 3.6: Comparison the impact of various weed control methods on the grain yield (t ha^{-1}) of wheat under field conditions. Grain yield data were obtained from Dookie Farm on the Dookie Campus, The University of Melbourne, where pre-emergence glyphosate (N-(phosphonomethylglycine) application is a common weed control practice, for comparison with microwave soil treatment. Note: Error bars represent the least significant difference (LSD) at 5% probability level.	49
Figure 3.7. Geometry of a horn antenna showing an equi-phase wave front in the flare opening of the antenna. The open-structure horn antenna with a waveguide feed inner dimension of $8.6 \times 4.3\text{cm}$ ($a \times b$) and an aperture inner dimension of $11 \times 5.5\text{cm}$ ($A \times B$) was used in this study for microwave irradiation of the soil.....	52
Figure 4.1: Thermal image captured through infrared camera (C2; FLIR Systems Inc; Wilsonville, Oregon, USA) after microwave irradiation of soil under the horn antenna (60.5 cm^2), evidenced the temperature increase of $80 - 90^\circ\text{C} \pm 5$	58
Figure 4.2: Microwave energy dissipated from pyramidal horn antenna for soil irradiation based on FT-DT simulation.	58
Figure 4.3: Microwave energy density from pyramidal horn antenna for soil irradiation based on FT-DT simulation.	58
Figure 4.4: Relationship between leaf area index and fertile tillers at the maximum tiller development stage.	62

Figure 4.5: Comparison of plant growth characteristics particularly tiller density per plant, randomly uprooted plant from microwave treated plots (left) and untreated control plots (right).	64
Figure 4.6: Influence of pre-emergence microwave treatment of soil for weed management on grain mineral accumulation of rice crop.	66
Figure 4.7: Influence of pre-emergence microwave treatment of soil for weed management on grain minerals accumulation of direct-seeded rice crop.	66
Figure 5.1: Diagram of microwave weed killer used in this study for pre-sowing soil treatment. See; Brodie et al. (2017) for details of this machine.	72
Figure 5.2: High resolution infrared thermal image of microwave treated plot depicting the temperature increase and retained in soil for weed seeds deactivation. These images were captured with thermal camera (C2; FLIR Systems Inc.; Wilsonville, Oregon, USA) from both study locations and randomly selected raw images were post-processed in MATLAB® for spatial temperature distribution forecasting. 77	
Figure 5.3: Pictorial comparison of randomly picked rice plants from Dookie (left image) and Jerilderie (right image) trial sites in response to microwave soil treatment for weed management. Plants from microwave treated plots had a higher number of tillers and dark green expanded leaf area compared with plants from untreated control plots.	82
Figure 6.1: Effect of pre-sowing microwave irradiation of soil on the tiller density of wheat. Note: Error bars represent the Least Significant Difference at 5% probability level.	87
Figure 6.2: Effect of pre-sowing microwave irradiation of soil on the dry biomass yield of wheat. Note: Error bars represent the Least Significant Difference test at 5% probability level.	88
Figure 6.3: Effect of pre-sowing microwave soil treatment on the grain yield of wheat crop. Note: Error bars represent the least significant different test at 5% probability level.	90

Figure 6.4: Change in mean wheat grain yield induced by microwave soil treatment, compared to the equivalent control treatment, as a function of applied nitrogen.	90
Figure 6.5: Linear relationship between grain yield and dry biomass yield of wheat ($r^2 = 0.86$).	91
Figure 7.1: Effect of microwave soil treatment on grain yield of wheat crop under various levels of isotopic nitrogen application measured under two years of study. Note: Error bars indicate a least significant different test at 5% probability level.....	103
Figure 7.2: Effect of microwave soil treatment on grain yield of wheat crop under various levels of isotopic nitrogen application measured under two years of study. Note: Error bars indicate a least significant different test at 5% probability level.....	103
Figure 7.3: Effect of microwave soil treatment on the on the nitrogen derived from fertilizer (Ndff%) under various level of isotopic nitrogen application (^{15}N) in wheat dry biomass (straw + grain) over two years. Error bars indicate a least significant difference (LSD) at 5% probability level.	104
Figure 7.4: Effect of microwave soil treatment on the rate of total nitrogen accumulation (mg g^{-1} ; $^{14}\text{N} + ^{15}\text{N}$) under various level of isotopic nitrogen application (^{15}N) in wheat dry biomass (straw + grain) over two years. Error bars indicate a least significant difference (LSD) at 5% probability level.	104
Figure 7.6: Residual impact of microwave soil treatment on the wheat grain yields in the consecutive seasons. The soils exposed to 120 s of microwave energy only at first season (160 days) prior to crop sowing. Error bars indicate the least significant different test at 5% probability level.	106
Figure 7.5: Residual impact of once-off microwave soil treatment on the dry biomass yield of the wheat crop in the consecutive seasons. The soils exposed to 120 s of microwave energy only at first season (160 days) prior to crop sowing. Error bars indicate the least significant different test ay 5% probability level.....	106
Figure 7.7: Abundance of ammonia oxidizing bacteria (AOB; above) and archaea (AOA; below) after 400 days of microwave soil treatment (soil samples was collected at 85% physiological crop maturity) under various levels of isotopic nitrogen	

application. Note: <i>amoA</i> gene copy number are plotted on a log ₁₀ scale. Error bars indicate a least significant different test at 5% probability level.....	107
Figure 7.8: Relationship between grain yield and aboveground N accumulation at maturity in response to microwave soil treatment of at 160 and 440 days after microwave soil treatment.....	110
Figure 7. S1: Pictorial comparison of the impact of microwave treated and untreated control soil on the wheat growth and development over two years of studies in glasshouse. In both years of study, the higher number of fertile tillers, spike length, expanded dark0green flag leaves from microwave treated pots are indicative of increased soil fertility following microwave soil treatment.	113
Figure 7. S2: Pictorial depiction of the impact of microwave treated (left pot) and untreated control soil (right pot) on the wheat growth and development at 440 days after microwave soil treatment. The expanded dark green flag leaves and number of fertile tillers from microwave treated pots clearly reflect the changes in soil fertility following soil heating. Also, at this stage soil samples were collected to evaluate the total abundance of ammonia oxidizing bacteria and archaea in response to microwave soil heating for 70 – 80 °C.	114
Figure 7. S3: Impact of microwave soil treatment on the photosynthetic rate of wheat crop. Note: Error bars represent the least significant difference test at 5% probability level.....	115
Figure 8.1: Infrared thermal images of microcosm treated through microwave energy under horn antenna of microwave weed killer depicting the gradual increase in soil temperature. Note: the untreated control soil (0 s; A), 30 s exposure (B), 60 s exposure (C) and 90 s exposure (D). Image was captured within 2 – 3 s after microwave soil heating and temperature loss during this time was negligible. The raw images were post-processed in MATLAB for temperature forecasting....	124
Figure 8.2: Principle coordinate analyses of Bray-Curtis dissimilarity index-based variation in the community composition with microwave soil heating treatment over two-time points.....	125

Figure 8.3: Variation in bacterial diversity against microwave soil heating. Shannon richness calculated from the 16S rRNA gene sequence data for the different treatment combinations at two-time points.	126
Figure 8.4: Relative abundance of the predominated bacterial phyla (Fennimore et al.) and genera (top) that significantly changed over time in response to microwave soil heating intensities. Affiliation displayed at each taxonomic level are the finest known affiliation of each taxon.	128
Figure 8.5: Influence of microwave soil heating on the total abundance of the AOB (A), AOA (B), AOA:AOB (C) and 16S rRNA bacterial gene (D). The log-transferred gene copies number per gram soil are plotted against the various doses of soil microwave treatment monitored at two times points.	130
Figure 8. S1: Microwave weed killer, designed by the University of Melbourne researcher – Dr. Graham Brodie (grahamb@unimelb.edu.au), used in this study to treat the microcosm under one of the four horn antennas.	138
Figure 8. S2: Cross-sectional infrared thermal image of the microcosm treated under the horn antenna of the microwave weed killer prototype. To further understand the heating pattern in a microcosm: a cross-sectional thermal image of the microcosm was taken in a separate experiment; where bi-sectioning of microcosm was done, and a plastic sheet adhered between two separate parts of a microcosm to avoid disturbance in a soil profile after heating for thermal imaging.	139
Figure 8. S3: Observed and Shannon Diversity index in response to microwave soil treatment.	140
Figure 8. S4: 16S amplicon data – OTUs Distribution (A) representing a sequencing read distribution (B) representing rarefied reads to 30, 745 reads per sample of analysis.	140
Figure 8. S5: Community composition of microwave heated soil over time. Explanation of split PCoA according to taxa response to microwave soil treatment. The phyla responsible for distinguishing between 60 and 90s on day 28 and the rest, particularly at 10cm, are firmicutes, as they are heat-resistant. Bacteroidetes seem	

to be important in distinguishing these microcosms on day 0 (see top right of plot)	
.....	141

List of Tables

Table 2.1: Precise review of microwave-based weed management under different scenarios.	21
Table 3.1: Effect of pre-sowing microwave (2.45 GHz; 600 W; 120 s; 560 J cm ⁻²) irradiation of sandy loam moist soil on weed parameters in dryland wheat. Within a row and column, different letters indicate significant difference at 5% probability level.	44
Table 3.2: Effect of pre-sowing microwave (2.45 GHz; 600 W; 120 s; 560 J cm ⁻²) irradiation of sandy loam moist soil for weed management on yield components of the dryland wheat. Note: Within a row and column, different letters indicate significant difference at 5% probability level.	44
Table 4.1: Effect of pre-emergence microwave irradiation of clay based moist soil for 120 s using horn antenna with aperture dimensions of 110 × 55 mm on weed parameters in rice crop. Superscripts sharing different letters are significantly differ at 5% probability level.	60
Table 4.2: Influence of pre-emergence microwave (2.45 GHz; 120 s; 560 J cm ⁻²) irradiation of clay based moist soil for weed management on yield components of the rice crop. Superscript sharing different letters differs significantly at 5% probability level.	63
Table 5.1: Physico-chemical properties of soil at the experimental sites. Properties was assessed by Nutrient Advantage Laboratory Services, Victoria, Australia.	74
Table 5.2: Effect of pre-sowing microwave (2.45 GHz; 2 kW; 60 s; 500 J cm ⁻²) irradiation of soil on weeds parameters of rice crop at both experimental sites. Note: Entries with different letters in rows are statistically significant different from each other at 5% probability level.	79
Table 5.3: Influence of pre-sowing microwave soil heating for weed seedbank depletion on rice productivity at two agro-ecological zones of the Australia. Note: Different letters in a row depicting a significant difference at 5% probability level.	81

Table 7.1: Comparison of N use efficiency in response to microwave soil treatment under different rates of applied nitrogen. This was measured only for first season crop.	102
Table 8. S1: Physico-chemical properties of the soil used in the study. Soil physico-chemical properties were measured by Nutrient Advantage Laboratory Services, Werribee, Victoria, Australia, before MW treatment.....	138

List of Mathematical Symbols and Abbreviations

β_o	wave number of MW field (m^{-1})
N_r	Rate of applied nitrogen
PEP	Partial factor productivity of nitrogen
E_o^2	Electric field strength at the soil surface (V m^{-1})
t_f	MW Heating time (s)
ε_o	Permittivity of free space
a	Waveguide width (m)
A	Width of horn antenna aperture (m)
B	Length of horn antenna aperture (m)
b	Waveguide height (m)
c	Speed of light (m s^{-1}) in free space
E_a	Electric field strength in horn antenna aperture (V m^{-1})
E_p	Electric field strength at a point in front of the antenna (V m^{-1})
f	Frequency (GHz)
h	Convective heat transfer coefficient at the surface of the material ($\text{Wm}^{-1} \text{K}^{-1}$)
k	Dielectric loss factor of the heated material
j	Complex operator $\sqrt{-1}$
K	Thermal conductivity of the material ($\text{W m}^{-1}\text{K}^{-1}$)
n	Amplitude scaling factor for simultaneous heat and moisture movement
P	Microwave power (W)
R_o	Geometric length of horn antenna's flare pyramid (m)
x'	Horizontal distance from centerline of waveguide in aperture plane (m)
x, y, z	Cartesian coordinates of center point of horn antenna aperture
y'	Vertical distance from centerline of waveguide in aperture plane (m)
Y_o	Yield without N application

ΔY	Increase in grain yield with an N application
Y_m	Yield from microwave treated pots at a particular nitrogen rate
Y_c	Yield from untreated control pots at a particular nitrogen rate
Y_∞	The limit of yield when applied nitrogen become very large
α	Microwave attenuation factor in soil (m^{-1})
γ	Combined heat and moisture diffusivity of coefficient of the soil
κ''	Dielectric loss factor of the soil
ω	Angular frequency of the microwaves (Rad s^{-1})
μ_o	Magnetic permeability of free space (H m^{-1})
$ML\ ha^{-1}$	Mega liter per hectare
$t\ ha^{-1}$	Tones per hectare
s	Seconds
h	Hour
<i>FAO</i>	Food and Agriculture Organization

Chapter 1 – Introduction

Farming systems are expected to incorporate new environment-friendly pest management technologies for sustainable crop production (Reeve, 2011). However, the adoption of new technologies in existing farming system has been challenging. Despite this, closing yield gaps is crucial to overcome the globally increasing pressure for food production (Chapagain and Good, 2015), and this can be conquered by optimizing on-farm management strategies (Yang et al., 2008). Considering this, the FAO (2009) predicted that a 70% increase in agriculture production is needed to feed 9.6 billion people by 2050. Additionally, in the world's major food crops – rice, wheat and maize – the rate of yield increase is declining (Fischer and Connor, 2018). Therefore, the sustainable intensification of agricultural production – higher crop production with less resources, while eliminating the negative impact of agriculture on the environment (Godfray et al., 2010) – is essential to meet the future challenges of food supply. Integrative and ecologically safe pest management strategies are one of the key elements of sustainable intensification (Pretty and Bharucha, 2014).

1.1. Importance of Weed Management

Numerous biotic and abiotic factors are constantly impacting on the cropping environment. Among those, weeds are a major hindrance in crop production (Chauhan et al., 2017). As a botanical competitor, they adopt the similar trophic level for food production as crop plants, and therefore competition for resources results in a considerable reduction in the crop yield (Swanton et al., 2015; Ramesh et al., 2017). In a particular region, the extent of weed competitiveness depends on the collective influence of soil (fertility and seedbank), crop management (agronomic practices), and environmental conditions (Chauhan et al., 2012; Swanton et al., 2015). Weed constraints are generally approached from two basic points of view: either control or management (Ghersa et al., 2000). Control strategies are associated with the reduction of weed densities, while management approaches emphasize keeping weed densities below the economic threshold level to meet the attainable yield for sustainable crop production (Radosevich et al., 1997). An effective weed management strategy needs to

simultaneously integrate two important goals: (1) short-term prevention of crop yield reduction due to weed competition; and (2) eliminating the soil weed seedbank size, to minimize the regeneration of weeds in the following cropping years (Chauhan et al., 2017).

Weed seedbanks in soil potentially regulate the persistence of weed problems in agricultural crop production systems and therefore reducing these seedbanks is critical in sustainable intensification. Regeneration strategies of numerous widespread weed species represent opportunities for understanding the species composition of a particular area and ultimately this will aid in developing suitable management approach to diminish seedbank persistency (Wilson et al., 1985). This directly reflects the size and capabilities of the seedbank to develop new weeds and this scenario varies from species to species and from field to field (Buhler et al., 1997), which is entirely dependent on the cropping history and management approaches.

In agricultural land, the seedbank size can vary from 0 to 1 million seeds m^{-2} of diverse species (Thompson et al., 1997). In a particular region, the occurrence of a few dominant species can represent the primary weed problem (70 – 90% of the entire seedbank) in a crop production system because of their resistance to management practices (Wilson et al., 1985). For example, wild radish (*Raphanus raphanistrum* L.) and annual ryegrass (*Lolium rigidum* L.; contribution to seedbank - 2041 seeds m^{-2}) are two important yield reducing weeds in no-till wheat farming systems of Australia. Because of their short-life span, early seed rain is potentially accumulated into the seedbank. Similarly, barnyard grass (*Echinochloa crus-galli* L.) is the problematic herbicide-resistant weed of the rice growing regions of the world. It can produce between 7000 – 20,000 seeds plant^{-1} , depending on the timing of germination within the rice crop and a considerable portion of these seeds can become a part of the soil seedbank, which can range between 0 – 36,080 seeds m^{-2} (Bagavathiannan et al., 2011). Therefore, seed production is a key element of long-standing weed population dynamics (Davis et al., 2003). The contribution of the weed seedbank to the long-term plasticity of weed infestations imposes another challenge in this discipline. However, the reduction in the weed seedbank capacity to regenerate weeds seems indispensable, especially in conservation agriculture systems, for sustainable weed management. Therefore, an ecologically sound and viable weed management technology is required to tackle these

constant problems in agricultural production systems. The weed species of tropical and temperate regions and their capacity to resist herbicides are very different. Further, different chemical herbicides are required to manage weeds in different farming systems, while glyphosate is used universally to facilitate no-till agriculture. An environmentally-friendly non-chemical weed management system to achieve the broad-spectrum activity in different farming systems (for example; dryland wheat and temperate rice) is still lacking.

1.2. Use of Herbicides for Weed Management

Inventions of numerous chemical herbicides for weed control have potentially revolutionized agricultural production (Shaw, 1964; Hamill et al., 2004). No-doubt, herbicide-based weed management systems are the potential driver of conservation agriculture and sustained cereal productivity (Thomas et al., 2007; Johnson et al., 2009) but the present and future challenges associated with herbicide use are having and will have a serious impacts on safe food production. Additionally, heavy reliance on herbicides has also resulted in the development of herbicide-resistant weeds. Over the past years, globally, 471 cases of herbicide-resistant weeds have been reported in 86 commercially important crops, and 250 weed species have developed resistance to 160 different chemistry herbicides (Heap, 2016).

However, the evolution of herbicide-resistant weeds, and ecological concerns with herbicide use, have motivated weed scientists to develop innovative technologies, which could be potentially integrated into existing management regimes, to sustain agricultural production by keeping weeds below threshold levels (Chauhan et al., 2017). Various non-herbicidal weed control techniques have been explored and developed. For example, Harrington and Powles (2012) developed a tool named the “Harrington Seed Destructor” to minimize the viable seed return to the soil at harvesting and this has been very well implemented in Australian crop production systems. Similarly, Gay et al. (2010b) successfully developed a steam-hood applicator for weed control, as an alternative to soil fumigants (i.e., Metham Sodium and Methyl Bromide) under field conditions. Likewise, there is increasing interest in the development of thermal weed

control methods in organic farming and potential methods have been reviewed for possible implementation by Peerzada and Chauhan (2018). In physically-oriented jump-started heating methods, for example thermal weed management, the time required to reach the target temperature is crucial. This regulates the efficacy and economics of the system. In the case of MW soil treatment, the rapid increase in temperature (*i.e.*, *real time heating*) distinguishes this technology from many classical heating methods (e.g., soil steaming, soil solarisation). The non-chemical weed control approaches have been tested for over a century and their broad-spectrum activity has been reviewed by Brodie (2018). Among those heating methods, based on literature assessment, microwaving and steaming can be effectively implemented for weed control in sustainable intensification.

1.3. Microwave Weed Control

It has been reported that microwave (MW) soil treatment can effectively control weed seedbanks (Nelson, 1996; Sartorato et al., 2006; Brodie et al., 2017). MW soil treatment can be defined as; MW heating of soil requires a suitable MW applicator/zapper (a multimode cavity for potted soil or an antenna for soil *in-situ* mounted to a magnetron/solid state microwave generator) to raise the soil temperature. While the application of electric current for pest management has been documented for over a century (dates back to 1880's; Diprose et al., 1984), numerous field investigation at many agricultural sites reported an appreciable impact of MW energy for weed management (Wayland et al., 1973; Davis, 1974). Several factors which collectively govern the efficacy of MW include: (1) the frequency used; (2) treatment duration; (3) weed type (grasses or broadleaf); (4) soil moisture; (5) seed moisture; and (6) soil-seed interaction. These are minor limitations for the MW-based weed management systems. In particular, the soil structure, moisture content and penetration depth required for seedbank depletion decide the amount of MW energy needed for operation.

From a practical perspective, in 1971, the Phytex Corporation designed and developed several MW weed killers for field trials. Among them, the Zapper III was the biggest weed controller, which was equipped with two hydraulically maintained 30 kW MW (2.45 GHz) generators, powered by an on-board 155 kW diesel generator (Diprose et al., 1984). This machine was widely tested to predict the feasibility of this system; however, considering the energy and time required for treatment and associated cost, the

researchers (Davis et al., 1971; Wayland et al., 1973; Hightower et al., 1974; Wayland et al., 1975) came to conclusion, without denying the potential of MW heating for weed management, that MW cannot be commercially applied for weed control on a large scale. Relevant to this, Nelson (1996) estimated that, if 1500 J cm^{-2} is the desired MW energy for soil treatment of a 1 m wide strip with ground speed of 1 km h^{-1} ; therefore, for this purpose a 4167 kW of MW power would be required and the cost associated with this would be USA\$3 W⁻¹. In addition, there are some safety issues associated to MW weed control technologies and these issues must be taken into account by the commercialization authorities of this technology. Even after the successful demonstration of numerous studies; however, a quest for practical application remained a dilemma until now. The MW energy application cost is probably the main reason for abandoning the idea of MW weed and soil treatment. However, all these studies were published before the evolution of herbicide-resistant weeds and did not consider the toxic and residual impacts of herbicides use in the food chain.

Later, Brodie (2008; 2012; 2014; 2015a; 2016; 2017) led this dormant project toward completion by rigorously-testing the MW efficacy for herbicide-resistant weed control. The conclusive statement from Brodie's experimentation is that pre-emergence application of MW energy effectively deactivates the weed seedbank within a temperature range of 75 – 85 °C while far less MW exposure (2.45 GHz; 2 kW; 5 – 10 s for majority of weeds at 2 – 3 leaf stage) is needed for post-emergence weed suppression. Additionally, Brodie designed a MW prototype for weed zapping between crop rows and this system can also be used for pre-emergence MW energy application for seedbank viability depletion. However, the efficacy of this innovative technology for herbicide-resistant weed management under field condition remains unexplored for sustainable agricultural production. Overall, the successful demonstration of this prototype for weed management will aid in the development of a commercial scale MW weed killer. Therefore, to understand this effect under field conditions a consolidated research effort would be needed to explore how heating impacts the weed seedbank of the dryland wheat and temperate rice crop and its ultimate effect on soil fertility and crop productivity.

1.4. Impact of Microwave Soil Treatment on Soil Properties and Biota

In this context, pre-emergence MW soil treatment for weed seedbank devitalization will also impose some impact on the soil properties. Soil is a complex heterogenous environment (Lu et al., 2014); therefore, the influence of transient temperature-induced changes on the soil nutrient profile and biologically important taxa are of interest and should be explored. Relevant to soil nutrient, it has been reported that MW soil heating facilitates organic nitrogen (N) mineralization (Wainwright et al., 1980; Yang et al., 1990) and ultimately increases the plant available N. The availability of indigenous soil N for crop productivity is an integral component of the farming system (Cassman et al., 1998). The higher N mineralization with soil heating (Glass et al., 2008) will benefit the N use efficiency of crops. Increases in crop productivity through MW soil heating has been reported previously (Gibson et al., 1988; Brodie et al., 2015a). However, the exact phenomenon behind this scenario has not been rigorously explored yet. Changes in N within a crop management strategy seems important and this would reflect the influence of a single management approach on crop productivity. In this regard, a study is needed to explore the heating influence on soil N behaviour and its impact on the long-term productivity of soil.

Additionally, a project cannot deny and ignore the soil microbiota as initially suggested by Nelson (1996) when consider the MW soil heating for killing the weed seedbank. The bacterial community's resistance, immediate response to heating disturbance (Hodgson et al., 2015), resilience, and recovery process following MW heating (Shade et al., 2012), may favour the practicability of this technology. In general, soil heating is detrimental to microorganisms (O'Brien et al., 2018); however, numerous safeguarding strategies within the soil microaggregates can harbour the biologically important taxa from heating disturbance (Nie et al., 2014). Relevant to indigenous N supply for crop growth, the role of ammonia nitrifying bacteria and archaea is critical. They are responsible for nitrification in the majority of soils (Banning et al., 2015). However, their response to MW soil heating is largely unclear but the literature (mostly evaluated by classical cultural-dependent techniques) suggests that they are resistant to MW energy (Vela et al., 1976).

In this view, the extent of temperature sensitivity varies from species to species (Bollen, 1969) and complete soil sterilization at ≤ 200 °C is not achievable with MW heating (Bárcenas-Moreno and Bååth, 2009). In an earlier glasshouse soil sterilization study, Eglitis and Johnson (1970) demonstrated that dielectric heating of the soil within a temperature range of 86 – 101 °C was effective to control the fungi without any serious damage being caused to nitrogen-fixing bacteria. Moreover, Vela et al. (1976) documented that soil nitrifying bacterial groups survived much higher dosage levels of MW exposure ($40,000 \text{ J cm}^{-2}$) than those needed for soil seedbank deactivation ($180 - 350 \text{ J cm}^{-2}$) (Wayland et al., 1973). Similarly, Ferriss (1984) reported that Prokaryotes showed higher resistivity to MW soil heating than Fungi. Recently, Nunes et al. (2018) employed various microwaving doses (2 – 3 minutes) for soil bacterial community shift and reported that there was no significant effect of heating on the potentially active bacteria. Finally, she confirmed the survival of nitrogen-fixing bacteria, after an exposure of 2 – 3 min of soil microwave heating, using high-throughput sequencing analysis. Overall, the adaptability of soil microbiota to management regimes gives a framework for understanding the potential of the system (i.e., MW weed management).

For this reason, the proposed peak soil temperature for weed seedbank suppression is in the range of 75 – 80 °C. However, it is expected that, this temperature regimes will alter the soil biota to some extent. The extent of immediate bacterial response to soil heating is relevant and mostly unexplored in the case of MW soil heating. However, a part of this thesis has explored the effect of MW soil heating on the bacterial community shift and total abundance of ammonia oxidizing bacteria (AOB) and archaea (AOA) using quantitative polymerase chain reaction (qPCR) and Next-generation Sequencing.

1.5. Research Aims

The main aim of this thesis is to explore the impact of MW soil heating for weed management on dryland wheat and temperate rice productivity under field conditions. Additionally, regarding crop productivity, a further evaluation on how soil heating influences the N use efficiency and long-term soil productivity is considered. Finally, the effect of MW-induced transient heat disturbance on total abundance of ammonia oxidizing bacteria and archaea and bacterial community composition is assessed.

1.6. Hypothesis

Considering all these contexts, therefore, the hypotheses of this study are that:

1. Pre-sowing MW soil heating reduces the seedling emergence of the main herbicide-resistant weed species in dryland wheat cropping;
2. It is possible to implement a microwave-based weed management strategy as an alternative to herbicide treatment in a temperate rice production system in Australia;
3. Soil heating for weed management also enhances indigenous N supply for sustainable production; and
4. Ammonia oxidizers are resistance to soil heating and the transient heating aids in shaping the soil's biologically import taxa.

1.7. Thesis Overview

In this thesis, following this introduction, **Chapter 2** highlights the weed problems in crop production systems, outlines possibilities of new thermal weed control methods and reviews diverse literature on the use of MW technology for weed management and its additional benefits on soil productivity. Therefore, **Chapter 3** demonstrates the effectiveness of pre-sowing MW soil treatment for weed management in a dryland wheat crop under field conditions and provides a preliminary economic evaluation of MW weed control compared to herbicide application for weed management and this has been published in the Transaction of the ASABE. Consequently, **Chapters 4** (published in the Transaction of the ASABE) and **5** explore the practical application of MW soil treatment to reduce the weed seedbank's regeneration capacity in rice farming systems at two agroecological zones of Australia (published in Crop and Pasture Science). Furthermore, **Chapters 6** (published in the Journal of Microwave Power and Electromagnetic Energy) and **7** (published in the Transaction of the ASABE) elaborate the effect of MW soil heating on wheat crop productivity, N use efficiency and highlight the persistence impact of soil heating on grain yield. In relation to soil heath, **Chapter 8** (published in the European Journal of Soil Science evaluates the effect of MW soil treatment on the soil bacterial community shift based on the 16S rRNA amplicon sequencing and qPCR to determine the total abundance of bacteria (16S rRNA gene copy

number) and ammonia oxidizers (*amoA* gene copy number). Finally, **Chapter 9** evaluates the practicability of MW weed management technology, based on the results of experiments presented in the all the chapters, for sustainable crop production and gives some directions for further research needed in this area.

Chapter 2 – Literature Review¹

2.1. Weeds: An Important Yield Limiting Factor

Cereal production is highly influenced by the biotic and abiotic factors. Among these factors, weeds are one of the most important biological constraints in cereals productions all over the world. They significantly affect the crop growth, development and finally economic yield through resource competition. In this view, Oerke (2006) estimated the maximum crop yield losses, 34%, is caused by weeds compared to other pests. The extent of crop yield losses is entirely depended on weed density (Cousens et al., 1987). Given this, in Western Australia, 7 – 90 plants m⁻² of capeweed (*Arctotheca calendula* L.) can reduce wheat yield up to 28 – 44%. In addition, annual ryegrass (*Lolium rigidum* L.) has significant impact on crop yield losses in conservation agriculture of Australia (Chauhan et al., 2012). In many direct-seeded rice growing countries, the yield losses due to weeds have been estimated: in Australia 10 – 30% (Pratley et al., 2004); India 20 – 90% (De Datta, 1981); Vietnam 10 – 50% (Chin and Mortimer, 2002); USA 10 – 30% (Hill et al., 1994) and in Europe 55 – 60% (Oerke et al., 1994). Therefore, a reducing weed pressure is essential for a sustainable crop production and closing yield gaps. For this, a single weed control method may not be enough to minimize the weed pressure and may cause shifting in the weeds to herbicide-resistance in farming systems.

2.2. Herbicide Resistant Problem

According to WSSA (2008), the herbicide resistance is a genetic ability of weed to survive and reproduce following the application of chemical which is normally lethal to wild type plants. Globally, there are 400 weed species that have developed resistance to herbicides and annually 9 new weed biotypes are reported as being herbicide resistant (Heap, 2014). The overall number of herbicide resistant species in crops is given in Figure 2.1. There are nine different mechanisms which weeds can adopt to escape the negative impact of herbicides and develop resistance. Cross-

¹Most of the information presented in this chapter has been published in a book chapter: <https://protectau.mimecast.com/s/DXozC4QZNwC4ql0ktOKDpR?domain=mts.intechopen.com>

resistance in weed flora implies resistance to two or more weedicides of the same or different chemistry because of one resistant mechanism (RM; Beckie and Tardif, 2012). However multiple resistances in individual weed species is generally characterized by the presence of two or more RM's. These mechanisms might be the mutation at the site of action (SOA) of herbicides, target site, or change in metabolism and translocation (non-target site), which reduces the phytotoxic effect of herbicides on their SOA (Beckie et al., 2012). Metabolic resistance is more commonly found in monocot (grasses) than in dicot (broadleaf) weeds (Beckie et al., 2012).

Therefore, herbicide-resistance in weeds is the greatest threat to sustainable productivity of agricultural commodities in industrialized countries. In addition, the glyphosate resistance cases in Australian farming systems has been increasing exponentially as presented in Figure 2.2 and 2.3. In Australian context, the glyphosate application is a major agriculture input for weed management. In case of ryegrass and barnyard grass, the early maturity and seed set make them more vulnerable for management. If a germination capacity of these seedbank declines with a novel management practice, which could indeed reduce the application of glyphosate for weed control and ultimately can sustain the crop production. Therefore, there is a present need of an alternative weed management strategy in exiting cropping system.

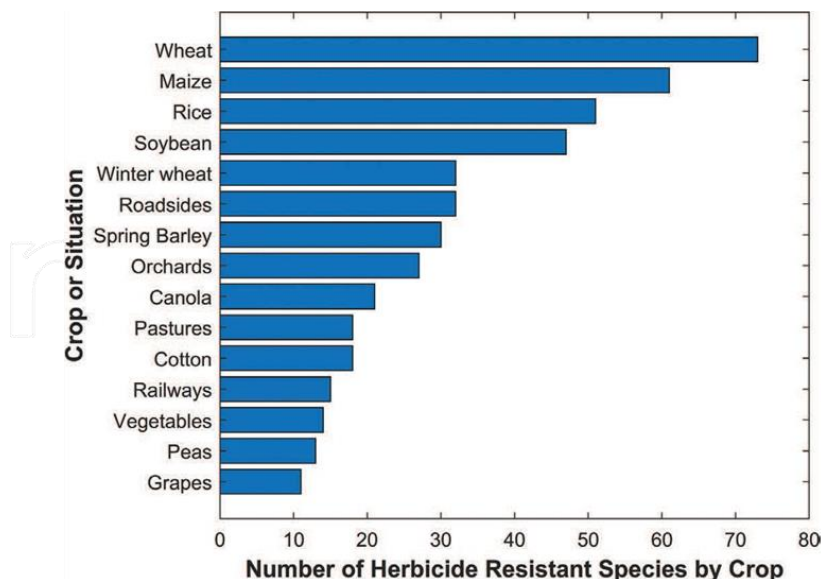


Figure 2.1: The overall number of herbicide-resistant species by crops. Source: Heap (2016).

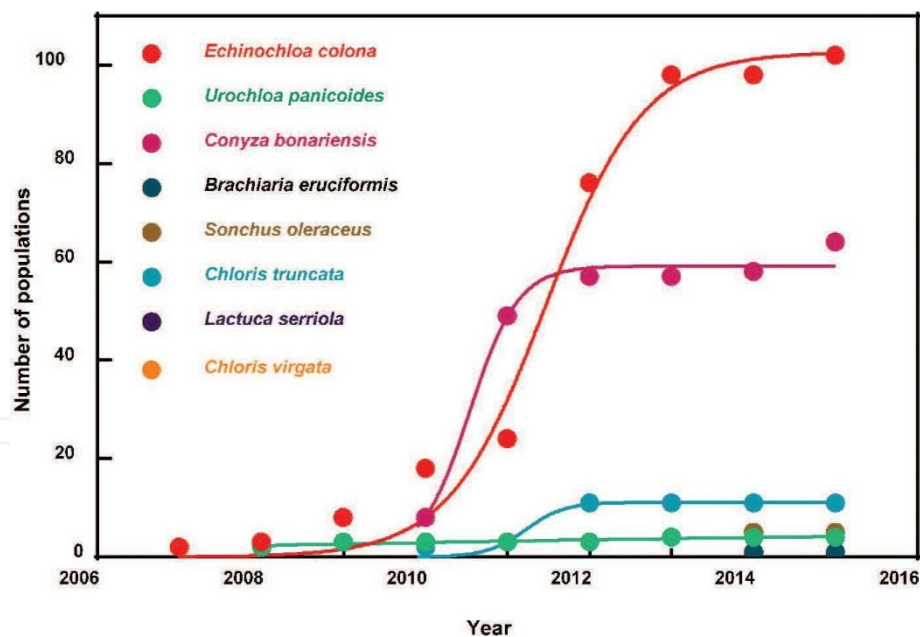


Figure 2.2: Confirmed cases of glyphosate resistance in summer weeds of Australia. Source: Australian glyphosate sustainability working group (<https://www.glyphosateresistance.org.au/>).

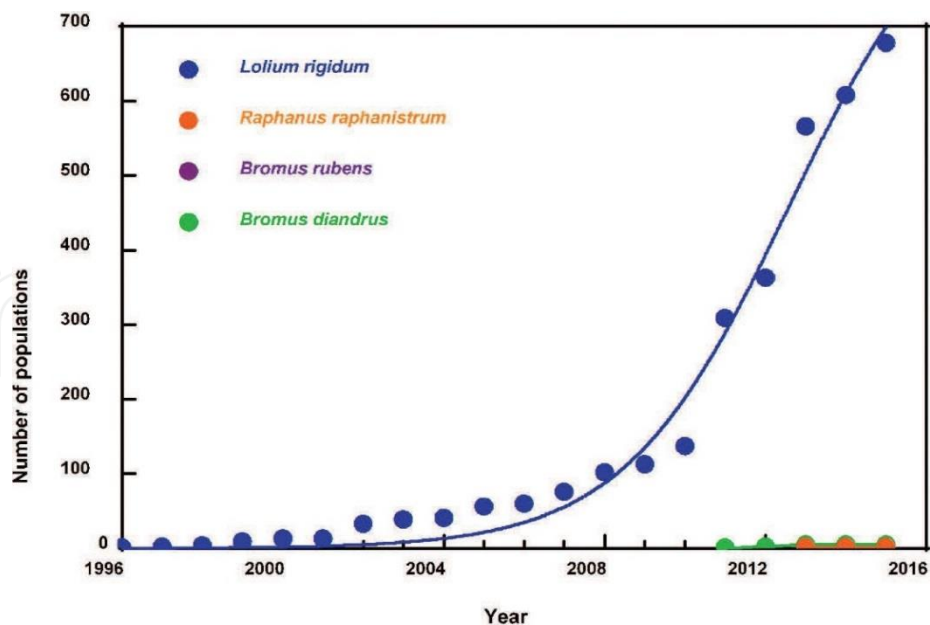


Figure 2.3: Confirmed cases of glyphosate resistance in winter weeds of the Australia. Source: Australian glyphosate sustainability working group (<https://www.glyphosateresistance.org.au/>).

2.3. Impact of Thermal Technologies for Herbicides Resistant Weed Management

Herbicide-resistant weeds and concerns about safe food production provide a strong framework for the development of new innovative technologies to minimize the multi-dimensional weed-induced problems in agricultural production. For this, a knowledge of the geographic weed distribution and weed control measures of that region helps in shaping region-oriented weed management technologies. For example, the resistiveness of barnyard grass of monsoon paddy to glyphosate (South-East Asia) would be quite different to temperate paddy environments (Australia and Italy), in general.

Initially, remote sensing technologies facilitate the decision for more specifically applying weed management control measures. Treatments need only be applied, in agricultural land, where they are deemed to be required for herbicide-resistant weed management. This could benefit the environment and minimize the operational (localized tillage and spray) cost (Shaw, 2005); however, in practice, precision weed management is entirely focused on safe application methods (shielded sprayer and optical-sensor sprayer for weed and crop distinguish) of existing chemistry herbicides. Additionally, an Australian University (Queensland University of Technology) developed a field robot named “AG-BOT II” to support precision weed management by detecting and classifying weeds in the field. Once AG-BOT II has classified weeds, its robotic precision system selects the best weed control methods, whether it be chemical, mechanical and a thermal measure. The researcher claimed that AG-BOT II would be effective in reducing the weed control cost by 90%, which would therefore save about AU\$1.3 billion for the farming sector annually (Anonymous, 2016).

Moreover, reduction in weed seed return to the soil weed seedbank could effectively reduce the long-term weed problem under field conditions. Most of the seed return occurred at crop harvesting, therefore, to deal with this, a semi-government Australian firm has developed a non-chemical weed control tool called the “Harrington Seed Destructor (HSD)” to minimize seed accumulated into seedbank at harvesting. This system has been effectively implemented in the Australian context (Harrington and Powles, 2012). Keeping in view the vulnerability of farming systems to the weed

seedbank, a multimode weed control approach, including both chemical and non-chemical approaches, is needed to sustain food production. From a non-chemical perspective, interest in thermal weed control methods has been increasing. Therefore, the following sections represent the potential of numerous thermal weed control methods for herbicide-resistant weed management;

2.3.1. Flaming

Flame weeding is broadly used to control weeds, as an alternative to chemicals. Of the several kinds of applicator, the tractor-mounted flame weeder has been extensively used for weeding in various cropping regimes. Ascard (1994) tested the flame weeder under field conditions for three years to develop a dose-response relationship between applied energy (liquid petroleum gas (LPG) doses; kg ha⁻¹) and percent weed control. Based on Ascard calculation, a total load of 159.3 kg LPG ha⁻¹ is needed for 95% reduction in the white mustard (*Sinapis alba* L.) plant density (607 plants m⁻²) at 4 – 6 leaves stage. Neeson (2008) reported that a second-hand tractor-mounted propane gas flame weeder costs about AUD\$ 12,000 – 15,000 and gas cost about AUD\$ 80 ha⁻¹ for weed management in an Australian organic rice farm. This technique has an advantage over chemical application and can effectively control herbicide-resistant weeds (Wszelaki et al., 2007); however, the flaming process contributes considerably to increase in CO₂ concentration, given that, combustion of 60 kg propane ha⁻¹ emits 188.9 kg CO₂ ha⁻¹ (Ulloa et al., 2011). Using an open flame in field conditions also constitutes a significant fire hazard.

2.3.2. Steam Treatment

Soil steaming has been reconsidered for weed management in recent years. Sheet steaming is the most common steam applicator which involves covering the soil with a heat resistance membrane. Saturated steam is pumped under the sealed edges of the thermally resistance sheet to penetrate the soil surface for weed control (Gay et al., 2010a). For designing and testing, Gay et al. (2010b) tested a small hooded applicator (15 × 15 cm) connected to a steam generator (8.5 kW nominal duty cycle) for soil steaming. In their experiment, 300 s of steam application to the soil surface achieved equilibrium in a temperature gradient, with a maximum temperature of 100 °C. With this exposure, the average weed density was less than 50 plants m⁻² compared to un-steamed soil with 400 plants m⁻². They proposed that soil steaming can

effectively control weeds and disinfect the soil; however, it requires sufficient energy investment for steam generation.

2.3.3. Solarisation

McGovern et al. (2013) proposed that soil solarisation had an appreciable impact on soil-borne pathogens and weeds and had been evaluated over the last three decades as a non-chemical approach in many countries. The use of double-layer polyethylene sheets has increased the efficacy of this weed control method by increasing the temperature gradient by 3 – 10 °C compared with a single layered sheet (Barakat and AL-Masri, 2012). Solarisation can kill a wide range of weed species by breaking seed dormancy and by heating-induced devitalization of young seedling (Soumya et al., 2010). Soil solarisation is best suited to integrated weed management (McGovern et al., 2013).

2.3.4. Electrocuting

The use of electric current for soil disinfestation has long been proposed and attempted (Diprose et al., 1984). Diprose and Benson (1984) reported that electrical methods of killing weeds fall into two main types; spark discharge and direct contact. Both of these methods needs high voltages (20 kV) for effective weed control. Depending on the voltage used, contact time and weed phenology regulate the damage severity (Vigneault et al., 1990). Diprose et al. (1980) reported that short exposure (20 s) of the high alternating voltage of 5 kV, applied by two electrodes, can potentially kill annual beet in pots. Various plant species require a different range of current; however, 0.5 – 1.0 A was enough for killing of 1.0 – 1.4 m tall vegetation. Furthermore, they tested a tractor-driven system with a capacity of 8 kV voltage and covering speed of 1.6 km h⁻¹ and found that annual beet was 75% killed with this exposure. Electrocutation has been successfully used to kill weeds both in the laboratory and under field conditions and offers advantages over chemical and mechanical weed control methods (Diprose et al., 1980); however, this approach needs a very high voltage for effective weed control (Brodie, 2018) and user safety remains a serious concern.

2.3.5. Infrared Radiation

Radiative heat transfer is referred to electromagnetic energy transfer from adjacent hot material to the target (Brodie, 2018). Infrared radiation systems use gas burners to heat up ceramic and metal surfaces, which then transfer heat to the ground for disinfection scenarios. The relationship between lethal temperature and holding time of this lethal temperature by a system (plant and weed seedbank) regulates the denaturing of plant cells (Levitt, 1980). Laboratory-Based research of Parish (1990) identified that a “medium-wave tubular fused quartz infrared emitter” was the most effective tool for infrared weed management. Compared with a flame weeder, there is no profound effect of wind on the infrared burners, and the target area coverage is much larger. In either burners, for 100% weed control a total load of 120 kg ha⁻¹ of propane is required and much higher temperature (1350 °C) is needed in the case of a flame weeder compared to the infrared radiator (770 °C) (Ascard, 1998). For efficient weed control, an energy density of 200 – 400 kJ m⁻² is needed in the case of medium-wave infrared energy (Parish, 1990). Numerous investigations reported that, the infrared flame weeder (Figure 2.4) is inferior compared to flame weeders; however, Parish (1990) and Ascard (1998) agreed that the difference in weed control effect was based on the dose, heating time, the speed of the applicator, burner height above target, plant size, density and type (monocot or dicot).

2.3.6. Ultraviolet Radiation

Ultraviolet radiation falls in the range of 100 – 400 nm and can be distinguished into three groups, based on wavelength (Brodie, 2018). When plants are subjected to ultraviolet radiation, most of the energy is observed into the thin outer layer (0.1 – 0.2 mm) of plant tissues and thus results in heating of plant material with a similar pattern to other thermal weed control methods like flame weeding (Andreasen et al., 1999). Moreover, regrowth after ultraviolet radiation was observed by Andreasen et al. (1999), which suggests that multiple treatments for effective weed control are required. No doubt, these thermal weed management technologies have positive impacts on sustainable weed control; therefore, a novel solution to herbicide-resistant weeds is still in question. To this end, a possible alternative – microwave-based solution – is explained in following sections.

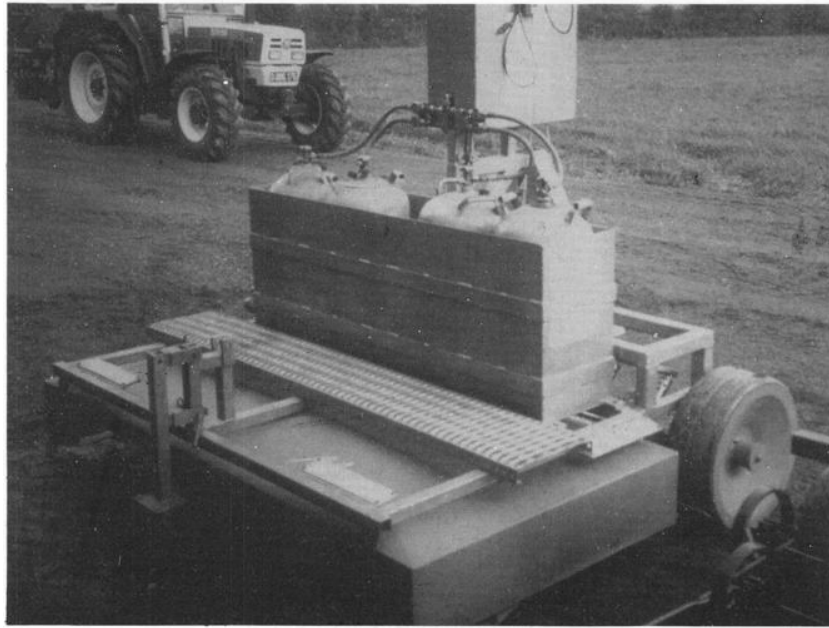


Figure 2.4: The earlier design of the infrared flame weeder for pre- and post-emergence weed management under field conditions; adapted from Parish (1990).

2.4. Microwave and it's Interactive Mechanisms

Microwaves (MW; the core of this thesis) are non-ionizing electromagnetic waves with a frequency of between 300 MHz and 300 GHz and a wavelength range of 1 m to 1 mm, respectively. Biological systems are electro-chemical in nature (Kothari et al., 2012) and a mixture of organic and dipole molecules, i.e. H_2O , arranged in different geometries (Brodie et al., 2012b). Interest in the study of the interactions of ultra-high frequency electromagnetic energy with complex biological systems dates to the 19th century (Ark and Parry, 1940). In 1924, Antonin Gosset was the first person who explored the bio-stimulation effect of MW energy, when he, along with his co-worker, used high frequency electromagnetic waves to treats tumours in plants with no injury to the plants (Bren, 1996). The interactions of MW with living systems are characterized as atomic, molecular, cellular and subcellular (Kothari et al., 2012).

The basic consideration in measuring the influence of MW irradiation on living systems is the determination of the induced electromagnetic field and its spatial distribution. The bio-effects of microwave treatments can be described solely by differences in temperature profile between MW and conventionally heated systems

(Kozempel et al., 2000). The energy of MW photon is 0.0016 eV at 2.45GHz (Kappe et al., 2012). This is not enough energy to break the structure of organic molecules (Yaghmaee and Durance, 2005). The basic interactive mechanism of MW with biological system is rotation of polar molecules (i.e. H₂O, Proteins and DNA) by the induced electric field (Takashima et al., 1984). The rotation occurs 2.45 billion times every second for 2.45 GHz waves. The MW interaction is entirely based on the dielectric heating mechanisms while many studies have also reported the non-thermal effects of MW energy application.

2.4.1. Microwave Weed Management

MW weed management technology could be used to replace or supplement traditional methods of weed control for crop production in agriculture. The precise history of microwave-based weed management is presented in Table 2.1 and Figure 2.5 shows early MW weed killer design. The promising efficacious application of microwave heating in agricultural systems can substitute the hazardous, toxic and environmentally-unsafe chemicals that are used to kill weeds (Wayland et al., 1978). The increasing trend in the use of MW energy as a tool to control weeds is mainly because of herbicide resistance in various weed species (Heap, 2016) and their long-lasting degrading impact on the environment (Cox, 1995). MW heating is not influenced by wind direction and speed, therefore prolonging the application periods compared to traditional methods of herbicides spraying (Diprose et al., 1984). Ayappa et al. (1991) reported that the most important features of microwave heating are its accurate control, diminutive start-up time and volumetric heating. The energy density is the most important factor in plant mortality rather than exposure time, two options during weed management through microwave energy become evident; increased exposure to diffuse microwave energy or deliberate application of a strongly focused MW pulse to quickly debilitate the plants (Brodie, 2012). MW radiation, which triggers dielectric heating of plant tissues, is inaugurated by the MW's electric field. This internal heating ultimately kills or debilitates the plant (Sartorato et al., 2006). In another study, Bigu-Del-Blanco et al. (1977) treated 2-day old seedling of maize with MW energy at a frequency of 9 GHz for time periods of 22 – 24 h. The authors revealed that more exposure time of MWs even at very low energy densities significantly dehydrates the maize plants and retarded their growth.

In contrast, the recent research on fleabane and paddy melon (Brodie, 2012) has concluded that a short exposure (≤ 5 s) of high intensity MW heating was enough to hinder plant growth. The plant tissues, which were subjected to MW energy rapidly dehydrated. Relevant to this, Whatley et al. (1973) stated that minimum moisture level in soil attenuated the MW transmission less than high moisture content; the authors suggested that pre-emergence MW treatment for weed control should be worked out when the top soil layer (1 – 2 cm) contains relatively low moisture.

