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Weed management options for Victorian councils - Alternatives to glyphosate

Final Report

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Research Report Title:	Final Project Report
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Project No. and Title:	RM38031 MAV Bräu L Weed management strategy at Victorian Councils - alternatives to glyphosate

Preliminary synopsis

Application of glyphosate-based herbicides has been a common method for weed control in most settings, including agriculture, parklands, urban environments and at households. Glyphosate has become the subject of much interest due to advances in understanding of its potential off-target toxicity, particularly in humans where it potentially may lead to carcinogenesis. This project sought to identify and assess the efficacy of potential alternative non-glyphosate-based weed management strategies. The dot points below summarise the outcomes from this study:

- Glyphosate was observed to significantly reduce weed coverage for up to 12 weeks with no evidence of negative impacts on soil profile, arthropod or microbial populations.

- Glufosinate significantly reduced weed coverage for up to 12 weeks with no evidence of negative impacts on soil profile, arthropod or microbial populations. The efficacy of glufosinate compared to glyphosate varied, and it was not as effective as glyphosate across all seasons. Compared with glufosinate, the percentage weed coverage was significantly lower for the following glyphosate treatments: 4 weeks post treatment for spring at Vermont South, 12 weeks post treatment for summer at Vermont South, 4 weeks post autumn treatment at Aspendale, and 12 weeks post autumn treatment at both sites. Compared to glyphosate, glufosinate is approx. twice as costly (AU$0.21/L for glufosinate compared to AU$0.10/L for glyphosate) and seasonal application rates may need to be higher and/or more frequent.

- Imazapyr has shown to be an effective broad-spectrum herbicide that kills established weeds and has preemergence effects. Imazapyr significantly reduced weed coverage 12 weeks and beyond from the first application at both sites. There were no notable alterations to the soil microbiome or arthropod communities associated with imazapyr treatment. There are potential issues with off-target effects due to its ability to readily diffuse through soil, residual

activity, and cost (AU$2.02/L). Imazapyr use would require large buffer zones from waterways, plant life that is to be retained and wait times of over 3 months before planting.

- Steam was found to be an effective short term to long term weed reduction strategy based on cumulative effects observed. However, the steam treatment caused alterations in soil microbe populations, reducing overall microbial diversity. Based on this, steam would be recommended as a chemical-free alternative for small-scale targeted applications where the environment is altered in such a way that soil microbial ecosystem services are of minor significance, such as concrete walkways, kerb and channel guttering, asphalt driveways and car parks. Accessibility also needs to be taken into account for steaming due to the size of the steam units (width up to 2.52 m, weight of up to 2.6 tonnes). There are also high capital costs and potentially high on going operational costs associated with the steam units.

- Clove oil, pine oil, nonanoic acid, acetic acid + hydrochloric acid, prodiamine and MCPA + dicamba treatments had varying short term effects on percentage weed coverage and showed no capacity to significantly reduce weed coverage 12 weeks post application or beyond. Based on the results of this study, there were no notable alterations to soil profile, microbial communities or arthropod communities associated with these products.

1.0 Introduction

Introduced invasive plant species (weeds) are controlled to maintain and preserve native flora and fauna in urbanised areas and revegetated habitat zones, prevent damage to infrastructure and to maintain aesthetically pleasing streetscapes and parklands. There are many forms of weed control with the current most-used strategy being the application of glyphosate-based herbicides (Global Industry Analysts 2011).

Glyphosate-based herbicides have become the most common choice for weed control based on cost, ease of application, target specificity and high efficacy in killing a broad range of weeds. Glyphosate was originally perceived as having low toxicity towards animals, however, recently it has been suggested that glyphosate may lead to carcinogenesis in humans (Buhl et al 2010). In 2016, the International Agency for Research on Cancer (IARC) published a report that classified glyphosate as a Group 2A agent (probable carcinogens), classifying glyphosate as being probably carcinogenic to humans.

Classification of agents as Group 2A (probable carcinogen) agents is applied when there is limited evidence of carcinogenicity in humans as well as sufficient evidence of carcinogenicity in experimental animals. Agents (substances and exposure circumstances that pose a risk) may also be classified as Group 2A if there is inadequate evidence of carcinogenicity in humans along with sufficient evidence of carcinogenicity in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Agents may also be classified as Group 2A based solely on limited evidence of carcinogenicity in humans. A complete list of Group 2A (probable carcinogens) is available through the American Cancer Society (https://www.cancer.org/cancer/cancer-causes/general-info/known-and-probable-human-carcinogens.html). The American Cancer Society does not determine if something is carcinogenic or classify agents based on their carcinogenicity. The

American Cancer Society presents the classifications determined by the IARC and the US National Toxicology Program (NTP).

The Australian Pesticides and Veterinary Medicines Authority (APVMA) initially conducted a comprehensive review of a glyphosate in 1997, which set Australia's health-based guidance values at a level that remains protective; with concluding outcomes being "that all registered glyphosate products are safe provided they are used as per the label instructions". In 2016, following the outcomes of the IARC assessment for glyphosate use, the APVMA reviewed the IARC assessment report and other relevant scientific information and concluded that there is currently no scientific reason to reconsider the registration of glyphosate. This means at present the APVMA advises that "Glyphosate is registered for use in Australia, and APVMA approved products containing glyphosate can continue to be used safely according to label directions".

The use of glyphosate has become an increasingly sensitive topic since 2018 when frequent users of the glyphosate based "Round up" in America pursued legal compensation from the company Bayer after they were diagnosed with non-Hodgkin's lymphoma. Increased media focus and subsequent community focus on council use of glyphosate for the management of weeds has prompted the need to engage an independent review of the options available in the market.

1.1.0 History and background of glyphosate and its common practice use

Since its release in 1974, glyphosate (N-(phosphonomethyl)glycine) has become the world's most common commercial synthetic phosphonate herbicide (Annamalai, 2020). Glyphosate was initially developed to reduce the reliance on other herbicides that would cause crop damage, had lower efficacy, allowed development of resistance, and posed health risks to humans (Antoniou et al 2016). Glyphosate is the active ingredient in as many as 500 weed killers and herbicide products (Australian Farmers 2018). Glyphosate is highly valued as a herbicide due to its rapid soil binding, biodegradation,

non-volatility, stability in favourable conditions including sunlight, complete solubility in water, easy application on crops, and is less toxic than a range of other broad spectrum herbicides (Borggaard and Gimsing 2008; Valavanidis 2018).

1.2.0 Glyphosate mode of action and toxicity

Glyphosate is applied on plant foliage where it is absorbed through cuticles, then taken up through the symplast via phosphate carrier channels (proteins) within the cell membrane (Chalifour et al 2014). Glyphosate moves through phloem, in a pathway similar to other photoassimilates, which are produced in photosynthetically active tissues, and then it migrates towards growth and storage tissues including roots, tubers, rhizomes, young leaves and meristematic zones (Christoffoleti et al 2004). Glyphosate accumulates in plant organs with high rates of metabolism and growth, including root nodules, root tips and shoot apices (Cakmak et al 2009). Glyphosate and aminomethylphosphonic acid (AMPA) are readily taken up from soil by plants through root tissues due to their chemical similarities.

After application to foliage, glyphosate breaks down into by-products including AMPA. Due to the chemical similarity with glyphosate, AMPA is able to compete with glycine for biological sites and pathways. This affects chlorophyll biosynthesis and photosynthetic process resulting in plant death (Chalifour et al 2014).

In humans, previous studies have suggested that dermal absorption of glyphosate is poor, where a maximum of 2.2% to 2.6 μg/cm^2 of glyphosate is absorbed across the skin, with peak absorption occurring 8 hours after administration (Buhl et al 2010). It has also been shown that glyphosate is non-volatile, with absorption from inhalation exposure deemed as not significant and not posing a threat (Buhl et al 2010). Glyphosate exposure is monitored by measuring the AMPA concentration in urine and faeces (Buhl et al 2010). It is perceived that there is little absorption of glyphosate during digestion (Buhl et al 2010). The impact of glyphosate metabolism on human health was found to be minimal

when high ratios of AMPA were detected in human patients' blood serum 8 h (22.6 µg/mL glyphosate to 0.18 µg/mL AMPA) and 16 h (4.4 µg/mL glyphosate to 0.03 µg/mL AMPA) post ingestion (Buhl et al 2010).

1.3.0 Effects of glyphosate on the soil microbiome

The soil microbiome is comprised primarily of bacterial and fungal communities (Reid and Wong 2005). Microorganisms play a vital role maintaining soil health; where they can support plant growth through nutrient cycling and many other processes, including soil structure, pH and water retention (Jansson and Hofmockel 2018). The microbiome of soils is vital as it regulates the molecular form of carbon form that is to be released. For example, carbon can be released as either CO_2 or CH_4, or, retained in the soil (Jansson and Hofmockel 2018). A study conducted by Fomsgaard et al 2008, demonstrated that agricultural soil amended with phosphorus fertilisers are high in unbound glyphosate. This is due to soil sorption sites being occupied by competing phosphate ions causing glyphosate to remain in the soil solution, leaving it vulnerable to the uptake by plant roots and associated rhizosphere microbial community alike.

A long-term study has identified that prolonged exposure of soil microorganism to glyphosate has led to fungal community dominated by undesirable plant pathogenic *Fusarium* spp. (Krzysko-Lupicka and Sudol 2008). A study conducted by Means (2004) showed a significant increase in the number of *Fusarium* spp. colony numbers within two weeks post glyphosate usage. The recommended rates of glyphosate usages include application once within a 24 h period, when weather is not windy or raining. Once glyphosate is applied to susceptible plants it can result in heavy colonisation of roots by soilborne fungi, mainly the *Fusarium* variety (Johal and Rhane 1984). Infection of plants by these pathogens contributes towards their death and could adversely affect subsequent plantings and the overall soil microbiome.