Van Wambeke et al. (1983) and Benz et al. (1984) reported that for MW irradiated soil samples, seeds, fungi and nematodes could be effectively controlled with a short exposure; however, the efficacy of this short exposure was highly influenced by soil texture, exposure time (s), soil depth and soil moisture content. Davis et al. (1971) conducted an experiment to evaluate the effect of MW on the seedling survival percentage of twelve species. They described that the 48 hour germinated seedling indicates no survival after short exposure of MW energy and concluded that susceptibility of young seedlings to MW heating was highly correlated with moisture content and absorption of energy. Davis et al. (1971) proposed that the specific mass and volume of crop seeds were positively correlated to seed mortality during MW heating. This might be due to the “radar cross-section²” as mentioned by Wolf et al. (1993), caused by weed seeds to transmitting MW. More radar cross-section enables the seed to interrupt, and thus absorb more MW energy. This seems to be the cause of death (Nelson et al., 1970). The use of electromagnetic radiations for post-emergence control of broad leaves and grasses is the least energy consuming process available for MW weed control (Wayland et al., 1978).

In a separate concept, Brodie et al. (2009) stated that based on MW energy calculation for seeds and plants on the sandy soil surface, more energy was required to kill dry seeds as compared to the previously emerged plants. The actual energy requirement on huge scale would depend on plant density and three-dimensional microwave distribution. Hence, the total energy required for weed management might be significantly reduced if weed seeker systems are employed to control the activation of the MW unit. Thermal runaway, caused by the temperature-dependence of the material properties, is common in

²Reflection of MW energy by target object – in this case, the ability of seedbank to reflect the MW energy based on their geometry and moisture content.

dielectric heating (Metaxas and Meredith, 1983; Nelson, 1996). Total energy and time exposure could be dramatically reduced if thermal runaway can be induced in weed plants throughout MW irradiation treatment. If this were possible, analogous energy requirements to those related with traditional chemical weed control method could be achieved. This temperature-time interaction for pest management can only be discovered and understood through more research into the MW heating in agriculture.



Figure 2.5: The Zapper III (60 kW; microwave weed killer) developed by Phytox Corporation to zap weeds and soil treatment under field conditions.

Table 2.1: Precise review of microwave-based weed management under different scenarios.

Microwave Frequency	Power Level	Irradiation Duration	Treatment Scenario	Target Weed Species	Percent Weed-Seed Destruction	Reference
39 MHz	–	4 – 37 s	Pre-emergence	Hard Red Winter Wheat	50% seed mortality	Nelson and Walker (1961)
2.45 GHz	600 W	60 s	Pre-emergence (Dry, 4 h soaked and 46 h germinated seeds)	<i>Zea mays</i> , <i>Arachis hypogaea</i> , <i>Prosopis juliflora</i> , <i>Cucumis sativus</i> , <i>Brassica</i> spp., <i>Rumex crispus</i> , <i>Echinochloa colonum</i> , <i>Amaranthus</i> sp., <i>Gossypium</i> <i>hirsutum</i> , <i>Glycine max</i> , <i>Sorghum vulgare</i> and <i>Triticum</i> <i>vulgare</i>	17% reduction in germination in dry seeds but 100% in case of moist seeds at 10 s of exposure	Davis et al. (1971)
2.45 GHz	600 W	8 s	Post emergence (Aquatic weed)	Duckweed (<i>Wolffia punctat</i>)	50%	Champ et al. (1973)
2.45 GHz	2 – 4 kW	Varying exposure Time (not mention properly)	Pre and Post emergences	Johonsongrass Morininglory Redroot Pigweed Texas panicum Barnyardgrass Sunflower London rocket Rigseed euphrobia	For post-emergence MW treatment 309 J cm ⁻² energy was required for 100% control (Field Conditions) while for pre- emergence MW weed control 73 J/cm ² gave 85 – 100% control (Glass house conditions)	Wayland et al. (1973)
2.45 GHz	45 – 720 J cm ⁻²	No information	Pre-emergence	London rocket (13 cm deep in soil profile) and Sunflower (2.5 cm seeded depth)	87% for London rocket and 93% for Sunflower	Menges and Wayland (1974)
2.45 GHz	100 – 750 W	120 – 1200 s	Pre-emergence	Clover and Turnip	60 – 78% reduction in seeds germination	Hightower et al. (1974)
2.45 GHz	0.1 – 1.5 kW	Varying Exposure Time	Pre-emergence of seeds in soil	Black Medic, Barnyard grass, Foxtail purslane, Redroot pigweed, Large crabgrass,	50%	Rice and Putnam (1977)
2.45 GHz	–	360 s	Pre-emergence	<i>Brassica napus</i> , <i>Linum usitatissimum</i> , <i>Avena fatua</i> ,	85 – 95%	Bhartia et al. (1977)
9 GHz	10 – 30 mW cm ⁻²	22 – 24 hours	Post emergence	<i>Zea mays</i>	100% growth inhibitions	Bigu-Del-Blanco et al. (1977)
2.45 GHz	1.2 kW	5 – 45 s	Pre-emergence	Trifolium and Medicago	85% reduction in germination	Crawford (1977)
2.45 GHz	500 W	30 s	Pre-emergence	<i>Avena fatua</i>	60% (based on seed moisture)	Diprose et al. (1984)
2.45 GHz	1.5 kW	0, 10, 20, and 30	Pre-emergence	Wild Oat & Wheat	90 – 100%	Lal and Reed (1980)
2.45 GHz	–	120 s	Pre-emergence	<i>Avena satvia</i> and native weed seeds	Reduced weed seeds emergence	Barker and Craker (1991)
2.45 GHz	900 W	4, 8, 16, 32, 64, 128 and 256 s	Post emergence	<i>Abutilon theophrasti</i> , <i>Panicum miliaceum</i> , Lucerne and Rapeseed	Complete dehydrating of plants	Sartorato et al. (2006)
2.45 GHz	800 W	120, 240, 420 and 960 s	Pre-emergence	Rubber vine, Parthenium and Bellyache bush	88% (Rubber vine), 67% (Parthenium) and 94% (Bellyache bush) mortality at 960 s irradiation	Bebawi et al. (2007)
2.45 GHz	0.10 – 1.24 kWh m ⁻²	30 – 300 s	Pre and Post emergence	<i>Malva parviflora</i> and <i>Triticum aesteivum</i>	100% destruction of tested specie at 0.65 kWh m ⁻²	Brodie et al. (2007)
2.45 GHz	700 W	120, 240, 320 and 720 s	Pre-emergence treatment of soil	<i>Lolium perenne</i> and <i>Lolium rigidum</i>	100% seed mortality was achieved at 240 s of MW irradiation	Brodie et al. (2009)
2.45 GHz	750 W	5, 15, 30 and 60 s	Pre and Post emergence	Prickly Paddy melon	100% debilitation of plants	Brodie et al. (2012a)
2.45 GHz	2 kW	5, 10, 15, 30, 60 s	Post emergence	Ryegrass and Wild Radish	100% Mortality	Brodie and Hollins (2015)

2.4.2. Microwave Toxicity to Weed SeedBank

Controlling weed seedbanks is crucial for sustainable weed management. Reduction in the weed seedbank is an essential approach to overcome weed invasion (Kremer, 1993). Soil heating has potential to retard the germination capacity of the dormant seedbank up to various depth in top soil layer (Gay et al., 2010b). In this view, Barker and Craker (1991) conducted an experiment to find out the effect of MW energy on oats and weed seeds in soil at different moisture levels. It can be concluded that seed susceptibility to MW treatment is totally dependent on the temperature. The important factors include frequency (Shehzad et al.), power (W), temperature (K) and exposure time (s). The soil temperature, which ranged between 75 – 80 °C, significantly inhibited the germination of the weed seed bank. MW energy can also destroy the weed reproductive plant parts and their seeds that are covered up by soil at a depth of several centimetres (Diprose et al., 1984; Brodie et al., 2007). Numerous factor influences MW efficacy, among those, the geometry of seeds (i.e., ellipsoids or even spheres) and the MW frequency has huge impact in MW-based weed management scenario (Brodie, 2008). As seed structure varies from seed to seed; therefore, the calibration of MW doses would be required to tackle the herbicide resistance weeds in different cropping systems. Additionally, Brodie et al. (2009) tested the impact of different soil temperature levels on the dry seeds of ryegrass, wheat and oat, and found 80 – 100 °C can effectively control seed germination capacity regardless of moisture content as presented in Figure 2.6.

Moreover, Nelson (1996) stated that selective heating of seeds through MWs *in situ* cannot be practicable. Olsen (1975) discovered selective heating during his research work on the inhibition of seed germination in MW treated soil. This exciting phenomenon depends on two factors: power absorption and thermal insulation. The absorption of power also depends on various factors including the dielectric properties, shape and size of the irradiated material. This assumption has later been experimentally proved by Brodie et al. (2007), who examined the effect of MW energy on young Marshmallow seedlings, using a prototype MW system (Whatley et al., 1973; Menges and Wayland, 1974).

In another study, Wayland et al. (1973) treated wheat and radish seeds *in situ* at 25 mm depth and moisture content of soil was 6.5%. He found that MW was toxic to seeds with

a threshold of just 10 J cm^{-2} energy density. Increasing energy density was more effective to reduce the germination percentage of seeds than increasing exposure time (s) for some species. In field conditions, appropriate soil moisture facilitates MW energy absorption (Whatley et al., 1973; Menges and Wayland, 1974). In a separate study, Menges and Wayland (1974) compared three effective herbicides (methazoal, propachlor and perfluidone) with MW at energy densities of $45 - 720 \text{ J cm}^{-2}$ for weed control in an onion crop. They found that all the chemicals damaged the onion crop (85%) while MW induced injury to onion crop was 18%. They observed that MW at 360 J cm^{-2} significantly reduced the number of viable weed seeds. Therefore, an effective weed management depends on reducing the soil weed seedbank (Burnside et al., 1986).

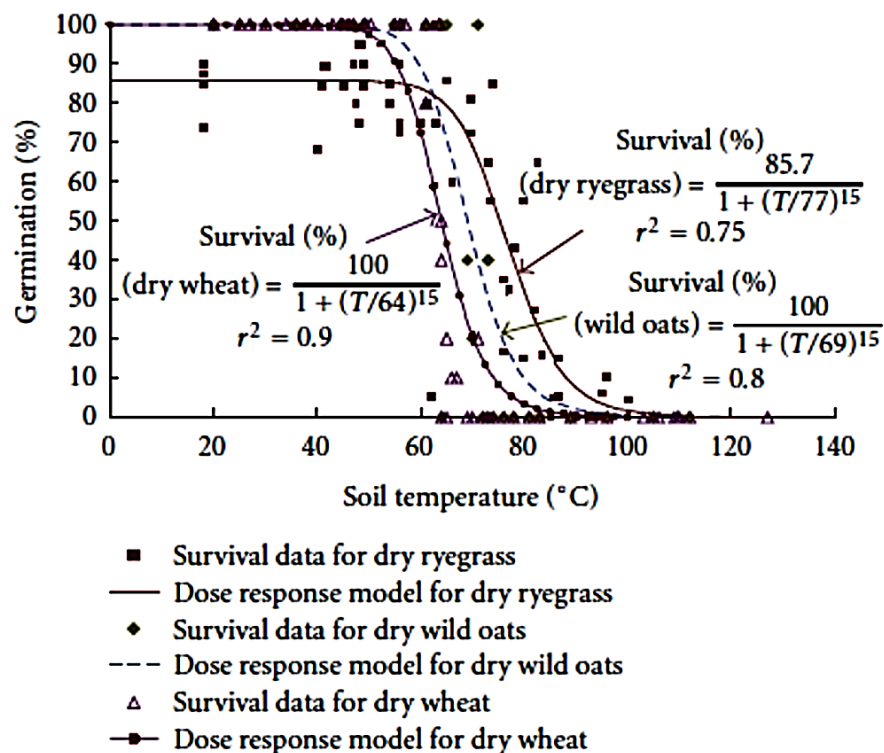


Figure 2.6: Impact of various doses of soil temperature increase following microwave soil treatment on the germination capacity of ryegrass, wheat and oat seeds, revealing the importance of soil-seed moisture of microwave efficacy. Source: Brodie et al. (2009).

2.4.3. Effect of Microwave Heating on Soil Nutrient Dynamics

Soil heating-induced change in the soil organic matter (SOM) also alters the plant growth limiting nutrients and elements (O'Brien et al., 2018). Notably, two important conditions, which manipulate thermal weed control are the heating temperature and time. In the case of MW weed management, 80 °C is the maximum threshold temperature required to kill almost 60 – 80% weed seedbank in top soil (0 – 6 cm) layer regardless of crop regimes (Brodie, 2018; Khan et al., 2018). However, there will be some effect of this short-term heat disturbance on the soil nutrient profile. The dynamics of key soil nutrients is explained by the knowledge of size and turnover rate of organic constituents, such as C-N compounds, cellulose and hemicellulose and lignin (Stevenson, 1994). The soil-MW interaction is a function of various soil properties such as texture, moisture, salinity, bulk density, temperature (Dobson et al., 1985; Hallikainen et al., 1985; Brodie et al., 2012b) and thermal conductivity of soil, which decide the fate of organic contents degradation and distillation (González-Pérez et al., 2004). The response of the soil nutrient profile, following MW heating, is not very well investigated. Given that, Cooper and Brodie (2009) investigated the effect of different durations of MW treatment and soil depth on soil nutrient status and pH. The MW treatments of soil had no significant effect on N, phosphorus, potassium and sulphate concentrations in all the treatment combination, but they reported an increase in nitrite concentration after 120 s of MW soil treatment. The nitrate reduction in the irradiated soil could be the principle cause of nitrite formation (Cawse and Crawford, 1967), in their study.

In another study, Wainwright et al. (1980) tested the effect of MW soil treatment (20 s; 20 g soil at 40% water content) on nitrification rate and sulphur oxidation of organic loamy soil. It was found that MW did not have a strong effect on the total bacterial count; however, a decrease in NO_3^- -N concentration of soil with heating reflects the susceptibility of ammonia oxidisers; although, an increase in NH_4^+ -N content in irradiated soil may favour the soil quality and ultimately plant growth and development. Additionally, this short exposure significantly stimulated S-oxidation. Similarly, Speir et al. (1986) examined the effect of MW energy (600 W; 2.45 GHz) on low fertility soil (100 randomly selected cores at depth of 50 mm) microbial biomass C, N, phosphorus and phosphatase activity. They reported that an increase in MW treatment duration (90 s)

dramatically increased the extractable N level ($106 \mu\text{g N g}^{-1}$ soil) but the microbial biomass C ($\mu\text{g C g}^{-1}$ soil) and phosphorus concentration declined with increasing irradiation duration (90 s). The higher flush in soil N is of microbial origin as MW has a biocidal effect (Shamis et al., 2008).

The fixation of NH_4^+ or K^+ in soil by inorganic colloids has been well documented (Kissel et al., 2008). In relation to this, Sawhney (1972) reported that the ion fixation in the clay lattice could be described by the expanding and contracting forces in the interlayer position. The contraction is due to electrostatic force of attraction between negatively charged clay mineral and positively charged ions and ion hydration causes the expansion. Fixation occurred when the force of attraction dominated the cations' hydration energy. Furthermore, Zagal (1989) pointed out that the mechanical effect induced by MW irradiation can stimulate the dispersion of inorganic colloids. This stimulation can increase the decomposition of non-biomass organic matter in soil and subsequently releases the fixed NH_4^+ .

Furthermore, Yang et al. (1990) tested the nutrient extractability effect of MW heating (0 – 240 s) on soil and then incubated for 0 to 12 days at 20°C . When fresh soil was exposed to MW energy a dramatic increase in the $\text{NH}_4^+\text{-N}$ concentration was observed for an extended treatment of 120 s. They concluded that this effect was partially from non-microbial processes, either from site exchange or from fixed position in inorganic collides (clay minerals). Additionally, decrease in $\text{NO}_3^-\text{-N}$ concentration of soil reflected the prevention of nitrification at high temperature. Alphei and Scheu (1993) evaluated the effect of various biocidal treatments on mull-structured soil (600 g) fauna, microbiota and nutritional dynamics over 120 days after heating. Of the tested treatment, MW (2.45 GHz, 700 W, 180 s) soil heating had a detrimental effect on soil fauna but did not significantly eliminate the soil microbial communities. They found increasing trends of $\text{NH}_4^+\text{-N}$ ($100 - 200 \mu\text{g g}^{-1}$ soil) with MW soil treatment compared to unheated soil ($\geq 100 \mu\text{g g}^{-1}$ soil); however, decreases in the $\text{NO}_3^-\text{-N}$ ($\geq 40 \mu\text{g g}^{-1}$ soil) content of the soil gave indication of no nitrification. Phosphorous content gradually increased over time.

The increase in soil C and N mineralization (Zagal, 1989) and $\text{NH}_4^+\text{-N}$ and sulphur oxidation was reported by Wainwright et al. (1980). In contrast; numerous studies documented that ionizing irradiation (γ -rays) treatment of soil effectively increased the

mineralization of $\text{NH}_4^+\text{-N}$ (Bowen and Cawse, 1964; Cawse and White, 1969). They proposed three possible pathways which may be responsible for the release of $\text{NH}_4^+\text{-N}$ from soil through irradiation: (1) Ammonia could be produced by the chemical action of ionizing radiation through a variety of biochemical processes from nitrogenous organic compounds, particularly deamination of amino acid (Maxwell et al., 1954) and proteins (Bennett and Garrison, 1959); (2) McLaren et al. (1957) pointed out that several enzymes were functional in irradiated soil including urease which is active during decomposition and produces ammonia; and (3) release of N from dead organisms due to subsequent cell lysis by irradiation (Eno and Popenoe, 1963).

2.4.4. Impact of Microwave Soil Heating on Soil Organic Matter

SOM is an aggregate of organic residues in soil at different degrees of humification (Stevenson, 1994). The various biopolymers are serially transferred to humus (fulvic acid, humic acid and humin) in soil through geological SOM development processes such as humification (Huang and Weber, 1997). Protein is the basic structural component of cell and cellular enzymes (Midigan et al., 2003). Approximately 5 – 25% of organic inputs are expected to accumulate in soil as proteins, peptides and free amino acids (Stevenson, 1982b). Amino acids typically incorporate about 10 – 20% of soil organic carbon and 30 – 40% of soil organic N (Stevenson, 1982b). Thermal denaturation of biopolymers induced by MW irradiation could increase the concentrations of free amino acids for succeeding turnover to the CO_2 and NH_4^+ pool. Moreover, Hur et al. (2013) tested the effect of MW soil treatment on SOM properties and its sorptive capacity for hydrophobic organic containments. A small amount of soil was treated in a lab-scale MW oven (700 W; 2.45 GHz) for 10 minutes in aerobic and anaerobic conditions with and without activated C (as a thermal catalyst). MW soil heating (aerobically with activated C) significantly facilitated the alteration of humification-like properties of SOM and its ability to bind the contaminants. They irradiated 5 g samples of soil in plastic tubes in aerobic and anaerobic conditions with activated C for 600 s in a MW oven. They pointed out that MW irradiation significantly alters the physical and chemical properties of SOM and increased its humification. In another study, Kim and Kim (2013) studied the influences of MW irradiation on the SOM properties. Kim treated a small amount of soil (5 g) with various levels of water content (0, 10, 20 and 30%), and reported a

maximum temperature of ≥ 140 °C at 30% water content. It was found that, MW soil heating considerably altered the molecular composition (C, H, O and N), chemical structure and humification-like properties of SOM. Relevant to this, the degradation of lignin's and hemicellulose constituents of the SOM starts at the temperature of 100 – 200 °C, and the complete disintegration of SOM does not start below 300 °C temperature (González-Pérez et al., 2004).

Independent of heating methodology, the following studies had reported the effect of heating on soil properties: Glass et al. (2008) studied the effect of high temperature, considering the severity of wild fires, on soil health. Ammonium was greatly increased with high temperature treatment and duration, and nitrate respond variably among various soil types but generally increased with high soil water content. It was concluded that, low-intensity fire ($100 \leq ^\circ\text{C} \leq 200$) can considerably alter the NH_4^+ and NO_3^- without any change in total N or C. Similarly, (Certini, 2005) explained the possible mechanisms and changes induced by fire on the soil quality. He reported that the transient changes in soil pH and available nutrients, after fire, depends on the severity and duration. Certainly, the extent of hydrophobicity associated with fire can reduce the soil water holding capacity and ultimately increase erosion. Although numerous studies reported an increase in SOM with fire and this would have enhanced the soil structure, which leads to an increase in water holding capacity. In contrast, Yi et al. (2016) reported that low-temperature remediation of oil-contaminated soil decreased the organic matter and total N while phosphorous, microbial count, enzyme activity (dehydrogenase) and water holding capacity increased. Based on these studies, soil heating has potential to alter the soil nutrient availability; therefore, the effect depends on the heating duration and intensity.

2.4.5. Response of Soil Microorganisms to Microwave Heating

Farming systems are expected to face new production and management techniques for sustainable food production. However, the influence of these new management practices on soil microbial composition and activity is unclear (Smith et al., 2016). For instance, the role of soil microbiota under increased environmental changes in protecting and regulating the key soil processes is poorly understood (Nemergut et al., 2014). The effect of MW heating on soil microbiota is still in question and has very limited literature. In this discipline, Vela et al. (1976) treated different types of soil to

check the instant effect of MW heating on soil microorganisms. Small soil samples (250 g) were subjected to 5, 10, 20 and 40 s of treatment in a 1 kW MW oven. This range of exposure achieved an overall temperature gradient of 80 °C. The results from all the soil types showed that only fungi colonies were significantly killed by MW irradiation. Vela found that soil nitrifying bacteria were highly resistant to MW energy applied at the rate 40,000 J cm⁻² to the soil surface. Bacteria could survive exposure of 480 s when in soil, and cannot bear a 20 s treatment in an isolated aqueous culture. However, their population reduced from 1.3×10^6 to 2.9×10^3 g⁻¹ soil. He collected soil from one of the pre-emergence weed management field experimental sites. The soil was irradiated with the Zapper III (60 kW; four independently controlled MW generators) with energy densities of 200, 400 and 800 J cm⁻² to check the microbiota damage and recovery. These tested energy densities were founded to be effective for killing weed population without any serious damage to soil microflora.

In a parallel study, Ferriss (1984) documented that the treatment time, soil moisture content and soil load collectively dictate the fate of soil microorganisms. In Ferriss' study, there was no pronounced effect of MW heating on the prokaryotes population, measured in colony-forming units (CFU), 1 day after soil treatment in a 625 W MW oven operating at 2.45 GHz. Additionally, Ferriss reported the relative susceptible ranking of soil biota to MW heating: nematodes > fungi > bacteria and thermophiles.

For soil steaming treatment, Bollen (1969) found a similar susceptible index of soil microflora to heating, independent of heating methodology. Ferriss conclusively stated that a small amount of soil (≤ 1 Kg) can easily be sterilized with MW heating; however, the complete sterilization of large soil volume is hard to achieve. The difference in temperature sensitivity of soil microflora (Bollen, 1969), suggest that fungi are more sensitive than bacteria (D'Ascoli et al., 2005). This triggers the competitive dynamics and reassembly scenario under extreme conditions. Transient heat (50 or 80 °C for 1 h) induced rearrangement of bacterial communities (Mendes et al., 2011), which ultimately favoured the rapid recovery of slow-growing thermophiles taxa (Voort et al., 2016). In the past, Wainwright et al. (1980) treated small amount of contrasting soil (20 g) in a 1 kW MW oven operating at 2.45 GHz for 10 – 30 s to check the instant effect of heating on soil microorganisms. This short-term heating progressively reduced the nitrification with an abrupt increase in soil NH₄⁺-N. Additionally, a 20-s exposure considerably

increased the sulphur oxidation (2 – 3-fold) compared to untreated control soil at day 28. They reported a rapid reduction in fungi, plated for the organic loam soil, from 30,000 g⁻¹ – 0 g⁻¹ with similar exposure time, without any profound detrimental effect on the heterotrophic bacteria measured at day 28 since MW soil treatment. Figure 2.7A and B depicting the survival of total and potentially active bacterial taxa at 120 s of MW, as observed by Nunes et al. (2018).

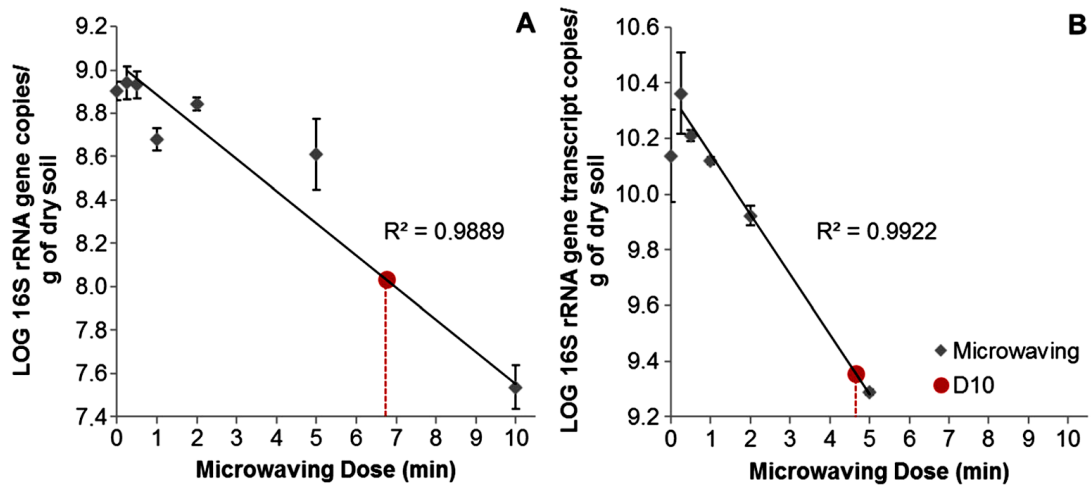


Figure 2.7: Influence of microwave soil treatment on the (A) total bacterial abundance and (B) potentially active bacterial abundance. Source: Nunes et al. (2018).

In the past, Lyon et al. (1978) heated small soil sample (25 g; organic loam) in a 1 kW MW oven (equivalent to 11 J cm⁻³ s⁻¹ dissipated in a 40 ml of water load) for a maximum of 30, 120 and 360 s. Fungal colonies were completely eradicated with 30 s exposure, although bacterial communities remained unaffected with this short-exposure. They found slight reductions in the bacterial colonies at 120 s and 360 s completely killed them all. For soil nematodes, O'Bannon and Good (1971) treated a root knot infected soil (90 g; 11% water content) in a 1.25 kW MW oven for 30 – 300 s and found that short exposure (≤ 30 s) severely hindered their activity. Similar reduction trends were found when larger soil loads (400 g) were treated for nematodes elimination.

To deal with field soil, Chen et al. (1995) checked the efficacy of MW soil heating on the total bacterial count and found that bacterial taxa were undetectable with soil heating of 6 minutes at 3.3% moisture content. The reduction pattern was 1.48×10^6 (with

2 minutes) to 0.021×10^6 (at 6 minutes) CFU g⁻¹ of soil. Therefore, the results of these previous studies give some insight into the interaction of MW energy with soil microorganisms; however, the limitations with these studies is the culture-dependent count of soil microbiota after soil heating and majority of soil bacteria are unculturable. Recently, Jurburg et al. (2017b) subjected 120 soil microcosms (50 g; 45% field capacity) to MW in an 800 W oven for 90 s, up to maximum temperature of 85.5 °C, to check the community shift after this transient heat disturbance over 50 days culture-independently. Jurburg found that the total potentially active bacteria, measured on the cDNA/DNA copies of 16S rRNA gene, reduced moderately following MW heating but reached pre-heating level at day 29, potentially revealed a succession after heating (stability phase).

In general, soil heating is detrimental to microorganisms (O'Brien et al., 2018); however, transient heat disturbance kills some species and favours others via various resistance mechanisms. In other domains, numerous studies reported the effect of MW heating on the disintegration of microbes in solid media, which were treated up to 7 – 9 min of MW energy (Woo et al., 2000; Zhou et al., 2010). MW induced disintegration of cells increases the amount of intracellular and extracellular components consequently increasing the soluble organic contents of soil. This is a direct effect of heating on the microbes in a less complex material; however, when MW energy is applied on a very complex substance like soil the disintegration scenario would have been changed. Temporary disturbance (i.e., heating in MW case) alters community composition by killing various vulnerable species (Lennon et al., 2012). Because of this, the newly available niches may favour the competitive dynamics like secondary succession (Jurburg et al., 2017a). In summary, there is a pronounced effect of MW heating on the soil microbiota. However, the rapid recovery of microbes had been reported by numerous studies after MW heating (Vela et al., 1976; Vela and Wu, 1979; Brodie et al., 2015b; Jurburg et al., 2017a; Jurburg et al., 2017b; O'Brien et al., 2018). Post-heating conditions stimulate the microbial community and it is mostly controlled by soil moisture and organic matter. Therefore, culture-independent research is needed to elucidate the effect of soil heating on the beneficial soil microbiota. Figure 2.8 shows the hypothetical model of response (recovery and resilience) of bacterial communities following soil disturbance (e.g. environmental changes, tillage, flood, drought and heat treatment etc.) with respect to time as described by Griffiths and Philippot (2013).

A part of this research has explored the effect of MW soil treatment on total abundance of ammonia oxidizing bacteria (AOB) and archaea (AOA) using quantitative polymerase chain reaction (qPCR). The ammonia monooxygenase α -subunit (*amoA* gene), carried by all known ammonia oxidisers, is involved in the first step of nitrification and used widely to study the community size of AOA and AOB. Ammonia oxidisers are responsible for the conversion of ammonia (NH_4^+) to nitrate (NO_3^- ; nitrification) via nitrite in terrestrial ecosystems (He et al., 2012; Banning et al., 2015). However, the relative contribution of AOA and AOB to soil nitrification is highly uncertain, AOA is found to be numerically higher than AOB but functionally less dominant (Banning et al., 2015). A comprehensive study is needed to understand the effect of MW heating on the soil beneficial microbiota particularly AOA and AOB and their role in soil nitrogen cycling. In addition, the bacterial community composition following MW soil heating would help in understanding the impact of MW soil heating intensities proposed for weed seedbank control. Overall, the responses of soil bacterial communities to MW soil heating would give an opportunity to calibrate the MW doses for seedbank control. Considering the spatial heterogeneity of soil, various heating intensities would be needed to tackle the herbicide-resistant weed in different cropping systems.

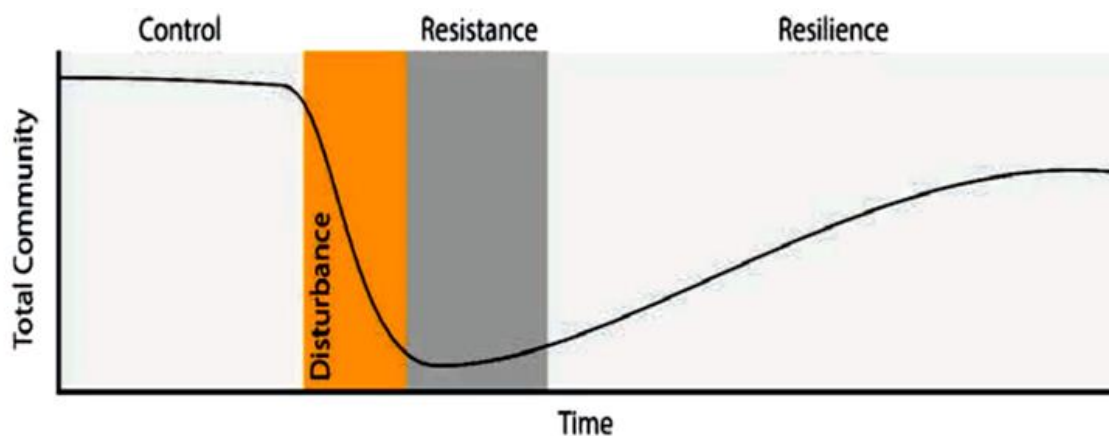


Figure 2.8: The hypothetical depiction of soil bacterial communities' responses to any disturbance regimes and their recovery trajectory according to time following disturbance. Source: Griffiths and Philippot (2013).

2.5. Summary

In the late 1990's, cases of herbicide-resistant weeds were reported in agriculture systems. Those rely heavily on the herbicides for weed control (for example, no-till farming systems). This led to a search of alternative methods for weed control to ensure the sustainability of crop production. In this discipline, thermal weed control methods provided a good opportunity for managing weeds as an alternative to herbicide applications. Among those thermal weed control methods, based on diverse literature, it can be concluded that pre- and post-emergence MW heating has the potential to minimize weed pressure. Despite this, MW technology did not achieve the commercialization scale. The heating intensities within a range of 75 – 80 °C can be employed to retard the germination capacity of the majority of the weed seedbank (Gay et al., 2010b; Brodie et al., 2018). In the case of MW soil treatment, this proposed temperature range (75 – 80 °C) can be achieved with 90 – 120 s of treatment as mentioned by Brodie (2008). The effectiveness of this temperature achieved through MW soil treatment under field conditions in different cropping system is still unexplored. The weed problem of different regions is quite different. Sub-tropical regions will have different weed species with different degrees of resistance than temperate regions, while temperature sensitivity of different weed species' seeds will not vary enormously regardless of seeds' moisture contents.

The question is; how can we implement a single technology for a diverse range of crop production systems? It can be postulated that if the germination capacity of the viable weed seedbank in top soil layer is reduced, it would indeed be helpful for sustainable weed management under field conditions. It is important to note that, soil heating will not only affect the weed seedbanks, but it will also influence the soil physical, chemical and biological properties as a whole. These assumptions are unclear and need rigorous research.

Therefore, the application of MW soil heating for weed control under field conditions is needed to understand the potential of this thermal weed management approach to tackle the herbicide-resistant weed emergence. Seedling emergence can be used to estimate the seedbank size and its viability (Bakker et al., 1996). This approach

has been employed in the following chapters to evaluate the effect of pre-sowing MW soil treatment on weed emergence under field conditions.

Chapter 3 – Microwave Soil Treatment Improves Weed Management in Dryland Wheat of Australia

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3.1. Abstract

Herbicide resistance has prompted the development of a chemical-free weed management practice in no-till farming systems. In this study, we examined the effect of pre-emergence microwave (MW) soil treatment for weed management in a no-till wheat production system in Australia. Once-off MW soil treatment (2.45 GHz, 600 W, 120 s,) was applied to corresponding plots, which were arranged in a randomized complete block design with five replicates. The MW energy was projected through a horn antenna with aperture dimensions of 5.5×11 cm into the top soil (0 – 6 cm) horizon. The applied MW energy density in the treated plot (2.6 m^2), which calculated by Simpson's numerical surface integral approximation, was approximately 560 J cm^{-2} . This achieved an increase in soil temperature of about $75^\circ\text{C} - 80^\circ\text{C}$. This temperature induced a 65% – 80% reduction in weed establishment through thermal devitalisation of the seedbank, compared to the untreated control plots. In addition, a substantial increase in wheat grain yield of 39.2% was achieved through MW energy application (7.8 t ha^{-1}), compared to non-MW treated conditions (5.6 t ha^{-1}). In summary, investment in this non-chemical weed management strategy promises to effectively control herbicide-resistant weeds and sustain dryland wheat yield.

Keywords. Soil, Microwave energy, Weed Suppression, Wheat

3.2. Introduction

Since the ancient era, wheat (*Triticum aestivum* L.) cultivation has performed a crucial role in sustaining human diets. In the Australian agricultural industry, wheat is an important grain crop with a total crop production area of 12.3 M ha and a production of approximately 23.7 M t annually (Statistics, 2016). The potential yield of the Australian wheat belt regions ranges from 4.5 to 11 t ha⁻¹ (Acuña et al., 2011); however, the present on-farm average yield is only about of 1.93 t ha⁻¹ (Christy et al., 2015). This yield gap can be minimized by optimizing on-farm management strategies (Yang et al., 2008), because, bridging yield gaps is crucial to meeting the global food demand (Chapagain and Good, 2015).

Numerous biotic and abiotic factors affect sustainable wheat productivity. Among them, weeds are one of the most important biological constraints in dryland wheat production regions of Australia. They significantly affect crop growth, development and final economic yield through resource competition. The weed survival mechanisms include seed production, distribution, soil seedbanks, seed dormancy and germination (Chauhan and Johnson, 2010). Among all these aspects, soil seedbank viability and longevity play a major role in weed establishment. Therefore, good weed management relies on the depletion of weed seedbanks in the top soil layer (Burnside et al., 1986).

In Australia, no-till farming relies on the use of chemicals for weed control, which has ultimately shifted weed flora to herbicides resistance. This is the greatest threat to sustainable productivity of agricultural commodities in industrialized countries. In time, globally, 471 unique cases of herbicide-resistant (species × site of action) have been documented in 86 crops with 250 weed species being resistant to 160 herbicides (Heap, 2016). Annually, nine new weed biotypes are reported as being herbicide-resistant (Heap, 2016). Considering this problem, various non-chemical weed control practices; for example, fire, flaming, grazing, soil fumigants, mechanical eradication, soil steaming and biocontrol agents have been used to control weeds (Gourd et al., 2002). However, all these methods of weed control have a limited spectrum of activity in crop production systems. Therefore, there is a present need for an alternative weed management strategy in existing cropping systems without sacrificing wheat grain yield and that is compatible with no-till production systems.

Microwaves (MW) are non-ionizing electromagnetic waves occupying a frequency range of $300 \text{ MHz} < f < 300 \text{ GHz}$ and the wavelength range of $1 \text{ mm} < \lambda < 1 \text{ m}$, respectively (Banik et al., 2003). MW absorption is primarily influenced by the dielectric and roughness properties of the target material (Ulaby et al., 1978). Soil is a complex three-dimensional heterogeneous substance. Therefore, in general, the absorption of MW energy projected into the soil can be affected by numerous unknown factors, consequently the electromagnetic impedance of soil is widely variable, even within relatively small spatial distributions. However, the soil gravimetric (θ_g) and volumetric (θ_v) moisture content (Nelson, 1996), bulk density (Hallikainen et al., 1985), organic matter content (O'Neill and Jackson, 1990), soil texture (Brodie et al., 2012b) and specific heat have historically been considered as key drivers of MW energy interactions with soil. The electric field of MW induces dipolar rotation of a polar molecules and internal physical resistance to molecular movement ultimately generates heat (Metaxas and Meredith, 1983). Accordingly, soil heating is the actual control mechanism in the case of a pre-emergence MW weed management program.

It has been reported that pre-emergence MW irradiation of soil may potentially minimize weed establishment (Nelson, 1996; Sartorato et al., 2006; Brodie et al., 2012a; Brodie et al., 2018). It can also destroy weed plants' reproductive parts and their seeds that are covered up by soil to a depth of several centimetres (Brodie et al., 2007). Additionally, Wayland et al. (1973) treated wheat and radish seeds *in situ* at 25 mm depth, with a soil moisture content of 6.5%. He found that MW was toxic to seeds with a threshold of 10 J cm^{-2} of energy density. Increases in energy density were more effective at reducing the germination percentage of seeds than increases in exposure time (s) for some species. Menges and Wayland (1974) compared post-emergence herbicides (methazol, propachlor and perfluidone) with MW energy densities between $45 - 720 \text{ J cm}^{-2}$ for weed suppression in an onion crop. They reported that MW (360 J cm^{-2}) irradiation significantly inhibited weed establishment. Additionally, minimum crop injury was noted in the case of MW (18%) compared to herbicide application (85%). Barker and Craker (1991) explored the effect of MW on oats and other weed seeds in soil at different moisture levels. They concluded that seed susceptibility to MW treatment is totally dependent on the soil temperature, and a soil temperature range between 75°C to 80°C significantly inhibited the germination of weed seeds.

Recently, the effect of MW soil treatment is again being explored for weed control and its effect on subsequent crop growth and development in Australia. All the previous experiments have yielded a profound impact of MW energy on weed establishment (Brodie et al., 2017; Khan et al., 2017a). However, the efficacy of this non-chemical weed control in a rain fed crop production system is still in debate. To narrow this knowledge gap, we hypothesised that the reduction of weed seedbank viability can minimize the weed pressure in modern agriculture systems. Therefore, the objective of this study was to evaluate the effect of pre-sowing MW soil treatment on weed establishment in a no-till rain fed wheat production system of Australia.

3.3. Materials and Methods

A field experiment was conducted between May and December 2016 at the Dookie Campus (36.395 °S, 145.703 °E) of the University of Melbourne, Victoria, Australia at an elevation of 189 m. The region has a temperate climate with an average annual rainfall of 575 mm and an average monthly temperature range of 9.4 °C to 20.9 °C (Australian Bureau of Meteorology). Soil at the experimental site is a sandy loam and classified as a Currawa Loam (Downes, 1949) or a Yellow Subnatic-Dystropic Sodosol (Isbell, 2016). Soil physico-chemical properties were measured by Nutrient Advantage Laboratory Services, Werribee, Victoria, Australia, before MW soil treatment. The soil properties (0 to 15 cm) were as follow: bulk density (1.6 gm cm^{-3}), Nitrate-Nitrogen 49 mg kg^{-1} , Phosphorus (Colwell) 31 mg kg^{-1} , pH (H_2O) 5.6, Electrical Conductivity (EC) 0.28 dS m^{-1} , and Cation Exchange Capacity $10.20 \text{ meq } 100 \text{ gm}^{-1}$. Historically, the paddock, which was used for our experiment, was used extensively to grow crops in a monocropping pattern with wheat-canola rotations.

An area of 75 m^2 was laid out in a randomized complete block design (RCBD) with five replications. The individual experimental plot size was $2.0 \times 1.3 \text{ m}$ and with a 0.5 m untreated buffer zone between each experimental plot. Additionally, a buffer zone of 10 m was maintained from all sides of the experimental plots to avoid any chemical drifting during the study period. The experiment consisted of two treatments: an untreated control ($\text{MW}_0 = 0 \text{ s}$) and a microwave treatment ($\text{MW}_1 = 120 \text{ s}$). The choice of 120 s for pre-emergence MW soil treatment for weed suppression was based on the profound effect from a previous field trial (Khan et al., 2017a). Regardless of the weed management

strategy, previous pot trials had recently reported good growth of crops, which were grown in a MW treated soil (120 s exposure; Khan et al., 2016).

A MW prototype was developed for soil irradiation (Figure 3.1). It was based on the magnetron of a commercial microwave oven (EMS8586V; Sanyo; Tokyo, Japan) operating at 600 W with a frequency of 2.45 GHz, which was assembled with a rectangular waveguide of 86×43 mm channelling the MW energy to an open-structured horn antenna with aperture dimensions of 110×55 mm. Because the prototype was free-standing, the antenna's aperture was always in direct contact with the soil surface when the MW system was operating; therefore, safety concerns about MW leakage were minimal. To ensure that safe operating conditions were maintained, MW leakage was measured periodically using a Tenmars TM-194 MW leakage detector, to ensure that the radiation was well below the maximum time average operator exposure to electric fields at 2.45 GHz is 137 V m^{-1} (Australian Radiation Protection and Nuclear Safety Agency 2002).

This experimental scenario was modelled using a Finite Difference Time Domain (FDTD) method to calculate the MW energy transfer from the antenna to the soil. As mentioned earlier, the electromagnetic impedance of soil varies widely due to moisture, texture, minerality etc.; therefore, impedance matching through antenna design is unviable. The choice of antenna design was based on earlier experimental work killing weed plants (Brodie et al., 2009) where it was revealed that some flare in the horn antenna helped project the energy onto the plants; however, a smaller aperture intensified the MW

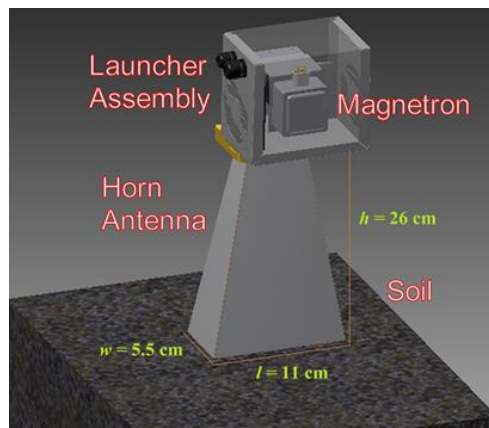


Figure 3.1: Schematic diagram of free-standing microwave prototype for soil irradiation used in this study under field conditions.

field intensity and therefore increased the efficacy for weed plant destruction (Brodie et al., 2012b). An electrical generator was used as the power source for the field experiment. Appendix 3.7 shows the simulation of MW energy transfer from the source to the target (soil in this case) via an open-structured horn antenna.

Under field condition, soil moisture in the top 1 – 2 cm is required to optimize MW effectiveness (Whatley et al., 1973). The soil was at 30% field capacity during MW irradiation for proper energy absorption. The MW treatment was performed using the free-standing MW prototype, which was connected to an electric generator. The horn antenna was oriented orthogonally to the soil surface and treated the soil for a defined period (120 s). The MW energy was applied to an area that corresponded with the horn antenna's aperture dimensions (11 cm × 5.5 cm), and the horn antenna was moved to an adjacent location on the soil surface that adjoin to the previously treated area (60.5 cm²) until the entire experimental plot (2.6 m²) was irradiated. Infrared thermal images, captured with an infrared camera (C2; FLIR Systems Inc.; Wilsonville, OR, USA) approximately 2 – 5 s after irradiation of the rectangular area (60.5 cm²) under the horn antenna, were post-processed in MATLAB to evaluate the temperature distribution. The experimental area remained undisturbed for 24 h after MW treatment, before planting the wheat crop, to ensure the soil returned to ambient thermal equilibrium conditions.

The Gregory wheat variety was sown using a small plot seeder, with row to row spacing of approximately 25 cm at a seeding rate of 60 kg ha⁻¹. Planting occurred in the last week of May 2016. All the agronomic practices, except herbicides application, were followed as recommended for the Goulburn Valley Wheat Belt region of Northern Victoria, Australia. Diammonia Phosphate (80 kg ha⁻¹) and potassium (60 kg ha⁻¹) were applied to the entire experimental area at the time of sowing. Three split doses of nitrogen, equivalent to a total rate of 120 kg ha⁻¹, using urea, were applied during the growing season. The number of tillers, fresh biomass and dry biomass were measured for a randomly selected 0.25 m² quadrats, drawn from each plot, for early growth assessment in response to MW soil heating and weights were converted to tonnes per hectare. At physiological maturity, the crop was manually harvested from whole plots for yield assessment. After harvesting, the crop biomass was dried in an air circulation oven (Nabertherm; TR1050 27124; Germany) for 24 h at 65 °C and final crop yield was converted into tonnes per hectare (t ha⁻¹). Weed density and biomass accumulation was

counted from whole experimental plot areas (2.6 m²) at maximum tillering stage and dry biomass, after drying in an oven (Nabertherm; TR1050 27124; Germany) for 24 h at 65 °C, of weeds were assessed at crop harvest. Harvest index was calculated by using equation (3.1);

$$\text{Harvest Index} = \frac{\text{Grain Yield}}{\text{Above ground biomass Yield}} \times 100 \quad (3.1)$$

The obtained data were subjected to normality assessment and a single factor Analysis of Variance (ANOVA) using MATLAB R2015b (The Mathworks Inc., Natick MA, USA) was applied to determine statistically significant means, and a least significant difference (LSD) test was used to compare the treatment means at a 5% probability level.

3.4. Results

Pre-emergence MW weed management relies on the temperature distribution into soil. The applied MW energy density in the treated plots, accounting for MW energy reflections from the soil in the absence of using any wave-guide tuning, was approximately 560 J cm⁻². This energy application induced an increase in the vertical temperature profile of soil (0 – 6 cm), which was approximately 75 ± 5 °C, evidenced through thermal images (Figure 3.2), and a thermometer. The highest temperature in the MW heated soil was along the centre line of the horn antenna and between 0 – 6 cm below the soil surface. The power dissipated below 6.5 cm of soil depth was minimal; Finite Difference Time Domain (FDTD) analysis has confirmed an energy distribution on the soil surface (Figure 3.2 and 3.3) that matched well with the thermal image. Similar power distributions in soil have been confirmed by Brodie et al. (2007) for killing seeds to a depth of about 6 cm into the soil. The same range of the soil temperatures has been extensively reported to debilitate the weed seedbank *in situ* (Davis et al., 1971; Gay et al., 2010b; Vidotto et al., 2013) for weed suppression.

The weed seedbank is a resting place of dormant seeds in the top soil horizon. Various biotic and abiotic factors have tremendous effect on seed viability. Among the many abiotic factors, however, soil temperature has an ability to debilitate weed seeds *in situ* (Ascard, 1994; Kolberg and Wiles, 2002; Melander and Jorgensen, 2005). Therefore,

it was hypothesized that the projection of non-ionizing energy into a top soil layer may cause thermal devitalisation of weed seeds. The results of this field study have strongly supported our hypothesis. The pre-emergence application of MW energy fed through a horn antenna into the soil significantly (ANOVA; $p < 0.01$) reduced weed density, weed fresh weight and dry weight in the wheat crop (Table 3.1) compare to untreated control plots.

The overall reduction in weed density, weed fresh weight and dry weight achieved through MW application in the dryland wheat crop were 62.9%, 89% and 90% respectively. In addition, the application of MW into soil for pre-emergence weed control significantly increased the dryland wheat productivity (Table 3.2) either through limited weed-crop competition or some effects on soil nutrient characteristics. Early crop weight gain is considered to be a crop-stability trait in dryland agricultural systems. The 50.9% gain in fresh weight and 42.4% gain in dry weight were acquired from the MW treated plots. Observation on the wheat growth and weed suppression in response to MW treated and untreated plots are shown in a Figure 3.4. Tiller density is the main contributor of final grain yield. The maximum number of tillers that developed in the MW treated plot (387 m^{-2}) was significantly higher compared to the untreated control plots (268 m^{-2} ; Table 3.2). Finally, the 7.8 t ha^{-1} grain yield, which was harvested from MW treated plots, compared favourably with the yield from the untreated control soil (5.6 t ha^{-1}).

3.5. Discussions

3.5.1. Effect of Microwave Soil Treatment on Weed Establishment

MW treatment caused soil temperature to rise quickly, which is the main benefit of MW heating. Heat transfer within the soil matrix leads to weed seed devitalisation. In this study, the experimental plots were highly invaded by wild oat (*Avena sativa* L.), wild radish (*Raphanus raphanistrum* L.), rye grass (*Lolium rigidum* L.), cape weed (*Arctotheca calendula* L.) and some unidentified weeds during the study period. We considered that weed emergence is a direct indication of weed seedbank size, because most weeds are reproduced through seeds. The survival percentage of ryegrass seeds, which is a problematic herbicide-resistant weed of grain production regions of Australia (Powles and Howat, 1990), under MW treatment, was tested by Brodie et al. (2009).

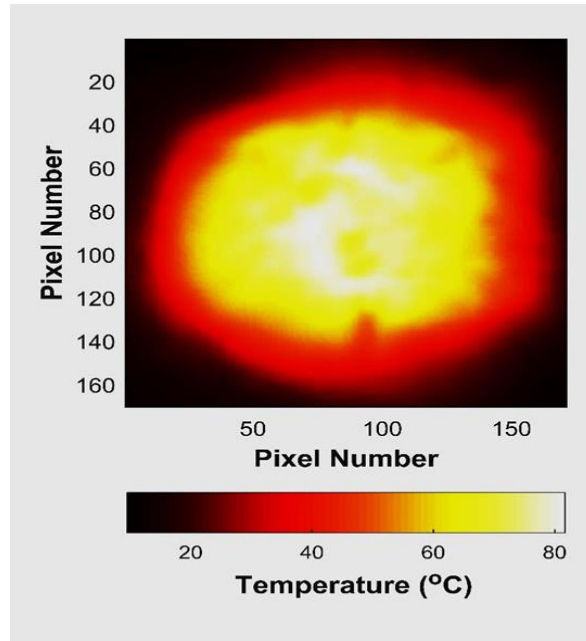


Figure 3.2: Thermal image captured with an infrared camera (C2; FLIR Systems Inc; Wilsonville, Oregon, USA) and post-processed in MATLAB[®] R2015b (The Mathworks Inc., Natick Massachusetts, USA), after 120 s of microwave irradiation of soil under the horn antenna (60.5 cm²), evidenced the average temperature increase of $75 \pm 5^\circ\text{C}$.

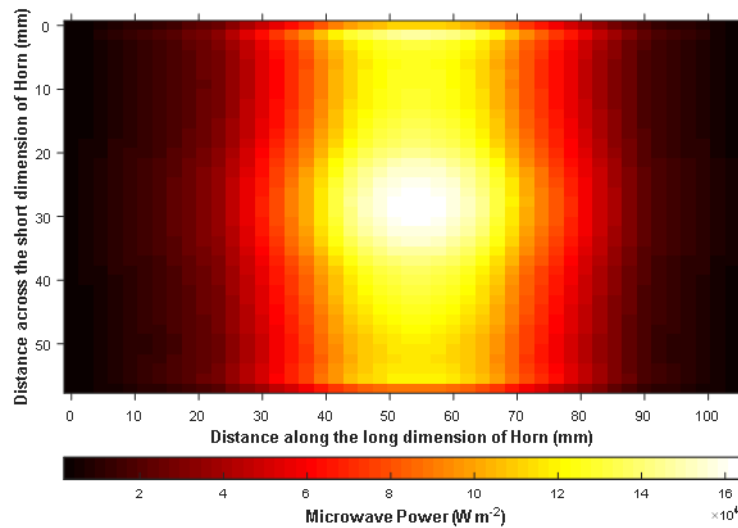


Figure 3.3: Power intensity in the top 0.5 cm of soil exposed to microwave radiation through a horn antenna (5.5×11 cm) for 120 s using a 600 W microwave sources. Note: Orientation of the Horn is such that the long dimension is parallel to the vertical axis.

Regardless of seed dormancy factors, Brodie reported that soil moisture has a direct influence on MW energy absorption for the prevention of ryegrass seed germination. Seeds were therefore completely killed at 80 °C – 90 °C of soil temperature. Similar reduction in weed establishment, including ryegrass, has found in the present study.

The relationship between weed dry weight accumulation at early crop growth stage and crop tiller development is given in Figure 3.5. Overall, energy of a MW system for a weed management program has a direct relationship with application duration. Therefore, for a pre-emergence weed control under field conditions, Wayland et al. (1973) reported an energy level of $80 < E_A \text{ (J cm}^{-2}\text{)} < 160$, which is quite low compared to the present study. In contrast, for a post-emergence weed control, Sartorato et al. (2006) tested the efficacy of MW energy on seedling of *Abutilon theophrasti* and *Panicum miliaceum*. They reported that an energy range of $101.5 < E_A \text{ (J cm}^{-2}\text{)} < 343.3$ gave significant reduction in dry weight (approximately 90%). However, it is highly unlikely that a certain set of MW energy may give a same control spectrum, because soil moisture (Nelson, 1996) and seed geometry (Davis et al., 1971; Brodie, 2008) have a considerable influence on MW absorption. These vary according to cropping system. Recently, in a direct seeded rice crop, MW energy of 560 J cm^{-2} for pre-emergence weed control was tested by Khan et al. (2017a) under field conditions. They reported that an application of MW energy into soil induced a 70% – 80% reduction in weed seedling emergence and establishment. In the past, less MW energy had been used to kill weed seeds; therefore, for example, Menges and Wayland (1974) compared the three effective herbicides (methazoal, propachlor and perfluidone) with MW ($45 - 720 \text{ J cm}^{-2}$) for weed control in an onion crop.

They found that MW radiation at 360 J cm^{-2} significantly reduced the number of viable weed seeds and less crop injury was observed in the case of MW energy (18%) compare to chemical application (85%). Interestingly, Wayland et al. (1975) studied an inhibitory effect of MW ($35 - 325 \text{ J cm}^{-2}$) on weed seedbanks in sandy loam soil under field conditions. They found a 91% reduction in seed germination of broadleaved weed species and 81% reduction in seed germination of grasses with an energy threshold of

Table 3.1: Effect of pre-sowing microwave (2.45 GHz; 600 W; 120 s; 560 J cm⁻²) irradiation of sandy loam moist soil on weed parameters in dryland wheat. Within a row and column, different letters indicate significant difference at 5% probability level.

Weed Parameters	Treatments		LSD (p=0.05)	Percentage Change
	Microwave Treated	Untreated Control		
Weed Density (plants plot ⁻¹) ^[i]	25.6 a	71.8 b	20.8	-62.9%
Weed Fresh Weight (t ha ⁻¹) ^[ii]	1.7 a	15.5 b	8.5	-89.0%
Weed Dry Biomass (t ha ⁻¹) ^[ii]	0.2 a	2.0 b	1.3	-90.0%
Weed Dry Biomass (t ha ⁻¹) ^[iii]	0.7 a	2.3 b	1.2	-69.5%

^[i] Data collection at early crop growth establishment from whole plot (2.6 m²).

^[ii] Data collection at maximum tillering stage (60 days after sowing; one quadrat (0.25 m²) from each plot).

^[iii] Data collection at crop harvesting (160 days after sowing) from whole plot (2.6 m²).

Table 3.2: Effect of pre-sowing microwave (2.45 GHz; 600 W; 120 s; 560 J cm⁻²) irradiation of sandy loam moist soil for weed management on yield components of the dryland wheat. Note: Within a row and column, different letters indicate significant difference at 5% probability level.

Wheat Parameters	Treatments		LSD (p=0.05)	Percentage Change
	Microwave Treated	Untreated Control		
Number of Tillers (m ⁻²) ^[i]	387 a	268 b	62.0	44.4%
Fresh Biomass Weight (t ha ⁻¹) ^[i]	30.8 a	20.4 a	10.4	50.9%
Dry Biomass Weight (t ha ⁻¹) ^[i]	4.7 a	3.3 b	1.5	42.4%
Dry Biomass Weight (t ha ⁻¹) ^[ii]	19.7 a	14.8 b	4.8	33.1%
Grain Yield (t ha ⁻¹) ^[ii]	7.8 a	5.6 b	2.3	39.2%
1000 Grain Weight (g) ^[ii]	45.9 a	42.8 b	3.2	7.2%
Harvest Index	39.9 a	37.6 a	6.1	6.1%

^[i] Data collection at maximum tillers stage (60 days after sowing).

^[ii] Data collection at crop harvesting (160 days after sowing).

183 J cm⁻². Similar reduction patterns in weed establishment were detected in the present field study (Table 3.1).

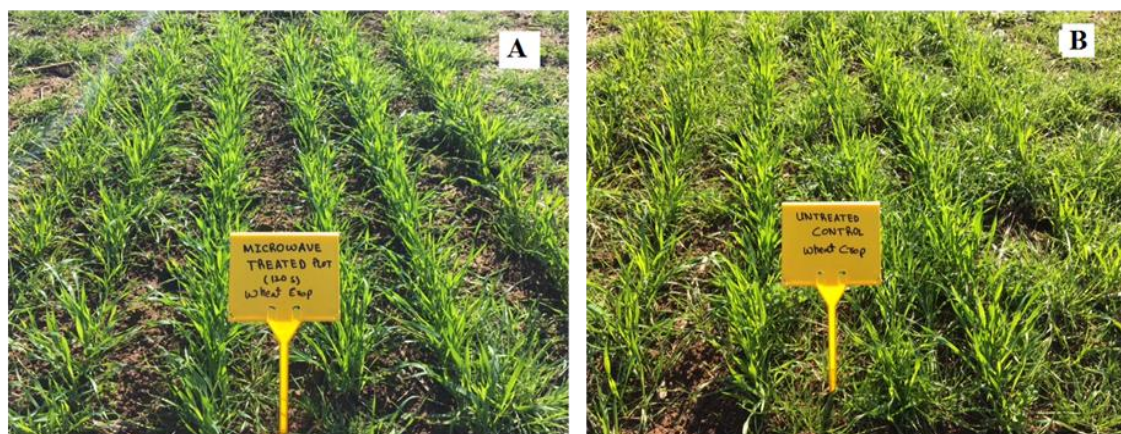


Figure 3.4: Visual comparison of weeds emergence in a microwave treated plot (A) and an untreated control plot (B). The dryland wheat crop rows (25 cm apart) were clean of weeds (MW treated plots) and highly invaded by weeds (untreated control plot).

Independent of soil heating methodology, various studies also reported the profound effect of high temperature for weed management. In this view, Gay et al. (2010b) reported on a soil steaming experiment with various duration (0, 6, 8 and 10 min) in a soil, to depths of about 1.5 – 16.5 cm, giving a temperature gradient of 100 °C to 37 °C (decreasing with depth), for weed control in a lettuce crop. They found an average weed density of less than 50 plants m⁻² in the case of soil steam treated plots compared to un-steamed control plots (400 plants m⁻²). In a separate discipline, Vidotto et al. (2013) found that exposure of a soil-seed mixture to high temperature gradually decreased seed germination. Almost all the tested weed species seeds were completely devitalized through soil thermal treatment at a temperature between 70 °C – 80 °C. The same temperature distribution was achieved through MW application in the present study, which might have a degrading effect on the weed seedbank and ultimately led to a significant weed reduction. Therefore, based on previous findings and the results of this study, it may be possible to minimize the weed pressure through MW irradiation of soil in no-till wheat production systems of Australia. However, a further research effort is

needed to understand the long-term effect of MW soil irradiation for weed control in crops.

3.5.2. Influence of Microwave Soil Irradiation on Wheat Productivity

Wheat production is strongly influenced by weed control methods. In this study, we examined the effect of MW soil treatment for weed management. In addition to weed suppression, MW soil treatment significantly increased the growth and development of the wheat crop. However, the reason for this good growth is still in question. Numerous previous studies have reported concurrent effect of MW energy on soil nutrient dynamics: Yang et al. (1990) tested the nutrient extractability effect of MW on soil. When fresh soil was exposed to MW energy, a dramatic increase in the $\text{NH}_4^+\text{-N}$ concentration was observed for MW treatments lasting 120 s. They concluded that this effect was partially from non-microbial processes, either from site exchange or from fixed position in inorganic clay minerals. Relevant to this, Hur et al. (2013) demonstrated that MW irradiation of soil can enhance the binding efficiency of hydrophobic organic compounds with soil organic matter.

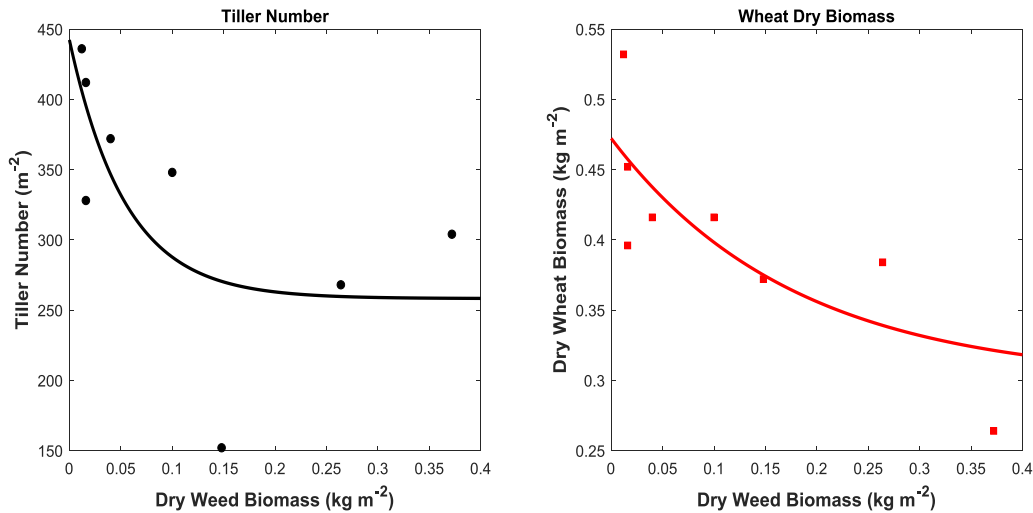


Figure 3.5: Relationship between the number of wheat tillers and dry weed biomass (left) at early crop growth stage and wheat dry biomass production as a function of dry weed biomass (right).

They irradiated 5 g samples of soil in plastic tubes in aerobic and anaerobic conditions with activated C for 600 s in a lab-scale MW oven (2.45 GHz) operated at 700W. They reported that MW irradiation significantly alters the physical and chemical properties of soil organic matter and increased its humification.

In another study, Kim and Kim (2013) studied the influences of MW irradiation on the soil organic matter properties. They reported that thermal cracking induced by irradiation potentially alters the molecular composition (C, H, O and N), chemical structure and humification of soil organic matter. Based on these previous findings, we assumed that thermal denaturation of recalcitrant humic substance induced by MW irradiation may increase the concentrations of free amino acids that are then converted to CO₂ and ammonia (NH₄⁺), which could have substantially increased wheat productivity in the present study. Therefore, in addition to weed control, future research should also elucidate the effect of MW soil treatment on soil nutrient dynamics and associated microbial activity. Less weed-crop competition for vital resources may be the possible cause of good crop growth in the MW scenario, because weeds have a direct impact on wheat production (Meulen and Chauhan, 2017). Relevant to crop weight gain, the current findings are favourably supported by Gibson et al. (1988). They reported that MW soil treatment markedly enhanced the shoot weight of birch (*Betula pendula* L.) seedlings; the maximum shoot weight (84 mg) was attained through exposure to MW (600 W; 120 s) compared to the untreated control soil (25 mg).

In the present study, the final incremental increase of 33.1% in dry biomass production and 39.2% in grain yield was attained through MW energy application into the soil for weed management. This is strongly supported by (Khan et al., 2017a); who examined the effect of pre-sowing MW application on weed establishment in a direct seeded rice system and found a 34% increase in a grain yield of the rice crop. Furthermore, Brodie et al. (2015a) also reported that an application of MW energy (560 J cm⁻²) into soil significantly increased wheat and canola growth compare to non-MW treated conditions. However, these results suggest that an increase in soil temperature through non-ionizing energy might have influenced the soil nutrient content, which could be the possible reason for the higher yield in MW treated plots.

But, this assumption still needs to be clarified. To determine the most likely reason for the increased crop growth in MW treated soil, Khan et al. (2016) conducted an interactive study of MW and nitrogen; which is an important yield limiting nutrient, on yield characteristics of wheat. They reported that regardless of nitrogen application, MW soil irradiation significantly (ANOVA; $p < 0.001$) increased the dry biomass (175%) and grain yield (92%) of wheat compared to untreated control soil. Substantial wheat grain yield response to various weed control methods under field conditions is given in Figure 3.6. Grain weight is an important quality related parameter. In the present study, we found that MW energy application into soil sustainably increased the 1000-grain weight approximately 7.2% compared to the untreated control. However, harvest index was not statistically significantly altered by the MW soil treatment, which clearly suggests that the changes in biomass production and grain yield are associated with good growth and healthy grains.

3.5.3. Economic Impact of Microwave Soil Irradiation on Profitability

In Australian agricultural industries, the total estimated direct cost of weed management and loss in crop productivity, due to weeds, was estimated to be about AU\$4 billion annually (DAFF, 2006). In term of present cost, the combined direct and indirect cost of chemical weed management may be approximately AU\$ 6.2 billion annually, which is between AU\$ 150 – 200 ha⁻¹ across the cropping regions of the country. Therefore, based on a preliminary assessment done by Brodie et al., (unpublished) the inter-row application of MW energy through a horn antenna for weed management, which is approximately AU\$ 64.80 ha⁻¹, is less or at least comparable to chemical weed control methods. In contrast, the MW soil treatment for weed management, as reported in this field experiment, had a direct cost of approximately AU\$ 400 – 500 ha⁻¹. However, in the present study, the 39.2% increase in grain harvest in response to MW soil treatment gave a profit of approximately ≈AU\$ 550 ha⁻¹.

Additionally, considering the secondary benefits of MW soil treatment on soil nutrient availability (Kim and Kim, 2013) and its long-lasting effect of up to three cropping seasons (Khan et al., 2017b) with same yield potential, this could overcome the application cost and be regarded as a soil steaming and fumigation treatment under field conditions. Furthermore, the fuel cost associated with a pre-sowing MW weed

management has been previously estimated by Khan et al. (2017a), to be approximately 0.98 L diesel m⁻². Samtani et al. (2012) calculated the fuel cost for pre-sowing steam treatment for weed control and reported a diesel consumption of between 0.81 – 2.16 L m⁻². The fuel consumption, which the MW system used in the present investigation for soil heating, was comparable to or even better than the soil steaming used by Gay et al. (2010b) and Samtani et al. (2012). The comparison of various weed control methods on wheat grain yield (Figure 3.6), 1.5 t ha⁻¹ additional grain yield obtained through MW soil heating, was significantly different from pre-sowing herbicide application for weed management. This enhanced yield trend provided evidence of change in the soil nutrients profile through MW energy application and thus it impacted crop productivity. Therefore, in general, dry-land wheat farmers could have secured an additional ≈AU\$ 370 ha⁻¹ through MW based weed management.

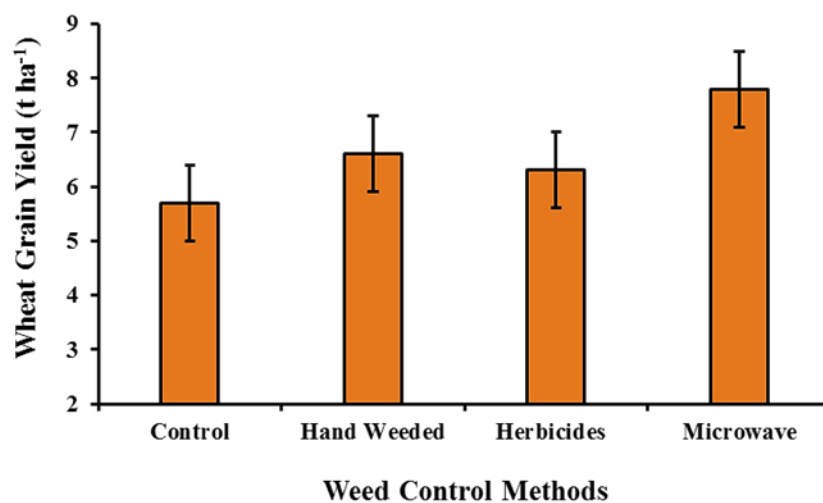


Figure 3.6: Comparison the impact of various weed control methods on the grain yield (t ha⁻¹) of wheat under field conditions. Grain yield data were obtained from Dookie Farm on the Dookie Campus, The University of Melbourne, where pre-emergence glyphosate (N-(phosphonomethylglycine) application is a common weed control practice, for comparison with microwave soil treatment. Note: Error bars represent the least significant difference (LSD) at 5% probability level.

3.6. Conclusions and Future Work

Based on the results of this field experiment, we conclude that pre-sowing MW irradiation of soil markedly decreased weed establishment in a dryland wheat cropping system and increased grain yield. However, two things remain unclear: whether a semi-commercial MW system could be employed to treat soil; and whether MW soil treatment could be implemented in an entirely different cropping system. Weed management in direct seeded rice system needs a novel approach to tackle the herbicide resistance problem. Rice is an important cereal, which grown in an anaerobic environment; therefore, it is important to explore whether MW soil treatment can be employed in this production system.

3.7. Appendix: Simulating Power Density from a Horn Antenna for Soil Irradiation

Microwave heating is directly governed by the dielectric properties of the soil and electrical field strength (E_o) of the MW source (Brodie, 2008), hence it is important to understand the distribution and intensity of the MW field on the soil surface. The direct measurement of MW electrical field intensity near the applicator is unsafe; therefore, it is crucial to estimate the field energy density using electromagnetic theory. The electric field in the rectangular waveguide has a sinusoidal distribution across the width of the waveguide. The maximum electric field strength (E_o) in a rectangular waveguide had been explained by Cronin (1995), and is given in equation 3.2:

$$E_o = \sqrt{\frac{8\pi f \mu_o P}{ab \sqrt{\left(\frac{2\pi f}{c}\right)^2 - \left(\frac{\pi}{a}\right)^2}}} \quad (3.2)$$

The MW's electric field distribution across the width of rectangular horn antenna, as calculated from the centre line of the waveguide, is given by equation 3.3:

$$E = E_o \cos\left(\frac{\pi}{a}x'\right) \quad (3.3)$$

There is some field expansion between the feeding waveguide and the antenna's aperture; therefore, the electric field in the antenna's aperture is:

$$E_a = E_o \cos\left(\frac{\pi}{A}x'\right) \cdot \frac{a}{A} \quad (3.4)$$

The determination of MW field intensity in front of the antenna, which has been applied to the soil, is crucial to understand. Based on the design of a horn antenna (Figure 3.7), this problem can be explained by Brodie and Hollins (2015):

$$E_p = \frac{E_a}{4\pi} \int_{-B/2}^{B/2} \int_{-A/2}^{A/2} \cos\left(\frac{\pi}{A}x'\right) \frac{e^{-j\beta_o\left(\sqrt{(x-x')^2+(y-y')^2+z^2} + \sqrt{R_o^2+(x')^2} + \sqrt{R_o^2+(y')^2}\right)}}{\sqrt{(x-x')^2+(y-y')^2+z^2}} \cdot dx' \cdot dy' \quad (3.5)$$

Where, E_a is electric field strength in horn antenna aperture (Vm^{-1}), E_p is electric field strength at a point in front of the antenna (Vm^{-1}), μ_o is the permeability of free space, P is the average MW power (W), a is the width of the wave guide (m), b is the height of the waveguide (m) and c is the speed of light (m s^{-1}), x' is the horizontal distance in the aperture plane from the centre line of the waveguide or aperture (m), A is the width of horn antenna aperture (m), B is the length of horn antenna aperture (m), β_o is wave number of MW field (m^{-1}), j is complex operator $\sqrt{-1}$, R_o is geometric length of horn antenna's flare pyramid (m), x , y , z are Cartesian coordinators of centre point of horn antenna aperture, x' is the horizontal distance from centre line of waveguide in aperture plane (m), y' is the vertical distance from centre line of waveguide in aperture plane (m).

Several options exist for solving equation (3.5). These include: explicit solutions of a simplified integral equation using Fresnel approximations; numerical integration of equation (3.5); and finite difference time domain (FDTD) analyses, which was originally developed by Yee (1966). The MW fields were simulated using the FDTD technique. A model of the horn antenna and soil was implemented in XFDTD (Remcom Incorporated version 7.5.1). Field intensities and power dissipated in the soil were post-processed using MATLAB (The Mathworks Inc., Natick, MA, USA)

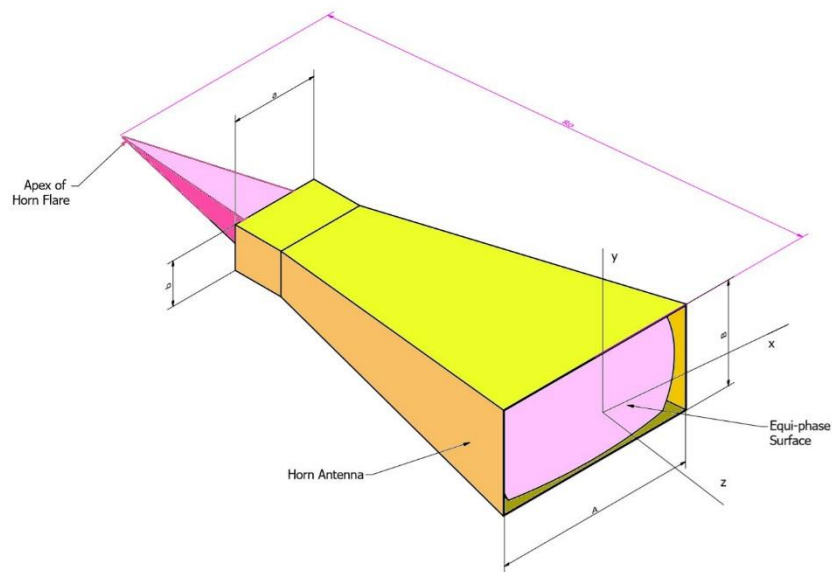


Figure 3.7. Geometry of a horn antenna showing an equi-phase wave front in the flare opening of the antenna. The open-structure horn antenna with a waveguide feed inner dimension of $8.6 \times 4.3\text{cm}$ ($a \times b$) and an aperture inner dimension of $11 \times 5.5\text{cm}$ ($A \times B$) was used in this study for microwave irradiation of the soil.

Chapter 4 – The Effect of Microwave Soil Treatment on Rice Production Under Field Conditions

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4.1. Abstract

Interest in chemical free weed management practices has increased to overcome the herbicide resistance in sustainable crop production systems. Here we examined the microwave as an alternative weed management strategy in direct seeded rice crop under field conditions. Pre-emergence microwave (2.45 GHz, 600 W, 120 s) irradiation of soil was assigned in randomized complete block design with five replications. The projection of 560 J cm^{-2} of microwave energy in to soil gave temperature gradient of $80 - 90^\circ\text{C}$ in top 5 – 6 cm of soil, which induced a 70 – 80% reduction in weed establishment through seed bank compared to untreated control and therefore, a 34% increase in the grain yield of rice (9.0 t ha^{-1}) was achieved, compared to the non-microwave scenario (6.7 t ha^{-1}). Microwave based weed control could be effective to manage the herbicide resistant weed biotypes in cropping system.

Keywords: *Microwave, Soil, Pre-emergence Weed Management, Rice,*

4.2. Introduction

Rice (*Oryza sativa* L.) is the staple food of 60% of the world's population (Mahajan et al., 2014), performs a significant role in the socio-economic constancy of the world and is grown in a vast range of agro-ecological conditions. In Australia, rice farming is done in Murray-Darling Basin, on an area of 70,000 ha, with an annual grain production capacity of 0.69 m t (Statistics, 2016). Direct seeding is a common sowing strategy of rice in Australia due to high labour costs and water shortages in direct seeded

rice systems. Weeds are one of the major biological constraints to increasing rice yield. Oerke (2006) estimated that globally 34% crop productivity losses are due to weeds. However, the dramatic decline in the production of rice due to weeds all over the world is 10.2% (Gianessi, 2013). The probability of yield loss in a direct seeded rice crop is high compared to transplanted rice (Rao et al., 2007).

Chemical and mechanical weed control methods are most commonly used in existing cropping systems (Batlla and Benech-Arnold, 2007). Agronomists generally use fire, flaming, grazing, soil fumigants, mechanical eradication, and biocontrol agents in various cropping system to control weeds below the economic threshold in the absences of weedicides (Gourd et al., 2002). In Australia, no-till farming practice has increased the use of chemicals for weed management, which has ultimately shifted the weed flora to herbicides resistance. Globally, 51 weed species are resistant to various chemical herbicides in rice crops (Heap, 2016).

Productivity losses of rice crops generally depend on climatic conditions, weed types, weed population density, rice variety, sowing methods, and weed management practices. The troublesome weeds of the Australian rice growing belt are barnyard grass (*Echinochloa crus-galli* L.), dirty dora (*Cyperus difformis* L.), arrowhead (*Sagittaria montevidensis* L.) and starfruit (*Damasonium minus* L.) (Pratley et al., 2004). Among all the weeds, Barnyard grass (*Echinochloa crus-galli* L.) is the major problematic bio-agent of rice (Norsworthy et al., 2007), and is also considered to be the main weed of several semi-aquatic cropping systems (Holm, 1997). It follows the C₄ photosynthetic pathway (Rao et al., 2007), and has indistinguishable morphology to rice at seedling stage which makes it extremely competitive with the rice crop. A 57% reduction in rice yield was documented with the population density of 9 plants m⁻² of barnyard grass (Maun and Barrett, 1986). Additionally, higher densities of barnyard grass can remove up to 80% of the soil nitrogen, especially at vegetative stages (Holm, 1997). Seed production is the key element of long-standing weed population dynamics (Davis and Liebman, 2003). The average seed production capacity of barnyard grass ranged from 20,000 – 73,000 seeds per plant (Bagavathiannan et al., 2011), and 60% of produced seed could become part of the weed seedbank. Therefore, effective weed management depends on reducing the soil weed seed bank (Burnside et al., 1986).

Microwaves (MW) are non-ionizing electromagnetic waves with a frequency range of $300 \text{ MHz} < f < 300 \text{ GHz}$ and the wavelength range of $1 \text{ m} < \lambda < 1 \text{ mm}$ (Banik et al., 2003). Interest in the interaction of MW energy with biological system dates back to the twentieth century (Ark and Parry, 1940). In 1924, *Antonin Gosset* was the first person who explored the bio-stimulation effect of MW energy, when he, along with his co-worker, used high frequency electromagnetic waves to treat tumours in plants with no injury to the plant (Bren, 1996). MW absorption is primarily influenced by the dielectric and roughness properties of the load (Ulaby et al., 1978). The absorption of MW energy projected into the soil can be affected by numerous unknown factors (Nelson, 1996). The propagation of MW energy through soil depends on the gravimetric (θ_g) and volumetric (θ_v) moisture content (Nelson, 1996), bulk density (Hallikainen et al., 1985), organic matter content (O'Neill and Jackson, 1990), soil texture (Brodie et al., 2012b) and specific heat of soil.

It has been reported that pre-emergence MW irradiation of soil may potentially minimize weed establishment (Davis et al., 1971; Wayland et al., 1973; Brodie et al., 2009; Hess et al., 2018). It can also destroy the weed reproductive plant parts and their seeds that are covered up by soil at a depth of several centimetres (Diprose et al., 1984; Brodie et al., 2007). In this view, Wayland et al. (1973) treated wheat and radish seeds *in situ* at 25 mm depth and moisture content of soil was 6.5%. He found that MW was toxic to seeds with a threshold of 10 J cm^{-2} of energy density. Increases in energy density were more effective at reducing the germination percentage of seeds than increases in exposure time (s) for some species. Davis et al. (1971) conducted an experiment to evaluate the effect of microwave on the seedling survival percentage of twelve crop and weed species. They described that the 48 h germinated seedlings showed no survival after a short exposure of microwave energy and concluded that susceptibility of young seedlings to microwave heating was highly correlated with moisture content and absorption of energy. In a different discipline, Menges and Wayland (1974) compared post-emergence herbicides (methazal, propachlor and perfluidone) with MW at energy density of $45 - 720 \text{ J cm}^{-2}$ for weed suppression in an onion crop. They reported that MW (360 J cm^{-2}) irradiation significantly inhibited weeds establishment. Additionally, minimum crop injury was noted in the case of MW (18%) compared to herbicides application (85%). Additionally, Barker and Craker (1991) explored the effect of MW on oats and weed

seeds in soil at different moisture levels. They concluded that seed susceptibility to MW treatment is totally dependent on the soil temperature, and soil temperature range between 75 – 80 °C significantly inhibited the germination of weed seeds.

Furthermore, Bigu-Del-Blanco et al. (1977) treated two days-old seedlings of maize with microwave energy at a frequency of 9 GHz with power density of 10 – 30 m W cm⁻² for time periods of 22 – 24 h. The authors revealed that more exposure time of microwaves even at very low energy densities significantly dehydrated the maize plants and retarded their growth. In contrast, recent research on fleabane and paddy melon has concluded that a short exposure (≤ 5 s) of high intensity MW (2 kW) heating was enough to dehydrate plant (Brodie et al., 2012b). Therefore, the application of microwave in agricultural systems has potential to substitute the hazardous, toxic and environmentally-unsafe chemicals used for weeds management (Wayland et al., 1975; Sartorato et al., 2006; Brodie et al., 2012b). Therefore, the objective of present study was to evaluate the effect of pre-emergence microwave irradiation of soil for subsequent weed management, germinated through seed bank, in direct-seeded rice crop under field conditions.

4.3. Materials and Methods

A field experiment was conducted between October 2015 and April 2016 at Dookie Campus (36.395 °S, 145.703 °E), The University of Melbourne, Victoria, Australia. The climate of this region is temperate with annual rainfall of 575 mm. Soil type of the experimental field area was classified as Red Chromosol (Downes, 1949; Isbell, 2016). Historically, the field is a grazing paddock with predominantly grass species. An area of 73.5 m² was excavated and manually levelled into a turkey-nest pond so the area could be flood irrigated to grow rice. The individual experimental plot sizes were 2.0 x 2.0 m, which were arranged in a randomized complete block design with five replications, and with a 0.5 m untreated buffer zone from all edges of the experimental plot. The experiment consists of two treatments: an untreated control (MW₀ = 0 s) and microwave treated (MW₁ = 120 s).

A MW prototype was developed for soil irradiation (see section 3.3; Figure 3.1). It was based on the magnetron of a commercial MW oven (EMS8586V; Sanyo; Tokyo, Japan) operating at 600 W with a frequency of 2.45 GHz, which was assembled with a

rectangular waveguide of 86×43 mm channelling the MW to a pyramidal horn antenna with aperture dimensions of 110×55 mm, which was connected to the electric generator for power source in field conditions. MW energy simulated according to Section 3.7 of Chapter 3. The applied MW energy density in the treated plots, accounting for MW energy reflections from the soil in the absence of using any wave-guide tuning, was approximately 560 J cm^{-2} .

In field condition, soil moisture in the top 1 – 2 cm is required to optimize MW effectiveness (Whatley et al., 1973). The dielectric constants of clay loam soils are directly proportional to volumetric moisture content at various MW frequencies (Hallikainen et al., 1985). The soil was lightly irrigated prior to MW treatment for proper attenuation. The MW treatment was performed by free-standing MW prototype, which was connected to electric generator, orthogonally to soil surface and treating the soil for a defined time of 120 s. Infrared thermal images, captured with an infrared camera (C2; FLIR Systems Inc; Wilsonville, OR, USA) immediately after irradiation of the rectangular area (60.5 cm^2) under the horn antenna, were used to evaluate the treatment. The vertical temperature profile in top 5 – 6 cm of soil was approximately $75 - 80^\circ \text{C}$, evidenced through thermal images (Figure 4.1), and a thermometer. Additionally, the energy dissipated into soil through horn antenna was simulated using FT-DT techniques is given in Figure 4.2 and 4.3. The experimental area remained undisturbed for 24 h after MW treatment. The experimental plot was then flooded to a depth of 5 cm and the pre-germinated seeds of the rice variety OPUS was broadcasted in bays at a seeding rate of 125 kg ha^{-1} .

Phosphorus (80 kg ha^{-1}), potassium (60 kg ha^{-1}) and zinc (4 kg ha^{-1}) were applied to the entire experimental area at the time of sowing. Three split doses of nitrogen, using urea, equivalent to a total rate of 120 kg ha^{-1} , were applied during the growing season. Netting was fixed over the experimental bay to reduce bird scavenging during the early stages of crop establishment. Irrigation for ‘OPUS’ rice variety was scheduled according to Ricegrower Association of Australia, Yanco, NSW. The leaf area index and chlorophyll content of the fully expanded flag leaf were measured using a Ceptometer (LP-80; Decagon Devices, Inc. Pullman; WA) and SPAD-502 ((Soil-Plant Analysis Development); Spectrum Technologies, Inc. Aurora, Illinois, USA)), respectively. The number of tillers, fresh biomass and dry biomass were measured for a randomly selected

0.25 m² quadrat drawn from each of plots. Biomass was dried in an air circulation oven (Nabertherm; TR1050 27124; Germany) for 24 h at 65 °C and final crop yield was converted into tone per hectare bases. Weed population and biomass accumulation was counted from whole experimental plot areas (4 m²). The obtained data were subjected to normality single factor Analysis of Variance (ANOVA) using MATLAB R2015b (The Mathworks Inc., Natick MA, USA) to determine statistically significant means, and a least significant difference (LSD) test was used to compare the treatment means at 5% probability level.

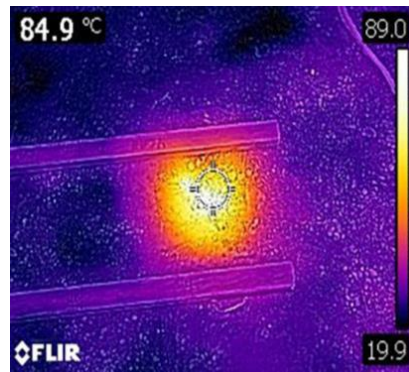


Figure 4.1: Thermal image captured through infrared camera (C2; FLIR Systems Inc; Wilsonville, Oregon, USA) after microwave irradiation of soil under the horn antenna (60.5 cm²), evidenced the temperature increase of 80 – 90 °C ± 5.

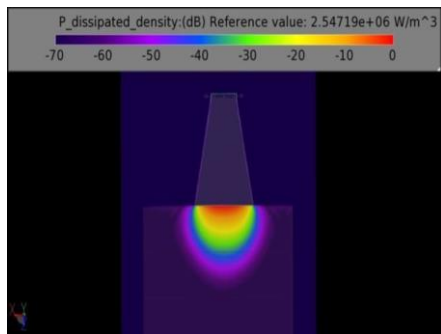


Figure 4.2: Microwave energy dissipated from pyramidal horn antenna for soil irradiation based on FT-DT simulation.

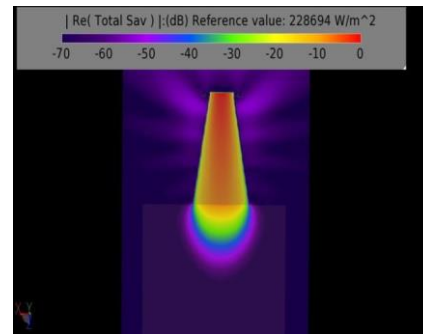


Figure 4.3: Microwave energy density from pyramidal horn antenna for soil irradiation based on FT-DT simulation.

4.4. Results and Discussion

4.4.1. Inhibitory Effect of Microwave on Weeds Establishment

The predominant weed species found at the experimental site were bermuda grass (*Cynodon dactylon* L.), barnyard grass (*Echinochloa crus-galli* L.) and dirty dora (*Cyperus difformis* L.). The MW (2.45 GHz, 600 W, 120 s) treatment of soil significantly (ANOVA; $P < 0.01$; Table 4.1) reduced the weed density ($17.6 \text{ plants plot}^{-1}$), weed fresh biomass ($156.4 \text{ g plot}^{-1}$) and weed dry biomass accumulation ($21.6 \text{ gm plot}^{-1}$) compared to the untreated control; $94.8 \text{ plants plot}^{-1}$, $612.8 \text{ g plot}^{-1}$ and $122.6 \text{ g plot}^{-1}$, respectively. The pre-emergence microwave irradiation of soil induced a 70 – 80% reduction in weed establishment. The majority of weed species are reproduced from seeds and vegetative parts, which are buried in the soil profile. Increase in the temperature gradient in the top layer of soil could reduce the viability of indigenous weed seeds.

In relation to this, Bødker and Noyé (1994) reported a 90% reduction in indigenous weed seedling emergence achieved through increasing the soil temperature to about 60°C , and a further 10°C increase in temperature gave substantial reduction of 99%. In the present study, an 82% reduction in weed dry weight was attained through application of MW energy (560 J cm^{-2}) to the soil. This is highly supported by findings of Sartorato et al. (2006), who estimated MW energy doses for 90% reduction in the dry weight of *Abutilon theophrasti* (101.5 J cm^{-2}) and *Panicum miliaceum* (343.3 J cm^{-2}). However, this energy for satisfactory post-emergence control is moderately low compared to the energy consumption in the present investigation. Additionally, they also considered that species sensitivity to MW energy probably depends on plant size, stem and leaf morphology. The effective energy range of $80 < \text{J cm}^{-2} < 160$ had been reported by Wayland et al. (1973) for pre-emergence weed control. Changing the power level of the MW system could be effective at reducing the irradiation duration by up to 50% for weed management. Given this, Crawford (1977) used a high-power MW system (2.45 GHz, 1.2 kW) for pre-emergence weed control with reduced irradiation duration of 5 – 45 s and detected 85% suppression in germination of *Trifolium* and *Medicago* species.

Table 4.1: Effect of pre-emergence microwave irradiation of clay based moist soil for 120 s using horn antenna with aperture dimensions of 110 × 55 mm on weed parameters in rice crop. Superscripts sharing different letters are significantly differ at 5% probability level.

Weed Parameters	Treatments		LSD (p=0.05)	Percentage Change
	Microwave Treated	Untreated Control		
Weed Density (plants plot ⁻¹)	17.6 ^a	94.8 ^b	37.7	-80%
Weed Fresh Weight (gm plot ⁻¹)	156.4 ^a	612.8 ^b	426.6	-74.6%
Weed Dry Weight (gm plot ⁻¹)	21.6 ^a	122.6 ^b	69.6	-82%

Similar weed suppression was achieved in this current study, which was 80% reduction in weed establishment. Lal and Reed (1980) evaluated the influence of MW irradiation of soil for 0, 5, 10, 20 and 30 s, with various moisture content, on seed germination percentage. They reported 90 – 100% reduction in the germination of irradiated seed. Hightower et al. (1974) investigated the effect of pre-emergence MW (2.45 GHz; 100 – 750 W) irradiation of soil, fed through a rectangular horn antenna (100 cm²; 120 – 1200 s), for germination inhibitions of four weed species; sorghum sudan, arrowleaf Clover, bluegrass and turnip, and found 60 – 78% reduction in seeds germination of clover and turnip. Davis et al. (1971) examined the seed susceptibility of 15 species to MW radiation (2.45 GHz; 600 W; 261 J g⁻¹) with respect to geometry, moisture content and chemical composition of the seed. They reported that reduction in germination was mediated by moisture percentage and internal biological factors. Wayland et al. (1975) examined the phytotoxicity of pre-emergence MW energy of 35 – 325 J cm⁻² application to weed seedbanks in sandy loam soil under field conditions. They achieved 91% control in germination of broadleaved weed species and 81% reduction in germination of grasses with energy threshold of 183 J cm⁻². Furthermore, they noted that there was no definite trend in selectivity between Broadleaved and grass species in response to MW energy application. Therefore, based on previous findings and the results of this study, which is first of its own for rice crop, it is possible to reduce the weed pressure in direct-seeded rice system through MW irradiation of soil in Australia, however, more consolidated research efforts are needed to understand the long-term effect of MW irradiation and weed control in rice.

4.4.2. Influence of Microwave Irradiation of Soil on Rice Growth

Rice productivity, like other broad-acres crops, is strongly influenced by weed management practices. The projection of MW (2.45 GHz; 120 s; 560 J cm⁻²) energy into soil for pre-emergence weed management significantly (ANOVA; $p < 0.01$; Table 4.2) increased the tiller density (419 m⁻²) and grain yield (9.0 t ha⁻¹) of rice, compare to the untreated control scenario 292 m⁻², and 6.7 t ha⁻¹, respectively. The significant difference was observed in case of dry biomass at maximum tillering stage (Table 4.2), but no statistically significant difference was observed at harvesting between MW treated plots (27.8 t ha⁻¹) and untreated control plots (22.8 t ha⁻¹). The higher crop productivity could be attributed to 70 – 80% reduction in weed establishment achieved through MW irradiation of soil, ultimately leaving more room for crop growth.

Thermal devitalisation of weed seedbanks in the vertical soil profile may be the possible cause of minimum weed interference with the rice crop. This was evidenced by (Vidotto et al., 2013), who explored the effectiveness of high temperature on seed viability of six weed species including *Echinochloa crus-galli* L., the problematic weed of rice growing regions globally. They stated that 80 – 100% germination reduction was achieved through raising the soil temperature to 79.6 °C. The same range of temperature regime (70 – 80 °C; see Figure 4.1) that was acquired by MW irradiation of the soil in the present study. This effectively induced an inhibitory effect on the weed population and therefore increased the rice crop yield. These results are strongly supported with findings of Brodie, (2016), who found that in pot trial maximum rice grain yield was attained with energy application of 600 J cm⁻² to soil before crop sowing. The increase in the crop dry biomass is also reported by Gibson et al. (1988) who demonstrated that shoot dry biomass of birch (*Betula pendula* L.) significantly increased in MW irradiated soil. Their experiment was to evaluate the effect of MW treatment of soil supplemented with two mycorrhizae on the birch seedling; the shoot dry biomass progressively increased with irradiation duration of soil, the highest dry shoot biomass was 84 mg for irradiation duration of 120 s with no *mycorrhizae* supplementation compared to non-irradiated soil (25 mg).

In present investigation, there was no statistically significant difference detected in leaf chlorophyll content, plant height and spike length according to MW soil heating for weed seedbank suppression. But, the crop leaf area index (4.0) was found to be significantly high (ANOVA; $p < 0.001$) in MW treated soil, suggesting a dense canopy development in response to MW soil treatment. The flag leaf area is a key component responsible for maximum photosynthetic rate and eventually help in higher biomass production, and there was a positive correlation detected between leaf area index and tiller production ($r^2 = 0.77$; Figure 4.4) in present study. To illustrate the difference between soil treatment, randomly selected plants were root out at maximum heading stage from treated and untreated plots separately is represented in Figure 4.5. Brodie et al. (2015a) demonstrated that MW energy (576 J cm^{-2}), applied to the soil, significantly increased the grain yield of wheat by 189% and in canola by 650% compare to the control. These results explain that MW treatment of soil could also enhance the growth and yield of rice. Furthermore, the possible reason for the increase in growth related parameters of rice may be due to the more availability of nitrogen and other nutrients to the plants. This postulate has recently been explored by Khan et al. (2016), who documented that pre-sowing MW irradiation of soil for 120 s significantly increased the

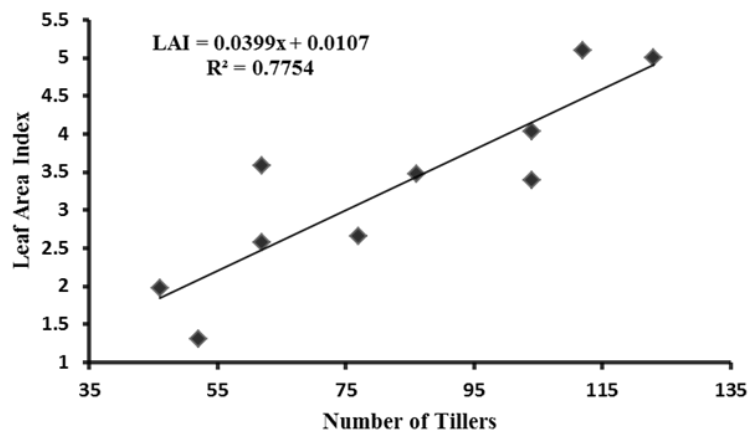


Figure 4.4: Relationship between leaf area index and fertile tillers at the maximum tiller development stage.

Table 4.2: Influence of pre-emergence microwave (2.45 GHz; 120 s; 560 J cm⁻²) irradiation of clay based moist soil for weed management on yield components of the rice crop. Superscript sharing different letters differs significantly at 5% probability level.

Rice Parameters	Treatments		LSD (p=0.05)	Percentage Change
	Microwave Treated	Untreated Control		
Plant Height	94.4 ^a	92.6 ^a	3.4	1.8%
Spike Length	16.7 ^a	16.1 ^a	2.2	3.7%
Chlorophyll Content (SPAD)	42.3 ^a	43.6 ^a	3.3	-3%
Leaf Area Index	4.0 ^a	2.6 ^b	1.5	56%
Number of Tillers (m ⁻²)	419 ^a	292 ^b	113.9	43%
Fresh Biomass Weight (t ha ⁻¹) [†]	46.3 ^a	25.1 ^b	9.4	84.6%
Dry Biomass Weight (t ha ⁻¹) [†]	10.1 ^a	5.6 ^b	2.1	80.5%
Dry Biomass Weight (t ha ⁻¹) [‡]	27.8 ^a	22.8 ^a	6.04	21.9%
Grain Yield (t ha ⁻¹) [‡]	9.0 ^a	6.7 ^b	2.04	34.38%

[†] = Data collection at maximum tillers establishment

[‡] = Data collection at crop harvesting

grain and biomass yield of wheat, and enhanced the agronomic efficiency of nitrogen, compared to non-microwaving conditions. It has also been demonstrated that MW irradiation markedly alters the physical and chemical properties of soil organic matter (SOM; Hur et al., 2013), and soil heating can enhance the humification of SOM (Kim and Kim, 2013). Relevant to this, SOM is aggregates of organic residues in soil at different stages of humification (Stevenson, 1994). Approximately 5 – 25% of organic inputs are expected to accumulate in soil as proteins, peptides and free amino acids (Stevenson, 1982b).

Amino acids typically incorporate about 10 – 20% of soil organic carbon and 30 – 40% of soil organic nitrogen (Stevenson, 1982b). Thermal denaturation of these biopolymers induced by MW irradiation could increase the concentrations of free amino acids for succeeding turnover to CO₂ and the ammonia pool (NH₄⁺), which might have led to higher yield of the rice crop in MW treated soil, in the present investigation. In this context, Brodie (2016) simulated the supplementary effect of soil MW energy application on crop yield. He stated that once-off MW irradiation for soil seedbank depletion gives a benefit of 1.5 full crops, but when this once-off treatment is coupled with 55% increase in crop productivity, MW treatment may give a cumulative advantage of 2.05 additional crops yields in a cropping system. This simulation has evidenced through the results of present study where we achieved an 80% reduction in weeds establishment (Table 4.1) and 34% increase in grain yield (Table 4.2).



Figure 4.5: Comparison of plant growth characteristics particularly tiller density per plant, randomly uprooted plant from microwave treated plots (left) and untreated control plots (right).