1.4.0 Alternative herbicide options for glyphosate

Organic herbicides have increased in availability and popularity, offering a potentially environmentally friendly and less toxic alternative. However, organic alternatives are not without issues, and like all herbicides precautions and risks need to be assessed prior to use. While there are many variations and alternatives to glyphosate, selecting those that maintain target specificity and reduce health risks to surrounding vegetation and humans needs to be fully considered.

For this study, an extensive list of alternative options to glyphosate was compiled and a shortlist of alternatives to glyphosate were selected for further trialling, which represented chemical, plant oil based organics, organic acid and physical management options. The chemical alternatives selected for testing were imazapyr, glufosinate, MCPA + dicamba and prodiamine. The organic plant oil based alternatives selected for testing were pine oil and clove oil. The organic acid based alternatives selected for testing were nonanoic acid and acetic acid + hydrochloric acid. The clove oil product also contained 40.4 g/L of acetic acid, in addition to the 40.4 g/L of plant-based clove oil. Steaming of weeds was selected as a non-chemical, physical weed eradication strategy for assessment against glyphosate. This is due to steaming increasingly being seen as an attractive option, given it knocks back weeds instantly and with higher success compared to manually hand-picking weeds.

1.5.0 Aims and objectives

Based on the drivers, the overall project goal is to:

1. Provide comparable data on the safety aspects (including increased or decreased risk), effectiveness, financial implications and potential long-term soil impacts of a range of methods available to manage weeds against the current product used which contains glyphosate.
2. Assess baseline parameters for referencing of microbial abundance and diversity in soils, establish “normal” physio-chemical conditions, and determine insect species abundance and

diversity and background flora to assess the impact of glyphosate and alternate weed management strategies on these.

2.0 Methods and Materials

2.1.0 Assessment of available weed products

In order to determine the trial alternatives a comprehensive assessment of currently available weed control products was conducted. A survey was undertaken of all currently used chemical herbicide alternatives to glyphosate looking at mode of action, solubility in water, poison schedule, resistance, effect on metabolism (plant and other organisms if known), flammability, availability for purchasing, contact effect, harmful residue, active constituent, specificity spectrum, residual/non-residual, exposure risk, common form and storage requirements. This working table (Appendix 1) was then used to further produce a shortlist of alternatives to glyphosate. This process was repeated with tables for organic herbicide alternatives separating each of the organic alternatives into categories of organic with chemical component (Appendix 2) and organic alternatives (Appendix 3). The assessment was also applied to manual strategies (Appendix 4) used to extract weeds, such as hand pulling weeds or steaming. From these lists, suitable and compatible herbicide treatment alternatives were chosen for trial against glyphosate.

2.2.0 Herbicide solution preparations and application strategy

Concentrated forms of glyphosate, pine oil, glufosinate, MCPA + dicamba, acetic acid + hydrochloric acid, prodiamine and imazapyr were diluted in water to recommended working concentrations (Table 1) as specified by manufacturers. For clove oil and nonanoic acid, preprepared working solutions were purchased (Table 1). For each weed management strategy and the untreated control, five replicate 1 m^2 quadrats were measured along separate transect lines and 200 mL of herbicide sprayed evenly across all plants within the quadrats. For steaming of weeds, a commercial weed steamer unit was used as per the manufacturer's directions. The temperature was set to 140 - 180 ^{0}C for 10 - 20 s per

0.015 m^2, to cover the entire 1 m^2 area of each quadrat. The weed management strategies were all applied four times over a 12-month period, with applications performed within the first month of each season (winter, spring, summer and autumn, respectively).

2.3.0 Treatment sites and design of treatment blocks for testing weed management strategies

Two sites were chosen to test the effects of the different weed control strategies. Site 1 was at Vermont South, Victoria, Australia (GPS coordinates: -37.860234, 145.198830), which had a heavy clay soil type. Site 2 was at Aspendale, Victoria, Australia (GPS coordinates: -38.012448, 145.090683), which had a sandy soil type. At each site, three blocks of 10 m x 20 m were selected. Within each block 11 transects were measured out and treatments performed within five separate 1 m^2 quadrats placed along the transects with 0.6 m spaces between each quadrat, giving five replicates along each transect for each of the three replicate blocks (Fig. 1). For each 1 m^2 quadrat, one soil sample (50 mL) was taken immediately before and 4 weeks after treatment with the different weed management strategies. At the time of collection soil samples were chilled on ice. Upon returning to the laboratory 10 mL of soil was taken out for doing counts of bacterial number and colony types and the remaining 40 mL stored frozen at -80 ^{0}C for subsequent DNA extractions.

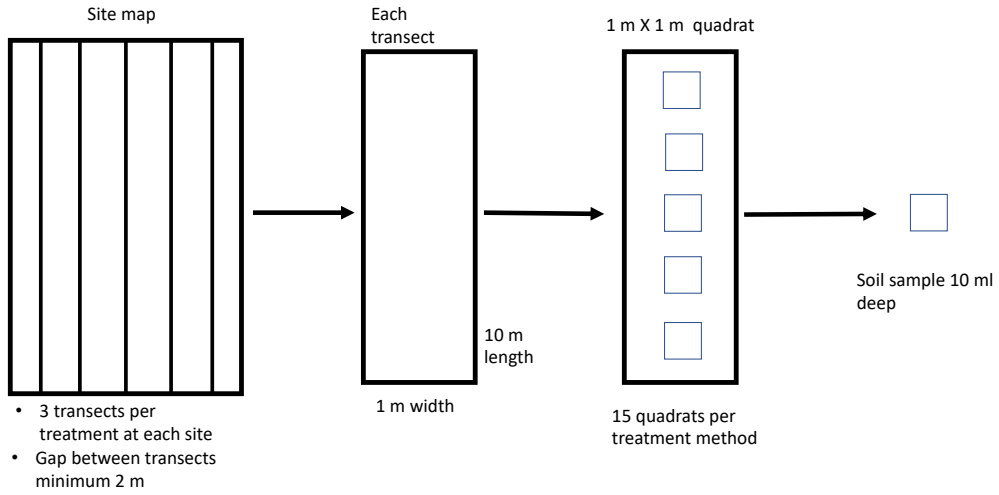


Figure 1. Overview of experimental design and trail sites for assessing the effects of the different weed management strategies. For each weed management strategy and the untreated control, five replicate 1 m^2 quadrats were measured along separate transect lines (2 trial sites, 3 replicate treatment blocks per site, 11 transects per block, each transect 10 m in length).

Table 1. Concentration of active ingredients and dilution factors for making working concentrations in 1 L volumes. For each product, a 1 L working solution was prepared and 200 mL of the working solution applied to each 1 m^2 quadrat. Levels of active ingredients specified may vary between products offered by different manufacturers or form of herbicide (granule, pre-diluted solution or concentrate).

	Glyphosate	Pine oil	Glufosinate	MCPA+ dicamba	Acetic acid + Hydrochloric acid	Prodiamine	Imazapyr	Nonanoic acid	Clove oil
Stock concentration	360 g/L	680 g/L	200 g/L	340 g/L MCPA + 80 g/L dicamba	900 g/L Acetic acid + 10 g/L hydrochloric acid	480 g/L	700 g/kg	36.8 g/L	40.4 g/L Clove oil + 40.4 g/L acetic acid
Dilution	10 ml/L	200 ml/L	5 mL/L	27 mL/L	90 mL/ L	40 mL/L	13 g/L	N/A	N/A
Final active concentration	36 g/L	136 g/L	2 g/L	9.18 g/L MCPA + 2.16 g/L dicamba	81 g/L Acetic acid + 0.9 g/L hydrochloric acid	19.2 g/L	9.1 g/L	36.8 g/L	40.4 g/L Clove oil + 40.4 g/L acetic acid

2.4.0 Total bacterial colony counts per gram of soil after herbicide treatment

For assessing colony forming units (CFU) of bacteria and diversity (based on different types of morphology observed), 4 weeks post treatment one gram of soil was weighted out from the 50 mL collected as described above (section 2.3). One gram of soil was weighed out and suspended in 10 mL of 1 x phosphate buffered saline (PBS) solution, in sterile 15 mL plastic tubes. The samples were mixed vigorously by vortexing for 3 min. Using aseptic technique, 100 µL of the soil suspension was transferred to a sterile microcentrifuge tube containing 900 µL of PBS. These samples were serially diluted a further eight times to reach a dilution factor of 10^{-9}. A volume of 100 µL from each sample of diluted soil was spread across the surface of solidified half-strength nutrient agar (50% NA) medium made up by diluting 37.5 g of nutrient agar medium (Thermo Fisher Scientific, Cat. No. CM0309) plus the addition of 7.5 g of agar into 1 L of water and the medium sterilised at 121 ^{0}C for 20 min. Under aseptic conditions, warm liquid medium was poured into 90 cm sterile petri dishes and the medium set and stored at 4 ^{0}C. After spread plating the diluted samples, they were set aside to dry at room temperature for 1 h, then incubated for 72 h at 22 ^{0}C. After the incubation period the number of colonies and number of different types of colonies (based on physiological and morphological traits) were counted. Samples (100 µL) of the serially diluted preparations were also spread plated on PDA medium, set aside to dry at room temperature for 1 h, then incubated for 72 h at 22 ^{0}C and the different types of fungi (based on morphology and physiology) assessed.

2.5.0 Extraction of total genomic DNA from soil samples and NGS sequencing

Extraction of total genomic DNA from soil samples for assessing bacterial and fungal diversity by subsequent sequencing was achieved using DNeasy PowerSoil Pro Kits (Qiagen, Cat. No. 47014) and following the kit protocol.

Sequencing of bacterial 16S rRNA and Fungal ITS regions, using next generation sequencing (NGS), was conducted by the Australian Genome Research Facility (AGRF). Results of sequencing data were

prepared by AGRF using the Greengenes database to determine species of bacteria based on 16S rRNA sequences and the UNITE database for species of fungi based on ITS sequences.

2.6.0 Assessment of invertebrates in quadrats treated with different weed management strategies

Using the full quadrat method (Cox et al 2017), 1 m^2 quadrats were divided into quarters (0.25 × 0.25 m) and all invertebrate species within this area were counted and identified based on morphology. Abundance values were multiplied by four to estimate the total abundance per quadrat. Pitfall traps were also used to capture arthropods. Following methods described by Work et al 2002, 4.5 cm diameter plastic cylinders, 15 cm in length, filled with ethylene glycol ~ 4 cm from the bottom, were placed centrally in three quadrats of each transect for each treatment (n = 15). After 7 days, the traps were collected, and the arthropods counted and classified to taxonomic order level based on morphology. Relative abundance for arthropods was calculated based on the average number observed from the two different assessment strategies and % relative abundance graphed (Zaller et al 2014).

2.7.0 Soil physical and chemical properties

Samples used for cumulative effect of weed management strategies were collected as follows: 10 core samples 5 cm in diameter and 10 cm in depth were collected from random quadrats for each of the 3 replicate transects. The 30 core samples for each treatment group were pooled and 300 grams weighed out into a plastic ziplock bag. Analyses of soil physical and chemical properties were performed by SWEP Analytical Laboratories (Keysborough, Australia) using methods devised by Mikhail (1980), Rayment and Lyons (2011), Peech et al (1962), and Ross and Wang (1993).

2.8.0 Data analysis and statistical methods

Assessment of percentage weed coverage was based on counting the total number of plants covering the area within each 1 m^2 quadrat of each transect and then the average coverage for each quadrat

of each transect calculated to give three independent counts for each trial site. Microsoft excel was used to prepare all data. Formatted data (using Excel) was imported into the statistical program SPSS for all statistical analysis. Probability plots were produced for all data to test for normal distribution. Analysis of variance (ANOVA) tests and Tukey's Post Hoc analyses were used to determine significant difference of means across the controls and multiple treatments for percentage plant coverage and microbial quantification and diversity data sets.

3.0 Results

3.1.0 Identification of non- glyphosate-based weed management alternatives

Over 50 different alternatives have been identified as possible alternative weed management strategies to glyphosate. From the approximately 50 alternative options, a shortlist of 9 alternatives were identified based on the following: cost, availability, known off-target toxicity, schedule poison classification, storage and handling requirements, flammability, solubility in water, ease of use, application requirements, residual time, specificity, exposure risks and efficacy on weeds typically found in parklands. The alternatives selected for testing against glyphosate were glufosinate, imazapyr, nonanoic acid, acetic acid + hydrochloric acid, clove oil, MPCA + dicamba, pine oil, prodiamine and steam (Appendices 1-3).

3.2.0 identification of dominant weed species at the two selected trial sites

Two sites were selected for testing the efficacy of the different weed management strategies. Site 1 was based in Vermont South, which had dense weed coverage and a heavy clay soil profile. The dominant weed plant species at Vermont South included: *Solanum nigrum* (Black Nightshade), *Brassica rapa L*., *Eleusine indica* (Crowsfoot), *Paspalum dilatatum* (Paspalum), *Cypress rotundus* (Nut Grass), *Digitaria sanguinalis* (Summer Grass), *Poa anua* (Winter Grass), *Romulea rosea* (Guilford Grass), *Trifolium rapens* (White Clover), *Medicago polymorpha* (Burr Medic), *Vicia sativa* (Common Vetch), *Sonchus olerachus* (Milk Thistle), *Gnaphalium sharcium* (Cudweed), *Taraxacum officinale*

(Dandelion), *Conzya spp.* (Fleabane), *Plantago laceolata* (Lambstongue), *Rumex Crispus* (Curled Dock), *Rumex obtusifolius* (Broad-leaf Dock), *Rumex conglomeratus* (Clustered Dock), *Oxalis pes-caprae* (Sour Grass) and *Nothoscordum inodorum* (Onion Weed). Site 2 was based in Aspendale and had a sandy loam soil type with a weed profile that include: *Solanum nigrum* (Black nightshade), *Brassica rapa L.* (Wild Cabbage), *Taraxacum officinale* (Dandelion), *Oxalis strica* (Sour Grass), *Nassella trichotoma* (Serrated Tussock), *Nassella trichomata* (Chilean Needle Grass), *Arctotheca calendular* (Cape Dandelion), *Pennisetum clandestrium* (Kikuyu), *Lycium ferocissimum* (African Boxthorn), *Ulex europaeus L.* (Gorse), *Echium plantagineum* (Paterson's curse) and *Cynodon dactylon* (Bermuda Grass).

3.3.0 Effect of weed management strategies on weed coverage 4- and 12-weeks post application

Seasonally, at each trial site (Vermont South and Aspendale), the effect each of the 10 weed management strategies had on total percentage plant (weed) coverage for each quadrat was assessed 4 weeks and 12 weeks post treatment (Fig. 2-5A-D).

3.3.1 Winter treatments

For winter treatments at Vermont South, 4 weeks after application of glyphosate, glufosinate and MCPA+ dicamba weed coverage was significantly ($p<0.05$) reduced by ~65% (Fig. 2A). Prodiamine treatment significantly reduced ($p<0.05$) weed coverage by ~30% and steam significantly reduced ($p<0.05$) reduced the coverage by over 95%. All other treatments had no significant effect on reducing weed coverage compared to untreated controls (Fig. 2A).

At Aspendale 4 weeks after application, glyphosate, pine oil, glufosinate and clove oil treatments reduced weed coverage significantly ($p<0.05$) by >90% (Fig. 2C). Treatment with acetic acid + hydrochloric acid reduced coverage significantly ($p<0.05$) by ~70% and steam reduced coverage

significantly ($p<0.05$) by over 90%. All other treatments had no significant effect on reducing weed coverage compared to untreated controls (Fig. 2C).

For winter treatments, after 12 weeks of regeneration (post treatment) at both sites, glyphosate and glufosinate significantly reduced ($p<0.05$) weed coverage by between 40-60% (Fig. 2B and D). At both sites after 12 weeks imazapyr significantly reduced ($p<0.05$) weed coverage by over 70% (Fig. 2B and D). After 12 weeks at both sites pine oil, clove oil, nonanoic acid, acetic acid + hydrochloric acid, prodiamine, MCPA + dicamba and steam treatments did not significantly alter weed coverage compared to untreated controls (Fig. 2B and D).

3.3.2 Spring treatments

The percentage weed coverage for quadrats treated with the different weed management strategies was assessed 4- and 12-weeks post application in spring (Fig. 3A – D). At Vermont South four weeks post treatment glyphosate, glufosinate, imazapyr and steam treatments significantly ($p<0.05$) reduced weed coverage per m^2 by over 70% compared to the untreated control (Fig. 3A).

At Aspendale four weeks post treatment glyphosate, pine oil, glufosinate, acetic acid + hydrochloric acid, clove oil, imazapyr and steam treatments significantly ($p<0.05$) reduced weed coverage per m^2 by over 20% to over 95% compared to the untreated control (Fig. 3C).

Twelve weeks post treatment, for spring treatments at both sites, glyphosate, glufosinate and steam significantly reduced ($p<0.05$) weed coverage by between 20-60% (Fig. 3B and D). At both site 12 weeks post treatment imazapyr significantly reduced ($p<0.05$) weed coverage by over 90% (Fig. 3B and D). Minimal changes in weed coverage per m^2 was measured at either site 12 weeks post treatment for pine oil, clove oil, nonanoic acid, acetic acid + hydrochloric acid, prodiamine and MCPA + dicamba steam at both sites (Fig. 3B and D).

3.3.3 Summer treatments

The percentage weed coverage for quadrats treated with the different weed management strategies was assessed 4- and 12-weeks post application in summer (Fig. 4A – D). Four weeks post treatment at Vermont South glyphosate, glufosinate, imazapyr and steam treatments significantly ($p<0.05$) reduced weed coverage per m^2 by over 80% compared to the untreated control (Fig. 4A).

At Aspendale four weeks post treatment glyphosate, pine oil, glufosinate, nonanoic acid, acetic acid + hydrochloric acid, clove oil, imazapyr and steam treatments significantly ($p<0.05$) reduced weed coverage per m^2 by over 20% to over 95% compared to the untreated control (Fig. 4C).

After twelve weeks post summer treatments at both sites, glyphosate, glufosinate and steam significantly reduced ($p<0.05$) weed coverage by between 20-60% (Fig. 4B and D).

3.3.4 Autumn treatments

The percentage weed coverage for quadrats treated with the different weed management strategies was assessed 4- and 12-weeks post application in autumn (Fig. 5A – D). At Vermont South four weeks post treatment glyphosate, glufosinate and imazapyr and steam treatments significantly ($p<0.05$) reduced weed coverage per m^2 by over 80% compared to the untreated control (Fig. 5A).

Four weeks post treatment at Aspendale glyphosate, pine oil, glufosinate, nonanoic acid, acetic acid + hydrochloric acid, clove oil, imazapyr and steam treatments significantly ($p<0.05$) reduced weed coverage per m^2 by over 20% to over 95% compared to the untreated control (Fig. 5C).