The future research work should focus on the cumulative effect of microwave on soil nutrient dynamics. The grain mineral concentration was assessed at harvesting and there was no significant difference in response to microwave irradiation of soil for weed management (Figures 4.6 and 4.7). These results suggest that increase in soil temperature through MW irradiation did not affect the quality characteristics of rice, and crop effectively utilize the yield-limited nutrients for higher yield.

4.4.3. Energy Analysis for Rice Field Trial at Dookie

The MW system used in the setup of the rice field trial used a modified microwave oven, feeding energy into a horn antenna, with an aperture dimension of 11 cm × 5.5 cm. The aperture of the antenna was in direct contact with the ground. This scenario was modelled using a Finite Difference Time Domain technique (FDTD), as originally developed by Yee (1966), to understand the transfer of energy from the antenna to the soil. In particular, this analysis revealed that the power transfer coefficient for the system (i.e. the amount of microwave power that was coupled into the soil as a fraction of the input microwave power), was 0.535. Poor coupling is caused by a significant impedance mismatch between the microwave system and the soil (Metaxas and Meredith, 1983). This coupling coefficient could be increased to almost 1.0 by using a stub-tuner in the wave guide to introduce enough admittance into the system to match the impedances (Griffin, 1976; Wang and Tiefenback, 2004).

Because microwave ovens use a half-wave voltage doubler circuit to power the magnetron and there are various other electrical power consuming elements in the oven, such as lamps, fans, and heating losses in the transformer and magnetron, the efficiency of the microwave system is probably between 40% and 45%; however, well designed microwave systems, operating at 2.45 GHz, can achieve efficiencies in excess of 75% (Patil et al., 2016). Industrial systems, operating at 922 MHz, usually achieve over 90% efficiency. Based on the poor coupling coefficient of the horn antenna and the poor efficiency of the microwave oven system used in the experiment, the overall energy transfer coefficient of the system was 0.214, assuming about 40% efficiency for the MW system. If a well-designed industrial quality system, which incorporated proper stub-tuning, was used for this experiment, the energy transfer coefficient could

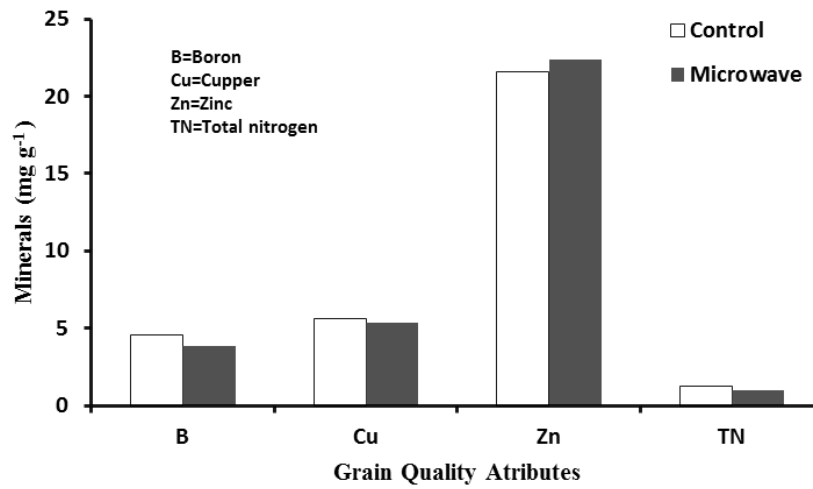


Figure 4.6: Influence of pre-emergence microwave treatment of soil for weed management on grain mineral accumulation of rice crop.

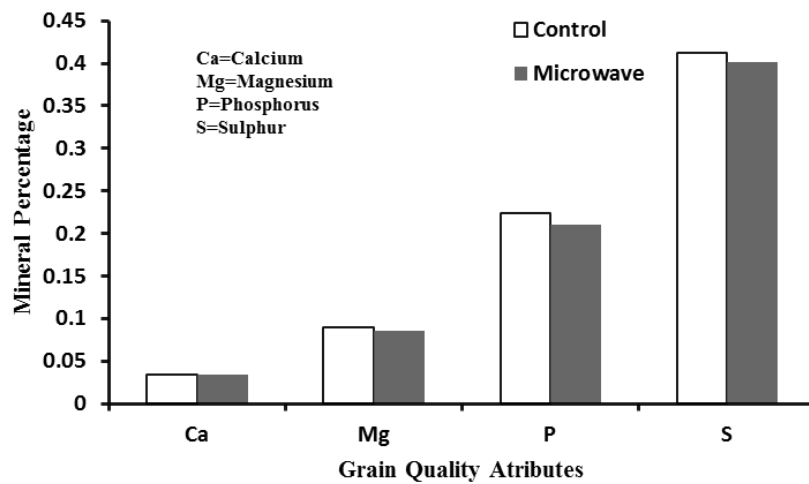


Figure 4.7: Influence of pre-emergence microwave treatment of soil for weed management on grain minerals accumulation of direct-seeded rice crop.

be 0.75, or higher, depending on the frequency chosen.

During the experiment, the portable electrical generator used to power the microwave system in the field, consumed 70 L of gasoline fuel. Because of the treatment regime, the need for cooling time between treatment events, and the need to move the microwave antenna between treatment events, the microwave system was only operational for 28% of the full time the generator was running; therefore about 19.6 L of fuel was consumed during the actual microwave treatment time needed to treat the 20 m² of soil for this experiment, or 0.98 L m⁻² of gasoline. Based on the earlier analysis of the energy transfer coefficient of the system, if a well-designed industrial quality system was employed to implement this treatment, the fuel consumption could effectively be reduced to about 5.5 L for the 20 m² treatment area, or 0.28 L m⁻² of gasoline.

MW soil treatments, such as was implemented in this experiment, should be considered as equivalent in operation as soil fumigation rather than being compared to a herbicide treatment. Steam treatment has been considered as an alternative to chemical treatment for soil fumigation. In a study by Samtani et al. (2012), they estimated the cost of steam treatment in their experiments to be between \$7,124 and \$19,000 ha⁻¹. Using their fuel cost of \$0.88 L⁻¹ for diesel fuel, their fuel consumption varied between 0.81 L m⁻² to 2.16 L m⁻² of diesel, therefore the MW treatment system is comparable or better than the steam treatment processes employed by Gay et al. (2010b) and Samtani et al. (2012).

4.5. Conclusion

Based on the result of this study, we infer that pre-emergence MW soil treatment could gain a wide acceptance for chemical-free weed management in a direct seeded rice cropping system. Although crop productivity was enhanced in this study, as was pointed out in the literature review, crop production in different agronomic zones may introduce unexpected constraints on the efficacy of the MW weed control technology. Further research is needed to understand the effectiveness of MW energy on soil seedbanks for weed management under different cropping regions and soil conditions.

Chapter 5 – Potential of Microwave Soil Heating for Weed Management and Yield Improvement in Rice Cropping

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5.1. Abstract

Non-herbicidal weed control techniques are needed for safe and sustained food production. In this study, we examined the impact of pre-emergence microwave (MW) soil treatment on rice productivity in two different agro-ecological zones of Australia. A once-off MW soil treatment was assigned in a randomized complete block design with five replicates at both study locations; Dookie, Victoria and Jerilderie, New South Wales (NSW). An experimental MW weed killer, which has four independently controlled 2 kW MW generators operating at 2.45 GHz, was used to treat the soil under field conditions. The applied energy density was approximately 500 J cm⁻². This energy achieved a temperature increase of 70 °C – 75 °C in top soil horizon (0 – 6 cm). Consequently, this transient soil heating induced on an average of 50 – 60% reduction in weed establishment compared to untreated control plots; however, there was no statistically significant difference found between MW and untreated control plots at both study locations. In addition to weed control, the MW soil treatment gave 20 – 52% increase in rice grain production. Furthermore, in case of MW soil treatment, collectively the agronomic efficiency of applied nitrogen was 10 times higher and irrigation water use efficiency (t MI⁻¹) was 50 – 100% higher compared to untreated control plots. In summary, investment in this non-chemical weed control technique can potentially reduce the weed

establishment and enhance rice productivity and thus this study clearly support the practicability of this technology.

Key words: Microwave heating, Soil temperature, Weed control, Rice, Yield

5.2. Introduction

Rice (*Oryza sativa* L.) is a profitable industry in Australia (Bajwa and Chauhan, 2017). The rice is cultivated in the Riverina region of the New South Wales (NSW), Australia, which is irrigated by two main rivers of Murray-Darling Basin; the Murrumbidgee and the Murray (Bajwa and Chauhan, 2017). In the country, rice is cultivated on an area of >0.5 m ha with an annual production of >0.8 m t (Statistics, 2016). Several biotic and abiotic aspects reduce rice productivity. Among them, weeds have significant growth retard impact on the rice production. In the region, pre- and post-emergence application of herbicides is a common practice for weed management. The *Echinochloa* species are the most problematic weeds of the Australian rice (Bajwa and Chauhan, 2017). Maun and Barrett (1986) reported a 57% reduction in rice yield with the *E. crus-galli* population of nine plants m⁻². Moreover, higher densities of *E. crus-galli* can potentially utilization the soil nitrogen up to 80%, especially at vegetative growth stages (Holm, 1997). Seed production is the basic survival and dominance traits of weed dynamics (Davis et al., 2003). Relevant to this, the size of the weed seedbank of *E. crus-galli* ranges from 0 – 36,080 seeds m⁻² with an average seed density of 1640 m⁻² (Bagavathiannan et al., 2011). Therefore, a good weed control approach relies on the devitalization of the viable weed seed bank in top 0 – 6 cm of soil.

Herbicides are the major input in the modern no-till farming for weed management, which has consequently shifted weed species towards herbicides resistance. Notably, this is the most significant threat to the safe and sustainable crop production in developed countries. In time, globally, 471 cases of herbicide-resistant had been reported in 86 commercially important crops, and 250 weed species developed resistance to 160 various chemistry herbicides (Heap, 2016). Annually, nearly 9 new weed species are being evolved as an herbicide-resistant (Heap, 2013). Given this, numerous non-herbicidal weed management strategies; for example, fire, flaming, soil fumigation, mechanical eradication and steam application have been widely considered and

developed for control weeds (Rask and Kristoffersen, 2007). However, all these non-chemicals methods have a limited control spectrum for weed control in cropping systems (Gay et al., 2010) and have one or the other adverse influences on the environment.

For all these reasons, therefore, there is a present need for an alternative weed control method in no-till cropping systems of developed countries for sustainable rice production. Microwave (MW; non-ionizing) soil treatment has been potentially proposed for weed control in broad-acre and high-value vegetable crops because of herbicides resistance (Brodie et al., 2017).

It has been explored that the pre-sowing MW soil heating can potentially control weeds infestation (Nelson, 1996; Sartorato et al., 2006; Brodie, 2012; Hess et al., 2018), kill the weed's reproductive parts, and their seeds up to a depth of 6 cm (Brodie et al., 2007). Barker and Craker (1991) tested a MW induced soil temperature regime of 75 °C – 80 °C for devitalization of the oats and various weed species seeds in soil with distinct moisture contents. It was founded that seed susceptibility to soil heating was controlled by soil moisture and temperature, and MW soil heating considerably inhibited the seed germinability. Similarly, Hess et al. (2018) tested the pre-sowing MW (various power × duration) application for seedbank depletion of wide range of species. It was found that a temperature gradient of 85 °C gave considerable reduction in the germination of all the tested invasive species. In addition to weed suppression, the MW soil treatment has shown increase in the crop growth and yield under controlled conditions (Gibson et al., 1988; Brodie et al., 2017; Khan et al., 2018). However, the reasons for long-term yield increasing benefits and effectiveness of this broad-spectrum weed control technique in real field conditions is still in question. We hypothesized that the rapid increase in temperature through MW soil treatment reduces the viability of top soil weed seedbanks. Therefore, the main objective of this field study was to evaluate the impact of pre-cropping MW soil heating to deactivate the seedbanks for weed management in rice production systems of two different agro-ecological zones of the Australia.

5.3. Materials and Methods

Two field trials were conducted during October, 2016 to April, 2017 in a randomized complete block design with five replications at two different locations. The

first location Dookie Campus of the University of Melbourne (36.395 °S, 145.703 °E) is a central grain-growing region of the Goulburn Valley, which is in the north of Victoria; part of this area grows temperate rice. This region has a temperate climate, and the 575 mm is an average annual rainfall with monthly temperature range of 9.4 °C – 20.9 °C . Soil at this location is medium clay and classified as an a Yellow Subnatric-Dystrophic Sodosol (Isbell, 2016). Historically, the same paddock has been used for sheep grazing and highly invaded with a numerous grass species particularly, panic grass (*Panicum capillare* L.). An area of 75 m² was evacuated and developed into a bay for growing rice. This field was manually leveled before microwave soil treatment, and the individual plot size was about 2.6 m² with a buffer zone of 0.5 m from all sides of the plot.

The second location at Old Coree, Jerilderie, New South Wales (35.36 °S, 145.75 °E) is on the rice research farm, which is a totally owned property of Rice Research Australia Pty. Ltd. (RRAPL) – SunRice™. This cropping area has a semi-arid climate with an average annual rainfall of 350 mm with hot summer (42.9 °C) and cool winter . Soil at this experimental site is a heavy clay and classified as a Grey Vertosol (Isbell, 2016). An area of 78 m² was laid out in a large rice paddock of approximately >5 ha for this MW trial. The actual plot size was 4 m² with a buffer zone of about 0.5 m² from all the sides of an experimental plot. Historically, the same paddock has been used for rice growing and was invaded with a semi-aquatic weed species, particularly barnyard Grass (*Echinochloa crus-galli* L.). Soil properties (0 – 10 cm) of both study locations are given in Table 5.1.

An experimental MW weed killer has been designed to eradicate the weeds between crop rows and for pre-emergence soil treatment (Figure 5.1). It has four independently controlled 2 kW MW (high energy) generators operating at 2.45 GHz. The trailer is powered by two onboard 7 kVA, 3-phase electrical generators (Brodie et al., 2017). The MW energy is channeled to the ground via waveguides (390 mm) and horn antennae with aperture dimensions of 55 × 110 mm. This weed killer can be used to treat the soil with high doses of MW energy. However, in this study, two-treatment durations of 0 (untreated control) and 60 s were assigned in a randomized complete block design with five replicates at both study locations. The selection of 60 s treatment duration was based on the finding of the previous field trial by Khan et al. (2017a) where they used a treatment duration of 120 s with a MW source of 0.7 kW (low energy) in this case, the

MW soil treatment of 60 s occurred when the trailer was stationary. The trailer was then moved forward by about 8 – 10 cm, depending on the dimensions of the horn antennae, and treatment was done again on the next small section of soil until the whole plot was treated.

After the completion of first run, the trailer was then moved back to start another treatment adjacent to previously treated strips at both study locations. The power distribution through a horn antenna was simulated according to section 3.7 of the Chapter 3. A 3-D model for a horn antenna and soil sample was implemented in XFDTD version 7.5.1 (Remcom, State College, PA, USA). MW field intensities and power dissipation into soil were post-processed using MATLAB (The Mathworks Inc., Natick, MA, USA). Infrared thermal images were taken using the infrared camera (C2; FLIR Systems Inc.; Wilsonville, OR, USA) immediately (2 – 5 s; temperature loss during that time was negligible) after MW soil heating of a small heated strips.

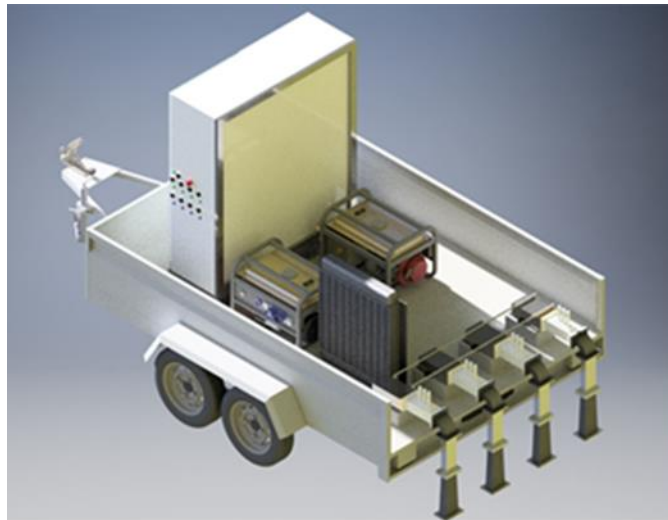


Figure 5.1: Diagram of microwave weed killer used in this study for pre-sowing soil treatment. See; Brodie et al. (2017) for details of this machine.

The raw image was post-processed in MATLAB (The Mathworks Inc., Natick MA, USA) to forecast the spatial temperature distribution into soil surface. At both locations, after MW soil treatments, the plots remained undisturbed for a few hours to allow the soil to cool until it reached equilibrium temperature, after which the rice variety YRM70 was sown with a small seed plotter at a seeding rate of 80 kg ha⁻¹ with a row-to-row distance of 25 cm. Diammonia phosphate was applied at the time of sowing at the rate of 80 kg ha⁻¹. Three split doses of nitrogen were applied during the growing period of rice at the rate of 125 kg ha⁻¹ using urea. Irrigation for rice growing at both locations was scheduled as per recommendation of the Ricegrower Association of Australia. At physiological maturity, the crop at both locations was manually harvested, two quadrats (0.25 m²) were taken from whole plots to minimize the edge effects, for yield assessment in response to MW soil heating.

After final crop harvesting, the biomass was dried in an air circulation oven (TR1050 27124; Nabertherm, Lilienthal, Germany) for 24 h at 65 °C, and the rice biomass and grain yield were measured. The yields were transformed into a tonne per hectare (t ha⁻¹) bases. Weed density and biomass accumulation were taken from whole experimental plot areas at both locations at the maximum tillering stage, and dry biomass of weeds, after drying in an oven (Nabertherm; TR1050 27124; Germany) for 24 h at 65 °C was assessed at crop harvest. The harvest index was calculated by using the following equation (5.1);

$$\text{Harvest Index} = \frac{\text{Grain Yield}}{\text{Biological Yield}} \times 100 \quad (5.1)$$

The data were subjected to normality assessment and single factor Analysis of Variance using MATLAB R2015b (The Mathworks Inc., Natick, MA, USA) to distinguish a statistically significant means, and a least significant difference (LSD) test was used to compare the treatment means at a 5% probability level. The scalar efficiency index of MW soil heating for weed control, modified from (Gay et al., 2010b) for steam application, was calculated the following mathematical equation, developed by Brodie (2018). The equation 5.2 was developed to forecast the scalar efficiency index (I) of MW soil heating for weed control under field conditions using an open structured horn antenna;

$$I = \frac{n\omega\epsilon_0\kappa''E_0^2}{64t_fk\gamma\alpha^4} (e^{4\gamma\alpha^2t_f} - 1) \quad (5.2)$$

Where,

ω is the angular velocity of the MW field (rad s^{-1}),

ϵ_0 is the permittivity of free space,

κ'' is the dielectric loss factor of the soil,

E_0 is the electric field strength at the soil surface (Vm^{-1}),

t_f is the MW heating time (s),

k is the thermal conductivity of soil ($\text{Wm}^{-1}\text{K}^{-1}$),

γ is the combined heat and moisture diffusivity coefficient for the soil,

α is the MW attenuation factor in the soil (m^{-1}).

Table 5.1: Physico-chemical properties of soil at the experimental sites. Properties was assessed by Nutrient Advantage Laboratory Services, Victoria, Australia.

Soil Properties	Locations		Units
	Dookie, Victoria	Jerilderie, New South Wales	
pH (1:5 H ₂ O)	5.6	7.0	–
Organic Carbon	1.2	0.77	%
Nitrate-Nitrogen	3.6	<0.55	mg kg ⁻¹
Ammonium-Nitrogen	3.2	10	mg kg ⁻¹
Phosphorus (Colwell)	15	55	mg kg ⁻¹
Electrical Conductivity	0.7	0.6	dS m ⁻¹
Cation Exchange Capacity	5.21	21.8	cmol (+) kg ⁻¹
Available Potassium	200	340	mg kg ⁻¹
Calcium/Magnesium Ratio	2.5	0.76	–
Zinc	4.7	0.47	mg kg ⁻¹
Sodium% of Cations	8.3	8.6	%

5.4. Results and Discussion

5.4.1. Spatial Distribution of Temperature into Soil

Spatial soil temperature distribution is a crucial contributor to the MW weeds control programme. In this study, we used a MW trailer for pre-sowing soil treatment, as this trailer has designed to zap the weeds between crop rows; therefore, it was a slightly difficult to cover the whole plot area because of inter-antenna distance (19.5 cm). However, independent of that, second-run of MW trailer alongside the previously treated strip gave good soil coverage. The horn antennas of this disinfestation machine project the energy into the soil; this flow goes up to 6cm depending on soil moisture and structure. The applied MW energy density was approximately 500 J cm^{-2} in the treated section. Therefore, the overall energy dissipation gave a temperature range of about $70^{\circ}\text{C} - 75^{\circ}\text{C}$ evidenced through infrared thermal images at both locations (Figure 5.2). Similar temperature range has been previously reported for pre-sowing weed management in different cropping systems (Brodie et al., 2009; Gay et al., 2010b; Khan et al., 2017a). Notably, the temperature fluctuation below soil surface can change the disinfestation scenario; which is mainly governed by soil moisture and organic matter (Nelson, 1996) these two parameters are the primary drivers of MW interaction into the soil. The temperature of untreated control plot was approximately $25^{\circ}\text{C} \pm 4$ at both locations.

5.4.2. Inhibitory Effect of Heating on Weed

In no-till agriculture, depleting weed seedbanks is the fundamental strategy of all weed control measures. In this study, we hypothesised that soil heating through non-ionizing energy can kill the viable weed seedbanks *in-situ*. However, despite an average weed density reduction of 60% in the MW treated plots compared to the untreated plots, the data analysis revealed that the MW soil heating (2.45 GHz; 60 s) did not significantly reduce the weeds in direct-seeded rice crop at both study locations (Table 5.2). We considered seedling emergence as a direct indication of soil seedbank (Price et al., 2010) of the experimental plots. At Dookie, the predominant weed species were dirty dora (*Cyperus difformis* L.), barnyard grass (*Echinochloa crus-galli* L.), bermudagrass (*Cynodon dactylon* L.) and panic grass (*Panicum effusum* L.). Similar weed flora had

been founded earlier at the same experimental site in direct-seeded rice crop (Khan et al., 2017a). At Jerilderie, in the entire trial plots, only barnyard grass (*Echinochloa crus-galli* L.) was the dominantly presented species, which is the glyphosate-resistant weed of the Australian rice industry <http://www.glyphosateresistance.org.au>, and the weed management at this rice research farm is mainly relying on the application of the pre-sowing herbicides.

At Jerilderie, the MW trial was conducted on to a small portion of a rice-field (approximately >5 ha) without any defined border; however, it is likely that the barnyard grass seeds might have migrated with wind/water into the experimental plots after MW treatment, which ultimately might not have given a definite decreasing trend of weeds against MW energy application. Despite this, Khan et al. (2017a) reported the profound effect of pre-emergence MW energy application of 560 J cm^{-2} for substantial weed control in a temperate rice crop. They used a similar horn antenna connected to a domestic modified MW-oven operated at 0.7 kW for soil treatment and found a 70% – 80% reduction in overall weed establishment, therefore, this energy is quite comparable to the present study. Interestingly, Brodie et al. (2017) reported that young seedling of barnyard grass was debilitated entirely with MW energy exposure of 280 J cm^{-2} during post-emergence MW treatment. In addition to that, the soil at Jerilderie site is loamy clay, and the MW absorption in clayey soil is higher (Brodie et al., 2012b) because of higher water holding capacity, however, during MW treatment the soil moisture was quite low (<5%) at the treatment time, which may have affected the MW penetration and attenuation below the soil surface, ultimately achieving less than the threshold temperature for weed seed killing. Interestingly, thermal image (Figure 5.2), captured during the experiment, depicted a lethal temperature range for the weed seedbanks. However, the limitations of these images are temperature fluctuation below the soil surface. Surface reflection minimizes the MW penetration which ultimately gives a sub-lethal temperature range for seed devitalization. Generally, the power of a MW tool for weed management has a direct relationship to treatment time. Therefore, Wayland et al. (1973) suggested an energy density level of $80 < E_A (\text{J cm}^{-2}) < 160$ for a pre-emergence weed management under field conditions regardless of seedbank size.

Although, this energy range is relatively low compared to the present investigation. Additionally, seed burial depth, species composition, and seed dormancy have strong influenced on the efficacy of any pre- and post-emergence weed management strategy in field situations.

In contrast, Sartorato et al. (2006) examined the effectiveness of post-emergence MW energy application on a young seedling of velvetleaf (*A. theophrasti* L.) and wild millet (*Panicum miliaceum* L.) weed species. In their study, an energy density of $101.5 < E_A (\text{J cm}^{-2}) < 343.3$ gave a 90% reduction in dry weight of tested species. Although, it is highly unlikely that a certain sets of MW dose and treatment duration may provide the same control spectrum; because soil moisture, which directly leads to the dielectric properties of soil (Nelson, 1996) and seed structure (Davis et al., 1971; Brodie, 2008) have a considerable influence on MW interaction with target (seedbank) under field conditions. These vary in accordance with cropping systems and management approaches. In the past, a smaller amount MW doses had been widely attempted to debilitate weed seedbanks; therefore, for example, Menges and Wayland (1974) compared the MW soil treatment ($45 - 720 \text{ J cm}^{-2}$) and various chemistry pre-emergent

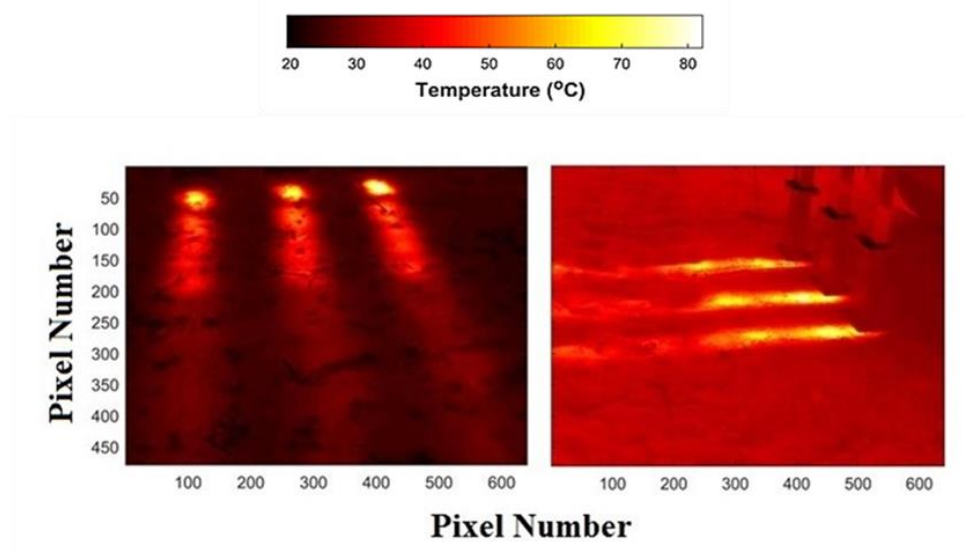


Figure 5.2: High resolution infrared thermal image of microwave treated plot depicting the temperature increase and retained in soil for weed seeds deactivation. These images were captured with thermal camera (C2; FLIR Systems Inc.; Wilsonville, Oregon, USA) from both study locations and randomly selected raw images were post-processed in MATLAB® for spatial temperature distribution forecasting.

herbicides (methazole; 2.2 kg a.i. ha⁻¹, propachlor; 9.0 kg a.i. ha⁻¹ and perfluidone; 3.4 kg a.i. ha⁻¹) for weed suppression and degree of crop damage (onion). Of the tested pre-emergent chemicals, only methazole gave 99% reduction in London rocket and 72% reduction in sunflower with 85% crop injury.

While MW soil treatment at 360 J cm⁻² gave 94% reduction in London rocket and sunflower was completely killed with minimum damage of the onion crop (18%) compared to hand-weeded plots (17%). Wayland et al. (1973) heated seeds of wheat and radish crop in soil (6.5% moisture content) at 25 mm depth. He reported that MW was toxic to seeds with a threshold level of just 10 J cm⁻². Therefore, all these studies tested the added seed into soil against MW energy; the natural soil-seed mixture is sometimes hard to kill with a specific range of temperature under distinct agro-ecological zones as the results of present investigation has shown, which therefore leads to additional attention for an understanding of MW × seed-biology × soil interaction. Based on these results; we, therefore, assumed that for 70% – 80% weed control through MW energy, the complete coverage of an area is needed, because if weed emerges even from the small untreated or slightly heated band, this could have been competed more aggressively with the main crop as the temperature below lethal dose enhances seed germination (Radzevičius et al., 2013).

Therefore, as this technology is in initial stages of development, we need to understand these critical aspects for future development. Furthermore, in this study, the heating efficiency index (measured using equation 5.2) of soil treated for 60 s using 2 kW, 2.45GHz MW source channelling via horn antenna (aperture dimensions 110 × 55 mm) was 80, which is twice of that soil steaming for weed control, as estimated by Gay et al. (2010b). In other domains, therefore independent of soil heating methodology, numerous studies had reported the debilitating effect of similar temperature range on soil seedbanks (Melander and Jorgensen, 2005; Gay et al., 2010b).

Table 5.2: Effect of pre-sowing microwave (2.45 GHz; 2 kW; 60 s; 500 J cm⁻²) irradiation of soil on weeds parameters of rice crop at both experimental sites. Note: Entries with different letters in rows are statistically significant different from each other at 5% probability level.

Weed Parameters	Treatments		LSD (p=0.05)	P-Value	Percentage Change
	Microwave Treated	Untreated Control			
Weed Density (plants plot ⁻¹) ^[i]	53 a	134 a	189.1	0.26	-60%
Weed Density (plants plot ⁻¹) ^[ii]	40 a	42 a	21.3	0.78	-4%
Weed Density (plants plot ⁻¹) ^[iii]	81 a	85 a	89.2	0.92	-4%
Weed Fresh Weight (t ha ⁻¹) ^[iv]	0.6 a	1.6 a	715.7	0.35	-57%
Weed Dry Weight (t ha ⁻¹) ^[iv]	0.2 a	0.5 a	242.7	0.41	-54%

^[i] Data collected at early crop growth establishment from the whole plot (2.6 m²) at Dookie, Victoria.

^[ii] Data collected at early crop growth establishment from the whole plot (4 m²) at Jerilderie, NSW.

^[iii] Data collected at crop harvesting from the whole plot (4 m²) at Jerilderie, NSW.

^[iv] Data collected at crop harvesting from the whole plot (2.6 m²) at Dookie, Victoria.

5.4.3. Rice Productivity in Response to Microwave Soil Heating

The MW soil treatment for pre-emergence weed suppression gave a substantial increase in rice productivity under field condition at both locations (Table 5.3). At Dookie, there was no statistically significant effect of the MW soil treatment on crop growth and yield parameters; however, it gave 51.1% increase in mean grain yield over the control plots. Apparently, At Dookie, the low night temperature induced flower sterility, which may have considerably affected the tillers fertility. Despite this high mean yield, there was an enormous variation in the dry biomass and grain yield among the experimental plots. In contrast, at Jerilderie, the dry biomass and grain yield significantly (ANOVA; $p < 0.001$) increased in response to MW soil heating with an overall grain productivity of the 9.21 t ha⁻¹. This finding is in agreement with previous studies (Khan et al., 2017a; Brodie et al., 2018; Khan and Brodie, 2018). In this study, the substantial increase (20 – 52%) in rice grain yield over untreated control plots, suggesting a direct indication of change in yield-limiting nutrient availability after MW soil heating. The increased yield response through MW soil treatment can be explained by two most likely mechanisms; (1) thermal degradation of soil organic matter (Kim and Kim, 2013; O'Brien et al., 2018) may favour the soil health, and (2) increase in NH₄⁺ concentration for plant uptake (Zagal, 1989; Yang et al., 1990). Given these reasons, presumably the thermal degradation of recalcitrant humic substance induced by MW soil irradiation might have

been increased the amount of free amino acids for succeeding turnover to C and NH_4^+ pool, which might have substantially increased rice grain yield in the present investigation regardless of weed suppression. However, these assumptions still need to be explored in controlled experiments; because, this transient heat disturbance ($\geq 75^\circ\text{C}$) neither strongly alters the soil properties (O'Brien et al., 2018) nor complete sterilization (Voort et al., 2016). Moreover, Figure 5.3 shows the comparison in rice growth in response to MW soil heating for weed suppression at both study locations.

Additionally, crop yield response to applied nitrogen has received considerable interest in irrigated rice farming system (Cassman et al., 1998). Therefore, at both locations, the agronomic efficiency of applied nitrogen was 10 times higher compared to the unheated control plots. The increase in indigenous nitrogen supply for plant uptake and accumulation in response to MW soil heating has been reported by (Khan et al., 2019). Relevant to this, for example, if a farmer treats the soil with MW energy for weed management this could optimize the nitrogen contribution from the soil's indigenous resources for crop productivity and therefore minimize the nitrogen-based fertilizers, and eventually this would reduce the farm inputs. In addition, the irrigation water is the main input in Australian rice industry. Globally, current estimates showed that an average of 0.003 – 0.005 ML of water is required for 1 kg of rice production, which is about 2 – 3 times higher than water consumption of other cereal crops (Yao et al., 2017). The cost of water in Australia is about AU\$ 200 – 300 ML^{-1} , which is consequently increasing the production cost of rice in Australia regardless of direct-drill farming, which saves flooding for almost 35 days. Furthermore, the rice production in Australia consumed approximately 2.5 – 3 ML ha^{-1} with an average grain harvest of about 1.4 t ML^{-1} however, in the case of MW soil treatment the production is about 3 t ML^{-1} at Jerilderie, which is around 100% higher irrigation water use efficiency compared to untreated control plots while at Dookie the irrigation water use efficiency was 52% higher because of MW soil heating.

Table 5.3: Influence of pre-sowing microwave soil heating for weed seedbank depletion on rice productivity at two agro-ecological zones of the Australia. Note: Different letters in a row depicting a significant difference at 5% probability level.

Rice Parameters	Dookie Location 1					Jerilderie Location 2				
	Treatments		LSD (p=0.05)	P-Value	Percentage Change	Treatments		LSD (p=0.05)	P-Value	Percentage Change
	Microwave	Untreated				Microwave	Untreated			
	Treated	Control				Treated	Control			
Number of Tillers (m ⁻²)	385 a	267 a	187	0.15	44.1%	480 a	418 a	145.2	0.29	14%
Dry Biomass Weight (t ha ⁻¹)	16.90 a	14.0 a	4.2	0.14	20.7%	19.80 a	17.05 b	0.57	<0.001	16.1%
Grain Yield (t ha ⁻¹)	3.88 a	2.56 a	1.76	0.12	51.5%	9.21 a	7.63 b	0.65	<0.001	20.7%
Harvest Index	22.3 a	17.4 a	8.08	0.19	6.1%	46.77 a	44.59 a	4.29	0.26	6.1%
¹ Water Use Efficiency (t M L ⁻¹)	1.3	0.85	–	–	–	3.07	2.54	–	–	–
² Partial Factor Productivity of Nitrogen (kg grain kg N ⁻¹)	31.04	20.42	–	–	–	73.68	61.04	–	–	–

¹Water use efficiency was calculated based on the change in grain yield per unit application of water. **Note:** Irrigation water volume was about 3 Ml ha⁻¹ as per the recommendation of Ricegrowers Association of Australia.

²Partial factor productivity of nitrogen (PFP) = $\frac{Y_0}{N_r} + \frac{\Delta Y}{N_r}$, the change in crop yield with nitrogen application was calculated according to Cassman et al. (1998). Furthermore, PFP can be considered as an agronomic efficiency of applied of nitrogen. **Note:** Applied nitrogen during cropping period was 125 kg N ha⁻¹ at both study locations.

Therefore, considering the economic return, this technology has potential to increase the overall farm profitability.



Figure 5.3: Pictorial comparison of randomly picked rice plants from Dookie (left image) and Jerilderie (right image) trail sites in response to microwave soil treatment for weed management. Plants from microwave treated plots had a higher number of tillers and dark green expanded leaf area compared with plants from untreated control plots.

5.5. Conclusions

Based on these finding, we conclude that there is a clear indication of MW energy killing the weed seedbanks under field conditions; however, for a substantial reduction in weed emergence, a complete coverage of the cropping area is needed in case of pre-sowing seedbank depletion. Furthermore, the reduction in weed establishment through an experimental MW weed killer strongly supported the commercialization and development of MW system for pre- and post-emergence weed control to tackle herbicides resistance. For a practicability perspective of this weed management technology, this study highlights that MW soil treatment can potentially be applied for weed control in rice production system; however, it is apparent from the data presented in this chapter that MW soil treatment provides additional benefits to the crop, beyond a reduction in weed emergence. Therefore, future research work is needed to examine the impact of MW soil treatment on soil nutrient characteristics and soil biology.

Chapter 6 – Effect of Microwave (2.45 GHz)

Treatment of Soil on Yield Components of Wheat

(*Triticum aestivum* L.)

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6.1. Abstract

Pre-sowing microwave (MW) irradiation (2.45 GHz) of soil for 120 s was investigated for sustainably enhancing wheat yield. The two-factor factorial glasshouse experiment was conducted to explore the bio-stimulation effect of MW energy on wheat crop growth and yield. The 175% increase in dry biomass and 96% incremental increase in grain yield of wheat were obtained through the application of 2.45 GHz MW energy to soil at N₁ (0 mg N pot⁻¹) compared to non-microwave treated soil. The MW irradiation of soil holds potential to significantly enhance wheat productivity.

6.2. Introduction

Microwaves (MW) are high frequency electromagnetic waves in the range of 300 MHz – 300 GHz and have recently been developed as an effective physical irradiation technique on account of its fast and effective transmittance, high heat-producing efficiency, cost efficiency, and low risks associated with its usage (Newnham et al., 1991). Interest in the interaction of MW energy with biological system dates back to the 20th century (Ark and Parry, 1940). In 1924, Antonin Gosset was the first person who explored the bio-stimulation effect of MW energy, when he, along with his co-worker, used high frequency electromagnetic waves to treat tumours in plants with no injury to the plant (Bren, 1996).

MW absorption is primarily influenced by the dielectric and roughness properties of the load (Ulaby et al., 1978). It has been demonstrated that MW treatment of soil

significantly reduced viability of the weed seed banks (Bebawi et al., 2007; Brodie et al., 2007; Brodie et al., 2012b) enhanced humification of soil organic matter (Kim and Kim, 2013), increased soil organic carbon and nitrogen mineralization (Zagal, 1989), and release of non-exchangeable NH_4^+ inorganic colloids (Zagal, 1989). However, the contribution of all these nutrient availability resources have potential to increase the soil productivity.

Wheat (*Triticum aestivum* L.) is the most important grain crop in Australia. In 2015 the total area under wheat crop production was 12.3 m ha with the productivity of about 23.7 m t (Statistics, 2016). The potential grain production capacity of major Australian wheat belt regions ranges from 4.5 – 11 t ha⁻¹ (Acuña et al., 2011); however, the present on-farm average wheat grain yield is about of 2.7 t ha⁻¹ (Christy et al., 2015). This yield gap can be conquered by optimizing on-farm management strategies (Yang et al., 2008). Narrowing yield gaps are crucial to overcome the globally increasing pressure for food production (Chapagain and Good, 2015).

Soil nitrogen (N) availability is a major yield limiting factor for sustainable crop productivity in Australian agriculture systems. N is the building block of life molecules such as proteins, RNA, DNA and various plant hormones and is a crucial macro-nutrient for plant growth and development (Wang and Schjørring, 2012). It is also involved in essential biochemical processes (Cai et al., 2012). Abeledo et al. (2008) reported that wheat yield variability could be reduced through attentive management of N. Soil organic nitrogen is an aggregate of amino acid (40%), peptide, proteins, Pyrines (35%; i.e. heterocyclic nitrogenous compounds), amino sugar (6%), and ammonia 19%; (Schulten and Schnitzer, 1997).

Furthermore, Reddy et al. (1998) documented that MW treatment dramatically increased germination in wheat. It has been demonstrated that MW treatment unlocks certain nutrients into the soil solution; markedly increases the nitrification efficiency (Zieliński and Zielińska, 2010); and has a minute effect on the root nodulation by *Rhizobium* species in the soil (Eglitis and Johnson, 1970). The mechanical effect induced by MW irradiation can stimulate the dispersal of inorganic colloids. This stimulation can increase the decomposition of non-biomass organic matter in the soil (Zagal, 1989). The bio-stimulation effects of MW energy on soil nutrient dynamics and their major

contribution to crop productivity are not well investigated. A glasshouse study was conducted to evaluate the effect of microwave (2.45 GHz) soil treatment on yield components of wheat with the hypothesis that microwave treatment of soil increases wheat yield by pasteurizing the soil and unlocking some nutrients in the soil to be readily available to the crop.

6.3. Materials and Methods

A two-factor factorial glasshouse experiment was conducted to examine the impact of microwave treatment of soil on the wheat crop in a completely randomized design with ten replicates of each treatment combination. The experiment was conducted at Dookie Campus of The University of Melbourne (36° 23' S, 145° 42' E). The factors include the different levels of applied nitrogen and two microwave exposure times. Soil, referred to locally as Dookie clay loam/Gowangardie loam (Downes, 1949), which is a red ferrosol soil (Isbell, 2016) with a pH of 5.6, was collected from the top 5 cm of a research field near Mount Major Lane, Dookie, Victoria. The soil was placed in polyethylene bags and immediately transfers to the laboratory. Stones and plant debris were removed, and the soil was sieved with a 4-mm mesh to ensure homogenization of the soil for microwave irradiation.

The average soil load in each experimental pot (diameter-25 cm) was 8.5 kg and the small black plastic pot with a diameter of 13 cm, 13 cm height was used to treat the soil in a microwave oven. The microwave treatment times were $MW_0 = 0$ s (Untreated Control) and $MW_1 = 120$ s. This particular exposure time (120 s) was selected based on the work conducted by Cooper and Brodie (2009), who documented that 120 s exposure of MW heating to agricultural soil at various vertical depths increased the nitrite concentration. A microwave oven (EMS8586V; Sanyo; Tokyo, Japan) with cavity dimensions of 370 × 380 × 210 mm operating at 2.45 GHz and rated power level of 1100 W was used for soil irradiation. The calibrated output power of microwave oven was 929.0 W. The soil temperature for each microwave treatments was measured in the centre of the pot at the depth of 2 – 5 cm by using the liquid-in glass thermometers immediately after microwave exposure. The average soil temperature was 85 – 90 °C. The temperature of all the untreated control soils was 25±2.1°C.

The dual label ammonium nitrate was purchased from Shanghai Research Institute of Chemical Industry, Shanghai, China. The four concentrations of nitrogen ($N_1 = 0$ (0 mg N pot⁻¹), $N_2 = 50$ (22.5 mg N pot⁻¹), $N_3 = 100$ (45 mg N pot⁻¹), and $N_4 = 150$ mg ¹⁵N L⁻¹ (67.5 mg N pot⁻¹)), were prepared using the isotopic ammonium nitrate (¹⁵NH₄¹⁵NO₃) with 10.4 atom% ¹⁵N enrichment. For clay loam the isotopic N solution delivery volume is approximately 1 ml per 18.8 cm³ of soil (Davidson et al., 1991). The 450 ml of isotopic N solution of various concentrations was applied to each experimental unit after MW treatment. The soil remained undisturbed for one day (24 h) after MW irradiation treatment and on the next day pots were sown with the wheat variety Gregory (20 seeds per pot were sown. We achieved 90–95% seed germination in all the potted trials.) and optimum growing conditions were maintained throughout growing period. The pots were irrigated after every three days at field capacity. The crop was manually harvested to measure the number of tillers per pot, above ground dry biomass and grain yield per pot. The obtained data were analysed using the software MATLAB R2015b (The Mathworks Inc., Natick Massachusetts, USA). Significance between treatment means was detected by Analysis of Variance and Least Significant Difference (LSD) test was used to compare the treatments means (at 5% level).

6.4. Results

6.4.1. Number of Tillers per Pot

Wheat grain yield is positively influenced by the tiller productivity (Shanahan et al., 1985). Optimum N application increases tiller fertility (YosefTabar, 2013). The data showed (Figure 6.1) that MW energy exposure significantly increased the number of tillers of wheat in all N concentrations of MW treated soil as compared to the untreated soil. The maximum number of tillers (19.1 pot⁻¹) was recorded at N_4 in MW treated pots. The N_2 (14 pot⁻¹) and N_3 (14.8 pot⁻¹) were statistically similar to each other in MW conditions. The lowest number of tillers (10.8 pot⁻¹) was recorded in treatments where no nitrogen was applied in untreated pots. There was no statistically significant difference between MW and untreated control pots for number of tillers at N_1 . An example of the reproductive development stages (tillers and spike length) of wheat in the N_2 treatment, for randomly selected MW treated and untreated control pots, is presented in Figure 6.4.

6.4.2. Dry Biomass Yield (g pot⁻¹)

Biomass accumulation is of great importance for enhancing the final grain yield of wheat (Villegas et al., 2001). The examination of data shows (Figure 6.2) that MW treatment of soil significantly increased the dry matter production compared with the controls in all the levels of nitrogen application. The maximum (24.9 gm pot⁻¹) dry matter harvest was recorded in MW treated soil at N₄ followed by N₃ (17.6 gm pot⁻¹). The lowest biomass yield (6.3 gm pot⁻¹) was measured in non-MW conditions at N₁. The linear relationship between grain yield ($r^2 = 0.86$) and dry biomass accumulations of wheat is revealed in Figure 6.5.

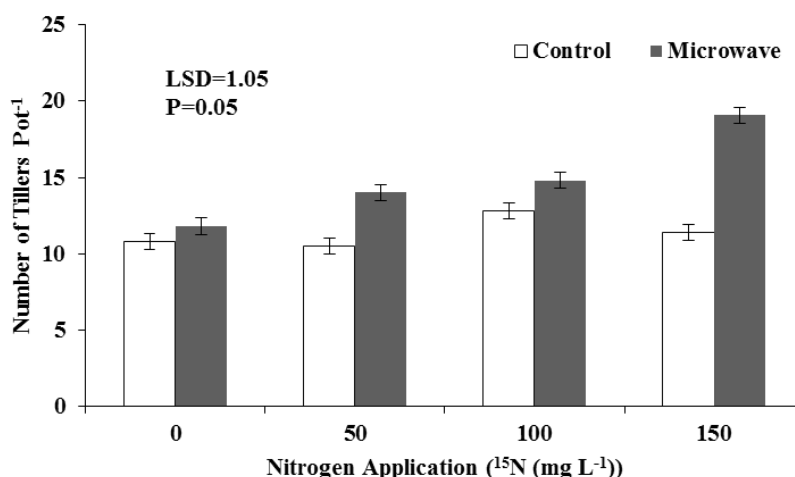


Figure 6.1: Effect of pre-sowing microwave irradiation of soil on the tiller density of wheat. Note: Error bars represent the Least Significant Difference at 5% probability level.

6.4.3. Grain Yield (g pot⁻¹)

MW (2.45 GHz) soil treatment significantly increased the grain yield of wheat under different levels of nitrogen application in the glasshouse (Figure 6.3). The maximum grain yield (11.0 g pot⁻¹) was obtained at N₄ in MW treated pots. It was interesting to observe that grain yield (5.6 g pot⁻¹) was more in MW treated pots where no N (N₁) was applied, compared to N₂ (4.0 g pot⁻¹) and N₃ (5.2 g pot⁻¹) in untreated soil. The lowest grain yield (2.0 g pot⁻¹) was achieved at N₁ in non-MW conditions. There was

a strong relationship detected ($r^2=0.99$) between the extent of yield increase due to MW soil treatment and the applied nitrogen (Figure 6.4). The relationship between applied nitrogen and the mean increase in wheat grain yield per pot due to MW soil treatment can be explained by equations 6.1 and 6.2;

$$\frac{Y - Y_{\infty}}{Y_0 - Y_{\infty}} = e^{-aN} \quad (6.1)$$

And;

$$Y = \frac{Y_m - Y_c}{Y_c} \quad (6.2)$$

Where,

Y_m is the yield from MW treated pots at a particular nitrogen application rate,

Y_c is the yield from MW untreated pots at a particular nitrogen rate,

Y_{∞} is the limit of Y when the applied nitrogen becomes very large,

Y_0 is the Y when zero additional nitrogen is applied to the pots.

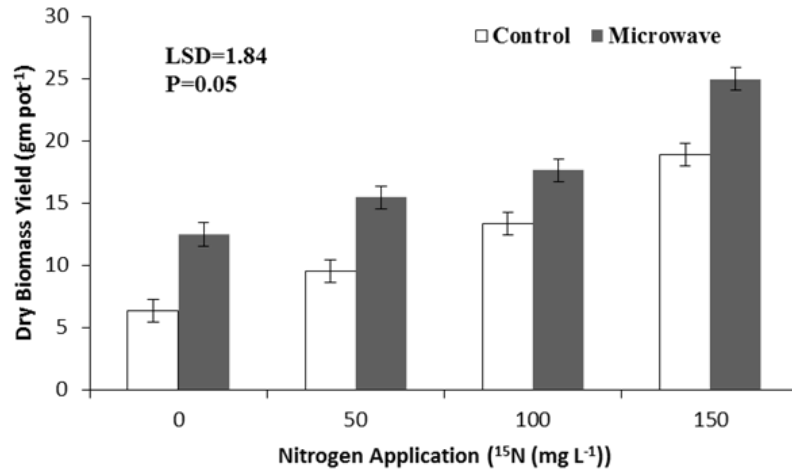


Figure 6.2: Effect of pre-sowing microwave irradiation of soil on the dry biomass yield of wheat. Note: Error bars represent the Least Significant Difference test at 5% probability level.

6.5. Discussion

The aim of this study was to evaluate the impact of MW soil treatment on crop growth and development. In general, the absorption of MW energy projected into the soil can be affected by numerous unknown factors (Nelson, 1996). The propagation of MW energy through soil is dependent upon the gravimetric (θ_g) and volumetric (θ_v) moisture content (Nelson, 1996), bulk density (Hallikainen et al., 1985), organic related parameters matter content (O'Neill and Jackson, 1990), soil texture (Brodie et al., 2012b) and specific heat of soil. The soil-microwave interaction depends on soil texture and moisture percentage (Brodie et al., 2012b). As the soil absorbs electromagnetic energy, its temperature increases which further leads to increase the thermal denaturation of biopolymers and turnover to plant available forms of nutrients.

In this study, the results showed that exposure of soil to 2.45 GHz MW radiation (120 s) significantly increased the number of fertile tillers, dry biomass and grain yield of wheat under all the applied N levels. Wheat growth is primarily influenced by the nitrogen status of soil. Higher availability of N at vegetative stages could enhance the accumulation of N into final grain yield. The MW treatment of soil dramatically increased wheat yield. An increment of 96% yield was observed in MW treated soil at N₁ (0 mg N pot⁻¹) level as compared to untreated control soil, which was consistent with the results reported by Brodie et al. (2015a). They reported that MW energy (576 J cm⁻²), applied to the soil, increased the grain yield of wheat by 189% and in canola by 650% compare to the control. These results explain that MW treatment of soil enhances the growth and yield of wheat. Furthermore, the possible reason of increase in growth related parameters of wheat may be due to the more availability of nitrogen and other nutrients to the plants.

The results are also in agreement with Wainwright et al. (1980), who found that microwave irradiation increased ammonium nitrogen (NH₄⁺-N) and sulphur oxidation (SO₄⁻²-S) in soil, which may trigger seedling emergence and growth. Cooper and Brodie (2009) documented that 120 s exposure of MW heating to agricultural soil, at various depths, increases the nitrite concentration. The nitrate reduction in the irradiated soil could be the principle cause of nitrite formation (Cawse and Crawford, 1967).

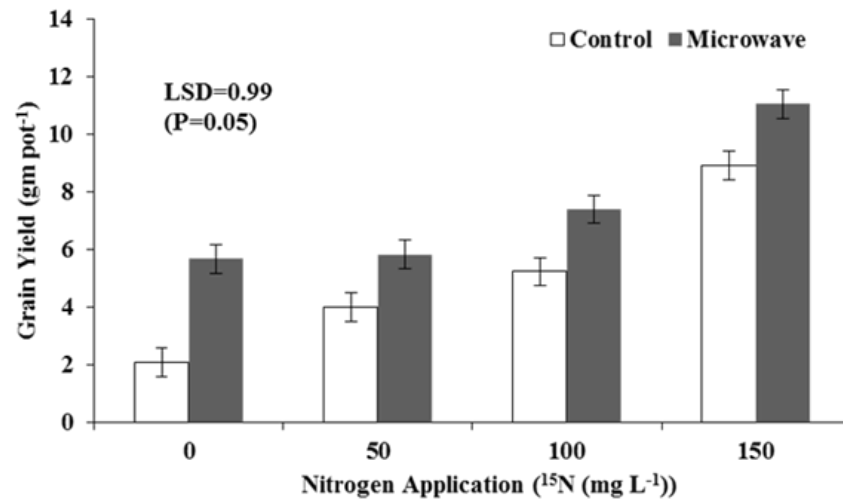


Figure 6.3: Effect of pre-sowing microwave soil treatment on the grain yield of wheat crop. Note: Error bars represent the least significant different test at 5% probability level.

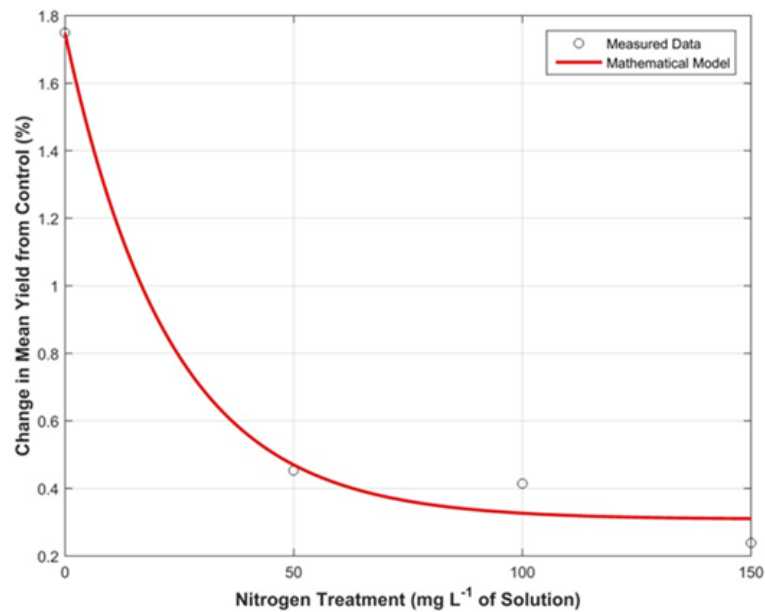


Figure 6.4: Change in mean wheat grain yield induced by microwave soil treatment, compared to the equivalent control treatment, as a function of applied nitrogen.

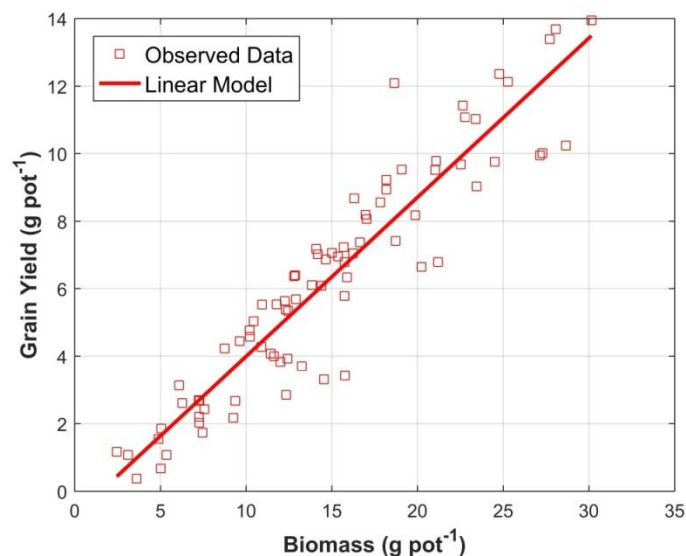


Figure 6.5: Linear relationship between grain yield and dry biomass yield of wheat ($r^2 = 0.86$).

The biomass production is governed by plant-N accumulation (Greenwood et al., 1990), and photosynthetic activity per unit leaf-N concentration (Sage and Percy, 1987). The dry biomass of wheat was considerably augmented through the MW irradiation of soil where no nitrogen was applied. This may be due to early availability of N for wheat growth from indigenous soil N resources through MW irradiation, which could enhance the conversion of accumulated N to dry biomass.

In this context, Speir et al. (1986) examined the effect of MW energy on low fertility soil (100 randomly selected cores at depth of 50 mm), microbial biomass, N, phosphorus, and phosphatase activity. They reported that an increase in MW treatment duration (90 s) dramatically increased the N level ($106 \mu\text{g N g}^{-1}$ soils) but the phosphorus concentration declined as treatment time increased. In this study, the increased growth of crop in response to soil heating is also in agreement with finding of Gibson et al. (1988), they demonstrated that shoot and root growth of birch (*Betula pendula* L.) was significantly increase in MW irradiated soil. Their experiment was to evaluate the effect of MW treatment of soil supplemented with two mycorrhizas on the birch seedling; the shoot growth progressively increased with irradiation duration, the highest dry shoot

weight was 84 mg for irradiation duration of 120 s with no mycorrhizal supplementation compare to non-irradiated soil (25 mg).

It has also been demonstrated that MW irradiation markedly alters the physical and chemical properties of soil organic matter (SOM; Hur et al., 2013) and enhances the humification of SOM (Kim and Kim, 2013). Relevant to this, SOM is aggregates of organic residues in soil at different degree of humification (Stevenson, 1994). Approximately 5 – 25% of organic inputs are expected to accumulate in soil as proteins, peptides and free amino acids (Stevenson, 1982b). Amino acids typically incorporate about 10 – 20% of soil organic carbon and 30 – 40% of soil organic nitrogen (Stevenson, 1982b). Thermal denaturation of biopolymers induced by MW irradiation could increase the concentrations of free amino acids for succeeding turnover to C and ammonia pool (NH_4^+), which may be attributed to higher yield of wheat in MW treated soil. Furthermore, Zagal (1989) pointed out the mechanical effect induced by MW irradiation can stimulate the dispersal of inorganic colloids thus; this stimulation can increase the decomposition of non-biomass organic matter in soil.

6.6. Conclusions

This study has illustrated that MW soil treatment can enhance crop growth and yield. To understand well the MW and soil interactions, future research should be focused on the impact of MW treated soil for unlocking the various N sources in soil, and soil nitrifying bacteria and their role to crop productivity. MW treatment of soil holds the potential to provide an alternative channel for efficient use of soil indigenous N by crop plants. However, there might be unknown factors contributing to final grain yield of wheat in MW soil treatment scenarios.

Chapter 7 – Microwave Soil Treatment Increases Soil Nitrogen Supply for Wheat Productivity

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7.1. Abstract

Herbicide-resistant weeds has promoted the development and adaption of new non-chemical weed management technologies for sustainable food production. Considering this, pre-sowing microwave (MW) soil treatment has potential to reduce the weed pressure in the no-till farming system. However, the dynamics of this transient heat disturbance on soil nutrient profile, uptake and accumulation in plant biomass, warrants further study. In this study, we examined the effect MW soil treatment on the recovery and accumulation of nitrogen (N) in wheat dry biomass using ^{15}N -pool dilution technique and temporal changes in yields were assessed by running sequential residual trials. Finally, the total abundance of ammonia oxidizing bacteria (AOB) and archaea (AOA) was assessed using qPCR. The MW soil treatment achieved a temperature of 75 – 85 °C; however, the transient heat disturbance did not significantly increase the N derived from fertilizer (Ndff%). The maximum Ndff (13%) achieved from untreated control soils compared to MW (8%), at higher N dose. Although, the total N accumulation in dry biomass increased 17% through MW soil heating compared to the control independent of the N application. Consequently, the grain yield supported by indigenous N supply was 152% higher with MW and this consistently sustained wheat productivity measured at 440 and 740 days since MW soil treatment. Additionally, there was no significant detrimental effect induced by MW on the abundance of AOB and AOA. Overall, the total abundance of AOA *amoA* gene copy number g soil^{-1} was higher than AOB. In summary, sustained yield response and N accumulation reflected a long-term reliability of MW soil heating and a more beneficial influence on the soil quality opens another research window for the application of MW technology.

Key words: Microwave energy, soil temperature, Nitrogen accumulation, Ammonia oxidizers, qPCR, Long-lasting effect, Wheat yields.

7.2. Introduction

Globally in agricultural soils, the availability of inorganic N is a key plant nutritional factor and accelerates net crop productivity (Banning et al., 2015). Optimizing the N contribution from soil organic matter can help to manage the fertilizer application. Relevant to this, SOM is an aggregate of organic residues in soil at a different degree of humification (Stevenson, 1994). Approximately, 5 – 25% of organic inputs are expected to accumulate in the soil as proteins, peptides and free amino acids (Stevenson, 1982a). Amino acids typically incorporate about 10 – 20% of soil organic carbon and 30 – 40% of soil organic N (Stevenson, 1982a). Soil organic N is a collective of amino acid (40%), peptide, proteins, Pyrimidines (35%; i.e., heterocyclic nitrogenous compounds), amino sugar (6%), and ammonia (19%; Schulten and Schnitzer, 1998). In general, these are the sources of the indigenous N supply, but it is unavailable to crop plants in most of the soils, which is depend on the SOM decomposition processes. However, the processes governing SOM decomposition and N mineralization appears most likely to contribute to the indigenous N supply for crop growth and development (Cassman et al., 1998). The amount and utilization of indigenous soil N can increase the N use efficiency and finally the grain productivity (agronomic efficiency; Cassman et al., 1998). Various factors affect SOM decomposition; however, soil thermal treatment indeed results in SOM decomposition (Certini, 2005; Kim and Kim, 2013; O'Brien et al., 2018). In the agriculture industry, new soil disinfection technologies have been introduced in the developed system and these have inevitably influenced soil quality.

Therefore, an adaptation of new technologies with minimum ecological impact has considerably influence on grain productivity. In the agriculture industry, Microwave (MW; non-ionizing energy) has recently emerged as a new technology to tackle herbicide-resistant weed (Brodie et al., 2017; Khan et al., 2017a) with minimum negative ecological impact. The pre- and post-emergence application of MW energy consistently induced 50 – 80% reduction in weed establishment, irrespective of cropping regimes (Brodie et al., 2017; Khan et al., 2017a). In addition to weed management, all the previous

trials showed good growth and enhanced yield of crops grown in MW treated soil. Regarding crop growth, Brodie et al. (2015a) found that MW (576 J cm^{-2}) treatment of soil significantly increased the grain yield of wheat by 189% and in canola pods, the yield was increased by 650% compared to the untreated control soil. Because of this, a curiosity has arisen: why are crops showing good growth and 30 – 50% increase in yield? The exact reason for this enhanced growth is still in question.

There is limited literature associated with this argument. For example, relevant to soil health, MW irradiated soil (90 s) can increase mineral N (Speir et al., 1986) after incubation of soils. In laboratory conditions, MW treatment released certain nutrients into the soil solution which markedly increased the nitrification efficiency (Zieliński et al., 2007), and had little effect on the *Rhizobium* species and other bacteria in the soil (Eglitis and Johnson, 1970). In addition, Wainwright et al. (1980) checked the effectiveness of MW energy on the mineral concentration of the soil (Morainic; Experiment IIIB) at two incubation times (2 and 14 days after treatment). They found higher organic N ($8.26 \mu\text{g N g}^{-2}$) content in MW treated soil compared to untreated control soil ($3.22 \mu\text{g N g}^{-2}$), however, the amount of $\text{NH}_4^+\text{-N}$ was substantially lower in the case of MW irradiation ($18.52 \mu\text{g N g}^{-2}$) compared to the untreated scenario ($34.07 \mu\text{g N g}^{-2}$) at 14 days after irradiation. In contrast, MW soil heating have potential to increase the $\text{NH}_4^+\text{-N}$ concentration (Yang et al., 1990) and this could be due to the release of interlayer ammonia and thermal degradation of organic compounds, but the exact mechanism for this nutrient behavior as a function of applied MW energy has still not been explored.

In addition, the long-term availability of yield changing nutrients, particularly N, depicts the sustainability of a system. However, the soil nutrient depletion factor needs to be considered in this scenario. A primary question needs to be addressed: with a reduced dose of N fertilizer application, to what extent can a system sustain grain yield without scarifying soil quality. However, the residual impact of a once-off MW soil treatment on crop growth and yield is still unclear. The lasting impact of applied MW energy on soil quality, in addition to weed management, would draw farmer's attention and interest in crop production systems.

In another domain, MW heating of soil can eradicate soil-borne fungi and pathogens with minimal reduction of prokaryotes (Ferriss, 1984). Relevant to N cycling microorganisms, ammonia oxidation by autotrophic AOB to nitrite (NO_2^-), which is the first step of nitrification, and subsequently to nitrate (NO_3^-) by nitrite oxidizing bacteria

is a crucial component of the global N cycle (He et al., 2012). Ammonia monooxygenase (*amoA*) enzyme, which is carried by all known AOB, has historically been considered to be solely responsible for terrestrial ammonia oxidation (Banning et al., 2015). The resource consumption pattern of AOB changes in various physio-chemical properties based soil (Ke and Lu, 2012). In general, soil temperature and pH considerably affect the soil biota. Numerous studies have reported the profound effect of temperature on reducing the soil nitrifying bacterial communities (Roux-Michollet et al., 2008; Tourna et al., 2008). Therefore, the effect of MW on soil biota, particularly AOB and AOA abundance for nitrogen cycling, have not yet been investigated independently. Considering all these aspects, we therefore hypothesized that MW soil heating facilitates some nutrient related processes and consequently ample substrate availability favors the activity of N cycling microbes. Finally, this leads to a long-lasting effect on soil nutrient availability for crop growth and development. Therefore, the objectives of this glasshouse pot trial were to: (i) evaluate the effect of MW soil treatment on recovery and accumulation of applied N in wheat over two years study; (ii) confirm the residual impact of MW soil treatment on growth and yield of following two seasons wheat crop; and (iii) elucidate the influence of MW soil irradiation on the total abundance of AOB and AOA.

7.3. Materials and Methods

7.3.1. Soil Collection

Soil (0 – 15 cm) was collected from a wheat paddock of the Dookie Campus (36° 37' S; 145° 70' E), the University of Melbourne, which is in the central grain-growing region of North Victoria, Australia. The mean annual rainfall of this region is 550 mm and mean annual temperature ranges from 9.4 – 20.9 °C . Soil for the trial was a brownish grey loam and was classified as Red Mesotrophic-Haplic Dermosol according to Australian Soil Classification (Isbell, 2016). Soil physico-chemical properties were measured by Nutrient Advantage Laboratory Services, Werribee, Victoria, Australia, before MW treatment. Soil properties (0 – 15 cm) were as follows: bulk density (1.6 g cm⁻³), Nitrate-Nitrogen 49 mg kg⁻¹, Phosphorus (Colwell) 31 mg kg⁻¹, pH_(H2O) 5.6, Electrical Conductivity (EC) 0.28 dS m⁻¹, Cation Exchange Capacity 10.20 meq 100 g⁻¹ and organic carbon (1.4%). Historically, the same paddock has been used extensively to grow crops in a monocropping pattern with wheat-canola rotations.

Particular care was devoted to soil sampling in order to provide a homogenous sample, particularly with regard to the soil characteristics. For this reason, the soil was sieved through a 4-mm mesh to minimize undesired effects of stones and clods of soil. However, this operation does not reflect an actual field situation, where the soil texture is highly irregular. Therefore, this is essential to guarantee the uniform experimental conditions, for MW soil heating. After sieving, samples of 8.5 kg ($\approx$ 45% water holding capacity) were thoroughly mixed and shifted into pots.

7.3.2. Microwave Soil Treatment

The soil was treated in a bench-scale MW oven (EMS8586V; Sanyo; Tokyo, Japan) with cavity dimensions of $370 \times 380 \times 210$ mm operating at 2.45 GHz and rated power level of 1.1 kW. The treatment consisted of 0 s and 120 s soil exposure duration. The selection of 120 s was based on a previous pot trial (Brodie et al., 2015), where the soil was treated before sowing and found to give good growth for wheat crops. The 1 kg of soil was sequentially treated in the microwave oven within a small pot and pooled into larger pots, to complete the total load of 8.5 kg of soil for each treated pot.

7.3.3. ^{15}N Soil Labelling

The ^{15}N pool dilution technique is a valuable tool to measure the N transformation fate in a soil-plant system (Murphy et al., 2003). The dual label ammonium nitrate ($^{15}\text{NH}_4^{15}\text{NO}_3$) with 10.4 atom% access was purchased from Sigma Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA). The four concentrations of isotopic N ($\text{N}_0 = 0$, $\text{N}_1 = 50$, $\text{N}_2 = 100$ and $\text{N}_3 = 150$ mg $^{15}\text{N L}^{-1}$) solution were prepared for labelling the soil N pool. The total N content in each concentration was N_0 (0), N_1 (22.5), N_2 (45) and N_3 (67.5 mg N pot^{-1}). For clay soils the isotopic N solution delivery volume is approximately 1 ml per 18.8 cm^3 of soil (Davidson et al., 1991). Based on this, a surface application of ^{15}N solution into the soil was selected to maximize the distribution uniform. A once-off 450 ml isotopic N solution, of various concentrations, was applied to the corresponding pots, which were arranged in a completely randomized design with ten replicates of each treatment combination, after MW soil treatment. In a uniform distribution scenario, an individual gram of the soil in a pot received about 0.0 $\mu\text{g N}$ (N_0), 2.64 $\mu\text{g N}$ (N_1), 5.29 $\mu\text{g N}$ (N_2) and 7.94 $\mu\text{g N}$ (N_3). The same amount of ^{15}N atom% has been used to study the recovery efficiency of an applied fertilizer (Davidson et al., 1991). After MW and ^{15}N soil labeling, all the pots were sown with wheat variety Gregory in the glasshouse, and

agronomic practices were maintained throughout growing periods. At physiological maturity, the crop was harvested for dry biomass and grain yield measurement, and N accumulation in wheat dry biomass.

7.3.4. Measurement of Nitrogen in Plant Biomass

The ^{15}N atom% abundance in the wheat dry biomass was measured using the Isotope Ratio Mass Spectrometry (Hydra 20–20, SerCon, Crewe, UK). Furthermore, the N concentration in wheat dry biomass was measured by using the micro-Kjeldhal procedure (VELP Scientifica s.r.l., Via Stazionne, 16, USMATE (MB), Italy). The N derived from fertilizer (Ndff%) was calculated by using the following equation (7.1) as described by Salamanca-Jimenez et al. (2017).

$$\text{Ndff (\%)} = \frac{\text{atom\% } ^{15}\text{N excess in plant dry biomass}}{\text{atom\% } ^{15}\text{N excess in fertilizer}} \times 100 \quad (7.1)$$

Furthermore, the partial factor productivity (PPF) is a useful measure of indigenous and applied N (Cassman et al., 1998), because it gives an integrative index of the total economic return relative to consumption of all the nutrients resources in the soil system. The PPF from applied N is the ratio of grain yield (Y) to the quantity of applied N and is given in equation (7.2). This was used to measure the agronomic efficiency of applied N and the indigenous N for grain yield according to following equation (7.2);

$$\text{PPF} = \frac{Y}{N_r} \quad (7.2)$$

Therefore, the grain yield is the total sum of applied (N_r) and indigenous N sources (Soil Organic N). So, the PFP can be explained as equation (7.3);

$$\text{PPF} = \frac{Y_0 + \Delta Y}{N_r} \quad (7.3)$$

Furthermore, the PFP can be described by following equation (7.4);

$$\text{PPF} = \frac{Y_0}{N_r} + \frac{\Delta Y}{N_r} \quad (7.4)$$

Where,

Y is the crop grain yield,

N_r is the applied Nitrogen for crop yield,

Y_0/N_r is the yield without nitrogen application (*i.e.*, *indigenous nitrogen supply for crop yield*),

$\Delta Y/N_r$ is the change in crop yield with nitrogen application (*often called as agronomic efficiency*).

A similar protocol was employed (from sections 7.3.1. to 7.3.4) in the second year to validate the impact of MW soil treatment on wheat yields and N accumulation in dry biomass.

7.3.5. Residual Experiments

After the completion of this first-year experiment, the soil remained undisturbed for 120 days in the same pots at room temperature, and soil moisture content was regularly monitored during this fallow period. The main purpose of this experiment was to evaluate the long-lasting effect of once-off MW soil treatment on crop yields, as MW soil heating assumed to maintain the soil fertility (Zagal, 1989; Yang et al., 1990). The same variety of wheat was sown to all the pots without any further application of MW energy or isotopic-N solution into the soil. However, Monoammonia phosphate (MAP; 150 kg ha⁻¹) was applied to each pot as a basal-dose for wheat production. The final dry biomass yield (g pot⁻¹) and grain yield were measured at crop harvesting. Furthermore, at 85% crop maturity, during this second trial, a small amount (50 g) of soil was sampled from each pot and immediately shifted to a freezer in the Dookie Campus laboratory and stored at -20 °C until further action was taken. After this second season trial, the soil was again preserved undisturbed in the same pots for another 120 days to further check the residual effect of MW energy on crop growth and development of same wheat variety during the third season. For this trial, only the final yield was taken into consideration for treatment effectiveness. For the first residual trial, the days since MW soil heating was 440 and for second trial 740 including 120 days fallow periods.

7.3.6. Nucleic Acid Extraction and qPCR

Soil DNA was extracted from 250 mg sub-sample using Power Soil™ DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) as per the manufacturer's instructions. The Mini Bead beater was used for cell lysis at 3000 rpm for 1 minute. All the DNA extracts were quantified using a Nanodrop™ ND2000c spectrophotometer

(NanoDrop Technologies, Wilmington, DE, USA) within the range of 10 – 115 ng μL^{-1} and the quality was ensured with 1% agarose gel electrophoreses.

The *amoA* functional gene of archaea and bacteria were quantified using a CFX96™ optical qPCR detection system (Bio-Rad, Laboratories Inc., Hercules, CA, USA). Each archaeal-*amoA* qPCR reaction, which was run in triplicate, of 20 μL contained 10 μL SensiFAST (Bio-Rad Laboratories, USA), 1 μL of each primer set (10 μM ; Arch-*amoA*F and Arch-*amoA*R) according to Francis et al. (2005), 2 μL of 10-fold diluted DNA template (10 – 115 ng μL^{-1}) and 6 μL of water. Each bacterial-*amoA* qPCR reaction of 20 μL contained 10 μL iTaq Universal SYBR GREEN Supermix (Bio-Rad Laboratories, USA), 1 μL of each primer set (10 μM ; *amoA*-1F and *amoA*-2R) according to Rotthauwe et al. (1997), 2 μL of 10-fold diluted DNA template (10 – 115 ng μL^{-1}) and 6 μL of water. The thermal cycling conditions for both archaeal and bacterial *amoA* gene quantification were used: 95 °C for 3 min followed by 40 cycles of denaturation at 95 °C for 5 sec, annealing at 60 °C for 30 sec and extension at 72 °C for 45 sec. Standard curves, generated in each qPCR reaction, were linear over six orders of magnitude (10^4 – 10^9 gene copies μL^{-1}) with r^2 values of 0.98 – 0.99. For all qPCR reactions, the efficiency was 85 – 100%.

7.3.7. Statistical Analysis

For all the trials, the obtained data were subjected to normality assessment, and a multiple variant Analysis of Variance was performed using MATLAB R2015b (The Mathworks Inc., Natick Massachusetts, USA) to determine statistically significant means, and a least significant difference (LSD) test was used to compare the treatment means at a 5% probability level. The *amoA* gene copy number data of AOA and AOB was transferred by $\log_{10}$ bases prior to analysis.

7.4. Results

7.4.1. Impact of Microwave Soil Treatment on Wheat Yields

The mean temperature of untreated control soil was 20 °C. Immediately after MW soil treatment, the soil temperature increased within a range of 75 – 85 °C. Therefore, MW soil treatment significantly increased the growth and development of the wheat crop at all the N levels compared to non-irradiated soil in both years of study (ANOVA; $P < 0.01$). In first year, the higher dry biomass yield obtained in MW soil treatment at N_3

(24.9 g pot⁻¹) followed by N₂ (17.6 g pot⁻¹) compared to untreated control soils, while the untreated control soils gave the lowest biomass yield (6.3 g pot⁻¹; Figure 7.1). The lowest biomass yield was measured in non-MW conditions at N₁ and surprisingly the grain yield from the MW treated soil without N applications pots was statistically similar to N₁ and N₂ in non-microwaving soils. This suggests that the contribution of indigenous soil N was considerably higher for biomass production in case of MW soil heating compared to untreated control soils. In second year, the effect of MW soil treatment on biomass production of wheat was significantly (ANOVA; P<0.001; Figure 7.1) higher compare to untreated control. The biomass yield at all the different rates of N application was statistically similar in both scenarios: MW and untreated control pots. The higher biomass yield obtained at N₃ (32.64 g pot⁻¹) in case of MW treated soil while lower biomass yield obtained at N₀ (19.21 g pot⁻¹) in untreated control soils.

Similarly, MW soil heating at up to 75 – 85 °C significantly increased the grain yield of wheat crop over two years of study (ANOVA; P<0.001; Figure 7.2). For the first year, the maximum grain yield (11.0 g pot⁻¹) was obtained at N₃ in MW treated pots compared to untreated control soil. It was interesting to observe that grain yield (5.6 g pot⁻¹) was slightly higher but statistically non-significant in MW treated pots where no N (N₀) was applied, compared to N₂ (4.0 g pot⁻¹) and N₃ (5.2 g pot⁻¹) in untreated soil. The lowest grain yield (2.0 g pot⁻¹) was recorded for the N₁ level in non-MW treated conditions. In the second year, the grain yield was not linearly related to the increase in isotopic N application rates. The N application did not significantly increase the grain yield in untreated control pots (ANOVA; P>0.05). In contrast, the sole effect of MW soil heating significantly increased the grain yield at N₀ (28.89 g pot⁻¹), and this trend was statistically similar to N₁ (28.46 g pot⁻¹) and N₂ (29.72 g pot⁻¹). Overall, the grain yield was higher in second year within all the treatment combination compared to first year (Figure 7.2).

7.4.2. Nitrogen Use Efficiency

The N derived from fertilizer (Ndff%) did not significantly change with MW soil treatment compared to untreated control soil over both years of study (ANOVA; P>0.05; Figure 7.3). In the first year, the maximum Ndff (13%) determined at N₃ in case of untreated control soil while the lowest Ndff achieved at N₁ level in the case of MW treatment (5%). Interestingly, there was no statistically significant difference between

MW treated and untreated control soils at N₂. In second year, the higher Ndff (23%) achieved at N₃ (23%) followed by N₂ in case of untreated control soils while the lowest Ndff (7%) attained at N₁ in case of MW soil treatments, suggesting that MW soil heating might have changed the uptake of plant available N and increased the indigenous N supply for crop accumulation. Furthermore, the total N accumulation in wheat dry biomass did not significantly change by MW soil heating (ANOVA; P=0.08; Figure 7.4). In first year, the overall N accumulation pattern was considerably higher in MW treated soil at N₁ (2.53%) and N₃ (2.70%) compare to untreated control soil. While in second year, the total N accumulation pattern decreased with increasing N rates, the maximum N accumulation observed at N₁ in both of treatments (MW (1.69%) and Untreated Control (1.65%)). In both years, the N % at N₀ from untreated control pots was higher than for MW treated pots (Figure 7.4). In order to understand the effect of soil heating on the yield increase, we tested the agronomic efficiency (i.e., change in crop yield per unit application of N). Furthermore, the higher agronomic efficiency ($\Delta Y N r^{-1}$) of applied N was achieved through MW soil heating (167 g grain g⁻¹ N applied) compared to untreated control soil (86 g grain g⁻¹ N applied) at N₁ in first year study (Table 7.1).

For residual trial (440 days after heating (DAH)), interestingly, the biomass yield from pots where no N was applied in the previous trial (160 DAH) but where soil was treated with MW energy produced the maximum above ground biomass (52 g pot⁻¹; Figure 7.5) and the lowest biomass yield was obtained where higher doses of N were initially applied (24 g pot⁻¹) to untreated control pots. There was a definite decreasing trend in biomass production with increasing amounts of applied N. The final grain yield showed similar patterns in response to MW energy application under all the N levels.

Table 7.1: Comparison of N use efficiency in response to microwave soil treatment under different rates of applied nitrogen. This was measured only for first season crop.

Nitrogen Rate (mg pot ⁻¹) (N _r)	Grain Yield (g pot ⁻¹) (Y)	Agronomic Efficiency (g g ⁻¹) ($\Delta Y N r^{-1}$)	Grain Yield (g pot ⁻¹) (Y)	Agronomic Efficiency (g g ⁻¹) ($\Delta Y N r^{-1}$)
	Control	Control	Microwave	Microwave
0	2.067	–	5.683	–
22.5	4.007	86	5.823	167
45	5.232	70	7.4	118
67.5	8.921	101	11.055	134

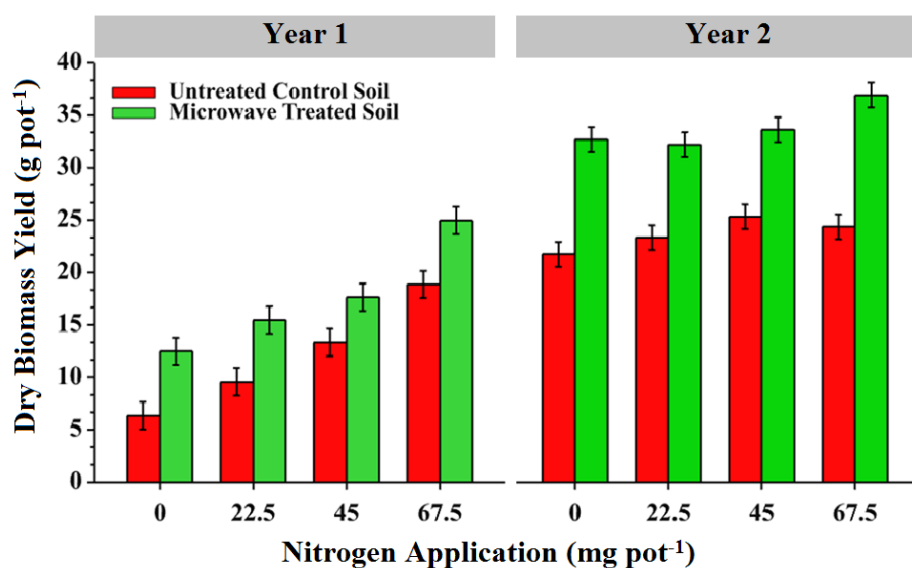


Figure 7.1: Effect of microwave soil treatment on grain yield of wheat crop under various levels of isotopic nitrogen application measured under two years of study.
Note: Error bars indicate a least significant different test at 5% probability level.

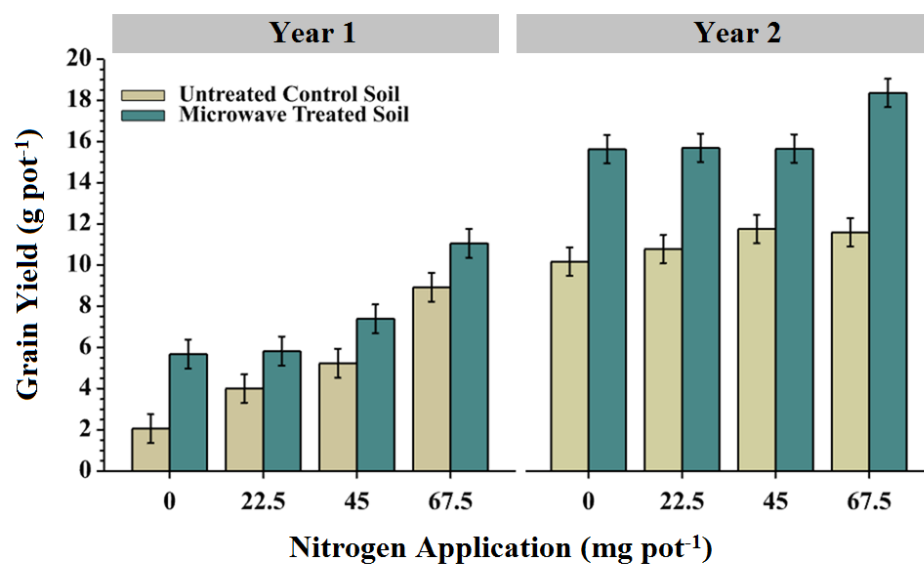


Figure 7.2: Effect of microwave soil treatment on grain yield of wheat crop under various levels of isotopic nitrogen application measured under two years of study.
Note: Error bars indicate a least significant different test at 5% probability level.

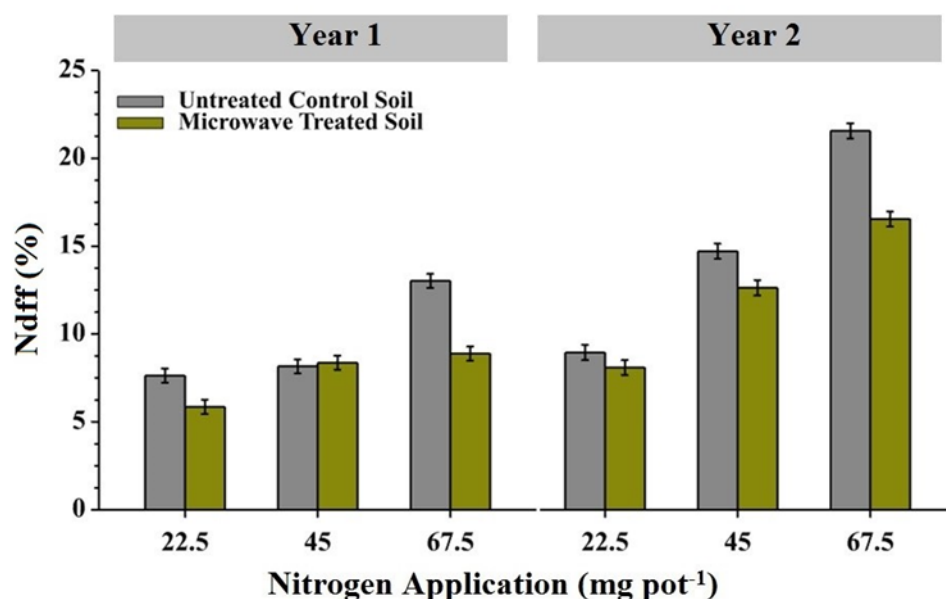


Figure 7.3: Effect of microwave soil treatment on the on the nitrogen derived from fertilizer (Ndff%) under various level of isotopic nitrogen application (¹⁵N) in wheat dry biomass (straw + grain) over two years. Error bars indicate a least significant difference (LSD) at 5% probability level.

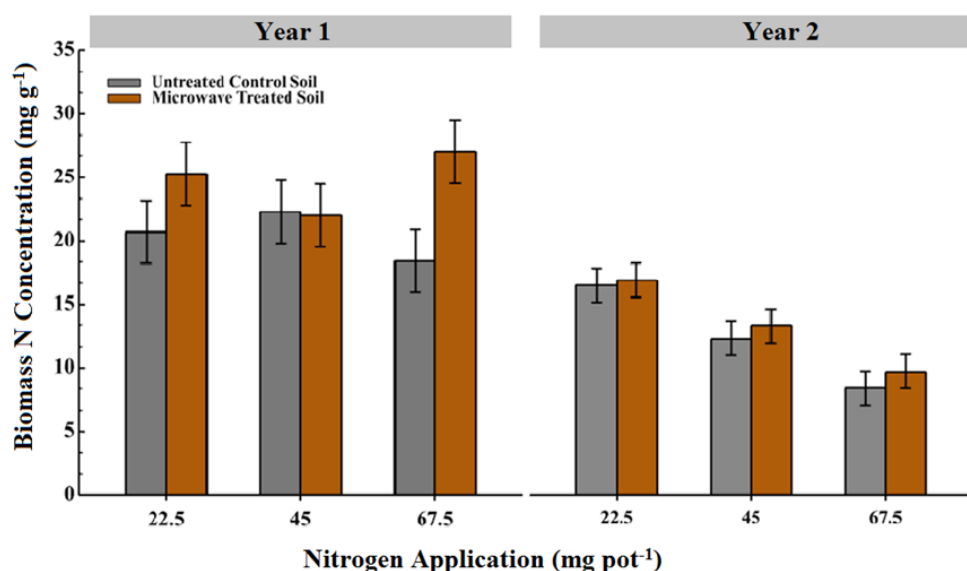


Figure 7.4: Effect of microwave soil treatment on the rate of total nitrogen accumulation (mg g⁻¹; ¹⁴N + ¹⁵N) under various level of isotopic nitrogen application (¹⁵N) in wheat dry biomass (straw + grain) over two years. Error bars indicate a least significant difference (LSD) at 5% probability level.

7.4.1. Residual Impact of Microwave soil treatment on Wheat Yields

The highest grain production (26.7 g pot⁻¹) was measured at N₀ where the soil was treated through MW energy, while the lowest grain yield (11 g pot⁻¹; Figure 7.6) was produced at the higher level of N application in non-MW conditions.

The grain yield was statistically similar under all the rates of N application in the second season from pots that were not treated with MW energy. This sustained-effect remained effective even after 440 days from MW soil exposure. Regardless of N application, the sole effect of MW soil treatment enhanced dry biomass (ANOVA; P<0.009) and grain yield (ANOVA; P<0.001) of the wheat crop even after approximately 740 days from the initial MW soil treatment (Table 1). For this trial, the MAP was applied as a basal-dose; therefore, the effect of the isotopic-N solution may have minimized during the third cropping season. The final dry biomass yield, of MW soil treatment, was about 36.7 g pot⁻¹ compared to the untreated control soil 31.9 g pot⁻¹, while the highest grain yield obtained through MW treatment was 16.6 g pot⁻¹ and lowest from non-MW conditions 12.8 g pot⁻¹. The MW × N interaction was not statistically significant in case of wheat productivity at day 720.

At 440 days, the dry biomass yield was 25% higher in case of MW soil treatment compared to untreated control soil. This trend became statistically indistinguishable at 740 days, suggesting that MW mediated changes in soil has no longer positive impact on crop productivity. It is important to note that, in the second and third residual trial a small amount of N was applied to all the pots to meet the crop N demand for growth and development. It implies that soil heating had positive impact on the soil fertility for the following cropping years.

7.4.2. Functional Gene Abundance

Irrespective of N application, the MW soil treatment did not significantly induce (ANOVA; P>0.05) a negative impact of the total abundance of AOB and AOA (Figure 7.7), which was quantified at 85% crop maturity of the second season trial. This suggests the possible recovery of ammonia oxidizers, after 400 days following MW irradiation, if MW heating initially induced some degree of mortality. Overall, the total abundance of AOB was comparably lower than AOA, suggesting that AOA recovered rapidly following heat disturbance than AOB. The total abundance of AOB *amoA* gene

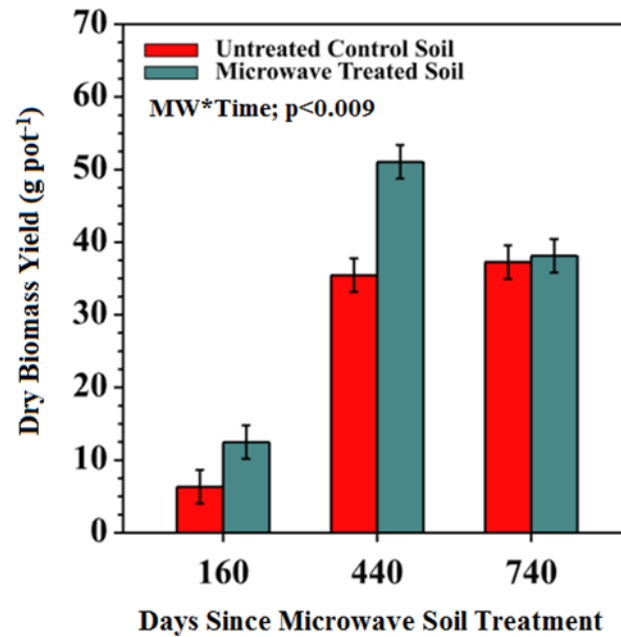


Figure 7.6: Residual impact of microwave soil treatment on the wheat grain yields in the consecutive seasons. The soils exposed to 120 s of microwave energy only at first season (160 days) prior to crop sowing. Error bars indicate the least significant different test at 5% probability level.

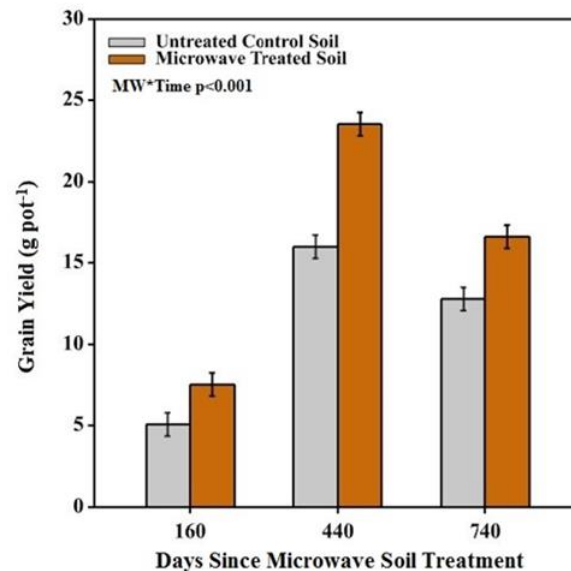


Figure 7.5: Residual impact of once-off microwave soil treatment on the dry biomass yield of the wheat crop in the consecutive seasons. The soils exposed to 120 s of microwave energy only at first season (160 days) prior to crop sowing. Error bars indicate the least significant different test ay 5% probability level.

copies g⁻¹ soil was 10⁵ while AOA total abundance varied between 10⁵ – 10⁶ *amoA* gene copies g⁻¹ soil.

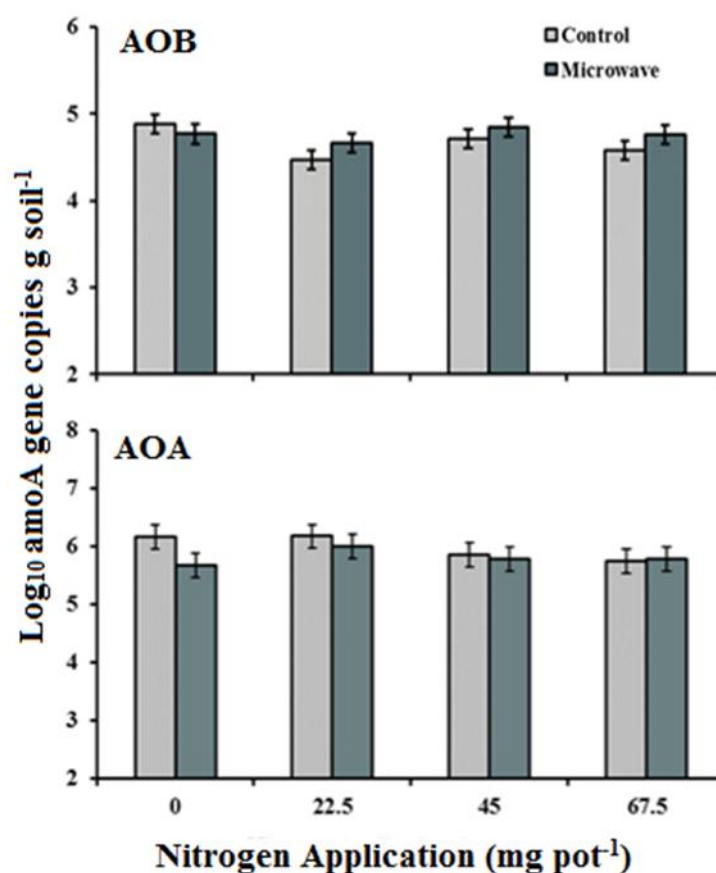


Figure 7.7: Abundance of ammonia oxidizing bacteria (AOB; above) and archaea (AOA; below) after 400 days of microwave soil treatment (soil samples was collected at 85% physiological crop maturity) under various levels of isotopic nitrogen application. Note: *amoA* gene copy number are plotted on a log₁₀ scale. Error bars indicate a least significant different test at 5% probability level.

7.5. Discussion

7.5.1. Crop Productivity and Nitrogen Accumulation in response to Microwave Soil Heating

The application of MW energy rapidly increased the soil temperature with an average range of 75 – 85 °C. This temperature affects the soil physico-chemical properties; however, the effects depend on peak temperature and duration. In this study,

the sole effect of MW soil treatment (Figure 7.6) consistently sustained the wheat crop yield for up-to three cropping seasons regardless of N application.

This increased yield response is highly supported with the finding of Gibson et al. (1988), who reported that shoot and root growth of birch (*Betula pendula* L.) was significantly increased in the soil that was treated with MW energy. In Gibson's experiment, the highest dry shoot weight of 84 mg was achieved for MW irradiation of soil for 120 s without any mycorrhizal supplementation compared to the non-irradiated soil (25 mg). The higher yield trend through pre-sowing MW soil treatment under field conditions was also reported elsewhere (Khan et al., 2017a).

Independent of heating methodology, these results are also consistent with Gay et al. (2010b), who reported that increase in soil temperature (80 °C) through steam application significantly increased the fresh biomass of lettuce at harvesting. The long-lasting effect of MW soil treatment on crop growth may be due to MW induced degradation of soil organic matter. This may have favored the soil fertility for a longer time. MW irradiation of soil has been demonstrated to increase the humification of SOM (Kim and Kim, 2013). Ample SOM may enhance the N immobilization-mineralization process *in situ*. So, this persistent effect of soil heating through MW soil treatment could reduce the amount of fertilizer needed in the cropping system, not only for the first crop but in subsequent crops in next two seasons.

In case of MW soil treatment, the grain yield supported from indigenous N supply ($Y_0 N r^{-1}$) was 171.8% higher compared to the untreated control soil in the first cropping season. Interestingly; in the second cropping season the $Y_0 N r^{-1}$ was two orders of magnitude higher than in the first season. The MW treatment of soil also exhibited a significant increase in the grain yield, where no N application was done. The possible reason for this may be higher N mineralization (indigenous soil N – this includes the biological N fixation, N mineralization from SOM, release of non-exchangeable NH_4 , residual leftover N from crop residues) and enhanced microbial activity during the fallow period as MW soil treatment has some influence on NH_4^+ -N mineralization (Zagal, 1989). Furthermore, the higher total N accumulation ($mg\ g^{-1}$) in wheat dry biomass in both seasons gave a direct indication of ample indigenous N supply for crop growth and development in response to MW soil heating. This response has been clearly reported by Cassman et al. (1998).

In addition, plants are more likely to accumulate the highly available nutrient concentration from the soil (Cassman et al., 1998). In Contrast, the N derived from fertilizer (Ndff%) was substantially higher in wheat dry biomass with an average range of 7 – 13% from untreated control pots compared to MW treated soil (5 – 8%). This suggests that the dependency of wheat growth was on applied N in the untreated pots. However, this can be explained by two most likely responses: (i) a dilution effect (Jarrell and Beverly, 1981), where plants produce higher yield but less accumulation of applied nutrient in dry matter, as in the case of MW soil heating; and (ii) pool substitution (Jenkinson et al., 1985), where the labelled N stands proxy for the unlabeled soil N pool via various displacement reactions. Considering the displacement reactions of added labelled N, MW energy may have induced the ammonia fixation and mineralization displacement reactions, and therefore minimized the uptake and recovery of labelled N in wheat dry biomass in the present study.

In general, the behavior of soil organic N within the soil treated through MW (non-ionizing) energy is still poorly understood. However, few studies had reported some controversial findings. Relevant to this, Alpehi and Scheu (1993) reported the survival of soil microorganisms; a higher concentration of $\text{NH}_4^+\text{-N}$, and phosphorus were released after 120 days of MW soil treatment. In another study, Speir et al. (1986) examined the effect of MW energy on low fertility soil (100 randomly selected cores at a depth of 50 mm), microbial biomass, N, phosphorus and phosphatase activity. They reported that an increase in MW treatment duration (90 s) dramatically increased the N level ($106 \mu\text{g N g}^{-1}$ soils), but the phosphorus concentration decreased relative to increasing MW treatment time. In addition, the increase in soil C and N mineralization (Zagal, 1989), $\text{NH}_4^+\text{-N}$ mineralization and sulphur oxidation was reported by Wainwright et al. (1980). In the light of all these findings, we argued that the rapid increase in soil temperature may have induced some degrading effects on the recalcitrant organic N pool and hydrolysable amino acid, which could have become a part of the liable organic-N pool in MW treated soil and subsequently plant available forms. This higher N concentration might have led to higher N uptake and accumulation in wheat dry biomass. Moreover, the grain yield is closely related to N accumulation in plant biomass in a curvilinear fashion (Cassman et al., 1998). In both years trial, our results showed a similar relationship of grain yield and aboveground N accumulation at crop maturity (Figure 7.8).