After twelve weeks post autumn treatments at both sites, glyphosate, glufosinate and steam significantly reduced ($p<0.05$) weed coverage by between 20-60% (Fig. 5B and D).

Fig. 2A. Effect of different weed management strategies at Vermont South 4 weeks post treatment - Winter

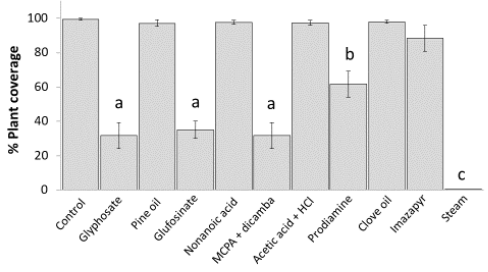


Fig. 2B. Effect of different weed management strategies at Vermont South 12 weeks post treatment - Winter

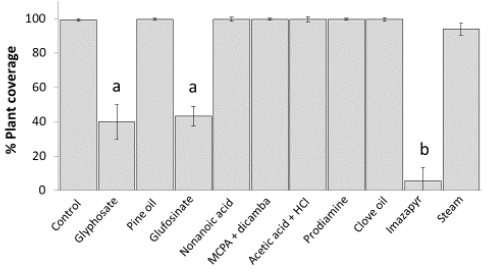


Fig. 2C. Effect of different weed management strategies at Aspendale 4 weeks post treatment - Winter

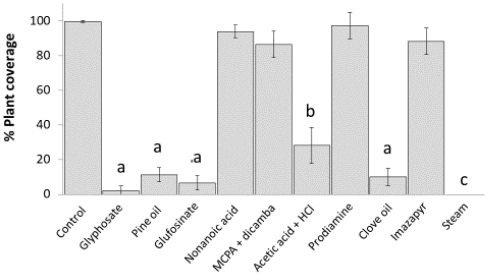
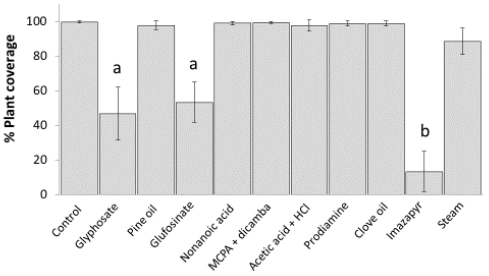


Fig. 2D. Effect of different weed management strategies at Aspendale 12 weeks post treatment - Winter

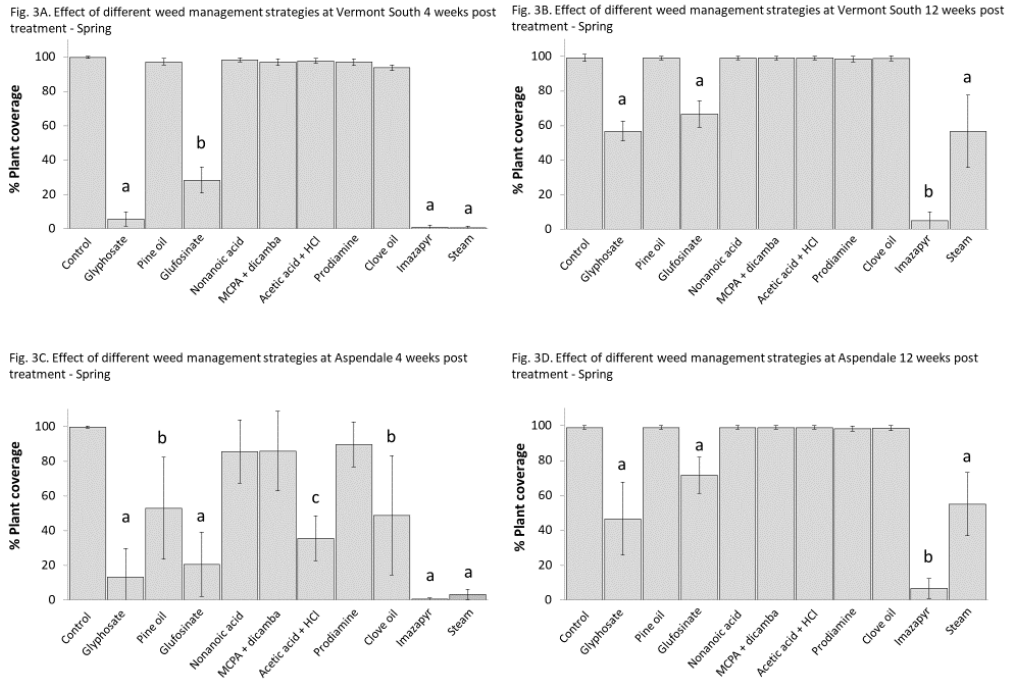


Figures 2A, 2B, 2C, 2D – Winter treatments - Effect of weed management strategies on average percentage coverage of weeds 4 weeks and 12 weeks post treatment

For **Fig. 2A**, "a" denotes significant difference ($p<0.05$) between glyphosate, glufosinate and MCPA + dicamba treatments compared to untreated control group at Vermont South. "b" denotes significant difference ($P<0.05$) between prodiamine treatment compared with all other treatment groups including the untreated "control" group at Vermont South. "c" denotes significant difference ($P<0.05$) between steam treatment compared with all other treatment groups including the untreated control group at Vermont South.

For **Fig. 2B** and **3D**, "a" denotes significant difference ($p<0.05$) between glyphosate and glufosinate treatments compared to untreated "control" group at both sites. "b" denotes significant difference ($p<0.05$) between imazapyr treatment compared with all other treatment groups including control group at both sites.

For **Fig. 2C**, "a" denotes significant difference ($P<0.05$) between glyphosate, pine oil, glufosinate and clove oil treatments compared with all other treatment groups including the untreated control group at Aspendale. "b" denotes significant difference ($P<0.05$) between acetic acid + hydrochloric acid treatment compared with all other treatment groups including the untreated control group at Aspendale. "c" denotes significant difference ($P<0.05$) between steam treatment compared with all other treatment groups including the untreated control group at Aspendale.

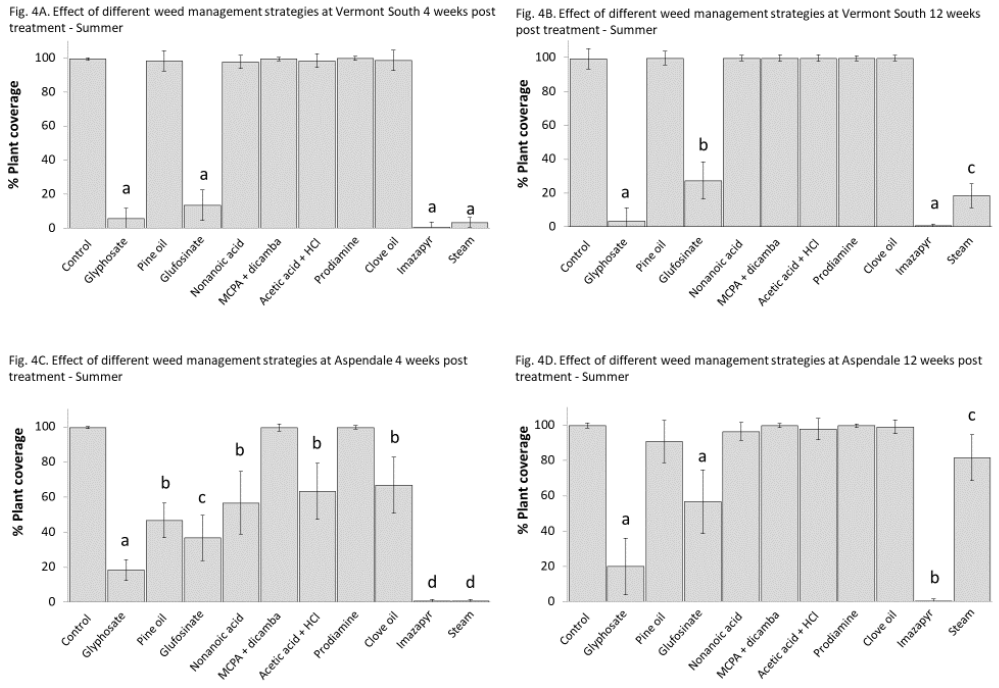


Figures 3A, 3B, 3C, 3D – Spring treatments - Effect of weed management strategies on average percentage coverage of weeds 4 weeks and 12 weeks post treatment

In **Fig. 3A,** "a" denotes significant difference ($p<0.05$) between glyphosate, imazapyr and steam treatments compared to untreated control group at Vermont South. "b" denotes significant difference ($P<0.05$) between glufosinate compared with all other treatment groups including the untreated control group at Vermont South.

For **Fig. 3B and D**, "a" denotes significant difference ($p<0.05$) between glyphosate, glufosinate and steam treatments compared to untreated control group at both sites. "b" denotes significant difference ($p<0.05$) between imazapyr treatment compared with all other treatment groups including control group at both sites.

For **Fig. 3C**, "a" denotes significant difference ($p<0.05$) between glyphosate, glufosinate, imazapyr and steam treatments compared with the untreated control group at Aspendale. "b" denotes significant difference ($p<0.05$) between pine oil and clove oil treatments compared to the untreated control group at Aspendale. "c" denotes significant difference ($p<0.05$) between acetic acid + HCl treatment compared to the untreated control and nonanoic acid, MCPA + dicamba, group at Aspendale.