Other factors leading to higher dry matter production are chlorophyll content, and higher photosynthetic activity and these two physiological parameters are closely related to each other. The maximum chlorophyll content with an average of range 58 – 64 was measured from the MW treated pots compared to untreated control soil with an average range of 42 – 56. Numerous studies had reported the leaf N status is associated with chlorophyll content (Peng et al., 1995; Peng et al., 1996; Cassman et al., 1998). In addition, the MW soil treatment increases the photosynthetic rate of crops (Unpublished data of Khan, M. J. and Brodie, G.), plants with highest photosynthetic rate ($18.73 \mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; $n=200$) were in MW treated pots compared to untreated control soil ($10.19 \mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) measured using an infrared gas analyser (LICOR-64; LI-COR Inc; Lincoln, Nebraska, USA). This considerable increase in photosynthetic activity clearly justifies the higher dry matter and grain yield in MW treated soil, because the plant N accumulation is tightly correlated to photosynthetic rate (Peng et al., 1995). This might have increased the high dry biomass production in the present study.

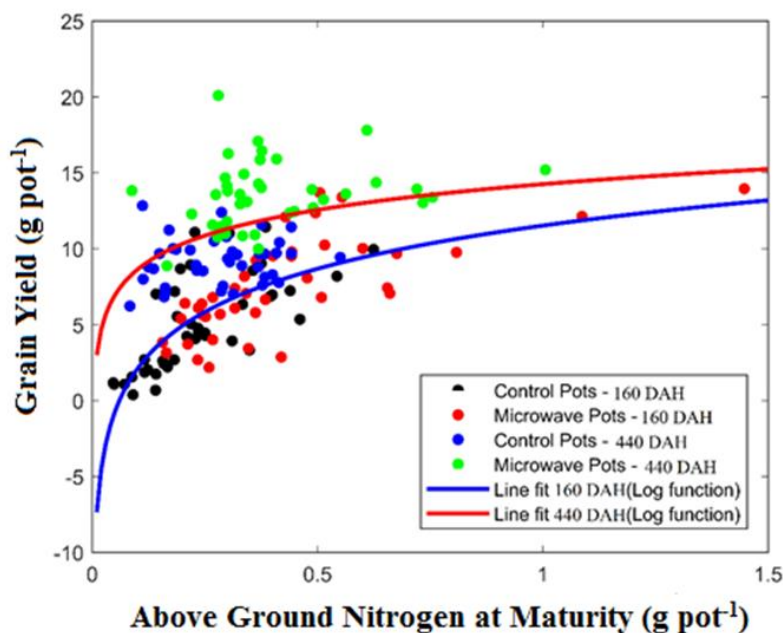


Figure 7.8: Relationship between grain yield and aboveground N accumulation at maturity in response to microwave soil treatment of at 160 and 440 days after microwave soil treatment.

7.5.2. Effect of Soil Heating on the Ammonia Oxidizers

In general, soil heating is detrimental to microorganisms (O'Brien et al., 2018). However, irrespective of heating methodology, the heat transfer mechanisms have a significant influence on the fate of microorganisms *in situ*. Relevant to N cycling microbes, inorganic-N availability depends on the mineralization and nitrification processes in-situ. Therefore, in this study, the resistance and resilience of AOB and AOA, based on the *amoA* gene abundance, in MW heated soil gave a preliminary indication that there is little to no detrimental effect of heating on the beneficial soil biota. Various factors limit the activity of ammonia oxidizers, among them, soil temperature and pH have considerable influence. In contrast, the higher availability of $\text{NH}_4^+\text{-N}$ considerably favors the population dynamics and activity of AOB over AOA (Banning et al., 2015). As mentioned earlier, the higher N accumulation in wheat dry biomass gives a reasonable estimate of indigenous N supply ($\text{NH}_4^+\text{-N}$), however, this higher substrate availability may have potentially favoured the activity of AOB as mentioned by Hink et al. (2018), as most of the studies reported that nitrification dominantly carried out by AOB in semi-arid agricultural soils than AOA (Banning et al., 2015). Therefore, these results are highly consistent with the work done by Vela et al. (1976), who reported that nitrifying bacteria were resistant to at least $40,000 \text{ J cm}^{-2}$ of MW energy applied to the soil surface. The energy applied to soil in this study was comparably less than Vela used.

Moreover, (Brodie et al., 2015b) reported the rapid recovery of soil bacterial population (culture dependently) within 35 days after MW soil treatment; however, we tested their community 400 days after MW soil treatment. In contrast, independent of heating methodology, soil steaming with the temperature gradient of about $80 - 100^\circ\text{C}$ immediately induced a 10-fold reduction in the population of AOB in an organic farming soil and recovery happened 60 days after soil steaming (Roux-Michollet et al., 2008). Furthermore, the soil heating at up to 80°C can change the bacterial community composition, and potentially favors heat-resistant tolerant species during recovery period (Voort et al., 2016). In general, the recovery of microorganisms is dependent on the soil conditions after heating, particularly the amount of SOM and moisture (O'Brien et al., 2018). As MW soil heating has potential to increase the soil carbon and N availability (Zagal, 1989). This could explain the survival and/or recovery of AOA and AOB in this study. Also, these preliminary results demonstrate that the MW soil heating ($75 - 80^\circ\text{C}$) can potentially be used for its primary application with minimal reduction in the

population of AOB and AOA. They also predict that the community size of ammonia-oxidisers in response to MW application may favor the fertilizers nitrogen use efficiency in cropping systems and can minimize the fertilizers application. Therefore, these preliminary findings have motivated that the further study of the effect of MW soil heating on the species composition of ammonia oxidizers, their functionality (nitrification rate), and why they seem to be resistance to MW heating is thus needed.

7.6. Conclusions

Based on the result of this study, we conclude that the MW soil heating can potentially enhance the indigenous N availability in the agriculture system, substantial increase and sustain the wheat yields for up to two cropping seasons in addition to weed management, which is the primary application of this technology. In addition, to avoid nutrient depletion, farmers need to apply a reduced dose of N fertilizer to MW treated soils in the following growing season. However, a further refinement of this new technology is needed to better understand the heating effect on the indigenous N supply for crop growth and the activity of N cycling microbes. Based on these preliminary results, the resistance of AOB to short-term heating suggests further research is needed to check the community composition and function (potential nitrification rate) with respect to sufficient temporal monitoring. It is conceivable that the temperature distribution into soil particles can alter the soil matrix and ultimately harbors the surviving species into newly available niches. From a practical perspective of this weed management technology, it can be inferred that the damaged community recovers to its pre-heating condition quickly following soil heating, and MW soil treatment can potentially be applied for weed control. Therefore, this assumption about how MW soil heating changes the overall bacterial community compositions and the total abundance of ammonia oxidisers needs further investigation.

7.7. Supplementary Material

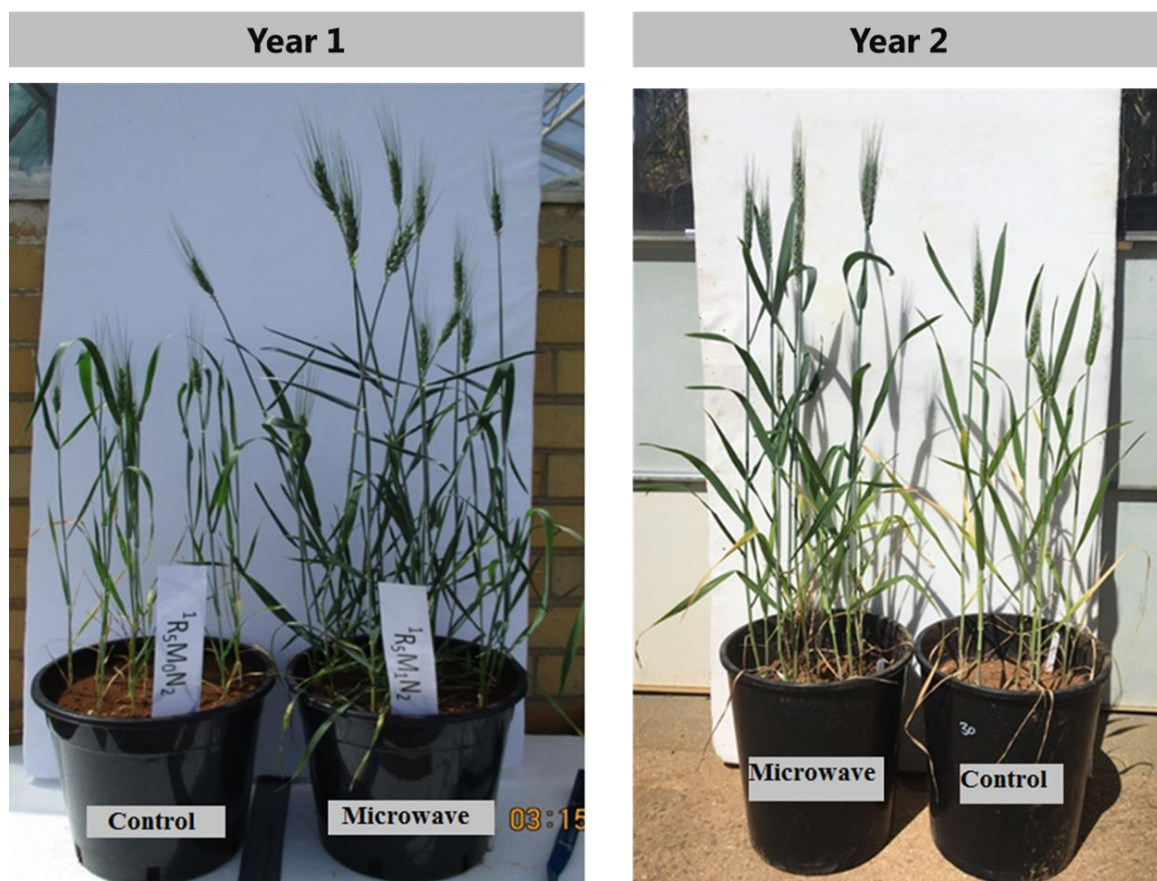


Figure 7. S1: Pictorial comparison of the impact of microwave treated and untreated control soil on the wheat growth and development over two years of studies in glasshouse. In both years of study, the higher number of fertile tillers, spike length, expanded dark green flag leaves from microwave treated pots are indicative of increased soil fertility following microwave soil treatment.

Residual Trial



Figure 7. S2: Pictorial depiction of the impact of microwave treated (left pot) and untreated control soil (right pot) on the wheat growth and development at 440 days after microwave soil treatment. The expanded dark green flag leaves and number of fertile tillers from microwave treated pots clearly reflect the changes in soil fertility following soil heating. Also, at this stage soil samples were collected to evaluate the total abundance of ammonia oxidizing bacteria and archaea in response to microwave soil heating for 70 – 80 °C.

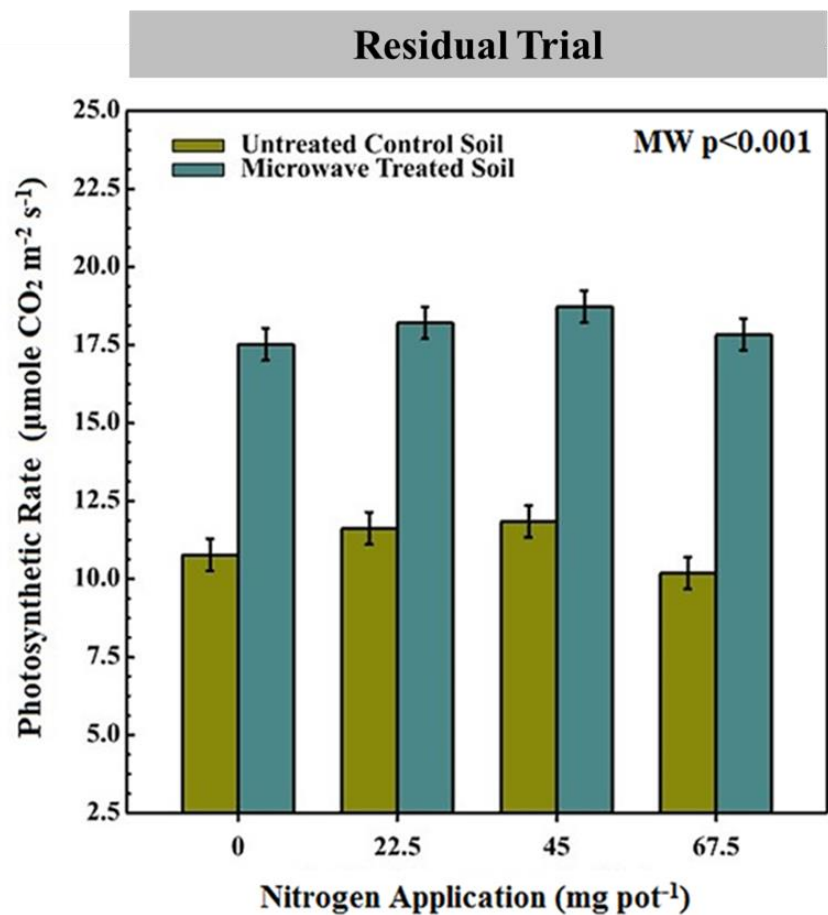


Figure 7. S3: Impact of microwave soil treatment on the photosynthetic rate of wheat crop. Note: Error bars represent the least significant difference test at 5% probability level.

Chapter 8 – Impact of Microwave Disinfestation Treatments on Bacterial Communities of No-till Agricultural Soils

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8.1. Abstract

Sustainable weed management demands new non-chemical technologies. Considering this, pre-sowing microwave (MW) soil heating potentially reduces weed establishment and substantially increases crop. However, the effect of this technology on the total soil microbial community in general, and on beneficial soil microbes such as ammonia oxidizers in particular, warrants further study. In order to check the effect of MW soil disinfestation treatment on the soil biota, indigenous soil microcosms were treated under the horn antenna of the MW disinfestation prototype for three distinct durations. Immediately after heating (T0), and 28 days after heating (T28) the soil was collected at two depths to determine the bacterial community responses based on the 16S rRNA amplicon sequencing and the total abundance of bacterial and ammonia oxidisers with qPCR. Bacterial community compositions differed according to treatment durations and fell into two categories: no effect at low heating intensities (0 and 30 s, 17 – 45 °C); and strong effect at high heating intensities (60 and 90 s, 65 – 78 °C). At T0 with high heating intensities, Proteobacteria decreased and Firmicutes increased significantly relative to low intensity treatment. At T28 with high intensities, Actinobacteria remained unchanged, Proteobacteria decreased and Firmicutes increased relative to low intensity treatment. The relative abundance of *Micromonosporaceae*, *Kaistobacter* and *Bacillus* increased significantly with high heating intensities and they remained dominant overtime. We did not detect any negative impact of MW soil heating intensities on the

total abundance of ammonia oxidisers overtime. Overall, ammonia oxidising bacteria (AOB) were more predominant than archaea (AOA). MW soil heating shaped the bacterial communities without any detected effect on the ammonia oxidisers. However, the bacterial community did not recover to their pre-heating conditions within the period studied. In summary, it is predictable that MW soil treatment can potentially be used for its primary application without damaging the soil beneficial bacterial communities.

8.2. Introduction

Modern agriculture is shifting towards sustainable practices for food production (Pretty and Bharucha, 2014). Among these practices, the development and adaption of non-chemical weed control techniques is one of the most pressing areas in need of development, due to the evolution of herbicide-resistant weeds (Heap, 2016). Microwave (MW) soil heating for weed management has effectively been proposed as an alternative to chemicals in the agriculture industry (Nelson, 1996; Sartorato et al., 2006; Brodie et al., 2009; Khan et al., 2017a). The phenomena behind the dielectric heating is that the electromagnetic field induces polarization of dipolar molecule, which results in the rapid increase of the temperature of the dielectric material due to internal resistance (Metaxas and Meredith, 1983; Diprose et al., 1984). In case of pre-emergence weed management, this rapidly increased temperature ultimately induces the devitalization of weed seedbank by generating micro-steam explosion in the seeds for mortality in top soil horizons and substantially preventing the emergence of weeds (Khan et al., 2017a; Khan et al., 2018). This characteristic of MW technology distinguishes from the numerous classical thermal methodologies, for example: soil steaming and soil solarization, for pest management. We have previously designed and developed a prototype to harness this property of MW energy by irradiating agricultural fields between crop rows for weed management (Brodie et al., 2018). This prototype has been successfully employed to minimize the regeneration capacity of the soil weed seedbanks. Recently, numerous field trials demonstrated that pre-sowing MW soil heating (75 – 80 °C; in top 0 – 8 cm) significantly reduced the weed establishment via seedbank devitalization in different cropping regimes of the Australia (Khan et al., 2017a; Brodie et al., 2018; Khan et al., 2018). The effects of this technology on soil microorganisms, which are important to plant productivity (Berendsen et al., 2012), is unknown. Soil is a complex heterogeneous environment (Lu et al., 2014),

harbouring a wealth of bacteria fungi, protozoans, nematodes, and arthropods which may be adversely affected by the MW weed disinfestation treatment.

Bacteria comprise the majority of the soil biota by biomass (Sánchez-Marañón et al., 2017). In general, soil heating creates a transient disturbance for soil bacterial communities, and may affect their composition and function (O'Brien et al., 2018). However, it is possible that the rapid growth and extreme diversity found in these communities' results in a rapid recovery of both community structure and function, resulting in no long-term negative effects. However, it is likely that the long-term effects of heating depend on the heating intensity. For example, Nunes et al. (2018) evaluated the tolerance range of a soil bacterial community exposed to different MW heating durations and reported a 90% reduction in total bacterial communities with 6.8 minutes of MW soil treatment. Additionally, they found tolerance to MW to be phylogenetically constrained: Actinobacteria were highly susceptible to heating, Proteobacteria showed intermediate tolerance, and Firmicutes exhibited the highest resistance. In another work, Jurburg et al. (2017a) demonstrated that in response to a 90 s MW treatment, the total potentially active bacterial community declined slightly, but the bacterial community underwent a succession of community shifts, approaching recovery 29 days after the heat disturbance.

Numerous studies have shown that pre-sowing MW soil heating for weed seedbank devitalization also increases crop growth and development either by reducing weed-crop competition for resources (Chauhan et al., 2017), or by altering the availability of yield-limiting nutrients (Gibson et al., 1988; Brodie et al., 2017; Khan et al., 2017a). However, the exact mechanism behind this phenomenon is still in question. The most likely explanation is that transient heat mediates the mineralization of organic nitrogen into ammonia (HN_3) (Zagal, 1989; Glass et al., 2008), and is subsequently converted to nitrate (NO_3^-) via nitrification, which could benefit crop productivity. In soil, the conversion of ammonia to nitrite, an intermediate step in nitrification, is performed exclusively by two small, phylogenetically-conserved groups containing the ammonia monooxygenase (*amoA*) gene: ammonia oxidizing Archaea (AOA) and Bacteria (AOB; Banning et al., 2015). The tolerance range and rate of recovery of ammonia oxidizers to MW treatment is unknown, but may be crucial to the maintenance of soil fertility following MW weed disinfestation. Previous studies have reported ammonia oxidizing

microbes to be a slow-growing and sensitive functional group (Leininger et al., 2006; Banning et al., 2015), suggesting that they could indeed be negatively affected by the MW treatment.

In order to fully understand the potential effects of the MW weed disinfection treatment and its role in shaping the soil microbiota, we exposed soil microcosms to the MW weed disinfection treatment at three MW durations and monitored the bacterial community composition (16S rRNA amplicon sequencing and 16S rRNA qPCR) as well as the total abundances of AOA's and AOB's (qPCR). We sampled the microcosms immediately after treatment (T0) and 28 days later (T28). Because the treatments were expected to be more extreme on the upper soil strata, we sampled the soil at 5 cm and 10 cm separately to evaluate the effectiveness of the MW treatment in regard to penetration depth for disinfection. We hypothesized that MW soil treatment would alter bacterial community based on taxa's temperature sensitivities proposed for weed seed depletion, and the damaged community would recover to its initial level within four weeks following heat disturbance. Additionally, we further hypothesized that the physiological adaptation of ammonia oxidisers to extreme events might help them to survive against this transient heat disturbance.

8.3. Materials and Methods

8.3.1. Soil Collection and Microwave Heating:

Soil was collected from a wheat paddock on temperate agricultural land at the Dookie Campus (36° 37' S; 145° 70' E), at The University of Melbourne, Australia. This region is the country's central grain growing belt, featuring a monocropping pattern with wheat-canola rotation in the Goulburn Valley, North Victoria, Australia. The mean annual rainfall is 550 mm and mean annual temperature ranges from 9.4 – 20.9 °C (Australian Bureau of Meteorology). The average load of inorganic nitrogen fertilizer for crop production in this region is 100 – 120 kg ha⁻¹ and herbicide application is the most common weed management practices. Soil at the sampling site was a brownish grey loam and was classified as Red Mesotrophic-Haplic Dermosol according to Australian Soil Classification (Isbell, 2016), with a field capacity of ≈53%. Soils were collected with cores (2 cm diameter; 0 – 10 cm), sieved (4-mm mesh size) and mixed thoroughly to

produce a composite sample and shifted to laboratory for microcosm establishment. Water content was measured by oven drying the soil at 105 °C for 24 h, for gravimetric moisture content measurement, and adjusted, with sterile distilled water, to 20% volumetric water content ($\approx$ 45% field capacity). Soil was placed in plastic microcosms (5×5×10 cm) and pre-incubated at 25 °C for three weeks in an incubator (Contherm Scientific Ltd.; Lower Hutt, New Zealand). To maintain the aerobic conditions, all the microcosms were aerated by opening lids every 3 days and moisture loss was maintained. Details of the soil properties are given in Table: 8. S1 (Supplementary Material).

Microcosms were treated under one of four open-structured horn antennas (5.5×11×36 cm) of a 2-kW MW system. This experimental system was designed to apply MW energy for pre- and post-emergence weed control between crop rows, with an inter-antennae distance of 19.5 cm. It has four independently controlled 2-kW MW generators, which are powered through two on-board 7 kVA 3-phase electrical generators (Supplementary Material; Figure 8.S1; Brodie et al., 2017). Microcosms were subjected to three treatment durations (30, 60 and 90 s) and sampled at two points following treatment (0 and 28 days) in quadruplicate. Irrespective of cropping systems, previous field trials demonstrated that MW soil treatment for 60 and 90 s was enough to get the necessary temperature for killing weed seedbanks (75 – 80 °C; Brodie et al., 2018), and these durations were thus selected for further study. A 30 s duration was selected as an intermediate treatment.

Thermal images were taken immediately (2 – 5 s) after soil heating by using the infrared thermal camera (C2; FLIR Systems Inc.; Wilsonville, OR, USA). The raw images of each MW treatment were post-processed in the MATLAB (The Mathworks Inc., Natick MA, USA) to evaluate the temperature distribution in microcosm. At each sampling point, a small core (1.5 cm in diameter) was collected at 0 – 5 cm and 5 – 10 cm depth from each replicate. For soil collection at T0, a small corer (1.5 cm in diameter) was used to evacuate the soil column at each depth. Immediately after sampling, to avoid the disturbance in soil profile, the corer positioned into microcosm exactly where the sampling occurred until the final sampling happened at T28. This final sampling was done with separate, but same sized, corer from both depths. The samples were immediately transferred to the laboratory and stored at – 20 °C.

8.3.2. DNA Extraction and qPCR

DNA was extracted from 0.25 g of soil with the Power Soil™ DNA Isolation Kit according to the manufacturer's instructions (MoBio Laboratories Inc., Carlsbad, CA, USA). DNA extracts were quantified using a Nanodrop™ ND2000c spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and their concentrations ranged between 5 – 20 ng μL^{-1} .

qPCR was used to quantify the absolute abundances of archaeal and bacterial *amoA* genes, as well as the total bacterial abundances. All qPCR reactions were performed in a CFX384™ optical qPCR detection system (Bio-Rad, Laboratories Inc.; Hercules, CA, USA), and each sample was measured in triplicate. The reaction for quantification of the archaeal-*amoA* gene contained 10 μL SensiFAST (Bio-Rad Laboratories, USA), 1 μL of each primer set according to Francis et al. (2005; 10 μM ; Arch-*amoA*F and Arch-*amoA*R), 2 μL of 10-fold diluted DNA template and 6 μL of water. Each bacterial-*amoA* qPCR reaction contained 10 μL iTaq Universal SYBR GREEN Supermix (Bio-Rad Laboratories, USA), 1 μL of each primer set according to Rotthauwe et al. (1997; 10 μM ; *amoA*-1F and *amoA*-2R), 2 μL of 10-fold diluted DNA template and 6 μL of water. The thermal cycling conditions for both archaeal and bacterial *amoA* gene quantifications were as follows: 95 °C for 3 min then 40 cycles of 95 °C for 5 s, 60 °C for 30 s and 72 °C for 45 s. Standard curves, generated in each qPCR reaction by using plasmids, were linear over six order of magnitude ($10^4 - 10^9$ gene copies μL^{-1}) with r^2 values of 0.98 – 0.99; efficiency was 85 – 100%. For the quantification of total bacteria, we quantified copies of 16S rRNA. Each 20 μL qPCR reaction consisted of 10 μL iTaq Universal SYBR GREEN Supermix (Bio-Rad Laboratories, USA), 1 μL of each primer set according to Suzuki et al. (2000; 10 μM ; 1369F and 1492R), 2 μL of 10-fold diluted DNA template and 6 μL of water. PCR thermal cycling conditions for 16S rRNA gene amplification were as follows: 95 °C for 10 min; followed by 40 cycles of denaturation at 95 °C for 15 s, annealing happened at 54 °C for 30 s, and extension at 72 °C for 30 s. Standard curves were generated for each qPCR reaction, which were linear over six orders of magnitude ($10^4 - 10^9$ gene copies μL^{-1}), with r^2 of 0.97 – 0.99 and efficiency of 80 – 100%.

8.3.3. 16S rRNA Sequencing

16S rRNA amplicon sequences were used to estimate the relative abundances within the soil bacterial community. Two samples per condition were sequenced. The V3-V4 regions of the 16S rRNA gene was amplified using primers 341F and 806R, as previously described (Yu et al., 2005). Paired-end sequencing was performed in an Illumina MiSeq sequencer (San Diego, CA, USA). Paired-end reads were merged with PEAR (v. 0.9.5; Zhang et al., 2013) and primers were trimmed. Reads were quality-filtered, full-length duplicates were removed, and chimeras were filtered against the “rdp_gold” database using USEARCH (v. 8.0.1623; Edgar, 2010; Edgar et al., 2011) and UPARSE (Edgar et al., 2011). Singletons were removed. Reads were clustered at 97% identity and assigned with the Greengenes database (Version 13_8, Aug 2013; DeSantis et al., 2006) in QIIME (v.1.8; Caporaso et al., 2010).

8.3.4. Statistical Analysis

All statistical analyses were performed in the R 3.4.0 software (Team, 2014) using the *phyloseq* (McMurdie and Holmes, 2013) and *vegan* (Oksanen et al., 2008) packages. qPCR data was transformed to $\log_{10}$ prior to analyses. Differences between gene copy numbers for AOA, AOB, and total 16S data were compared between treatments using ANOVA. Specific measurements are presented as *mean*±*standard deviation*.

Prior to the analysis of amplicon sequencing data, reads were randomly subsampled to 30 745 reads for sample (Supplementary Material; Figure 8. S3). The final dataset contained 6 154 prokaryotic OTUs. Alpha diversity was evaluated using Shannon’s diversity index, and differences between durations and time were tested with a two-way ANOVA, and Tukey post-hoc test was performed to determine changes in response to MW soil heating intensities. Beta diversity was evaluated with a principal coordinate analysis (PCoA) of Bray-Curtis distances. Clustering patterns of the resulting ordination were assessed using *adonis* and *betadisper* from the *vegan* package. For genus-level analyses, taxa were agglomerated by genus, and individual ANOVAs for the effect of time and MW duration were performed for each genus. Phyla and Genera for which $P < 0.001$ were selected, in order to detect the changes in relative abundance in response to combine effect of MW × penetration depth × sampling time (T0 and T28).

8.4. Results

8.4.1. Temperature Distribution in Soil

The mean temperature of the untreated control soil was 20 ± 2 °C (Figure 8.1A). Immediately after heating for 30, 60 and 90 s, soils exhibited sharp increases in temperature to 45.1 ± 2.1 , 64.3 ± 2.8 and 78.3 ± 2.1 °C, respectively, confirmed by infrared thermal images of the microcosm and liquid-in thermometer (Figure 8.1B-D). The peak temperature for treatment duration was retained for ≤ 20 s in the soils following heat disturbance. A cross-sectional infrared thermal image of the microcosms indicated that the heating intensity gradually decreased along penetration depth (Supplementary material; Figure 8. S2), and we categorized 0 – 5 cm depth as a high heating zone and 5 – 10 cm depth as a moderate heating zone based on temperature profile. Despite this, we detected a slightly higher reflection of MW energy in the moderate heating zone than in the high heating zone.

8.4.2. Bacterial Community Composition

We performed 16S rRNA amplicon sequencing in order to observe the effect of MW heating on the bacterial community. A visual inspection of the PCoA of Bray-Curtis distances between samples, revealed clustering of samples exposed to 0 – 30 s MW, and samples exposed to 60 – 90 s MW (Figure 8.2). The first axis represented 53.6% variation in community composition, and the second axis represented 13.8% variation. At T0, the soil exposed to 30 s MW soil heating treatment did not induce a significant change in the community composition and clustered closely to control samples, regardless of penetration depths. A similar pattern was found at T28. In contrast, microcosms exposed to 60 – 90 s MW clustered separately from untreated controls soils. Therefore, we categorized these durations into two groups: low-intensity (0 – 30 s) and high intensity (60 – 90 s) treatments. Community composition significantly clustered according to these MW heating intensity categories (*adonis*, pseudo-F = 26.3, $R^2 = 0.28$; $p < 0.001$), days after heating (*adonis*, pseudo-F = 17.7, $R^2 = 0.18$; $p < 0.001$), and the interaction of MW intensities $\times$ days after heating (*adonis*, pseudo-F = 14.56, $R^2 = 0.15$; $p < 0.001$) on the bacterial community composition (Figure 8.2). Community composition varied with penetration depth (*adonis*, pseudo-F = 3.22, $R^2 = 0.03$, $p = 0.034$; Figure 8.2). Further

explanation of bacterial community responses to soil heating is presented in Figure 8. S5. (Supplementary Material).

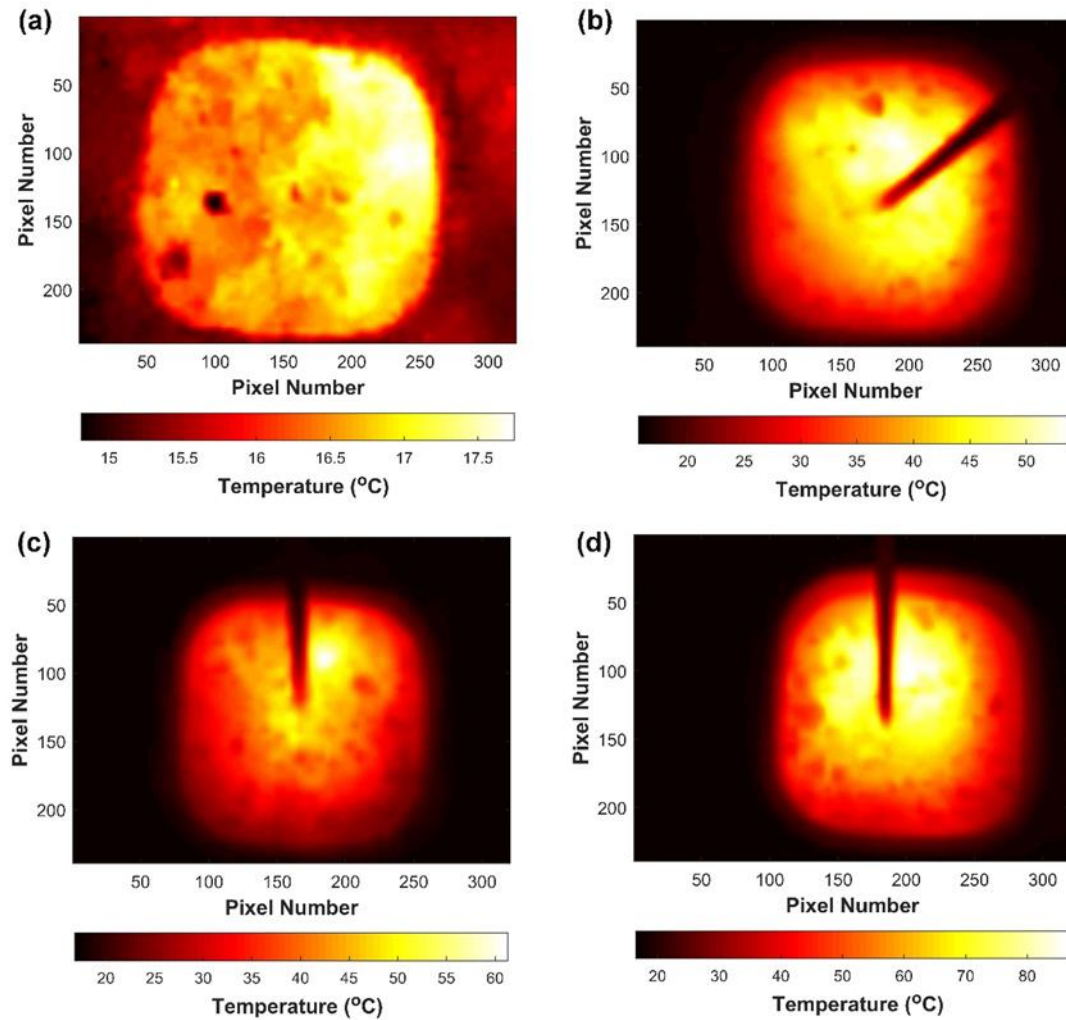


Figure 8.1: Infrared thermal images of microcosm treated through microwave energy under horn antenna of microwave weed killer depicting the gradual increase in soil temperature. Note: the untreated control soil (0 s; A), 30 s exposure (B), 60 s exposure (C) and 90 s exposure (D). Image was captured within 2 – 3 s after microwave soil heating and temperature loss during this time was negligible. The raw images were post-processed in MATLAB for temperature forecasting.

8.4.3. Bacterial Community Richness

High MW soil heating intensities significantly reduced bacterial community diversity relative to the low intensity treatments, evidenced by decreasing Shannon richness index (ANOVA; $p < 0.001$; Figure 8.3). At T0, the low heating intensities did not reduce the species richness significantly, while the high heating intensities greatly reduced the community richness relative to untreated soils for both depths 0 – 5 and 5 – 10 cm. The reduction in the richness with high intensity MW was still detected 28 days after exposure (Tukey post-hoc test; $p < 0.0001$ for comparison among low and high intensities at T28 for 0 – 5 cm and 5 – 10 cm depths). Additionally, the combination of MW heating intensity $\times$ days after heating had a significant impact on the community richness (ANOVA; $p < 0.001$; Figure 8.3). Immediately after heating (T0), the community diversity did not vary considerably with low heating intensity while the high intensities induced a 58% reduction in the richness (see Figure 8. S4; Supplementary Material).

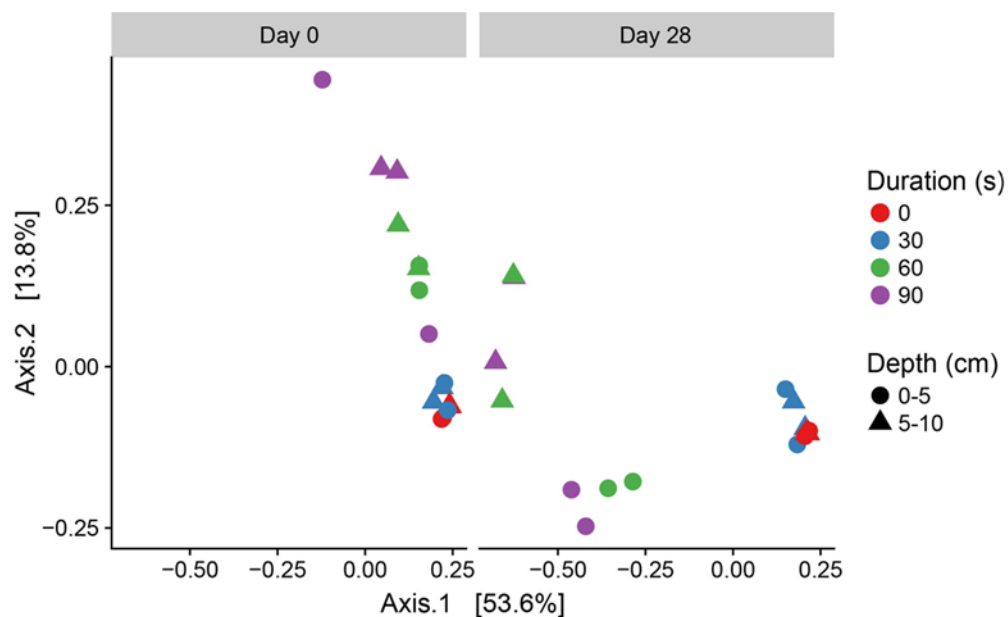


Figure 8.2: Principle coordinate analyses of Bray-Curtis dissimilarity index-based variation in the community composition with microwave soil heating treatment over two-time points.

In contrast, at T28, a considerable reduction in the community richness was observed with higher MW intensities at lower depths (5 – 10 cm) relative to low intensities (Figure 3). Notably, from T0 – T28, the low intensity heating did not significantly alter the bacterial diversity (Tukey post-hoc test; $p>0.55$). Further, a variation in community diversity along penetration depths was moderately significant for both tested heating intensities (ANOVA; $p<0.02$; Figure 8.3). At 0 – 5 cm depth for T0, the high intensity MW soil treatment significantly reduced the OTUs richness compared to the low intensity MW treatment (Tukey post-hoc test; $p<0.001$, comparison between low and high at T0), this variation in richness with high temperature treatment continued by T28 (Tukey post-hoc test; $p<0.001$, comparison between low and high at T28). Similarly, the community richness declined with the high intensity treatment at 5 – 10 cm depth. Overall, regardless of days after MW exposure and penetration depth, the low intensity MW soil treatment did not significantly reduce the richness (Tukey post-hoc test; $p>0.1$) than those high intensities treatment (Tukey post-hoc test; $p<0.0001$ for all comparison among low and high intensities treatment).

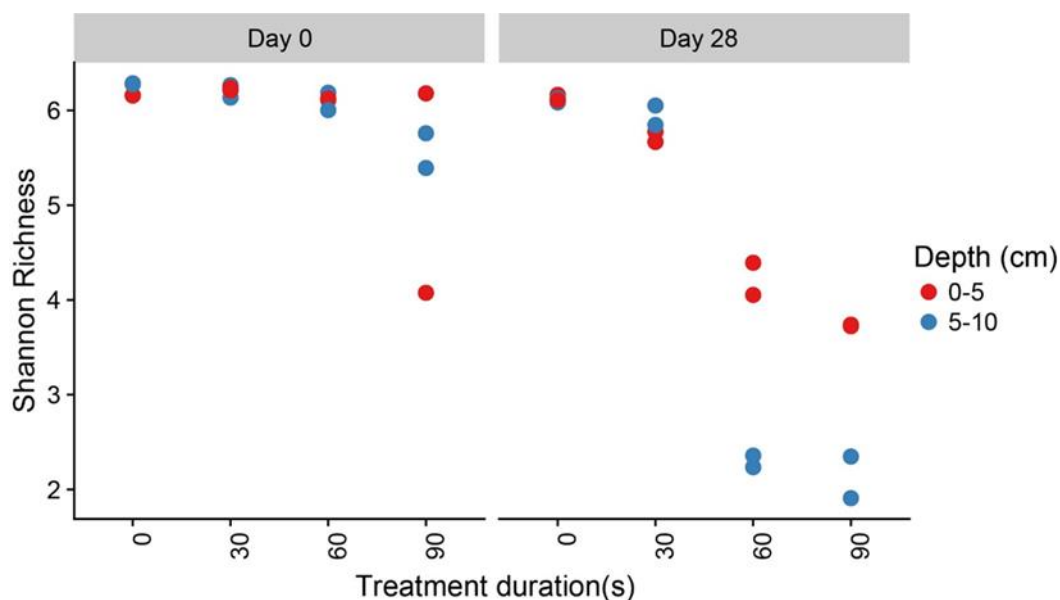


Figure 8.3: Variation in bacterial diversity against microwave soil heating. Shannon richness calculated from the 16S rRNA gene sequence data for the different treatment combinations at two-time points.

8.4.4. Bacterial Responses

The change in bacterial community in response to the combine effect of MW soil heating intensities and penetration depth for disinfestation scenario was assessed at the phylum and genus levels. Of all the examined phyla, Actinobacteria (37.3%) was the predominant phylum followed by Proteobacteria (26.7%), Gemmatimonadetes (7.9%), Acidobacteria (7.2%) and Firmicutes (6.1%) associated to all the treatment combinations. Immediately after heating (T0), only Proteobacteria and Firmicutes significantly varied according to heating intensities $\times$ depth (ANOVA; $p < 0.001$; Figure 8.4). Proteobacteria showed a significant increase while Firmicutes exhibited a significant decrease in relative abundance with high intensities MW soil treatment relative to low intensities for 0 – 5 cm MW depths while at lower depth (5 – 10 cm) only Firmicutes exhibited a significant increase in relative abundance with high intensity MW.

At T28, all phyla except for Chloroflexi varied significantly according to heating intensities $\times$ depth (ANOVA; $p < 0.001$; Figure 8.4). The relative abundance of Proteobacteria increased at 0 – 5 cm depth, but decreased at 5 – 10 cm depth, and at this depth (5 – 10 cm), Firmicutes exhibited a significant increase in relative abundance with high intensities MW relative to low intensities. The high heating intensities decreased the relative abundance of Proteobacteria and Acidobacteria while those heating intensities increased the relative abundance of the both Actinobacteria and Firmicutes relative to low intensities at 5 – 10 cm depth. Further, at T28, the relative abundance of the TM-7 significantly increased (ANOVA; $p < 0.001$; Figure 8.4) with high intensities in 0 – 5 cm zone and this increased trend continued for lower (5 – 10 cm) depth relative to low intensities.

Moreover, the MW soil treatments significantly influenced the relative abundance of several genera. At T0, among the 10 most abundant genera, only *Bacillus* and an unclassified *Gaiellaceae* significantly increased in the high intensity treatments (ANOVA; $p < 0.001$; Figure 8.4). Similarly, the unclassified *Gaiellaceae* exhibited a consistent increased trend in the relative abundance with high intensities MW soil treatment at 5 – 10 cm depth compared to low intensities at 0 – 5 cm depth. In contrast, at T28, of the 10 most abundant taxa, *Kaistobacter*, *Micromonosporaceae*, *Bacillus*, *Gaiellaceae*, *Rhodospirillaceae*, *TM7-1*, *Myxococcales* and *Gemm-1* varied significantly

according to heating intensities $\times$ depth (ANOVA; $p < 0.001$; Figure 8.4). The high intensity MW soil treatment at 0 – 5 cm depth significantly increased relative abundance of the *Micromonosporaceae*, *Kaistobacter* and TM7-1, while at 5 – 10 cm depth the relative abundance of *Kaistobacter* significantly declined and the community dominated by *Micromonosporaceae* and *Bacillus* at this depth. Notably, the TM7-1 maintained relatively higher abundance at all depths over time following the high intensity MW treatments.

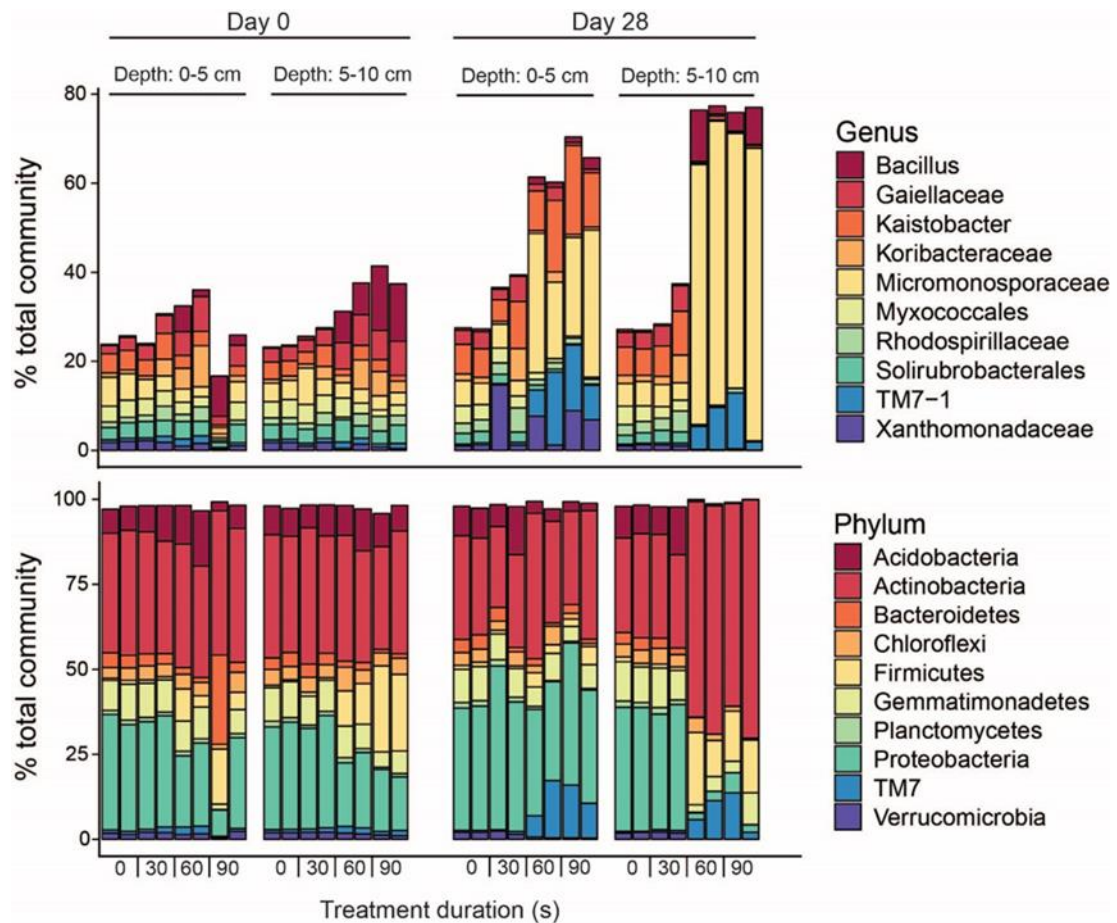


Figure 8.4: Relative abundance of the predominated bacterial phyla (Fennimore et al.) and genera (top) that significantly changed over time in response to microwave soil heating intensities. Affiliation displayed at each taxonomic level are the finest known affiliation of each taxon.

8.4.5. Taxonomic and Functional Gene Abundances

The average abundance of 16S rRNA bacterial gene for untreated control soil was $10^{10} \pm 0.42$ sd gene copies g^{-1} soil. Overall, during experiment no significant difference detected in total 16S rRNA abundance in any of the studied factors (Tukey post-hoc test; $p > 0.05$; Figure 8.5D).

Similarly, MW soil treatments had no detectable effect on *amoA* gene abundances at any time point (Figure 8.5). At T0, the AOB total abundance ranged between $10^{7.9} \pm 0.25$ sd to $10^{7.8} \pm 0.34$ sd *amoA* gene copies g^{-1} soil from low to high intensity MW soil treatments respectively, at 0 – 5 cm depth. Lower numbers of AOB *amoA* genes were detected at the 5 – 10 cm depth, with an average abundance of $10^{7.5} (\pm 0.43$ sd) and $10^{7.4} \pm 0.46$ sd *amoA* gene copies g^{-1} soil for low and high intensity treatments (t-test; $p = 0.53$), respectively. At T28, AOB *amoA* abundance ranged between $10^{7.7} \pm 0.28$ sd – $10^{7.9} \pm 0.38$ sd gene copies g^{-1} soil (Figure 8.5A).

At T0, the average AOA population slightly decreased between high ($10^7 \pm 0.39$ sd) to low ($10^{6.4} \pm 0.43$ sd *amoA* gene copies g^{-1} soil) intensity MW soil treatment irrespective of penetration depth (one-tailed t-test; $p = 0.30$). At T28, the high intensity MW soil treatment significantly changed the total abundance of AOA relative to low intensities (Tukey post-hoc test; $p < 0.002$; Figure 8.5B). Also, the total abundance of AOA varied significantly along depths between the high and low intensities MW soil treatment (Tukey post-hoc test; $p < 0.0001$; Figure 8.5B) but did not show any significantly changed pattern over time from T0 – T28 (Tukey post-hoc test; $p = 0.54$). Overall, the AOB were more abundant than AOA (Figure 8.5C).