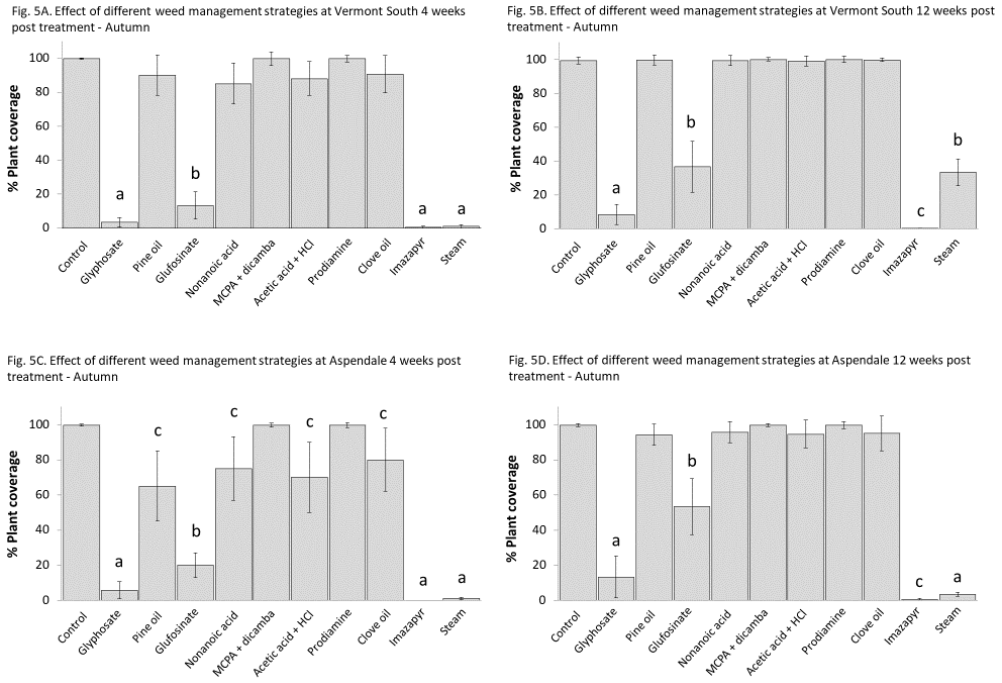
Figures 4A, 4B, 4C, 4D – Summer treatments - Effect of weed management strategies on average percentage coverage of weeds 4 weeks and 12 weeks post treatment

For **Fig. 4A**, "a" denotes significant difference ($p<0.05$) between glyphosate, glufosinate, imazapyr and steam treatments compared to the control Vermont South site sites.

For **Fig. 4B**, "a" denotes significant difference ($p<0.05$) between glyphosate and imazapyr treatments compared to the control, pine oil, glufosinate, nonanoic acid, MCPA + dicamba, acetic acid + HCl, prodiamine and clove oil treatments at Vermont South. "b" denotes significant difference ($p<0.05$) between glufosinate and all other treatment groups except for steam. "c" denotes significant difference ($p<0.05$) between steam and all other treatment groups except for glyphosate and glufosinate.

In **Fig. 4C**, "a" denotes significant difference ($p<0.05$) between glyphosate compared to the control and all other treatments except for glufosinate. "b" denotes pine oil, nonanoic acid, acetic acid + HCl and clove oil treatments as being significantly different ($p<0.05$) to the control but not significantly different from each other or glufosinate. "c" denotes significant difference ($p<0.05$) between glufosinate compared to the control MCPA + dicamba, prodiamine, imazapyr and steam treatments. "d" denotes significant difference ($p<0.05$) between imazapyr and steam compared to the untreated control and other treatment groups at Aspendale.

In **Fig. 4D**, "a" denotes significant difference ($p<0.05$) between glyphosate and glufosinate treatments compared to the control. "b" denotes significant difference ($p<0.05$) between imazapyr and all other treatments. "c" denotes steam treatment as being significantly different compared to the untreated control at Aspendale.



Figures 5A, 5B, 5C, 5D – Autumn treatments - Effect of weed management strategies on average percentage coverage of weeds 4 weeks and 12 weeks post treatment

In **Fig. 5A**, "a" denotes significant difference ($p<0.05$) between glyphosate, imazapyr and steam treatments compared to untreated control group at both sites. "b" denotes significant difference ($p<0.05$) between glufosinate treatment compared with all other treatment groups except for glyphosate treatment.

For **Fig. 5B**, "a" denotes significant difference ($p<0.05$) between glyphosate and untreated control. "b" denotes significant difference ($p<0.05$) between glufosinate, and steam compared to the control. "c" denotes significant difference ($p<0.05$) between imazapyr and all other treatments.

For **Fig. 5C**, "a" denotes significant difference ($p<0.05$) between glyphosate, imazapyr and steam treatments compared to untreated control group at both sites. "b" denotes significant difference ($p<0.05$) between glufosinate treatment compared with all other treatment groups. "c" denotes significant difference ($p<0.05$) between pine oil, nonanoic acid, acetic acid + HCl and clove oil compared to the control, glyphosate, imazapyr and steam treatment groups at Aspendale.

In **Fig. 5D**, "a" denotes significant difference ($p<0.05$) between glyphosate and steam compared with the untreated control. "b" denotes significant difference ($p<0.05$) between glufosinate compared to the control. "c" denotes significant difference ($p<0.05$) between imazapyr and all other treatments.

3.4.0 Effect of weed management strategies on bacterial abundance and diversity in soil 4 weeks post treatment

Four weeks after winter and spring treatments with the different weed management strategies, no significant difference ($p<0.05$) in bacteria per gram of soil was observed between the soil samples from untreated controls and soils treated with different weed management strategies at either Vermont South or Aspendale trial sites (Table 3-10). Generally, the bacteria per gram of soil and diversity was lower in sandy loam samples from Aspendale compared to the CFU per gram of soil in the heavy clay from Vermont South (Table 3-6). No significant changes in CFU were determined between seasonal treatment groups. Colony forming units were approx. 10-fold higher at the Vermont South site for summer and autumn (Table 7 and 9).

Table 3. Number of bacterial colony forming units (CFU) per gram of soil and diversity of bacteria based on different phenotypes observed in clay soil from the Vermont South trial site treated with different weed management strategies – winter treatment. Samples were collected 4 weeks after treatment with the different strategies.

Treatment	Bacterial CFU (10^6)	Bacteria diversity
Control	5.1 ± 2.3	11
Glyphosate	3.1 ± 2.1	6
Glufosinate	3.7 ± 2.3	8
Pine Oil	8.8 ± 2.3	12
Clove Oil	8.3 ± 2.8	13
Imazapyr	3.7 ± 2.1	4
Prodiamine	4.7 ± 2.6	5
MCPA + dicamba	6.3 ±2.8	9
Acetic Acid + HCl	12 ± 5.8	18
Steam	9.6 ± 3.1	7
Nonanoic acid	5.7± 1.2	9

Table 4. Number of bacterial colonies forming units (CFU) per gram of soil and diversity of bacteria based on different phenotypes observed in sandy loam soil from the Aspendale trial site treated with different weed management strategies– winter treatment. Samples were collected 4 weeks after treatment with the different strategies.

Treatment	Bacterial CFU (10^6)	Bacteria diversity
Control	4.1 ± 1.9	7
Glyphosate	2.1 ±1.6	5
Glufosinate	2.3 ± 0.7	5
Pine Oil	2.1 ±1.3	6
Clove Oil	2.9 ± 1.5	5
Imazapyr	1.7 ± 1.0	5
Prodiamine	3.1 ± 1.8	6
MCPA + dicamba	4.1 ± 1.8	7
Acetic acid + hydrochloric acid	3.6 ± 1.3	5
Steam	5.2 ± 2.1	7
Nonanoic acid	2.7 ± 1.9	5

Table 5. Number of bacterial colonies forming units (CFU) per gram of soil and diversity of bacteria based on different phenotypes observed in clay soil from the Vermont South trial site treated with different weed management strategies – spring treatment. Samples were collected 4 weeks after treatment with the different strategies.

Treatment	Bacterial CFU (10^6)	Bacteria diversity
Control	8.7 ± 3.7	8
Glyphosate	7.2 ± 2.6	6
Glufosinate	8.4 ± 3.1	10
Pine Oil	7.9 ± 2.7	8
Clove Oil	7.9 ± 2.2	11
Imazapyr	7.6 ± 3.0	7
Prodiamine	8.4 ± 2.5	9
MCPA + dicamba	6.8 ± 2.7	12
Acetic Acid + HCl	7.9 ± 2.9	8
Steam	8.3 ± 2.6	8
Nonanoic acid	7.4 ± 3.1	10

Table 6. Number of bacterial colonies forming units (CFU) per gram of soil and diversity of bacteria based on different phenotypes observed in sandy loam soil from the Aspendale trial site treated with different weed management strategies – spring treatment. Samples were collected 4 weeks after treatment with the different strategies.

Treatment	Bacterial CFU (10^6)	Bacteria diversity
Control	3.9 ± 2.7	6
Glyphosate	4.1 ± 2.3	6
Glufosinate	3.7 ± 1.4	6
Pine Oil	3.2 ± 1.8	7
Clove Oil	3.3 ± 1.6	6
Imazapyr	3.7 ± 2.1	5
Prodiamine	4.5 ± 2.7	6
MCPA + dicamba	3.9 ± 2.0	6
Acetic acid + hydrochloric acid	4.0 ± 1.5	6
Steam	4.2 ± 2.8	6
Nonanoic acid	3.9 ± 2.6	6

Table 7. Number of bacterial colony forming units (CFU) per gram of soil and diversity of bacteria based on different phenotypes observed in clay soil from the Vermont South trial site treated with different weed management strategies – summer treatment. Samples were collected 4 weeks after treatment with the different strategies.