8.5. Discussion

The primary purpose of the MW weed management technology is to reduce the viability of the soil weed seedbank of the herbicide-resistant weeds as an alternative to chemicals in the agriculture industry. We previously observed that the temperature requirement for this task ranged between 75 – 80 °C for effective weed seedbank depletion (Brodie et al., 2018). Understanding the response of soil bacterial communities to this temperature range is crucial for the maintenance of ecosystem services provided by the soil biota. Therefore, a major aim of this study was to evaluate the effect of MW

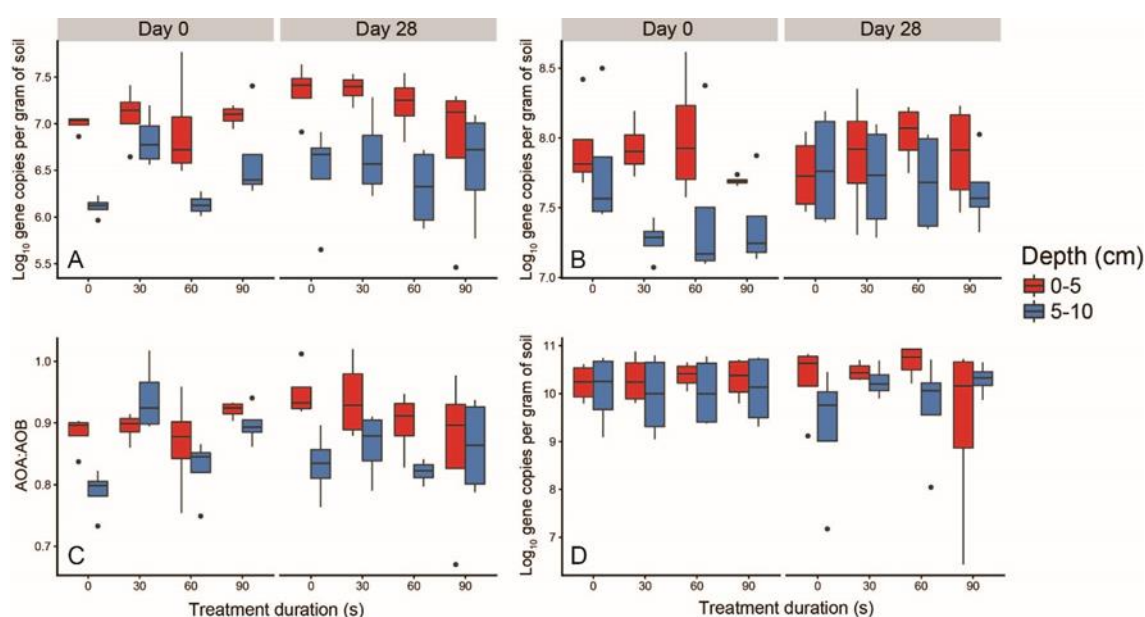


Figure 8.5: Influence of microwave soil heating on the total abundance of the AOB (A), AOA (B), AOA:AOB (C) and 16S rRNA bacterial gene (D). The log-transferred gene copies number per gram soil are plotted against the various doses of soil microwave treatment monitored at two times points.

soil heating on the soil bacterial communities and on the total abundance of AOB and AOA at two penetration depths. The temperature distribution, achieved through MW energy application in the top soil layer has been simulated elsewhere (Brodie, 2016), where it was reported that most of the MW energy attenuation occurred between 0 – 8 cm of soil. A similar temperature distribution pattern was found in the microcosm of this study. Generally, when the temperature equilibrium of soil is altered by MW heating, both MW energy diffusion and heat transfer happen, and the majority of MW energy attenuation occurs in the top few centimetres for disinfestation (i.e., pasteurization). The temperature distribution of deeper sites (for example, 5 – 10 cm in this study) depends on heat conduction, and during conduction, heat lost to the atmosphere causes cooler and hotter spots (*i.e., inconsistency in soil heating*) at lower penetration depths, as detected in the microcosm of the present study (please see Figure 8.S2). Soil heterogeneity, inconsistency in soil heating, preferential localization (inside and outside of soil particles) of soil biota, huge diversity in microbial community and inherent capacity to recover after

disturbance support the survival of microbial community including ammonia oxidiser (numerous studies observed this response, resistance).

Based on the Bray-Curtis PCoA, we categorised different MW heating durations into two groups: the low heating intensities, where soil exposed to 30 s did not induce a discernible effect on the bacterial communities and clustered closely to untreated control soil; and the high heating intensities, where 60 and 90 s treatments induced a significant divergence in bacterial communities, and clustered closely overtime. The observed pattern likely depended on temperature distribution, peak temperature retention into soil and bacterial differential recolonization patterns following heating, as mentioned by Neary et al. (1999) who studied fire effects on soil microbiota. In our experiment, the 30 s exposure raised soil temperature to 45 °C while in the 60 and 90 s soil treatments, soil temperature ranged between 65 – 78 °C.

In this study, the high heating intensities resulted in a divergence in the bacterial communities' composition (Figure 8.2). This is in agreement with findings of Russell (2003), where high temperature treatment (50 – 60 °C) dramatically altered the structure and physiological properties of the bacterial communities, resulting in a changed community structure. MW heating also induces cell wall rupture (Woo et al., 2000) therefore a complete cell lysis in a complex matrix like soil is somehow hard to achieve. In contrast, Pietik inen et al. (2000) reported that heating soils to 45 – 160 °C did not reduce the bacterial abundance in soils. Therefore, the soil heterogeneity and the inconsistent heating pattern of MW may explain the survival and/or reshuffling of bacterial communities at T28, with high heating intensities in this study (Figure 8.4).

Based on the bacterial richness, which is widely employed to understand the recovery of bacterial communities after certain disturbances or environmental changes (Shade et al., 2012), the communities disturbed with high intensity MW treatment did not recover to their pre-heating richness within the period studied (28 days; Figure 8.3). This suggests that the four weeks for bacterial colonization, following heat disturbance of 75 – 80 °C, appeared to be an insufficient duration for bacterial recovery. A similar pattern was observed in a recent study where the soil bacterial community declined with a 90 s MW treatment (800 W; 2.45 GHz), and subsequently underwent a successional shift approaching community recovery by 29 days after soil heating (Jurburg et al., 2017b). In

contrast, Voort et al. (2016) found higher community richness with 80 °C soil heating treatment for 1 h and argued that this was in agreement with the concept of ‘*intermediate disturbance hypothesis*’, which was proposed by Connell (1978). According to this hypothesis, species exhibit a trade-off between their ability to compete for survival/regrowth on available resources and their capacity to withstand a certain disturbance (Connell, 1978).

Bárcenas-Moreno and Bååth (2009) reported the effect of a range of heating intensities for soil sterilization on soil bacteria. They reported that, a soil temperature above 50 °C initially inhibited bacterial growth but recovery occurred within a few thereafter, while temperature above 200 °C completely inhibited bacterial growth. Therefore, soil temperature within a range of 70 – 80 °C can be considered as a moderate temperature range for soil disinfestation. In addition, Labeda et al. (1975) found that soil heating at 160 °C for 3 h did not completely sterilize the soil. In this investigation the peak temperature (65 – 78 °C) retention into soil was only ≤ 20 s; however, it is highly likely that heat-resistant bacterial community can survive the heating intensities proposed for disinfestation.

Our results show that, bacterial diversity declined significantly at lower penetration depth of MW energy into microcosm (Figure 8.3; also see Figure 8.S4). The lower soil depth should be classified as a moderate heating zone (Figure 8.S2) based on temperature distribution, because MW energy loses its intensity with increasing penetration depth (Nelson, 1996). Therefore, the reduction in community diversity at the lower depth (5 – 10 cm) is probably linked to the reflection of MW energy from the working surface beneath the samples. Thus, the reabsorption of this energy might have changed the soil temperature distribution at lower depths to regulate the higher bacterial communities’ mortality. This phenomenon is highly complex and needs sound simulation modelling for assessment but can be visualized in the cross-sectional thermal image of the microcosm (Supplementary Material; Figure 8.S2).

Our results indicate that, at T0 the relative abundance of Proteobacteria and Firmicutes varied significantly according to MW heating intensities while at T28 the abundant phyla Proteobacteria, TM-7, Firmicutes and Actinobacteria varied significantly in response to high heating intensities (Figure 8.4). In general, the immediate negative

impact of soil heating on bacterial communities is mortality (O'Brien et al., 2018), which is evident with a relative abundance of heat-sensitive to heat-tolerant bacterial taxa. In this view, a relative abundance of Actinobacteria, which have previously been identified as being moisture-dependent in another microcosm experiment (Jacquiod et al., 2013), increased with respect to days after heating. We did not observe a decrease in a relative abundance of this phylum with high intensities MW soil treatment at T0, indicating that they survived the transient heating disturbance because they were indistinguishable at this time point from untreated soil while Nunes et al. (2018) classified Actinobacteria as a heat-sensitive group. It is important note that, the downward movement of moisture along the MW penetration depth into microcosm might have changed the relative abundance of this phylum, as they are moisture dependent (Jacquiod et al., 2013). In a separate discipline, the heat-resistant slow-growing bacterial families, belonged to Actinobacteria and Acidobacteria phyla, can be stimulated by soil heating at 80 °C to protect the rhizosphere from disease-causing pathogens (Voort et al., 2016). During the microcosm experiment, Proteobacteria was the second most abundant phylum. Nunes et al. (2018) found that there was no appreciable detrimental effect of MW soil heating (≤ 180 s) on the beta-proteobacteria, which is in accordance with our findings. Finally, the relative abundance of Firmicutes increased at T28 with high heating intensities, which is widely reported as a heat-resistant bacterial phylum (Vos et al., 2011; Jurburg et al., 2017a).

In this study, the relative abundance of gram-positive spore-forming genus *Micromonosporaceae* increased according to high heating intensities of MW soil treatment at lower depths into microcosm. The heat-induced activation of this genus, with a 70 °C temperature application, has been reported previously (Hoskisson et al., 2000). In addition, Suarez et al. (1980) reported that the dormant spores of *Micromonospora* were resistant upon temperature exposure within a range of 75 – 90 °C in a culture-dependent study. The carbon compound degradation capacity of the genus *Micromonosporaceae* in dryland soils has been documented elsewhere (Yeager et al., 2017). *Micromonospora* species have also been reported to mediate a crop protection by producing the anti-fungal and anti-microbial compounds (Hirsch and Valdés, 2010). They also help in promoting nitrogen-fixing symbioses, root nodulation and phytohormones formation (Solans et al., 2006). Considering these services provided by *Micromonosporaceae*, it can be inferred that MW soil heating has positive impact on soil

functionality to support crop productivity. Further, it can explain the reason for a good growth of crops after MW soil treatment for up to two cropping seasons, as reported by Khan et al. (2017b).

Moreover, the increase in relative abundance of the genus *Kaistobacter* at T28 with high intensities, which belongs to the phylum alpha-Proteobacteria and few species under this subphylum, have potential to survive under extreme environmental conditions (Kumbhare et al., 2015). Additionally, this genus has been well known to regulate the carbon and nitrogen cycling (Rampelotto et al., 2013). This could explain the soil organic matter degradation after MW soil heating at up to 80 – 90 °C, reported by Kim and Kim (2013), which ultimately favours the survival and activity of bacterial communities.

Survival and reassembly of the microbial community in soil depends on the substrate availability and post-heating conditions (D'Ascoli et al., 2005; Bárcenas-Moreno and Bååth, 2009; O'Brien et al., 2018). Overall, the MW soil heating intensities appeared to be effective in shaping the bacterial communities by stimulating important taxa for soil health to enhance crop productivity, which is supporting our hypothesis.

We did not detect a negative impact of MW soil heating on the ammonia oxidisers over the period studied (28 days; Figure 8.5). These results supported our hypothesis that ammonia oxidiser are resistance to MW energy, which was initially argued by Vela et al. (1976). Vela et al. (1976) reported that nitrifying bacteria are resistance to at least 40,000 J cm⁻² of MW energy applied to the soil surface. In this study, the maximum applied energy was 2,500 J cm⁻² associated to high intensities MW treatment. This energy was measured by simulation modelling of the soil heated by MW energy challenged via horn antenna according to Brodie (2016). This is quite a low energy compared to tested energy by Vela et al. (1976) and can therefore justify the survival of ammonia oxidisers. Also, the resistance of prokaryotes to MW energy had been reported by Ferriss (1984). Ferriss concluded that soil moisture and treatment time decide the fate of soil microorganisms. In contrast, soil steaming with the temperature gradient of about 80 – 100 °C for 10 minutes induced a 10-fold reduction in the population of AOB in an organic farming soil (Roux-Michollet et al., 2008). In the present study the peak temperature exposure was less than 2 minutes. Moreover, the difference in response of ammonia oxidizers to MW and steam treatment could be explained through changes in the heat transfer mechanism

associated with both soil disinfestation strategies. Additionally, the same range of ammonia oxidisers had been reported in the Southern Australian soils regardless of the management practices (Jenkins et al., 2016).

In other domain, Shamis et al. (2011) exposed the bacterial cell to MW energy in a highly concentrated solution and found the low bactericidal effect of MW because the majority of energy absorption was by the surrounding water molecules. As soil is a highly complex material and the moisture content of the soil absorbs more MW energy than biological material, ultimately there is less microbial-MW interaction. Also, it is well known that, the soil temperature within a range of 80 – 100 °C can increase the amount of ammonia in the soil, independent of heating methodology. Substrate availability could therefore justify the ammonia oxidiser's survival following heating, as mentioned by Prosser and Nicol (2012).

We have previously achieved about 80% reduction in weed establishment when this same soil was treated with MW energy for 120 s under field conditions. This resulted in overall temperature in the top soil layer (0 – 8 cm) of 75 – 80 °C, and a 34% increase in wheat grain yield either through less weed-crop competition for resources and/or some effects on soil nutrients availability for crop growth (Brodie et al., 2017; Khan et al., 2017a; Khan et al., 2018). Interestingly, the results of this study provide the antagonistic effect: that the weed seeds are susceptible but ammonia oxidisers are resistant to MW soil heating. However, this pattern could be explained by number of factors: (1) variation in microbial distribution into the soil matrix (soil type, soil moisture, soil pH and agricultural strategies); (2) specific mass and volume of seeds (much higher than microbes); (3) uneven MW energy distribution into soil causes hot and cold spots and (4) treatment times and energy levels.

Furthermore, the complete sterilization of soil at lower temperature (<200 °C) is not achievable (Bárcenas-Moreno and Bååth, 2009). Although, the temperature sensitivity of microbial groups widely differ (Bollen, 1969), and fungi are more sensitive than bacteria (D'Ascoli et al., 2005). The difference in temperature sensitivity could have been explained by microbial habitat preferences within the soil aggregates. Bacteria preferably locate into the microaggregates (Nie et al., 2014) and, in contrast, fungal colonization occurs in macroaggregates (Kihara et al., 2012). For example, considering a soil

aggregate being heated by MW energy, the inside-out heat transfer pattern associated to MW heating can induce a slight change in the heating intensity in a soil particles. Therefore, this phenomenon potentially leads to hotter- and colder-spots because of enormous spatial soil heterogeneity. Given that, ammonia oxidisers are groups of the beta-proteobacteria families, which are commonly sensitive to heat; however, their survival after heating can be explained by their existence in safer spots (Voort et al., 2016). This safeguarding strategy could have harbored the ammonia oxidisers against a transient heat disturbance in the present study. Spatial distribution of ammonia oxidisers into soil particles, and preferential localization, can help protect them from extreme conditions (Richardson et al., 2002). Although, the physiological adaptation of microbial groups to certain sets of extreme events helps in distinguishing the bacterial taxa based on what extents they can survive the unexpected extreme conditions. However, this study provides evidence that AOB and AOA have potential to tolerate the transient heat disturbance of the soil proposed for seedbanks depletion. This assumption needs to be tested rigorously in future experimentations. Therefore, these findings should motivate further study of the effect of MW soil heating on the species composition of ammonia oxidizers, their functionality (potential nitrification rate), and why they seem to be resistant to MW heating is thus needed to further support the commercialization of this technology for sustainable weed management.

Overall, these results demonstrate that the MW soil heating can potentially be used for its primary application with little to no negative impact on the population of AOB and AOA. Despite this, the results of this study depict that four weeks is insufficient time for maximum recovery of the bacterial communities following MW soil heating. This implies that soil heating for 75 – 80 °C enormously changes the bacterial communities and recovery requires more time.

8.6. Conclusions

Based on these findings, we conclude that MW heating intensities proposed for weed management had a strong effect on the community change without significantly influencing the ammonia oxidizers abundance. However, the resistance of ammonia oxidizer to transient heating suggests further research to check the community composition and function (potential nitrification rate) with respect to sufficient temporal investigation

to understand the recovery regimes of the damaged communities with MW soil heating. It is conceivable that the spatial differentiation in temperature distribution into soil matrix can alter the preferential colonization spots which ultimately harbors the surviving species for their growth and activity following heat disturbance. For a practical perspective of this innovative weed management technology, this study highlights that MW soil treatment can potentially be applied for weed control without significantly damaging the biologically important taxa and helps in shaping the bacterial community for soil health to maintain ecosystem services. Finally, the heat-induced triggering of important dormant taxa responsible for carbon decomposition and disease suppression by retarding the growth of soil-borne pathogens warrants further research in this discipline.

8.7. Supplementary Material

Table 8. S1: Physico-chemical properties of the soil used in the study. Soil physico-chemical properties were measured by Nutrient Advantage Laboratory Services, Werribee, Victoria, Australia, before MW treatment.

Soil Properties	Dookie Victoria	Units
Texture	Clay	–
pH _(1:5 H₂O)	5.7	–
Organic Carbon	1.4	%
Nitrate-Nitrogen	37	µg g ⁻¹
Ammonium-Nitrogen	2.0	µg g ⁻¹
Phosphorus _(Colwell)	180	µg g ⁻¹
Electrical Conductivity	0.6	dS m ⁻¹
Cation Exchange Capacity	7.90	cmol (+) kg ⁻¹
Available Potassium	340	µg g ⁻¹

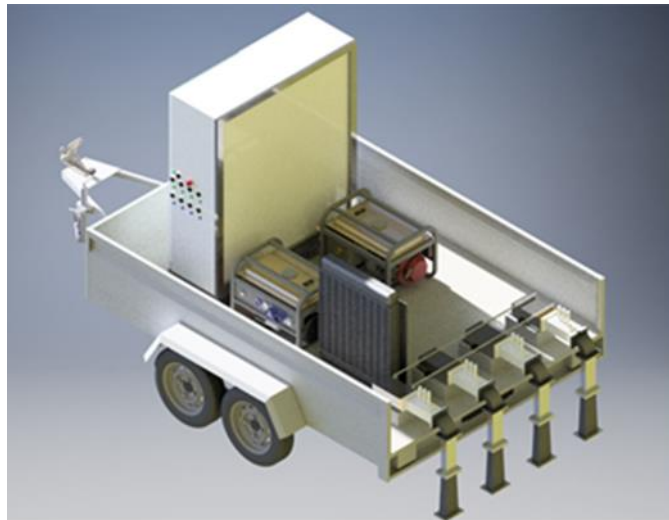


Figure 8. S1: Microwave weed killer, designed by the University of Melbourne researcher – Dr. Graham Brodie (grahamb@unimelb.edu.au), used in this study to treat the microcosm under one of the four horn antennas.

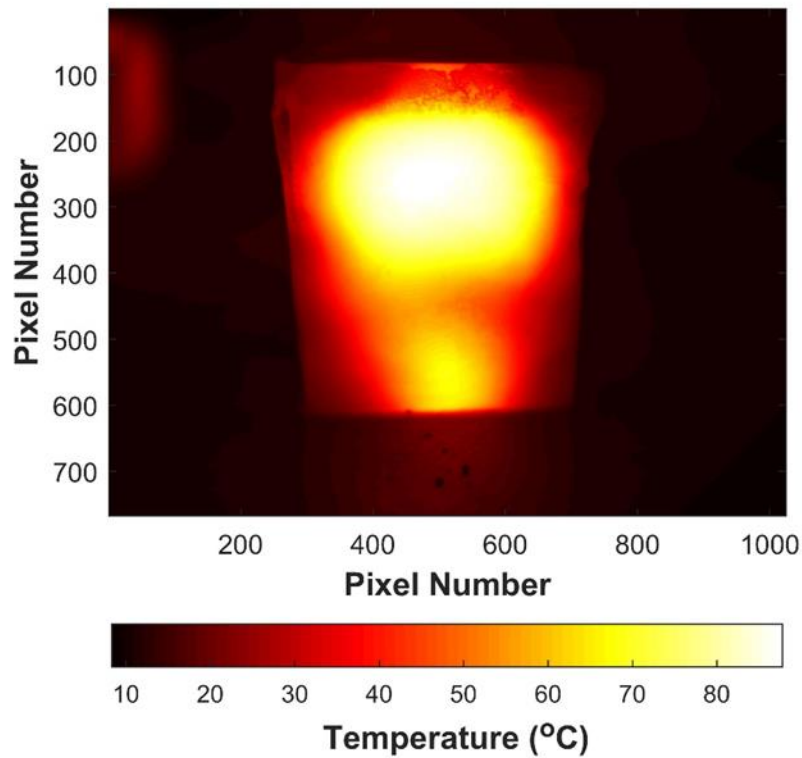


Figure 8. S2: Cross-sectional infrared thermal image of the microcosm treated under the horn antenna of the microwave weed killer prototype. To further understand the heating pattern in a microcosm: a cross-sectional thermal image of the microcosm was taken in a separate experiment; where bi-sectioning of microcosm was done, and a plastic sheet adhered between two separate parts of a microcosm to avoid disturbance in a soil profile after heating for thermal imaging.

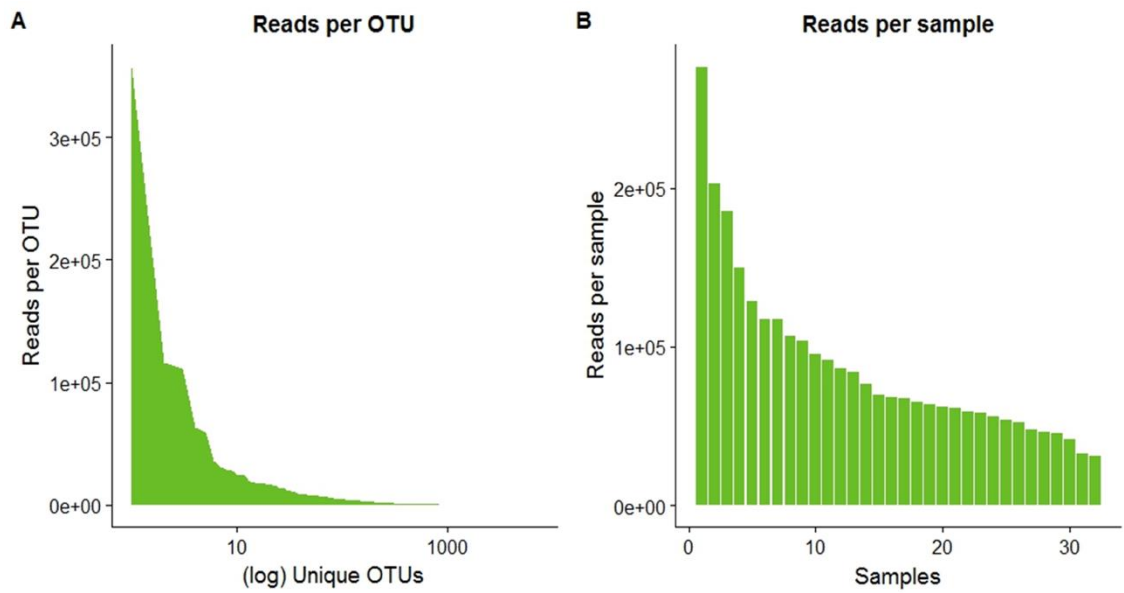


Figure 8. S3: 16S amplicon data – OTUs Distribution (A) representing a sequencing read distribution (B) representing rarefied reads to 30, 745 reads per sample of analysis.

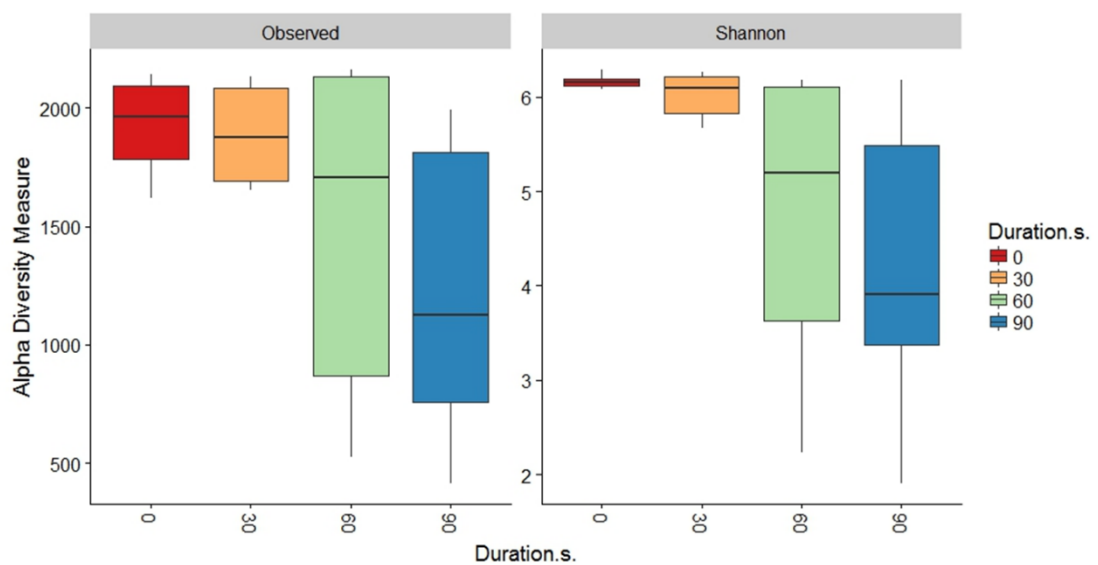


Figure 8. S4: Observed and Shannon Diversity index in response to microwave soil treatment.

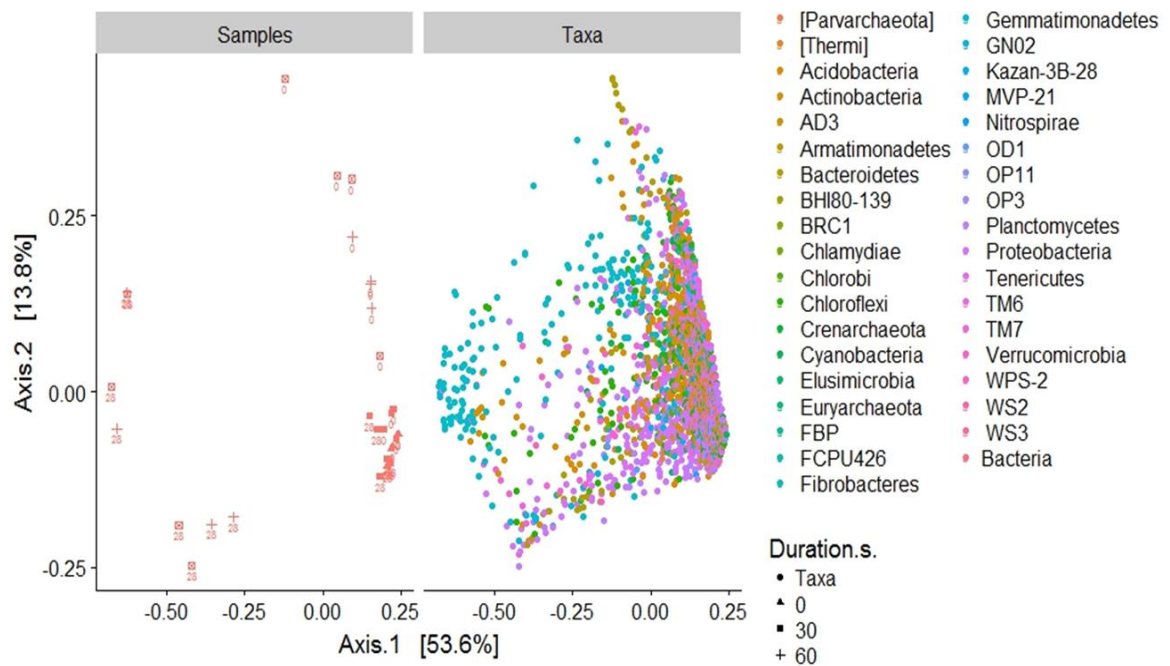


Figure 8. S5: Community composition of microwave heated soil over time. Explanation of split PCoA according to taxa response to microwave soil treatment. The phyla responsible for distinguishing between 60 and 90s on day 28 and the rest, particularly at 10cm, are firmicutes, as they are heat-resistant. Bacteroidetes seem to be important in distinguishing these microcosms on day 0 (see top right of plot)

Chapter 9 – General Discussion and Conclusions

9.1. Microwave Soil Heating for Weed Seedbank Control

This thesis sought to identify the potential impacts of MW soil treatment for weed management as a novel alternative to herbicides in no-till farming systems. The reasons for the development and adaptation of this innovative physical weed management technology are the evolution of herbicide-resistant weeds (Heap, 2016), heavy reliance on old chemistry herbicides (Johnson et al., 2009), and the lack of diversity in weed management approaches (Chauhan et al., 2017). In addition, herbicide application is considerably deteriorating the cropping environment and can result in unwanted herbicide residue. Agricultural inputs are one of the potential drivers of climate change and farming systems are shifting towards sustainable and/or integrated pest management. Depleting soil weed seedbanks is an effective control measure to support sustainable weed management (Buhler et al., 1997). In view of this, the reduction in the regeneration capacity of soil weed seedbanks as a result of MW soil heating, before cropping, can help to overcome weed pressure (Sartorato et al., 2006; Brodie et al., 2009). Therefore, in this research project, MW soil treatment was employed to deplete herbicide-resistant weed seedbanks, which was the main hypothesis of this research. The advantage of MW technology is the rapid increase in soil temperature (Metaxas and Meredith, 1983; Nelson, 1996) for soil disinfestation scenarios, and its unique volumetric and real-time soil heating behaviour distinguishes this technology from the numerous classical thermal pest management technologies (for example, soil steaming and soil solarization) (Brodie, 2016; Hess et al., 2018).

Weed seedling emergence can be used to estimate the viability of weed seedbanks, according to Buhler et al. (1997); however, the complete emergence of an entire seedbank, in a single cropping season, is unlikely. Despite this, most of the soil weed seedbank can germinate in a single season, depending on the suitability of growing conditions and levels of seed dormancy and hard seededness. In many cases, seedling emergence can be used to indicate the viable weed seedbank size. Therefore, the effects of pre-sowing MW soil treatment, to deactivate the regeneration capacity of the soil weed

seedbank, are presented in **Chapters 3** and **4**. In this work, soils were exposed to 120 s of MW energy, channelled via open-structured horn antenna (**Chapter 3; Figure 3.1**), to give a temperature range of 70 – 80 °C in the top soil layer (0 – 6 cm) under field conditions for weed seedbank control. This temperature range is widely reported for weed seedbank control (Brodie et al., 2009; Vidotto et al., 2013), and gave a considerable reduction in weed establishment in these investigations, which strongly supported our hypothesis. In addition, the MW energy, channelled via a horn antenna (see **section 3.7**), ranged between 500 – 560 J cm⁻². This energy range for killing weed seedbanks has been reported in numerous studies (Wayland et al., 1973; Wayland et al., 1975; Sartorato et al., 2006; Brodie et al., 2009). Notably, this energy range gave equivalent weed control in two contrasting farming systems (dryland wheat and anaerobic rice), suggesting a broad-spectrum of activity for this technology.

It is evident that MW soil heating with the horn antenna in direct contact with the soil surface appeared to be effective for weed management in two different cropping systems: dryland wheat (**Chapter 3**); and temperate rice (**Chapter 4**). There was a 60% reduction in ryegrass and wild radish (*Raphanus raphanistrum* L.) seedling emergence in the MW treated plots. Both of these species have developed resistance to a number of herbicides in Australian cropping systems (Heap, 2016). In the Australian context, annual ryegrass (*Lolium rigidum* L.) resistance management is a huge concern in farming systems (Boutsalis et al., 2012; Broster et al., 2013); however, the implementation and/or integration of MW technology for weed management can potentially provide an alternative solution for resistance management. Therefore, MW soil treatment can be applied to control the weed seedbank for sustainable weed management in an agricultural industry.

Furthermore, in **Chapter 5**, a 2-kW MW weed killer, designed for weed zapping between crop rows (Brodie et al., 2018), was tested to deactivate the weed seedbank under field conditions at two different agro-ecological zones of Australia. It was further validated that pre-sowing MW soil treatment potentially reduced the weed establishment (50 – 60% reduction; **Table 5.2**) under field conditions. Also, it was identified that horn antenna distance from soil surface, soil moisture and complete coverage of soil are important factors affecting the efficacy of MW soil treatment for weed management under field condition. In addition, equal distribution of the maximum temperature is required for

good seedbank control under field conditions. Considering this, the colder spots, which were alongside the MW treated strips, can become a favourable environment for weed seed emergence, as a low temperature exposure stimulates weed seedbank germination (Radzevičius et al., 2013). Overall, based on a scalar efficiency index (**equation 5.2; section 5.4.2**), MW soil treatment appeared to be effective (30 times more effective) for weed seedbank control, compared to other thermal soil treatment options, such as soil steaming. Considering a stepwise heating methodology as proposed in **Chapter 3, 4 and 5**, travelling speed experiments are needed to understand the efficacy of MW soil treatment for weed seedbank control under field conditions. Development of new MW weeder with higher power can overcome the operational speed of MW soil irradiation for weed seedbank control. These assumptions need to be explored in future experimentation.

9.2. Crop Productivity

In general, reducing weed pressures can improve crop yields (Cousens et al., 1987) and minimize crop yield gaps. Closing yield gaps in the world's major food crops is crucial to meet future food demand (Fischer and Connor, 2018). The adaptation of new pest-management technologies is needed to minimize yield gaps in sustainable intensification farming systems (Reeve, 2011). Sustainable weed management is one of the key elements of sustainable intensification (Pretty and Bharucha, 2014). This research indicated that pre-sowing MW soil treatment significantly increased crop yields, either through less weed-crop competition for resources or through the positive impacts of soil heating on soil fertility to sustain crop yields. Overall differences in yields between MW treated and untreated control plots were 30 – 40%, regardless of cropping systems, as presented in **Table 3.2 and 4.2**. In addition, **Figure 3.6** shows the effectiveness of MW soil treatment for weed management on grain yields of wheat crop (1.5 t ha⁻¹ additional grain yield) compared to herbicide application for weed management. This is indicative of soil fertility changes in response to MW soil heating, in addition to the reduced weed pressures on yields. Relevant to this, the heat mediated changes in soil nutrient availability is widely reported (Yang et al., 1990; Glass et al., 2008; Gay et al., 2010b; O'Brien et al., 2018), and a soil temperature within a range of 70 – 120 °C does not completely degrade the soil carbon and N stocks (Glass et al., 2008).

In addition, **Table 5.2** shows the increase in rice grain yields in response to MW soil treatment, relative to untreated control plots at two agroecological zones of Australia, supporting the idea of ecological adaptability of this technology for weed management, and increased crop yields. Considering the overall resource use efficiency, in the case of MW soil treatment, the agronomic efficiency of applied N was 10 times higher and irrigation water use efficiency (t MI^{-1}) was 50 – 100% higher, compared to untreated control plots, as evident in **Chapter 5**. In most of the important food crops, the agronomic use efficiency of applied N varied between 30 – 40 kg grain N kg^{-1} (Cassman et al., 1998); however, MW soil treatment gave 60 – 70 kg grain N kg^{-1} , suggesting stimulation of the indigenous soil N supply for crop productivity.

This assumption was further validated by N accumulation in dry biomass as a function of MW soil treatment (70 – 80 °C) over two years in a glasshouse study, as presented in **Chapter 7**. The effective soil indigenous N supply can sustain crop productivity and help in optimising the N fertilizer usage for sustainable crop production (Cassman et al., 1998). The release of an N flush, following the thermal treatment of the soil, can be explained by two important mechanisms: (1) the fixed N between clay particles can be released upon heat disturbance; and (2) the death of heat-sensitive bacterial communities can increase the organic N pool. These could both increase the availability of indigenous N supply for further transformation to plant available N to facilitate crop growth and development. In this debate, knowledge of the impact of any crop management approach on the overall soil N stock is deemed important to understand the long-term reliability of the system for crop productivity. It is interesting to note that, if transient soil heating increases crop growth and development without N application, as observed in **Figure 7.5** and **7.6**, it could deplete the soil nutrient stocks in low SOM soils (for example, in many Australian soil types). To minimize the risk of N depletion, it would be advisable to apply a reduced amount of N fertilizers for the following cropping seasons. But, a dose-response experiment would be required to determine how much N can be lowered for the same yield response in various crops. It was further evident that the residual impact of one-off MW soil treatment on the wheat grain yields, for up to two consecutive seasons (440 and 740 days after MW soil treatment) with a defined cropping gap (120 days between each cropping periods), confirmed the long-term positive impact of soil heating on soil fertility (**Figure 7.7**), suggesting that soil heating may have changed

and/or reshaped soil microbial community to sustain soil health. These controlled studies supported our hypothesis that soil heating increases soil fertility and sustainability. But again, the nutrient depletion scenario should be considered and timely soil nutrient profile assessment after MW soil treatment (if farmers go for a residual impact) is highly recommended. Furthermore, the enzymatic activity related to the increased N availability and transformation in response to MW soil heating, should be investigated in future experimentation.

9.3. Soil Bacterial Community Responses to Heat Disturbance

In general, soil heating is detrimental to microorganisms (O'Brien et al., 2018). Despite this, rapid growth and extreme diversity within the bacterial communities can help them to survive any disturbance and/or management regime (Shade et al., 2012). However, the response of soil bacterial communities depends on the intensity and frequency of the disturbance. Transient heat disturbance has been shown to influence bacterial community structure, and the communities change during the recovery period (Jurburg et al., 2017a). A preliminary data presented in **Figure 7.7**, confirmed that this MW soil heating did not induce a detrimental impact on soil ammonia oxidisers. Furthermore, **Chapter 8** demonstrates that, the heating intensities, which were proposed for weed seedbank depletion, had a strong impact on the bacterial community changes over time, without damaging the ammonia oxidisers. Once-off MW soil treatment changed the bacterial communities with the triggering of biologically important taxa in particular, – *Micromonosporaceae* and *Kaistobacter* as presented in **Figure 8.4**. Those taxa have the potential to decompose organic matter (Abdulla and El-Shatoury, 2007), and can retard pathogen growth as a biocontrol agent (Hirsch and Valdés, 2010), which is an important service provided by soil biota. Based on the capacity of these taxa, it can be inferred that the MW soil heating may have been induced a positive impact on soil health. This can explain the reason for a positive residual impact of MW soil treatment on wheat yield as observed in **Figure 7.5** and **7.6**. These species may have facilitated soil nutrient cycling to sustain the soil fertility for crop productivity. But this would not always be the case. The bacterial communities of different soils will respond differently. Instead, the triggering dormant species, as we observed in this study (**Figure 8.4** above right panel), it could provide a microbial-dependent pathway for soil cleanliness (i.e., pathogen

control). MW soil heating is non-selective, therefore, the microbial communities (bacterial and fungal) would shift and change based on temperature sensitivity of each species and this response will vary in every soil type depending on the cropping history and management regimes. It also depends on soil heterogeneity and inconsistent heating behaviour of MW.

In regard to other treatment options, soil steaming, solarisation and fumigation are common practice for soil disinfestation, before cropping, to facilitate the production of high value crops. Certainly, many soil fumigants are effective in minimizing pathogens (Xie et al., 2015); however, the ozone-depleting nature of these chemicals provoked a considerable debate in the farming communities. In the Australian context, Metham Sodium is commonly used to retard the growth of numerous disease-causing pathogens for strawberry and tomato production. Based on MW soil treatment, as developed for weed management in this investigation, it can also be inferred that MW soil heating could be applied as an alternative to soil fumigation for high-value vegetable and fruit crop production. In this case, MW mediated soil disinfestation can be achieved in two ways: (1) the direct soil heating effect can kill the majority of pathogens, because pathogens are more sensitive to heating than bacteria (D'Ascoli et al., 2005); and (2) the stimulation of genera belonging to Actinomycetes, following MW soil heating, has the potential to retard the pathogens' regrowth, because soil heating mediates microbial interactions for ecosystem services (Voort et al., 2016). Also, MW has better and more rapid heating capacity for soil disinfestation, compared with soil steaming and soil solarisation.

In addition, the bacterial community's recovery and reassembly depends on the substrate availability and post-heating conditions (O'Brien et al., 2018). In this view, the release of proteaceous compounds upon the death of heat-sensitive microbes can increase the availability of decomposable organic substances to facilitate the life style of heat-tolerant and/or surviving species, following soil heating. Relevant to soil health, soil bacterial communities play a major role in recycling the majority nutrients to maintain soil fertility (Berendsen et al., 2012).

In **Chapter 8**, a transient heat disturbance did not induce a negative impact on the total abundance of the ammonia oxidisers. It is important to note that, DNA remains in the soil upon cell lysis of heat-sensitive microbes, which might have influenced the

interpretation of these results. For this reason, a transcriptionally active ammonia oxidisers fractions is needed to be explored in future reaserch to understand the impact of MW soil heating on the beneficial microbiota. Despite this, the resistance of ammonia oxidisers to 40,000 J cm⁻² of MW energy applied to soil surface was reported by Vela et al. (1976) and the maximum energy required to achieve the temperature range of 75 – 80 °C was 2500 J cm⁻², which confirms the potential survival of ammonia oxidisers. Also, the spatial distribution of ammonia oxidisers into soil particles, and perferential localization, might have protected them from soil heating (Richardson et al., 2002). Therefore, the survival of ammonia oxidisers during MW soil treatment appeared to be important to maintain the soil N cycling and its availability for crop productivity, and thus supporting our hypotheis. Hence, it is evidenced that MW soil heating has little to no obvious negative impact on the beneficial soil bacterial communities and this technology can therefore be used for its primary purpose of weed seedbank mangement. Thus, this research potentially favours the adaptability of this thermal weed management technology in modern farming systems and can be implemented as an alternative to soil steaming and soil solarisation for pathogen control.

9.4. Energy and Economic Analysis for Soil Treatment

MW soil treatment relies on energy transfer from an electromagnetic wave (operating in the ISM MW frequency bands – 922 MHz, 2450 MHz, or 5800 MHz) into the soil as the wave energy is absorbed by polar molecules (mostly water) in the soil. Consequently, MW soil treatment energises indigenous moisture in the soil and plant materials resulting in a very rapid rise in soil temperature accompanied by rapid steam generation which permeates through the soil matrix. During the first two field trials (dryland wheat (Chapter 3) and rice (Chapter 4)), we used a portable modified MW-oven system (2.45GHz, 0.7 kW, lower power for soil treatment) to treat the soil for weed seedbank control before cropping. Because MW ovens use a half-wave voltage double circuit to power the magnetron and there are some other energy consuming elements in an oven (fan, light, heating losses in transformer and magnetron). Based on the earlier analysis (see, **Section 4.4.3**), if a well-designed industrial quality system was employed to treat the soil before cropping, the fuel consumption could have reduced to lower level of 0.28L m⁻², which is probably equivalent to \$4060 ha⁻¹ for weed management under

field conditions (in case of pre-emergence treatment). Compared to herbicides, the pre-emergence MW soil treatment is costly while post-emergence inter-row treatment of MW for weed control (\$65 ha⁻¹; see, **Section 3.5.3**) is quite comparable with herbicide applications. The MW soil treatment was found to have significant beneficial effects on subsequent crop growth, beyond the simple control of weed competition. Without a well-designed system, MW soil treatment for broadacre crops would be impractical; however, we cannot deny the potential of MW soil treatment for weed seedbanks control.

For high-value crop production systems, the reliability of MW soil treatment is higher than present options, such as, Methyl bromide fumigation, which is currently used in the strawberry runner industry as a contract service at a cost of \$14,023 ha⁻¹. Both steam and MW soil treatment are in the late research and early development stage; therefore, estimates of costs for these alternatives are based on published information or recent field-based research efforts. Based on the best available literature (Goodhue et al., 2005; Fennimore et al., 2008), steam fumigation may cost approximately \$17,710 ha⁻¹. Based on recent field research at both 922 MHz and 2450 MHz frequencies, MW soil treatment may cost approximately \$8,950 to \$9,850 ha⁻¹ (Brodie et al., (unpublished data)), which suggests that MW soil treatment may be more cost competitive than other options, including the current practice of using soil fumigants. In conclusion, based on the best evidence available, steam fumigation will be approximately \$3,700 more expensive (Samtani et al., 2012) than the current methyl bromide fumigation; however, because it is a soil heating process, access to the treated fields will be within hours or a day, compared with the 21-day exclusion needed for chemical fumigation. On the other hand, MW soil treatment is also a soil heating strategy and provides the same ready access to the treated field; however, the estimated cost associated with this technology is between \$4,000 and \$5,000 cheaper than the current cost of methyl bromide treatment. This should provoke future research in this area.

9.5. Risk and Safety Issues with Microwave Weed Control Technology

According to the Radiation Protection Standard for Maximum Exposure Levels (Australian Radiation Protection and Nuclear Safety Agency, 2002), the maximum time average operator exposure to electric fields at 2.45 GHz is 137 V m⁻¹, while the exposure for the general public must be below 61.4 V m⁻¹ (Gourd et al.). A rigorous evaluation by

Brodie (Brodie, 2008; Brodie et al., 2009; Brodie and Hollins, 2015; Brodie, 2016; 2018; Brodie et al., 2018) indicated that the closest approach to the horn antenna arrangement for a MW weed killer, operating at 2.45 GHz and fed from a 2 kW MW system was: 3.75 m along the centre line of the antenna; 2.3 m from the antenna at right angles to the centre line of the antenna in the $x - z$ plane; and 1.25 m in the $y - z$ plane (see, **Appendix 3.7 and Figure 3.7**). Safe distances for the general public are: 7.0 m along the centre line of the antenna; 5.0 m from the antenna at right angles to the centre line of the antenna in the $x - z$ plane; and 3.0 m in the $y - z$ plane. These distances were determined by calculation, based on the field distribution from a horn antenna, shown in equation (3.5, **Appendix 3.7**); however, this equation does not include the aperture efficiency of the antenna or the influence of other structures associated with the MW weed killer. Therefore, Brodie (unpublished data) conducted a trial to assess the field distribution for soil treatment using the 2-kW MW prototype (see, **Figure 8.S1**, the same prototype used for field trials).

The MW field intensity was measured at the operating point near the front of the trailer, the hitching point for the trailer, and in the various directions from the horn antenna (i.e. at right angles to the centre line of the antenna in the $x - z$ plane and the $y - z$ plane). The field intensity was measured using a Tenmars TM-194 MW leakage detector. Limits of approach to the operating antenna were determined by moving the leakage detector from a point at 5 m from the antenna towards the antenna until the maximum exposure limit was reached. At this point the system was turned off and the distance from the leakage detector to the antenna was measured. Only one of the four MW generators was operating at any given time during the testing procedure, and all MW shielding was removed from the system so that the tests could be regarded as the worst-case scenario.

The maximum field strength measured in Brodie's experiment at the operating point for the trailer was 47.7 Vm^{-1} (*which is well below the exposure limits of general public*) and the proximity to the unshielded antenna for the public was determined to be at 2.8 m from antenna in the $x - z$ plane and at 1.5 m in the $y - z$ plane. This experiment confirms that the field strength, associated with the MW soil treatment (presented in this thesis), where the operator stands and where the MW trailer hitching point (*i.e., treatment area*) is located are well below the expected threshold of exposure. This suggests that the risk associated with using this MW weed killer system is low. However, future evaluation

is needed to better validate the risk scenarios and proper shielding should be designed into a new MW weed zapper.

9.6. Industrial Applications of this Research

Based on the findings of this study, it is concluded that;

1. MW soil treatment can potentially be applied as a novel tool for herbicide-resistant weed management for sustainable food production systems.
2. The proposed soil heating for seedbank depletion helps in facilitating the indigenous N supply for crop production, thus providing better N use efficiency.
3. MW soil heating can become a potential alternative to soil steaming, solarization and fumigation for pest (pathogens and weed seedbank) management in high-value vegetable productions systems, for example, in the strawberry and tomato industries.
4. MW soil heating does not completely sterilize the soil and helps in activating the biologically important dormant taxa to maintain the soil ecosystem services.

9.7. Future Research Work

This study has identified the following research ideas, which should be explored in future experiments:

1. Travelling speed experiments for soil treatments and post-emergence weed management trials under field conditions are needed to understand the efficacy of various doses of MW energy in different cropping systems.
2. Investigation of the responsible mechanisms for changes in soil properties following MW soil heating: characterization of soil organic matter; nitrogen; phosphorus and potassium.
3. Enzyme activity following soil heating would unravel the potential drivers of soil property changes in crop productivity.
4. The impact of MW soil heating on the total and potentially active bacterial communities, their recovery trajectories and their role and/or mechanisms for suppressing the pathogens to support high-value and organically produced crops should be explored.

5. The ability of *Micromonosporaceae* to rapidly regenerate after MW treatment and retard disease-causing pathogens, compared with chemical soil treatments, needs to be explored.

References

- "Australian Bureau of Meteorology - Climate data online. Canberra, ACT, Australia: Australian Bureau of Meteorology. Retrieved from <http://www.bom.gov.au/climate/data>".).
- Abdulla, H.M., and El-Shatoury, S.A. (2007). Actinomycetes in rice straw decomposition. *Waste Management* 27, 850-853.
- Abeledo, L.G., Savin, R., and Slafer, G.A. (2008). Wheat productivity in the Mediterranean Ebro Valley: Analyzing the gap between attainable and potential yield with a simulation model. *European Journal of Agronomy* 28, 541-550.
- Acuña, T.B., Dean, G., and Riffkin, P. (2011). Constraints to achieving high potential yield of wheat in a temperate, high-rainfall environment in south-eastern Australia. *Crop and Pasture Science* 62, 125-136.
- Alphei, J., and Scheu, S. (1993). Effects of biocidal treatments on biological and nutritional properties of a mull-structured woodland soil. *Geoderma*. 56, 435-448.
- Andreasen, C., Hansen, L., and Streibig, J.C. (1999). The effect of ultraviolet radiation on the fresh weight of some weeds and crops. *Weed technology*, 554-560.
- Anonymous (2016). *Weed-Slaying Robot Could Save Farm Sector \$1.3 Billion a Year*. [Online]. Available: <http://www.abc.net.au/news/2016-10-21/weed-killing-robotcould-save-billions/7954680> [Accessed].
- Ark, P., and Parry, W. (1940). Application of high-frequency electrostatic fields in agriculture. *The Quarterly Review of Biology* 15, 172-191.
- Ascard, J. (1994). Dose–response models for flame weeding in relation to plant size and density. *Weed Research* 34, 377-385.
- Ascard, J. (1998). Comparison of flaming and infrared radiation techniques for thermal weed control. *Weed Research-Oxford* 38, 69-76.
- Australian Radiation Protection and Nuclear Safety Agency (2002). "RPS3 Radiation Protection Standard for Maximum Exposure Levels to Radiofrequency Fields—3 kHz to 300 GHz.". (Yallambie, Victoria: ARPANSA).
- Ayappa, K., Davis, H., Crapiste, G., Davis, E., and Gordon, J. (1991). Microwave heating: an evaluation of power formulations. *Chemical Engineering Science* 46, 1005-1016.
- Bagavathiannan, M., Norsworthy, J., Smith, K.L., and Neve, P. (2011). "Modeling the evolution of barnyardgrass (*Echinochloa crus-galli* L.) resistance to glyphosate in

- cotton and its management implications", in: *Proc. Beltwide Cotton Conference*. (Atlanta, Ga, USA).
- Bajwa, A.A., and Chauhan, B.S. (2017). "Rice Production in Australia," in *Rice Production Worldwide*. Springer Nature: Basel Switzerland), 169-184.
- Bakker, J.P., Poschlod, P., Strykstra, R., Bekker, R., and Thompson, K. (1996). Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandica* 45, 461-490.
- Banik, S., Bandyopadhyay, S., and Ganguly, S. (2003). Bioeffects of microwave—a brief review. *Bioresource Technology* 87, 155-159.
- Banning, N.C., Maccarone, L.D., Fisk, L.M., and Murphy, D.V. (2015). Ammonia-oxidising bacteria not archaea dominate nitrification activity in semi-arid agricultural soil. *Scientific Reports* 5, 11146.
- Barakat, R.M., and Al-Masri, M.I. (2012). Enhanced Soil Solarization against *Fusarium oxysporum* f. sp. *lycopersici* in the Uplands. *International Journal of Agronomy* 2012, 1-7.
- Bárcenas-Moreno, G., and Bååth, E. (2009). Bacterial and fungal growth in soil heated at different temperatures to simulate a range of fire intensities. *Soil Biology and Biochemistry* 41, 2517-2526.
- Barker, A.V., and Craker, L.E. (1991). Inhibition of Weed Seed Germination by Microwaves. *Agronomy Journal* 83, 302-305.
- Batlla, D., and Benech-Arnold, R.L. (2007). Predicting changes in dormancy level in weed seed soil banks: implications for weed management. *Crop Protection* 26, 189-197.
- Bebawi, F., Cooper, A., Brodie, G., Madigan, B., Vitelli, J., Worsley, K., and Davis, K. (2007). Effect of microwave radiation on seed mortality of rubber vine (*Cryptostegia grandiflora* R. Br.), parthenium (*Parthenium hysterophorous* L.) and bellyache bush (*Jatropha gossypifolia* L.). *Plant Protection Quarterly* 22, 136.
- Beckie, H.J., and Tardif, F.J. (2012). Herbicide cross resistance in weeds. *Crop Protection* 35, 15-28.
- Beckie, H.J., Warwick, S.I., and Sauder, C.A. (2012). Basis for herbicide resistance in Canadian populations of wild oat (*Avena fatua*). *Weed Science* 60, 10-18.
- Bennett, W., and Garrison, W.M. (1959). Production of amide groups and ammonia in the radiolysis of aqueous solutions of protein. *Nature* 183, 889-889.
- Benz, W., Moosmann, A., Walter, H., and Koch, W. (1984). Wirkung einer Bodenbehandlung mit Mikrowellen auf Unkrautsamen, Nematoden und bodenbürtige Mikroorganismen. *Mitteilung BBA* 223, 128.

- Berendsen, R.L., Pieterse, C.M., and Bakker, P.A. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science* 17, 478-486.
- Bhartia, P., M. Lebell, and Baron., J. (1977). Weed control with electromagnetic fields. *Journal of Microwave Power* 12, 52-54.
- Bigu-Del-Blanco, J., Bristow, J., and Romero-Sierra, C. (1977). Effects of low-level microwave radiation on germination and growth rate in corn seeds. *Proceedings of the IEEE* 65, 1086-1088.
- Bødker, L., and Noyé, G. (1994). "Effekten af varmebehandling af overfladejord i nåletræssåbæde over for ukrudt og rodpatogene svampe.(Effect of heat treatment of surface-soil in raised seedbeds with conifers against weeds and root pathogens. With English summary)", in: *Proc. 11th Danish Plant Protection Conference/Pests and Diseases*. (Nyborg, Denmark).
- Bollen, G. (1969). The selective effect of heat treatment on the microflora of a greenhouse soil. *Netherlands Journal of Plant Pathology* 75, 157-163.
- Boutsalis, P., Gill, G.S., and Preston, C. (2012). Incidence of herbicide resistance in rigid ryegrass (*Lolium rigidum*) across southeastern Australia. *Weed Technology* 26, 391-398.
- Bowen, H., and Cawse, P. (1964). Some effects of gamma radiation on the composition of the soil solution and soil organic matter. *Soil Science* 98, 358-361.
- Bren, S.P.A. (1996). Historical introduction to EMF health effects. *IEEE Engineering in Medicine and Biology Magazine* 15, 24-30.
- Brodie, G. (2008). The Influence of Load Geometry on Temperature Distribution During Microwave Heating. *Transactions of the ASABE* 51, 1401-1413.
- Brodie, G. (2012). "Applications of Microwave Heating in Agricultural and Forestry Related Industries," in *The Development and Application of Microwave Heating*. InTech), 45-78.
- Brodie, G. (2014). Derivation of a Cropping System Transfer Function for Weed Management: Part 1 – Herbicide Weed Management. *Global Journal of Agricultural Innovation, Research & Development* 1, 11-16.
- Brodie, G. (2016). Derivation of a cropping system transfer function for weed management: Part 2. Microwave weed management. *Global Journal of Agricultural Innovation, Research & Development* 3, 1-9.
- Brodie, G. (2018). "The Use of Physics in Weed Control," in *Non-Chemical Weed Control*. Elsevier), 33-59.
- Brodie, G., Bootes, N., and Reid, G. (2015a). "Plant growth and yield of wheat and canola in microwave treated soil.", in: *IMPI's 49th Microwave Power Symposium*. (USA).

- Brodie, G., Gixti, M., Hollins, E., Cooper, A., Li, T., and Cole, M. (2015b). Assessing the Impact of Microwave Treatment on Soil Microbial Populations. *Global Journal of Agricultural Innovation, Research and Development* 2, 25-32.
- Brodie, G., Hamilton, S., and Woodworth, J. (2007). An assessment of microwave soil pasteurization for killing seeds and weeds. *Plant Protection Quarterly* 22, 143.
- Brodie, G., Harris, G., Pasma, L., Travers, A., Leyson, D., Lancaster, C., and Woodworth, J. (2009). Microwave soil heating for controlling ryegrass seed germination. *Transactions of the ASABE* 52, 295-302.
- Brodie, G., and Hollins, E. (2015). The Effect of Microwave Treatment on Ryegrass and Wild Radish Plants and Seeds. *Global Journal of Agricultural Innovation, Research & Development* 2, 16-24.
- Brodie, G., Khan, J., Gupta, D., Foletta, S., and Bootes, N. (2017). "Microwave Weed and Soil Treatment in Agricultural Systems". AMPERE Newsletter: Association for Microwave Power in Europe for Research and Education).
- Brodie, G., Khan, M.J., Gupta, D., and Foletta, S. (2018). Microwave Weed and Soil Treatment in Agricultural Systems. *Global Journal of Agricultural Innovation, Research & Development* 5, 1-14.
- Brodie, G., Ryan, C., and Lancaster, C. (2012a). The Effect of Microwave Radiation on Prickly Paddy Melon (*Cucumis myriocarpus*). *International Journal of Agronomy* 2012, 1-10.
- Brodie, G., Ryan, C., and Lancaster, C. (2012b). Microwave Technologies as Part of an Integrated Weed Management Strategy: A Review. *International Journal of Agronomy* 2012, 1-14.
- Broster, J., Koetz, E., and Wu, H. (2013). Herbicide resistance levels in annual ryegrass (*Lolium rigidum* Gaud.) and wild oat (*avena spp.*) in southwestern New South Wales. *Plant Protection Quarterly* 28, 126.
- Buhler, D.D., Hartzler, R.G., and Forcella, F. (1997). Implications of weed seedbank dynamics to weed management. *Weed Science*, 329-336.
- Burnside, O.C., Moomaw, R.S., Roeth, F.W., Wicks, G.A., and Wilson, R.G. (1986). Weed seed demise in soil in weed-free corn (*Zea mays*) production across Nebraska. *Weed Science* 34, 248-251.
- Cai, H., Lu, Y., Xie, W., Zhu, T., and Lian, X. (2012). Transcriptome response to nitrogen starvation in rice. *Journal of Biosciences* 37, 731-747.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., and Gordon, J.I. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7, 335-336.

- Cassman, K., Peng, S., Olk, D., Ladha, J., Reichardt, W., Dobermann, A., and Singh, U. (1998). Opportunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. *Field Crops Research* 56, 7-39.
- Cawse, P., and Crawford, D. (1967). Accumulation of nitrite in fresh soils after gamma irradiation. *Nature* 216, 1142-1143.
- Cawse, P., and White, T. (1969). Accumulation of nitrate in fresh soils after gamma irradiation. *The Journal of Agricultural Science* 72, 331-333.
- Certini, G. (2005). Effects of fire on properties of forest soils: a review. *Oecologia*. 143, 1-10.
- Champ, M., Davis, F., and Wayland, J. (1973). "Ultrahigh-frequency electromagnetic radiation utilized for aquatic vegetation control", in: *Proceedings of the... annual conference*).
- Chapagain, T., and Good, A. (2015). Yield and production gaps in rainfed wheat, barley, and canola in Alberta. *Frontiers in Plant Science* 6, 1-10.
- Chauhan, B.S., and Johnson, D.E. (2010). "The role of seed ecology in improving weed management strategies in the tropics," in *Advances in Agronomy*. Elsevier), 221-262.
- Chauhan, B.S., Matloob, A., Mahajan, G., Aslam, F., Florentine, S.K., and Jha, P. (2017). Emerging Challenges and Opportunities for Education and Research in Weed Science. *Frontiers in Plant Science* 8, 1-13.
- Chauhan, B.S., Singh, R.G., and Mahajan, G. (2012). Ecology and management of weeds under conservation agriculture: a review. *Crop Protection* 38, 57-65.
- Chen, S., Dickson, D., and Mitchell, D. (1995). Effects of soil treatments on the survival of soil microorganisms. *Journal of Nematology* 27, 661-663.
- Chin, D., and Mortimer, M. (2002). Weed management in direct-seeded rice in South Vietnam. *Direct seeding: research strategies and opportunities*. Los Banos, Philippines: International Rice Research Institute, 349-356.
- Christy, B., Riffkin, A., Clough, A., Norton, R., Stott, K., and O'leary, G. (Year). "Crop yield potential limited by nutrient status in the high rainfall zone of Southern Australia", in: *Proceedings of the 17th ASA Conference*. , (2015).
- Connell, J.H. (1978). Diversity in tropical rain forests and coral reefs. *Science* 199, 1302-1310.
- Cooper, A., and Brodie, G. (2009). The Effect of Microwave Radiation and Soil Depth on Soil pH, N, P, K, SO₄ and Bacterial Colonies. *Plant Protection Quarterly* 24, 67-70.

- Cousens, R., Brain, P., O'donovan, J.T., and O'sullivan, P.A. (1987). The use of biologically realistic equations to describe the effects of weed density and relative time of emergence on crop yield. *Weed Science* 35, 720-725.
- Cox, C. (1995). Glyphosate, part 1: toxicology. *Journal of Pesticide Reform* 15, 14-20.
- Crawford, A. (1977). Phytotoxicity threshold levels of microwave radiation for Trifolium and Medicago seeds. *Seed Science and Technology (Netherlands)*.
- Cronin, N.J. (1995). *Microwave and optical waveguides*. CRC Press.
- D'ascoli, R., Rutigliano, F.A., De Pascale, R.A., Gentile, A., and De Santo, A.V. (2005). Functional diversity of the microbial community in Mediterranean maquis soils as affected by fires. *International Journal of Wildland Fire* 14, 355-363.
- Daff (2006). "Weeds", (ed.) F.a.F. Australian Department of Agriculture. (Queensland, Australia).
- Davidson, E., Hart, S., Shanks, C., and Firestone, M. (1991). Measuring gross nitrogen mineralization, and nitrification by 15 N isotopic pool dilution in intact soil cores. *Journal of Soil Science* 42, 335-349.
- Davis, A.S., Dixon, P.M., and Liebman, M. (2003). Cropping system effects on giant foxtail (*Setaria faberi*) demography: II. Retrospective perturbation analysis. *Weed Science* 51, 930-939.
- Davis, A.S., and Liebman, M. (2003). Cropping system effects on giant foxtail (*Setaria faberi*) demography: I. Green manure and tillage timing. *Weed Science* 51, 919-929.
- Davis, F. (1974). "New techniques in weed control via microwaves", in: *Southeastern Nurserymen's Conferences Eastern Session/Western Session*).
- Davis, F., Wayland, J., and Merkle, M. (1971). Ultrahigh-frequency electromagnetic fields for weed control: phytotoxicity and selectivity. *Science* 173, 535-537.
- De Datta, S.K. (1981). *Principles and practices of rice production*. Int. Rice Res. Inst.
- Desantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., and Andersen, G.L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* 72, 5069-5072.
- Diprose, M., and Benson, F. (1984). Electrical methods of killing plants. *Journal of Agricultural Engineering Research* 30, 197-209.
- Diprose, M., Benson, F., and Hackam, R. (1980). Electrothermal control of weed beet and bolting sugar beet. *Weed Research* 20, 311-322.

- Diprose, M., Benson, F., and Willis, A. (1984). The effect of externally applied electrostatic fields, microwave radiation and electric currents on plants and other organisms, with special reference to weed control. *The Botanical Review* 50, 171-223.
- Dobson, M.C., Ulaby, F.T., Hallikainen, M.T., and El-Rayes, M.A. (1985). Microwave dielectric behavior of wet soil-Part II: Dielectric mixing models. *IEEE Transactions on Geoscience and Remote Sensing* GE-23, 35-46.
- Downes, R.G. (1949). *A soil, land-use and erosion survey of parts of the Counties of Moira and Delatite, Victoria*. Commonwealth Scientific and Industrial Research Organization.
- Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460-2461.
- Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194-2200.
- Eglitis, M., and Johnson, F. (1970). Control of seedling damping-off in greenhouse soils by radio frequency energy. *Plant Disease Reporter* 54, 268-271.
- Eno, C.F., and Popenoe, H. (1963). The Effect of Gamma Radiation on the Availability of Nitrogen and Phosphorus in Soil 1. *Soil Science Society of America Journal* 27, 299-301.
- Fao (2009). "Feeding the world, eradicating hunger. Background document for World Summit on Food Security", (ed.) F.a.A. Orginzation. (Rome).
- Fenner, M. (2012). *Seed ecology*. Springer Science & Business Media.
- Fennimore, S.A., Haar, M.J., Goodhue, R.E., and Winterbottom, C.Q. (2008). Weed control in strawberry runner plant nurseries with methyl bromide alternative fumigants. *HortScience* 43, 1495-1500.
- Ferriss, R. (1984). Effects of microwave oven treatment on microorganisms in soil. *Phytopathology* 74, 121-126.
- Fischer, R., and Connor, D. (2018). Issues for cropping and agricultural science in the next 20 years. *Field Crops Research* 222, 121-142.
- Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., and Oakley, B.B. (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Procc. of the National Academy of Sciences of the U. S. A.* 102, 14683-14688.
- Gay, P., Piccarolo, P., Aimonino, D.R., and Tortia, C. (2010a). A high efficiency steam soil disinfection system, part I: Physical background and steam supply optimisation. *Biosystems Engineering* 107, 74-85.

- Gay, P., Piccarolo, P., Ricauda Aimonino, D., and Tortia, C. (2010b). A high efficacy steam soil disinfestation system, part II: Design and testing. *Biosystems Engineering* 107, 194-201.
- Gelsomino, A., Petrovičová, B., Zaffina, F., and Peruzzi, A. (2010). Chemical and microbial properties in a greenhouse loamy soil after steam disinfestation alone or combined with CaO addition. *Soil Biol Biochem.* 42, 1091-1100.
- Ghersa, C.M., Benech-Arnold, R.L., Satorre, E.H., and Martinez-Ghersa, M. (2000). Advances in weed management strategies. *Field Crops Research* 67, 95-104.
- Gianessi, L.P. (2013). The increasing importance of herbicides in worldwide crop production. *Pest Management Science* 69, 1099-1105.
- Gibson, F., Fox, F.M., and Deacon, J. (1988). Effects of microwave treatment of soil on growth of birch (*Betula pendula*) seedlings and infection of them by ectomycorrhizal fungi. *New Phytologist* 108, 189-204.
- Glass, D.W., Johnson, D.W., Blank, R.R., and Miller, W.W. (2008). Factors affecting mineral nitrogen transformations by soil heating: A laboratory-simulated fire study. *Soil Science* 173, 387-400.
- Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., and Toulmin, C. (2010). Food security: the challenge of feeding 9 billion people. *Science*, 1185383.
- González-Pérez, J.A., González-Vila, F.J., Almendros, G., and Knicker, H. (2004). The effect of fire on soil organic matter—a review. *Environment International* 30, 855-870.
- Goodhue, R.E., Fennimore, S.A., and Ajwa, H.A. (2005). The economic importance of methyl bromide: Does the California strawberry industry qualify for a critical use exemption from the methyl bromide ban? *Review of Agricultural Economics* 27, 198-211.
- Gourd, T., Ferrell, T., Organic, P.B.P.F.C., and Market, P.Y.O.F. (2002). Controlling weeds using propane generated flame and steam treatments in crop and non croplands. *Final Report, Organic Farming Research Foundation, Santa Cruz, California, USA.*
- Greenwood, D., Lemaire, G., Gosse, G., Cruz, P., Draycott, A., and Neeteson, J. (1990). Decline in percentage N of C3 and C4 crops with increasing plant mass. *Annals of Botany* 66, 425-436.
- Griffin, E. (1976). Design of 3-screw tuners. *Electronics Letters* 12, 657-658.
- Griffiths, B.S., and Philippot, L. (2013). Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiology Reviews* 37, 112-129.

- Gupta, L., Intwala, K., Khetwani, K., Hanamshet, T., and Somkunwar, R. (2017). Smart Irrigation System and Plant Disease Detection. *International Research Journal of Engineering and Technology* 4, 2395-2356.
- Hallikainen, M.T., Ulaby, F.T., Dobson, M.C., El-Rayes, M.A., and Wu, L.-K. (1985). Microwave dielectric behavior of wet soil-part 1: Empirical models and experimental observations. *IEEE Transactions on Geoscience and Remote Sensing*, 25-34.
- Hamill, A.S., Holt, J.S., and Mallory-Smith, C.A. (2004). Contributions of weed science to weed control and management. *Weed Technology* 18, 1563-1565.
- Harrington, R.B., and Powles, S.B. (2012). Harrington seed destructor: a new nonchemical weed control tool for global grain crops. *Crop Science* 52, 1343-1347.
- He, J.-Z., Shen, J.-P., Zhang, L.-M., and Di, H.J. (2012). A review of ammonia-oxidizing bacteria and archaea in Chinese soils. *Frontiers in Microbiology* 3, 1-7.
- Heap, I. (2014). "Herbicide resistant weeds," in *Integrated pest management*. Springer), 281-301.
- Heap, I. (2016). "International Survey of Herbicide Resistant Weeds".).
- Hess, M., De Wilde, M., Yavercovski, N., Willm, L., Mesléard, F., and Buisson, E. (2018). Microwave soil heating reduces seedling emergence of a wide range of species including invasives. *Restoration Ecology* 26, 160-169.
- Hightower, N.C., Burdette, E., and Burns, C. (1974). "Investigation of the use of microwave energy for weed seed and wood products insect control". Georgia Institute of Technology).
- Hill, J., Smith, R.J., and Bayer, D. (1994). Rice weed control: current technology and emerging issues in temperate rice. *Australian Journal of Experimental Agriculture* 34, 1021-1029.
- Hink, L., Gubry-Rangin, C., Nicol, G.W., and Prosser, J.I. (2018). The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidisers for nitrous oxide emissions. *ISME Journal* 12, 1084-1093.
- Hirsch, A.M., and Valdés, M. (2010). Micromonospora: An important microbe for biomedicine and potentially for biocontrol and biofuels. *Soil Biology and Biochemistry* 42, 536-542.
- Hodgson, D., McDonald, J.L., and Hosken, D.J. (2015). What do you mean, 'resilient'? *Trends in Ecology & Evolution* 30, 503-506.
- Holm, L.G. (1997). *World weeds: natural histories and distribution*. John Wiley & Sons.

- Hoskisson, P., Hobbs, G., and Sharples, G. (2000). Response of *Micromonospora echinospora* (NCIMB 12744) spores to heat treatment with evidence of a heat activation phenomenon. *Letters in Applied Microbiology* 30, 114-117.
- Huang, W., and Weber, W.J. (1997). A distributed reactivity model for sorption by soils and sediments. 10. Relationships between desorption, hysteresis, and the chemical characteristics of organic domains. *Environmental Science & Technology* 31, 2562-2569.
- Hur, J., Park, S.W., Kim, M.C., and Kim, H.S. (2013). Enhanced binding of hydrophobic organic contaminants by microwave-assisted humification of soil organic matter. *Chemosphere* 93, 2704-2710.
- Isbell, R. (2016). *The Australian soil classification*. CSIRO publishing: Melbourne.
- Jacquioud, S., Franqueville, L., Cécillon, S., Vogel, T.M., and Simonet, P. (2013). Soil bacterial community shifts after chitin enrichment: an integrative metagenomic approach. *PLoS One* 8, 1-13.
- Jarrell, W., and Beverly, R. (1981). The dilution effect in plant nutrition studies. *Advances in Agronomy* 34, 197-224.
- Jenkins, S.N., Murphy, D.V., Waite, I.S., Rushton, S.P., and O'donnell, A.G. (2016). Ancient landscapes and the relationship with microbial nitrification. *Scientific Reports* 6, 30733.
- Jenkinson, D., Fox, R., and Rayner, J. (1985). Interactions between fertilizer nitrogen and soil nitrogen—the so-called ‘priming’ effect. *Journal of Soil Science* 36, 425-444.
- Johnson, W.G., Davis, V.M., Kruger, G.R., and Weller, S.C. (2009). Influence of glyphosate-resistant cropping systems on weed species shifts and glyphosate-resistant weed populations. *European Journal of Agronomy* 31, 162-172.
- Jurburg, S.D., Nunes, I., Brejnrod, A., Jacquioud, S., Priemé, A., Sørensen, S.J., Van Elsas, J.D., and Salles, J.F. (2017a). Legacy effects on the recovery of soil bacterial communities from extreme temperature perturbation. *Frontiers in Microbiology* 8, 1-13.
- Jurburg, S.D., Nunes, I., Stegen, J.C., Le Roux, X., Priemé, A., Sørensen, S.J., and Salles, J.F. (2017b). Autogenic succession and deterministic recovery following disturbance in soil bacterial communities. *Scientific Reports* 7, 1-11.
- Kappe, C.O., Stadler, A., and Dallinger, D. (2012). *Microwaves in organic and medicinal chemistry*. John Wiley & Sons.
- Ke, X., and Lu, Y. (2012). Adaptation of ammonia-oxidizing microorganisms to environment shift of paddy field soil. *FEMS Microbiol Ecol* 80, 87-97.
- Khan, M.J., and Brodie, G. (2018). "Microwave Weed and Soil Treatment in Rice Production," in *Rice Crop - Current Developments*. IntechOpen), 99 - 127.

- Khan, M.J., Brodie, G., and Gupta, D. (2016). Effect of microwave (2.45 GHz) treatment of soil on yield components of wheat (*Triticum aestivum* L.). *Journal of Microwave Power and Electromagnetic Energy* 50, 191-200.
- Khan, M.J., Brodie, G., and Gupta, D. (2017a). The Effect of Microwave Soil Treatment on Rice Production under Field Conditions. *Transactions of the ASABE* 60, 517-525.
- Khan, M.J., Brodie, G., Gupta, D., and He, J. (2017b). "Residual effect of microwave soil treatment on growth and development of wheat.", in: *Proc. IMPI 51st Symposium*. (Mechanicsville, VA: USA).
- Khan, M.J., Brodie, G.I., Gupta, D., and Foletta, S. (2018). Microwave Soil Treatment Improves Weed Management in Australian Dryland Wheat. *Transactions of the ASABE* 61, 671-680.
- Khan, M.J., Brodie, G.I., Gupta, D., and He, J. (2019). Microwave Soil Treatment Increases Soil Nitrogen Supply for Sustained Wheat Productivity. *Transactions of the ASABE* 62, 355-362.
- Kihara, J., Martius, C., Bationo, A., Thuita, M., Lesueur, D., Herrmann, L., Amelung, W., and Vlek, P.L. (2012). Soil aggregation and total diversity of bacteria and fungi in various tillage systems of sub-humid and semi-arid Kenya. *Applied Soil Ecology* 58, 12-20.
- Kim, M.C., and Kim, H.S. (2013). Artificial and enhanced humification of soil organic matter using microwave irradiation. *Environmental Science and Pollution Research* 20, 2362-2371.
- Kissel, D., Cabrera, M., Paramasivam, S., Schepers, J., and Raun, W. (2008). *Ammonium, ammonia, and urea reactions in soils: In; Nitrogen in Agricultural Systems*. American Society of Agronomy.
- Kolberg, R.L., and Wiles, L.J. (2002). Effect of steam application on cropland weeds. *Weed Technology* 16, 43-49.
- Kothari, V., Dholiya, K., and Patel, D. (2012). *Effect of low power microwave on microbial growth and metabolism*. Grin Verlag Ohg.
- Kozempel, M., Cook, R.D., Scullen, O.J., and Annous, B.A. (2000). Development of a process for detecting nonthermal effects of microwave energy on microorganisms at low temperature. *Journal of food processing and preservation* 24, 287-302.
- Kremer, R.J. (1993). Management of weed seed banks with microorganisms. *Ecological Applications* 3, 42-52.
- Kumbhare, S.V., Dhotre, D.P., Dhar, S.K., Jani, K., Apte, D.A., Shouche, Y.S., and Sharma, A. (2015). Insights into diversity and imputed metabolic potential of bacterial communities in the continental shelf of Agatti Island. *PloS One* 10, e0129864.

- Labeda, D., Balkwill, D., and Casida Jr, L. (1975). Soil sterilization effects on in situ indigenous microbial cells in soil. *Canadian Journal of Microbiology* 21, 263-269.
- Lal, R., and Reed, W. (1980). The effect of microwave energy on germination and dormancy of wild oat seeds. *Canadian Agricultural Engineering* 22, 85-88.
- Leininger, S., Urich, T., Schlöter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S., and Schleper, C. (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* 442, 806-809.
- Lennon, J.T., Aanderud, Z.T., Lehmkuhl, B., and Schoolmaster, D.R. (2012). Mapping the niche space of soil microorganisms using taxonomy and traits. *Ecology* 93, 1867-1879.
- Levitt, J. (1980). *Responses of Plants to Environmental Stress, Volume 1: Chilling, Freezing, and High Temperature Stresses*. Academic Press.
- Lu, Y., Lu, S., Horton, R., and Ren, T. (2014). An empirical model for estimating soil thermal conductivity from texture, water content, and bulk density. *Soil Science Society of America Journal* 78, 1859-1868.
- Lyon, A., Hackam, R., and Benson, F. (Year). "Partial soil sterilisation and soil and leaf moisture content measurement by microwave radiation", in: *Proc. 1978 British Crop Protection Conference-Weeds. November. Brighton, UK*, 491-498.
- Mahajan, G., Chauhan, B.S., and Kumar, V. (2014). "Integrated weed management in rice," in *Recent advances in weed management*. Springer), 125-153.
- Maun, M., and Barrett, S. (1986). The Biology of Canadian Weeds.: 77. *Echinochloa crus-galli* (L.) Beauv. *Canadian journal of plant science* 66, 739-759.
- Maxwell, C.R., Peterson, D.C., and Sharpless, N.E. (1954). The Effect of Ionizing Radiation on Amino Acids: I. The Effect of X-Rays on Aqueous Solutions of Glycine. *Radiation Research* 1, 530-545.
- McGovern, R.J., Chaleeprom, W., Chaleeprom, W., McGovern, P., and To-Anun, C. (2013). Evaluation of soil solarization and amendments as production practices for lettuce and vegetable soybean in northern Thailand. *Journal of Agricultural Technology* 9, 1863-1872.
- Mclaren, A., Reshetko, L., and Huber, W. (1957). Sterilization of soil by irradiation with an electron beam, and some observations on soil enzyme activity. *Soil Science* 83, 497-502.
- Mcmurdie, P.J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS one* 8, e61217.
- Melander, B., and Jorgensen, M.H. (2005). Soil steaming to reduce intrarow weed seedling emergence. *Weed Research* 45, 202-211.

- Mendes, R., Kruijt, M., De Bruijn, I., Dekkers, E., Van Der Voort, M., Schneider, J.H., Piceno, Y.M., Desantis, T.Z., Andersen, G.L., and Bakker, P.A. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332, 1097-1100.
- Menges, R., and Wayland, J. (1974). UHF Electromagnetic energy for weed control in vegetables. *Weed Science* 22, 584-590.
- Metaxas, A.A., and Meredith, R.J. (1983). *Industrial microwave heating*. IET.
- Midigan, M., Martinko, J., and Parker, J. (2003). "Brock: Biology of Microorganisms (10th edn.). Chapter 19". Pearson Education Inc., Prentice Hall).
- Murphy, D., Recous, S., Stockdale, E., Fillery, I., Jensen, L., Hatch, D., and Goulding, K. (2003). Gross nitrogen fluxes in soil: theory, measurement and application of ¹⁵N pool dilution techniques. *Advances in Agronomy* 79, 69-118.
- Neary, D.G., Klopatek, C.C., Debano, L.F., and Ffolliott, P.F. (1999). Fire effects on belowground sustainability: a review and synthesis. *Forest Ecology and Management* 122, 51-71.
- Neeson, R. (2008). "Organic rice and soybean production: A guide to convert to organic production. ").
- Nelson, S. (1996). A review and assessment of microwave energy for soil treatment to control pests. *Transactions of the ASAE* 39, 281-289.
- Nelson, S., Nutile, G., and Stetson, L. (1970). Effects of radiofrequency electrical treatment on germination of vegetable seeds. *Journal of the American Society of Horticultural Science* 95, 359-366.
- Nelson, S., and Walker, E.R. (1961). Effects of radio-frequency electrical seed treatment. *Agricultural Engineering* 42, 688-691.
- Nemergut, D.R., Shade, A., and Violle, C. (2014). When, where and how does microbial community composition matter? *Frontiers in Microbiology* 5, 497.
- Newnham, R., Jang, S., Xu, M., and Jones, F. (1991). Fundamental interaction mechanisms between microwaves and matter. *Ceramic Transactions* 21, 51-67.
- Nie, M., Pendall, E., Bell, C., and Wallenstein, M.D. (2014). Soil aggregate size distribution mediates microbial climate change feedbacks. *Soil Biology and Biochemistry* 68, 357-365.
- Norsworthy, J.K., Burgos, N.R., Scott, R.C., and Smith, K.L. (2007). Consultant perspectives on weed management needs in Arkansas rice. *Weed Technology* 21, 832-839.

- Nunes, I., Jurburg, S., Jacquioid, S., Brejnrod, A., Salles, J.F., Priemé, A., and Sørensen, S.J. (2018). Soil bacteria show different tolerance ranges to an unprecedented disturbance. *Biology and Fertility of Soils* 54, 189-202.
- O'bannon, J., and Good, J. (1971). Applications of microwave energy to control nematodes in soil. *Journal of Nematology* 3, 93.
- O'brien, P.L., Desutter, T.M., Casey, F.X., Khan, E., and Wick, A.F. (2018). Thermal remediation alters soil properties—a review. *Journal of Environmental Management* 206, 826-835.
- O'Neill, P., and Jackson, T. (1990). Observed effects of soil organic matter content on the microwave emissivity of soils. *Remote sensing of environment* 31, 175-182.
- Oerke, E.-C. (2006). Crop losses to pests. *The Journal of Agricultural Science* 144, 31-43.
- Oerke, E., Dehne, H., Schonbeck, F., and Weber, A. (1994). *Crop Production and Crop Protection: Estimated Losses in Major Food and Cash Crops*,. Amsterdam.: Elsevier.
- Oksanen, J., Kindt, R., Legendre, P., O'hara, B., Simpson, G.L., Solymos, P., Stevens, M.H.H., and Wagner, H. (2008). The vegan package. *Community ecology package*. [<http://r-forge.r-project.org/projects/vegan/>].
- Olsen, R. (1975). A theoretical investigation of microwave irradiation of seeds in soil. *Journal of Microwave Power* 10, 281-296.
- Parish, S. (1990). A review of non-chemical weed control techniques. *Biological Agriculture & Horticulture* 7, 117-137.
- Patil, N.G., Rebrov, E.V., Eranen, K., Benaskar, F., Meuldijk, J., Mikkola, J.-P., Hessel, V., Hulshof, L.A., Murzin, D.Y., and Schouten, J.C. (2016). Effect of the Load Size on the Efficiency of Microwave Heating Under Stop Flow and Continuous Flow Conditions. *Journal of Microwave Power and Electromagnetic Energy* 46, 83-92.
- Peerzada, A.M., and Chauhan, B.S. (2018). "Thermal Weed Control: History, Mechanisms, and Impacts," in *Non-Chemical Weed Control*. Elsevier), 9-31.
- Peng, S., Cassman, K.G., and Kropff, M.J. (1995). Relationship between leaf photosynthesis and nitrogen content of field-grown rice in tropics. *Crop Science* 35, 1627-1630.
- Peng, S., Garcia, F., Laza, R., Sanico, A., Visperas, R., and Cassman, K. (1996). Increased N-use efficiency using a chlorophyll meter on high-yielding irrigated rice. *Field Crops Research* 47, 243-252.

- Pietikaäinen, J., Hiukka, R., and Fritze, H. (2000). Does short-term heating of forest humus change its properties as a substrate for microbes? *Soil Biology and Biochemistry* 32, 277-288.
- Powles, S.B., and Howat, P.D. (1990). Herbicide-resistant weeds in Australia. *Weed Technology* 4, 178-185.
- Pratley, J., Flower, R., Heylin, E., and Sivapalan, S. (2004). Integrated weed management strategies for the rice weeds *Cyperus difformis* and *Alisma plantagoaquatica*. A report for the Rural Industries Research and Development Corporation (RIRDC) Project No USC A 20.
- Pretty, J., and Bharucha, Z.P. (2014). Sustainable intensification in agricultural systems. *Annals of botany* 114, 1571-1596.
- Price, J.N., Wright, B.R., Gross, C.L., and Whalley, W.R.D.B. (2010). Comparison of seedling emergence and seed extraction techniques for estimating the composition of soil seed banks. *Methods in Ecology and Evolution* 1, 151-157.
- Prosser, J.I., and Nicol, G.W. (2012). Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. *Trends in Microbiology* 20, 523-531.
- Radosevich, S.R., Holt, J.S., and Ghersa, C. (1997). *Weed ecology: implications for management*. John Wiley & Sons.
- Radzevičius, A., Sakalauskienė, S., Dagys, M., Simniškis, R., Karkleliene, R., Bobinas, Č., and Duchovskis, P. (2013). The effect of strong microwave electric field radiation on: (1) vegetable seed germination and seedling growth rate. *Zemdirbyste-Agriculture* 100, 179-184.
- Ramesh, K., Matloob, A., Aslam, F., Florentine, S.K., and Chauhan, B.S. (2017). Weeds in a changing climate: vulnerabilities, consequences, and implications for future weed management. *Frontiers in Plant Science* 8, 1-12.
- Rampelotto, P.H., De Siqueira Ferreira, A., Barboza, A.D.M., and Roesch, L.F.W. (2013). Changes in diversity, abundance, and structure of soil bacterial communities in Brazilian Savanna under different land use systems. *Microbial Ecology* 66, 593-607.
- Rao, A., Johnson, D., Sivaprasad, B., Ladha, J., and Mortimer, A. (2007). Weed management in direct-seeded rice. *Advances in Agronomy* 93, 153-255.
- Rask, A.M., and Kristoffersen, P. (2007). A review of non-chemical weed control on hard surfaces. *Weed Research* 47, 370-380.
- Reddy, M.B., Raghavan, G., Kushalappa, A., and Paulitz, T. (1998). Effect of Microwave Treatment on Quality of Wheat Seeds Infected with *Fusarium graminearum*. *Journal of Agricultural Engineering Research* 71, 113-117.

- Reeve, J.R. (2011). Toward sustainable agricultural systems in the 21st century. *Choice: Current Reviews for Academic Libraries* 48, 1109.
- Rice, R.P., and Putnam, A.R. (1977). Some factors which influence the toxicity of UHF energy to weed seeds. *Weed Science* 25, 179-183.
- Richardson, R.E., James, C.A., Bhupathiraju, V.K., and Alvarez-Cohen, L. (2002). Microbial activity in soils following steam treatment. *Biodegradation* 13, 285-295.
- Rotthauwe, J.-H., Witzel, K.-P., and Liesack, W. (1997). The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied Environmental Microbiology* 63, 4704-4712.
- Roux-Michollet, D., Czarnes, S., Adam, B., Berry, D., Commeaux, C., Guillaumaud, N., Le Roux, X., and Clays-Josserand, A. (2008). Effects of steam disinfection on community structure, abundance and activity of heterotrophic, denitrifying and nitrifying bacteria in an organic farming soil. *Soil Biology and Biochemistry* 40, 1836-1845.
- Russell, A. (2003). Lethal effects of heat on bacterial physiology and structure. *Science Progress* 86, 115-137.
- Sage, R.F., and Pearcy, R.W. (1987). The nitrogen use efficiency of C3 and C4 plants: I. Leaf nitrogen, growth, and biomass partitioning in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiology* 84, 954-958.
- Salamanca-Jimenez, A., Doane, T.A., and Horwath, W.R. (2017). Nitrogen use efficiency of coffee at the vegetative stage as influenced by fertilizer application method. *Frontiers in Plant Science* 8, 1-11.
- Samtani, J.B., Gilbert, C., Weber, J.B., Subbarao, K.V., Goodhue, R.E., and Fennimore, S.A. (2012). Effect of steam and solarization treatments on pest control, strawberry yield, and economic returns relative to methyl bromide fumigation. *HortScience* 47, 64-70.
- Sánchez-Marañón, M., Miralles, I., Aguirre-Garrido, J.F., Anguita-Maeso, M., Millán, V., Ortega, R., García-Salcedo, J.A., Martínez-Abarca, F., and Soriano, M. (2017). Changes in the soil bacterial community along a pedogenic gradient. *Scientific Reports* 7, 1-11.
- Sartorato, I., Zanin, G., Baldoin, C., and De Zanche, C. (2006). Observations on the potential of microwaves for weed control. *Weed Research* 46, 1-9.
- Sawhney, B. (1972). Selective sorption and fixation of cations by clay minerals: a review. *Clays Clay Miner* 20, 93-100.
- Schulten, H.-R., and Schnitzer, M. (1997). The chemistry of soil organic nitrogen: a review. *Biology and Fertility of Soils* 26, 1-15.

- Schulten, H.R., and Schnitzer, M. (1998). The chemistry of soil organic nitrogen: a review. *Biology and Fertility of Soils* 26, 1–15.
- Shade, A., Peter, H., Allison, S., Baho, D., Berga, M., Buergermann, H., Huber, D., Langenheder, S., Lennon, J., Martiny, J., Matulich, K., Schmidt, T., and Handelsman, J. (2012). Fundamentals of Microbial Community Resistance and Resilience. *Frontiers in Microbiology* 3.
- Shamis, Y., Taube, A., Mitik-Dineva, N., Croft, R., Crawford, R.J., and Ivanova, E.P. (2011). Specific electromagnetic effects of microwave radiation on *Escherichia coli*. *Applied and Environmental Microbiology* 77, 3017-3022.
- Shamis, Y., Taube, A., Shramkov, Y., Mitik-Dineva, N., Vu, B., and Ivanova, E.P. (2008). Development of a microwave treatment technique for bacterial decontamination of raw meat. *International Journal of Food Engineering* 4.
- Shanahan, J., Donnelly, K., Smith, D., and Smika, D. (1985). Shoot Developmental Properties Associated with Grain Yield in Winter Wheat 1. *Crop Science* 25, 770-775.
- Shaw, D.R. (2005). Translation of remote sensing data into weed management decisions. *Weed Science* 53, 264-273.
- Shaw, W.C. (1964). Weed science—revolution in agricultural technology. *Weeds* 12, 153-162.
- Shehzad, M.T., Murtaza, G., Shafeeque, M., Sabir, M., Nawaz, H., and Khan, M.J. (2019). Assessment of trace elements in urban topsoils of Rawalpindi-Pakistan: a principal component analysis approach. *Environmental Monitoring and Assessment* 191, 65.
- Smith, P., House, J.I., Bustamante, M., Sobocká, J., Harper, R., Pan, G., West, P.C., Clark, J.M., Adhya, T., and Rumpel, C. (2016). Global change pressures on soils from land use and management. *Global Change Biology* 22, 1008-1028.
- Solans, M., Vobis, G., Cassán, F., Masciarelli, O., Luna, V., and Wall, L. (2006). Producción de Fitohormonas por Rhizoactinomicetes; Posibles Efectos Sobre la Simbiosis. *Discaria trinervis-Frankia. XXVI Reunión de la Asociación Argentina de Fisiología Vegetal. Book of abstracts, PII* 74.
- Soumya, T., Nanjappa, H., and Ramachandrappa, B. (2010). Effect of soil solarization on weed count, weed dry weight and pod yield of groundnut. *Karnataka Journal of Agricultural Sciences* 17.
- Speir, T., Cowling, J., Sparling, G., West, A., and Corderoy, D. (1986). Effects of microwave radiation on the microbial biomass, phosphatase activity and levels of extractable N and P in a low fertility soil under pasture. *Soil Biology and Biochemistry* 18, 377-382.

- Statistics, A.B.O. (2016). *Agricultural Commodities* [Online]. Available: <http://www.abs.gov.au/Agriculture> [Accessed].
- Stevenson, F.J. (1982a). *Nitrogen in agriculture soils*. Madison WI.: Soil Science Society of America Inc., Crop Sciences Society of America Inc., and American Society of Agronomy Inc.,.
- Stevenson, F.J. (1982b). *Organic forms of soil nitrogen*. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.
- Stevenson, F.J. (1994). *Humus Chemistry: Genesis, Composition, Reactions*. New York: Wiley.
- Suarez, J.E., Barbes, C., and Hardisson, C. (1980). Germination of spores of *Micromonospora chalybeata*: physiological and biochemical changes. *Microbiology* 121, 159-167.
- Suzuki, M.T., Taylor, L.T., and Delong, E.F. (2000). Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. *Applied and Environmental Microbiology* 66, 4605-4614.
- Swanton, C.J., Nkoa, R., and Blackshaw, R.E. (2015). Experimental methods for crop-weed competition studies. *Weed Science* 63, 2-11.
- Takashima, S., Gabriel, C., Sheppard, R., and Grant, E. (1984). Dielectric behavior of DNA solution at radio and microwave frequencies (at 20 degrees C). *Biophysical Journal* 46, 29-34.
- Team, R., C. (2014). *R: a language and environment for statistical computing*. R foundation for Statistical Computing, Vienna, Austria. [Online]. <http://www.R-project.org/> [Accessed].
- Thomas, G., Titmarsh, G., Freebairn, D., and Radford, B. (2007). No-tillage and conservation farming practices in grain growing areas of Queensland—a review of 40 years of development. *Australian Journal of Experimental Agriculture* 47, 887-898.
- Thompson, K., Bakker, J.P., Bakker, J.P., and Bekker, R.M. (1997). *The soil seed banks of North West Europe: methodology, density and longevity*. Cambridge university press.
- Tourna, M., Freitag, T.E., Nicol, G.W., and Prosser, J.I. (2008). Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environmental Microbiology* 10, 1357-1364.
- Ulaby, F.T., Batlivala, P.P., and Dobson, M.C. (1978). Microwave backscatter dependence on surface roughness, soil moisture, and soil texture: Part I-bare soil. *IEEE Transactions on Geoscience Electronics* 16, 286-295.

- Ulloa, S.M., Datta, A., Bruening, C., Neilson, B., Miller, J., Gogos, G., and Knezevic, S.Z. (2011). Maize response to broadcast flaming at different growth stages: effects on growth, yield and yield components. *European Journal of Agronomy* 34, 10-19.
- Van Wambeke, E., Wijsmans, J., and D'hertefeldt, P. (Year). "Possibilities in microwave application for growing substrate disinfestation", in: *II International Symposium on Soil Disinfestation* 152), 209-218.
- Vela, G., and Wu, J. (1979). Mechanism of lethal action of 2,450-MHz radiation on microorganisms. *Applied and Environmental Microbiology* 37, 550-553.
- Vela, G., Wu, J., and Smith, D. (1976). Effect of 2450 MHz Microwave Radiation on Some Soil Microorganisms in situ. . *Soil Science* 121, 44-51.
- Verschaeve, L., and Maes, A. (1998). Genetic, carcinogenic and teratogenic effects of radiofrequency fields. *Mutation Research/Reviews in Mutation Research* 410, 141-165.
- Vidotto, F., De Palo, F., and Ferrero, A. (2013). Effect of short-duration high temperatures on weed seed germination. *Annals of Applied Biology* 163, 454-465.
- Vigneault, C., Benoit, D., and Mclaughlin, N. (1990). Energy aspects of weed electrocution. *Reviews of Weed Science* 5, 15-26.
- Villegas, D., Aparicio, N., Blanco, R., and Royo, C. (2001). Biomass accumulation and main stem elongation of durum wheat grown under Mediterranean conditions. *Annals of Botany* 88, 617-627.
- Voort, M., Kempenaar, M., Van Driel, M., Raaijmakers, J.M., and Mendes, R. (2016). Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression. *Ecology Letters* 19, 375-382.
- Vos, P., Garrity, G., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., and Whitman, W.B. (2011). *Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes*. Springer Science & Business Media.
- Wainwright, M., Killham, K., and Diprose, M. (1980). Effects of 2450 MHz microwave radiation on nitrification, respiration and S-oxidation in soil. *Soil Biol Biochem.* 12, 489-493.
- Wang, H., and Tiefenback, M. (2004). Waveguide stub tuner analysis for CEBAF application. *published in LINAC*.
- Wang, L., and Schjørring, J.K. (2012). Seasonal variation in nitrogen pools and ¹⁵N/¹³C natural abundances in different tissues of grassland plants. *Biogeosciences* 9, 1583.
- Wayland, J., Davis, F., and Merkle, M. (1973). Toxicity of an UHF device to plant seeds in soil. *Weed Science* 21, 161-162.

- Wayland, J., Merkle, M., Davis, F., Menges, R., and Robinson, R. (1975). Control of weeds with UHF electromagnetic fields. *Weed Research* 15, 1-5.
- Wayland, J.R., Davis, F.S., and Merkle, M.G. (1978). "Vegetation control". Google Patents).
- Whatley, T.L., Davis, F.S., and Merkle, M.G. (1973). *Effects of soil moisture on phytotoxicity of microwave fields.*: (Abs.) Proc. Southern Weed Science Society 26: 389.
- Wilson, R.G., Kerr, E.D., and Nelson, L.A. (1985). Potential for using weed seed content in the soil to predict future weed problems. *Weed Science* 33, 171-175.
- Wolf, W., Vaughn, C., Harris, R., and Loper, G. (1993). Insect radar cross-sections for aerial density measurements and target classification. *Transactions of the ASAE* 36, 949-954.
- Woo, I.S., Rhee, I.K., and Park, H.D. (2000). Differential Damage in Bacterial Cells by Microwave Radiation on the Basis of Cell Wall Structure. *Applied Environmental Microbiology* 66, 2243-2247.
- Wssa (2008). "Common and chemical names of herbicides approved by the WSSA. ". Weed Sceince Society of America).
- Wszelaki, A.L., Doohan, D.J., and Alexandrou, A. (2007). Weed control and crop quality in cabbage (*Brassica oleracea* (capitata group)) and tomato (*Lycopersicon lycopersicum*) using a propane flamer. *Crop Protection* 26, 134-144.
- Xie, H., Yan, D., Mao, L., Wang, Q., Li, Y., Ouyang, C., Guo, M., and Cao, A. (2015). Evaluation of methyl bromide alternatives efficacy against soil-borne pathogens, nematodes and soil microbial community. *PloS one* 10, e0117980.
- Yaghmaee, P., and Durance, T. (2005). Destruction and injury of *Escherichia coli* during microwave heating under vacuum. *Journal of Applied Microbiology* 98, 498-506.
- Yang, J.E., Skogley, E.O., and Schaff, B.E. (1990). Microwave radiation and incubation effects on resin-extractable nutrients: I. Nitrate, ammonium, and sulfur. *Soil Sciinec Society of American Journal* 54, 1639-1645.
- Yang, W., Peng, S., Laza, R.C., Visperas, R.M., and Dionisio-Sese, M.L. (2008). Yield gap analysis between dry and wet season rice crop grown under high-yielding management conditions. *Agronomy Journal* 100, 1390-1395.
- Yao, Z., Zheng, X., Liu, C., Lin, S., Zuo, Q., and Butterbach-Bahl, K. (2017). Improving rice production sustainability by reducing water demand and greenhouse gas emissions with biodegradable films. *Scientific Reports* 7, 39855.
- Yeager, C.M., Dunbar, J., Hesse, C.N., Daligault, H., and Kuske, C.R. (2017). Polysaccharide degradation capability of Actinomycetales soil isolates from a

- semi-arid grassland of the Colorado Plateau. *Applied and environmental microbiology*, AEM. 03020-03016.
- Yee, K. (1966). Numerical solution of initial boundary value problems involving Maxwell's equations in isotropic media. *IEEE Transactions on Antennas and Propagation* 14, 302-307.
- Yi, Y.M., Park, S., Munster, C., Kim, G., and Sung, K. (2016). Changes in ecological properties of petroleum oil-contaminated soil after low-temperature thermal desorption treatment. *Water, Air, & Soil Pollution* 227, 108.
- Yoseftabar, S. (2013). Effect of nitrogen and phosphorus fertilizer management on growth and yield of rice. *International Journal of Agriculture and Crop Sciences* 5, 1659.
- Yu, Y., Lee, C., Kim, J., and Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnology and Bioengineering* 89, 670-679.
- Zagal, E. (1989). Effects of microwave radiation on carbon and nitrogen mineralization in soil. *Soil Biology and Biochemistry* 21, 603-605.
- Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. (2013). PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 30, 614-620.
- Zhou, B.W., Shin, S.G., Hwang, K., Ahn, J.-H., and Hwang, S. (2010). Effect of microwave irradiation on cellular disintegration of Gram positive and negative cells. *Applied Microbiology and Biotechnology* 87, 765-770.
- Zieliński, M., Ciesielski, S., Cydzik-Kwiatkowska, A., Turek, J., and Dębowski, M. (2007). Influence of microwave radiation on bacterial community structure in biofilm. *Process Biochemistry* 42, 1250-1253.
- Zieliński, M., and Zielińska, M. (2010). Impact of microwave radiation on nitrogen removal and quantity of nitrifiers in biofilm. *Canadian Journal of Civil Engineering* 37, 661-666.