Treatment	Bacterial CFU (10^7)	Bacteria diversity
Control	8.2 ± 2.8	13
Glyphosate	8.8 ± 1.9	11
Glufosinate	7.6 ± 2.3	13
Pine Oil	7.2 ± 2.7	13
Clove Oil	6.9 ± 3.1	13
Imazapyr	7.6 ± 2.8	12
Prodiamine	7.0 ± 2.5	12
MCPA + dicamba	7.2 ± 2.9	12
Acetic Acid + HCl	6.8 ± 3.1	11
Steam	7.5 ± 2.8	9
Nonanoic acid	8.3 ± 2.8	12

Table 8. Number of bacterial colonies forming units (CFU) per gram of soil and diversity of bacteria based on different phenotypes observed in sandy loam soil from the Aspendale trial site treated with different weed management strategies– summer treatment. Samples were collected 4 weeks after treatment with the different strategies.

Treatment	Bacterial CFU (10^6)	Bacteria diversity
Control	5.3 ± 2.0	7
Glyphosate	4.8 ± 1.9	8
Glufosinate	3.8 ± 1.6	7
Pine Oil	4.1 ± 2.9	8
Clove Oil	5.8 ± 3.1	8
Imazapyr	4.2 ± 2.1	8
Prodiamine	6.1 ± 3.0	8
MCPA + dicamba	6.0 ± 2.7	7
Acetic acid + hydrochloric acid	5.7 ± 2.3	8
Steam	5.8 ± 2.6	8
Nonanoic acid	6.2 ± 2.3	7

Table 9. Number of bacterial colonies forming units (CFU) per gram of soil and diversity of bacteria based on different phenotypes observed in clay soil from the Vermont South trial site treated with different weed management strategies – autumn treatment. Samples were collected 4 weeks after treatment with the different strategies.

Treatment	Bacterial CFU (10^7)	Bacteria diversity
Control	8.3 ± 3.2	12
Glyphosate	8.5 ± 3.0	13
Glufosinate	8.0 ± 2.9	11
Pine Oil	8.3 ± 2.8	11
Clove Oil	9.2 ± 2.8	13
Imazapyr	8.1 ± 3.1	13
Prodiamine	7.9 ± 2.9	12
MCPA + dicamba	8.3 ± 2.6	12
Acetic Acid + HCl	9.0 ± 3.2	13
Steam	8.7 ± 2.9	13
Nonanoic acid	8.7 ± 2.8	12

Table 10. Number of bacterial colonies forming units (CFU) per gram of soil and diversity of bacteria based on different phenotypes observed in sandy loam soil from the Aspendale trial site treated with different weed management strategies – autumn treatment. Samples were collected 4 weeks after treatment with the different strategies.

Treatment	Bacterial CFU (10^6)	Bacteria diversity
Control	6.3 ± 2.7	9
Glyphosate	5.3 ± 2.3	8
Glufosinate	5.7 ± 2.5	9
Pine Oil	6.0 ± 2.6	9
Clove Oil	5.8 ± 2.7	8
Imazapyr	4.9 ± 2.0	8
Prodiamine	6.3 ± 3.0	7
MCPA + dicamba	5.8 ± 2.8	8
Acetic acid + hydrochloric acid	5.9 ± 2.5	9
Steam	6.2 ± 2.8	9
Nonanoic acid	5.7 ± 2.4	9

3.5.0 Effect of weed management strategies on arthropod relative abundance 4 weeks post treatment

Arthropod relative abundance varied across all treatments with no discernible link between a particular weed management strategy and relative abundance (Fig. 6-7). On average Hymenoptera was the most abundant order at both sites across all seasons. Relative abundance of Hemiptera was higher at Aspendale compared to Vermont South, particularly for “prodiamine”, “clove oil”, “imazapyr” and “steam” treatments.

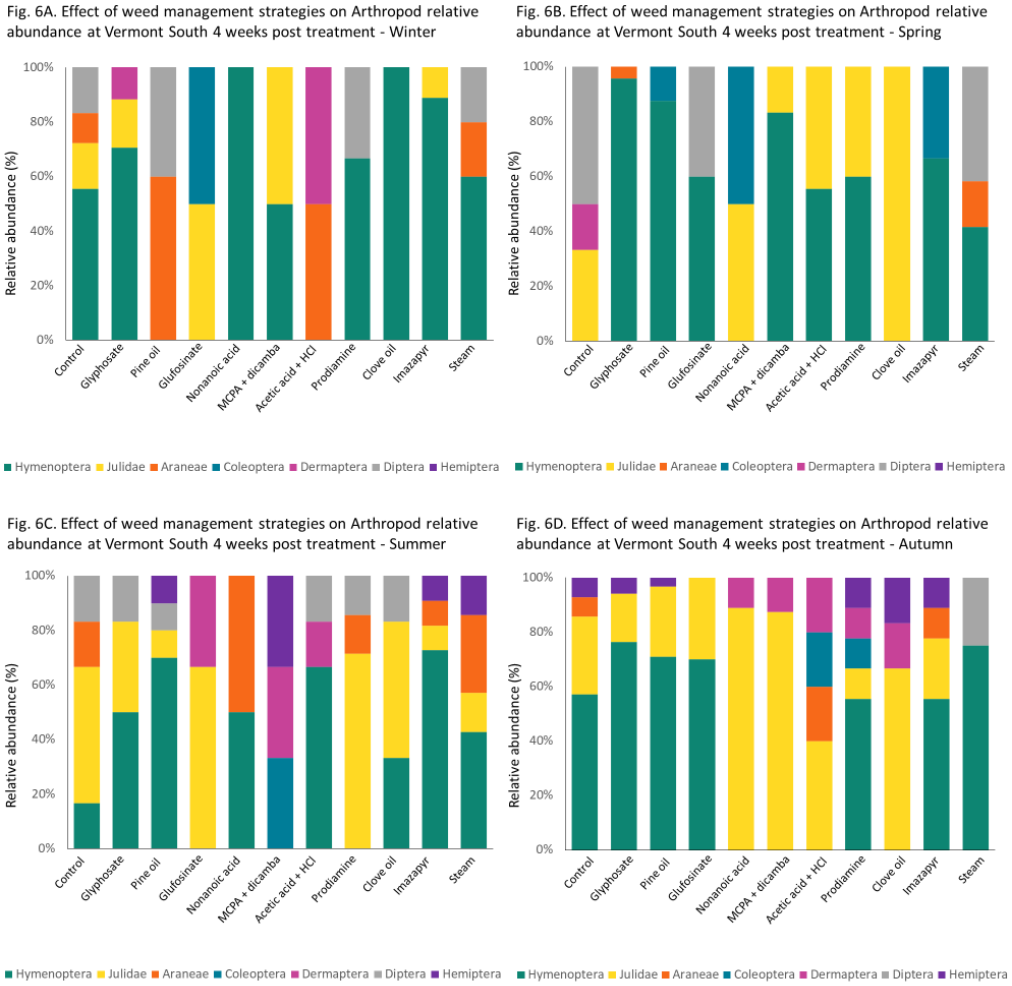


Figure 6. Effects of weed management strategies on relative abundance of Arthropod Orders enumerated at Vermont South for (**A**) winter, (**B**) spring, (**C**) summer and (**D**) autumn applications.

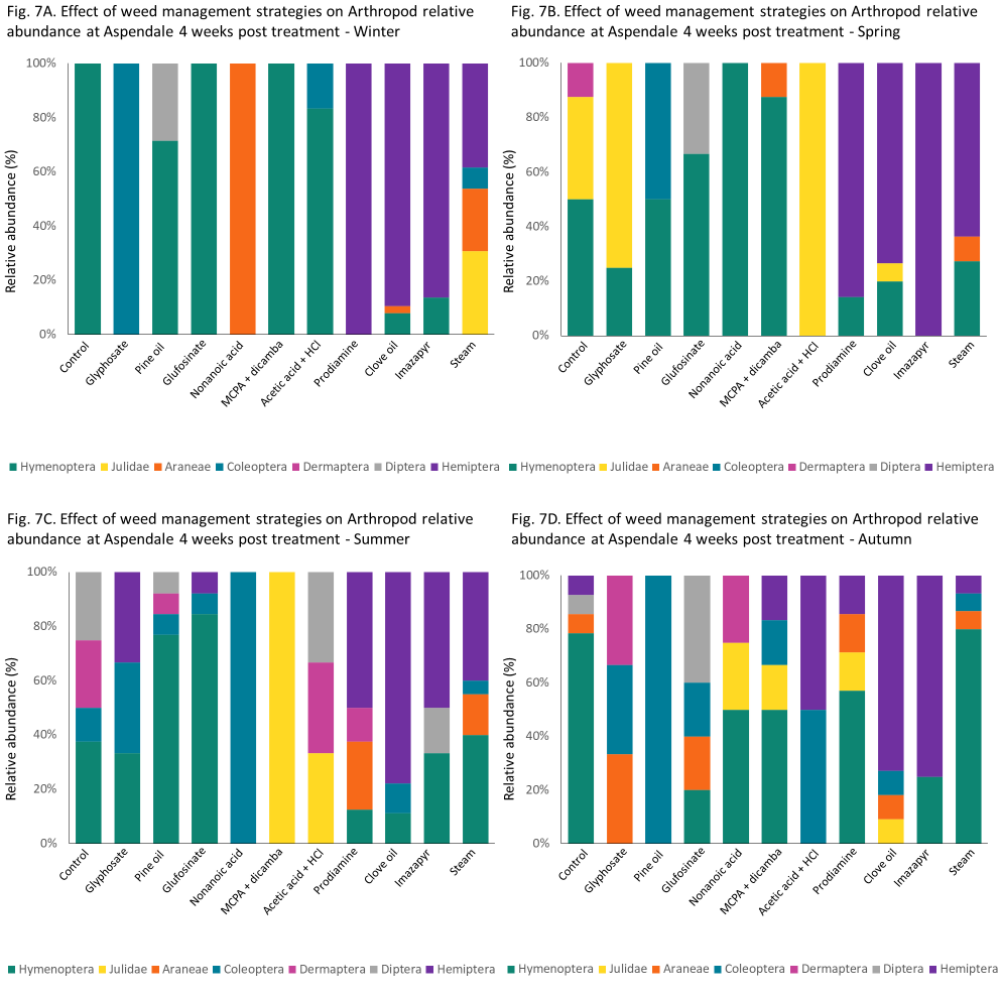


Figure 7. Effects of weed management strategies on relative abundance of Arthropod Orders enumerated at Aspendale for (**A**) winter, (**B**) spring, (**C**) summer and (**D**) autumn applications.

3.6.0 Effect of weed management strategies on bacterial diversity in soil 4 weeks post treatment

Sequencing of total 16S rRNA in soil samples taken 4 weeks after treatment with the different weed management strategies showed that the relative abundance of bacteria phyla was generally similar for all treatments compared to the control apart from the steam treatment groups at both sites across all seasons (Fig. 8A-D and 9A-D). The relative abundance of phyla did alter seasonally with increased Verrucomicrobia present in winter samples and increased Fibrobacteres present in spring samples (Fig. 8A, B and 9A, B). The spring glyphosate treatment showed to have increased relative abundance of cyanobacteria compared with other treatments (Fig. 8B). For soils from winter steam treatments, Proteobacteria were the most abundant phyla, with a general lower level of diversity (lower number of phyla) (F. 8A and 9A). Firmicute abundance increased in soils treated with steam in spring, whilst Proteobacteria abundance was reduced (Fig. 8B and 9B). Four weeks post summer treatments, a common seasonal trend was observed for microbial communities within the soil at both sites (Fig. 8C and 9C). For the imazapyr treated soil at Vermont South an increase in Gemmatimonadetes was observed in summer (Fig. 8C). Four weeks post summer application of steam, the overall diversity of bacteria phyla was reduced at both sites, with increased relative abundance of Firmicutes at both sites (Fig. 8C and 9C). For the bacterial communities four weeks post autumn treatment, at both sites a seasonal shift in community composition was observed, where the relative abundance of Actinobacteria increased, whereas the Fibrobacteres abundance reduced dramatically for all chemical treatments (Fig. 8D and 9D). Four weeks post autumn treatments the steam treatment at both sites showed to increase Fibrobacteres relative abundance (Fig. 8D and 9D).

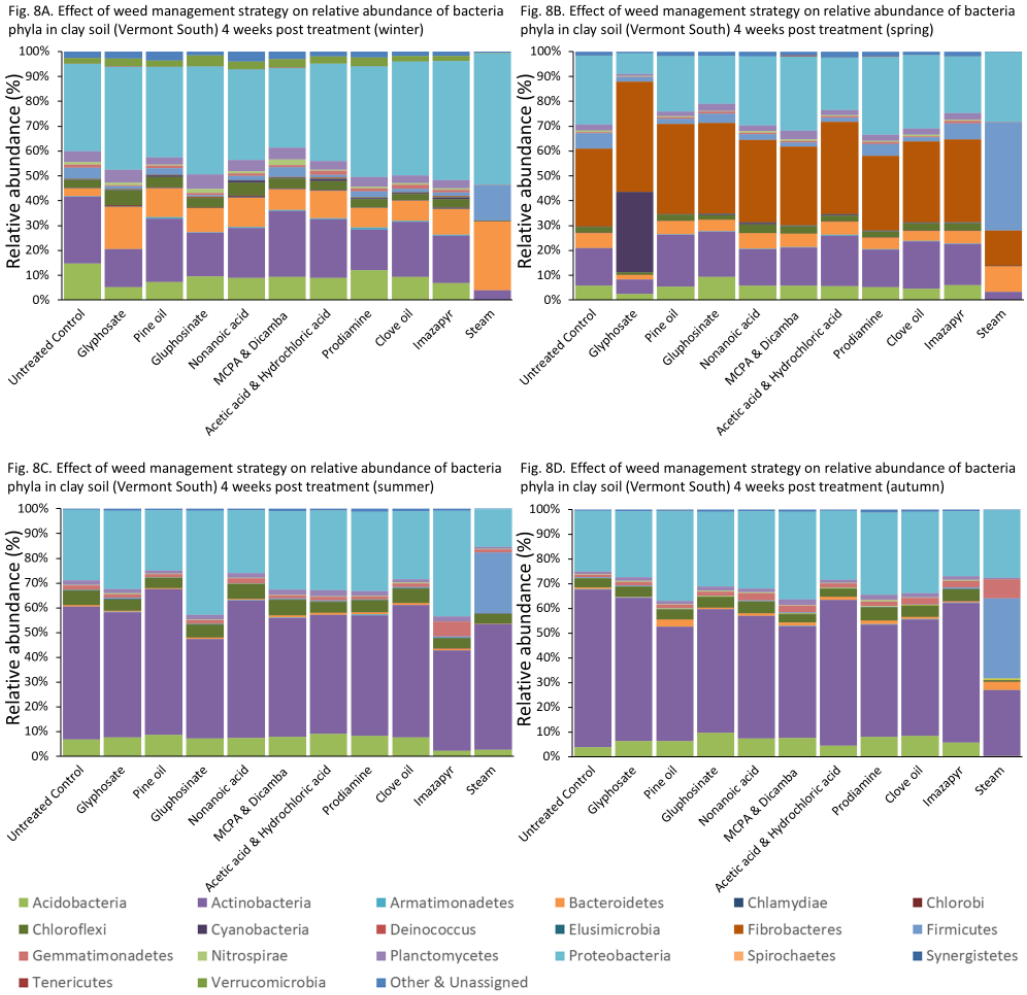


Figure 8. Effects of weed management strategies on relative abundance of bacteria phyla 4 weeks post treatment at Vermont South (heavy clay soil profile) for (**A**) winter, (**B**) spring, (**C**) summer and (**D**) autumn applications.

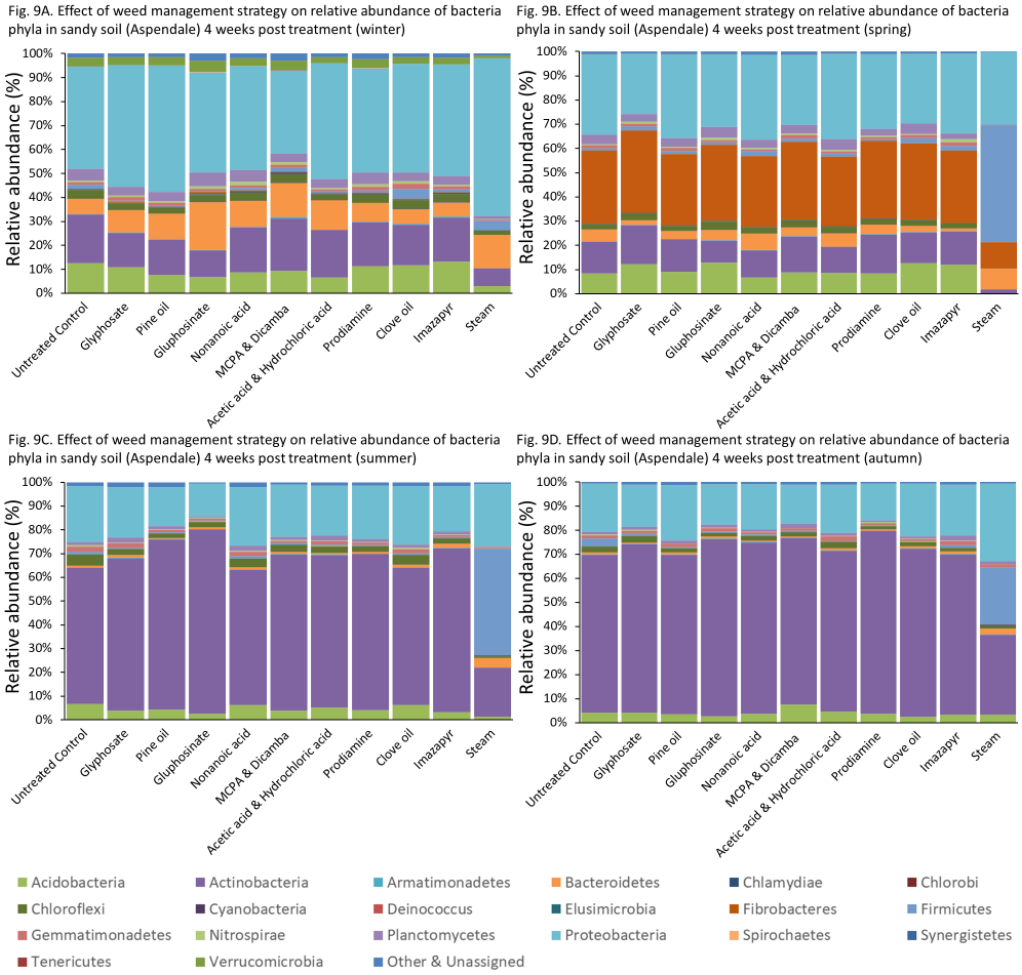


Figure 9. Effects of weed management strategies on relative abundance of bacteria phyla 4 weeks post treatment at Aspendale (sandy loam soil profile) for (**A**) winter, (**B**) spring, (**C**) summer and (**D**) autumn applications.

3.7.0 Effect of weed management strategies on fungal diversity in soil 4 weeks post treatment

Sequencing of total fungal ITS in soil samples taken 4 weeks after treatment with the different weed management strategies showed that the relative abundance of fungal phyla varied with the highest relative abundance generally being Ascomycota for both season at the two trial sites (Fig. 10A-D and 11A-D). The treatment of acetic acid + hydrochloric acid in winter at Vermont South increased Blastocladiomycota relative abundance, with a reduction in Ascomycota relative abundance also observed (Fig. 10A). Four weeks post winter steam treatment at Vermont South increased showed to increase the relative abundance of Mortierellomycota (Fig. 10A). For the steam treated areas 4 weeks post treatment at Aspendale, Blastocladiomycota relative abundance was seen to increase (Fig. 11A). For spring samples at both sites, the steam treatment reduced the number of different phyla present, particularly in spring samples were >80% of species present belonged to Ascomycota (Fig. 10B and 11B). For the summer round of treatments at both sites, there was an obvious seasonal associated change of the fungal community profiles, where a reduced amount of diversity was observed (Fig. 10C and 11C). For the summer treatment round, an increased relative abundance of Aphelidiomycota was observed at both sites (Fig. 10C and 11C). For the autumn treatment rounds, 4 weeks post treatment the relative abundance of fungi present Vermont South showed to have a higher proportion of Chytridiomycota (Fig. 10D).

3.8.0 Cumulative effect of weed management strategies on soil properties

The cumulative effects of the different weed management strategies on soil physical and chemical properties were assessed. Generally, there was no discernible changes in soil physical and chemical properties associated with the different treatments (Table 11 and 12). At Vermont South, higher levels of nitrogen (N) were measured in soils treated with glyphosate and imazapyr (70 ppm and 131 ppm respectively; see table 11). Higher levels of cobalt (Co) were measured in soils treated with steam (3.02 ppm; see table 11). For samples from Aspendale, higher nitrogen (N) levels were measured in soils treated with imazapyr (31 ppm; see table 12).

Figure 10. Effects of weed management strategies on relative abundance of fungi phyla 4 weeks post treatment at Vermont South (heavy clay soil profile) for (**A**) winter, (**B**) spring, (**C**) summer and (**D**) autumn treatments.

Figure 11. Effects of weed management strategies on relative abundance of fungi phyla 4 weeks post treatment at Aspendale (sandy loam soil profile) for (**A**) winter, (**B**) spring, (**C**) summer and (**D**) autumn treatments.

Table 11. Cumulative effects of the different weed management strategies on soil physical and chemical properties at Vermont South

Table11. Soil profiles for heavy clay soil at Vermont South	Control	Glyphosate	Pine oil	Glufosinate	Nonanoic acid	MCPA + dicamba	Acetic acid + Hydrochloric acid	Prodiamine	Clove oil	Imazapyr	Steam
pH (1:5 Water)	6.1	5.2	5.7	5.5	5.8	6	6.2	6.3	6.1	5.4	6.9
pH (1:5 0.01M $CaCl_2$)	5.56	4.56	5.14	4.86	5.23	5.37	5.72	5.7	5.48	4.79	6.35
Electrical Conductivity (EC)	91.4 µS/cm	183.0 µS/cm	96.0 µS/cm	104 µS/cm	86.8 µS/cm	46.2 µS/cm	102 µS/cm	73.8 µS/cm	109 µS/cm	207 µS/cm	48 µS/cm
Total Soluble Salt (TSS)	301.62 ppm	603.9 ppm	316.8 ppm	343.2 ppm	286.44 ppm	152.46 ppm	336.6 ppm	243.5 ppm	359.7 ppm	683.1 ppm	158.4 ppm
Available Calcium (Ca)	2260 ppm	1560 ppm	1550 ppm	1336 ppm	1472 ppm	1142 ppm	1512 ppm	1452 ppm	1576 ppm	1474 ppm	2320 ppm
Available Magnesium (Mg)	525.6 ppm	460 ppm	488.4 ppm	373.2 ppm	415.2 ppm	336 ppm	450 ppm	457.2 ppm	452.7 ppm	404.4 ppm	424.8 ppm
Available Sodium (Na)	25.99 ppm	16.9 ppm	22.954 ppm	10.764 ppm	16.86 ppm	12.052 ppm	17.73 ppm	18.65 ppm	18.08 ppm	15.04 ppm	31.05 ppm
Available Nitrogen (N)	6.77 ppm	70.7 ppm	7.92 ppm	27.7 ppm	8.59 ppm	1.7 ppm	7.58 ppm	8.3 ppm	8.81 ppm	131 ppm	8.2 ppm
Available Phosphorus (P)	17.0 ppm	42.8 ppm	24.7 ppm	35 ppm	24.3 ppm	17.6 ppm	28.4 ppm	23.6 ppm	27.6 ppm	36.5 ppm	13.5 ppm
Available Potassium (K)	530.4 ppm	499.2 ppm	522.6 ppm	464.1 ppm	600.6 ppm	365.43 ppm	577.2 ppm	534.3 ppm	585 ppm	432.9 ppm	289.38 ppm
Available Sulphur (S)	11.8 ppm	17.6 ppm	15.0 ppm	8.86 ppm	12 ppm	5.85 ppm	13.5 ppm	11.9 ppm	18 ppm	20.4 ppm	4.11 ppm
Available Copper (Cu)	8.52 ppm	7.89 ppm	30 ppm	16.6 ppm	13.5 ppm	9.1 ppm	7.33 ppm	7.73 ppm	6.21 ppm	17.4 ppm	3.99 ppm
Available Zinc (Zn)	45.6 ppm	47.1 ppm	37.6 ppm	36.4 ppm	76.9 ppm	33.6 ppm	43.1 ppm	33.5 ppm	35.2 ppm	36.4 ppm	20.5 ppm
Available Iron (Fe)	12 ppm	56.0 ppm	81 ppm	109 ppm	69 ppm	58 ppm	30 ppm	39 ppm	26 ppm	34 ppm	8 ppm
Available Manganese (Mn)	8 ppm	13 ppm	9 ppm	6 ppm	8 ppm	4 ppm	5 ppm	5 ppm	6 ppm	6 ppm	8 ppm
Available Cobalt (Co)	0.73 ppm	0.69 ppm	0.62 ppm	0.44 ppm	0.52 ppm	0.42 ppm	0.51 ppm	0.39 ppm	0.51 ppm	0.46 ppm	3.02 ppm
Available Molybdenum (Mo)	0.20 ppm	0.26 ppm	0.36 ppm	0.45 ppm	0.28 ppm	0.29 ppm	0.26 ppm	0.28 ppm	0.29 ppm	0.29 ppm	0.17 ppm
Available Boron	0.52 ppm	0.40 ppm	0.35 ppm	0.31 ppm	0.31 ppm	0.28 ppm	0.29 ppm	0.37 ppm	0.35 ppm	0.44 ppm	0.24 ppm
Total Organic Matter (OM)	17%	17%	13%	10%	13%	8%	13%	13%	13%	12%	13%
Total Organic Carbon (OC)	8.45%	8.50%	6.35%	5.20%	6.50%	3.99%	6.40%	6.50%	6.25%	6.50%	6.45%
Exchangeable Calcium (meq/100g of soil)	10.9	7.18	7.39	6.31	7.05	5.54	7.14	6.97	7.45	6.59	11.3
Exchangeable Magnesium (meq/100g of soil)	4.22	3.5	3.88	2.94	3.31	2.72	3.54	3.66	3.74	3.01	3.45
Exchangeable Sodium (meq/100g of soil)	0.109	0.0675	0.0952	0.0442	0.0702	0.0508	0.0729	0.0779	0.0743	0.0585	0.132
Exchangeable Potassium (meq/100g of soil)	1.31	1.18	1.28	1.12	1.47	0.909	1.4	1.32	1.42	0.992	0.724
Exchangeable Hydrogen (meq/100g of soil)	9.88	12.3	9.43	9.64	10.1	7.28	7.51	8	8.47	9.53	5.6
Cation Exchange Capacity (CEC)	26.4	24.2	22.1	20.1	22	16.5	19.7	20	21.2	20.2	21.2
Adjusted CEC	18	20	15.7	17.5	15.5	12.5	13.3	13.5	14.9	17.1	15.6
Exchangeable Sodium Percentage (ESP)	0.41%	0.28%	0.43%	0.22%	0.32%	0.31%	0.37%	0.39%	0.35%	2.90%	0.62%
Calcium / Magnesium Ratio (Ca/Mg)	2.58	2.05	1.9	2.15	2.13	2.04	2.02	1.91	1.99	2.19	3.28
Base Saturation Percentage (BSP)	64%	51%	58%	53%	55%	57%	63%	61%	61%	56%	74%

Table 12. Cumulative effects of the different weed management strategies on soil physical and chemical properties at Aspendale

Table 12. Soil profiles for sandy loam soil at Aspendale

	Control	Glyphosate	Pine oil	Glufosinate	Nonanoic acid	MCPA + dicamba	Acetic acid + Hydrochloric acid	Prodiamine	Clove oil	Imazapyr	Steam
pH (1:5 Water)	6.9	6.8	6.7	7.1	7	6.8	7	6.9	6.8	6.7	6.7
pH (1:5 0.01M $CaCl_2$)	6.36	6.33	6.23	6.64	6.5	6.32	6.45	6.36	6.3	6.23	6.21
Electrical Conductivity (EC)	43.7 µS/cm	48.3 µS/cm	44.9 µS/cm	49 µS/cm	51 µS/cm	40.8 µS/cm	43 ppm	38.6 µS/cm	35.2 µS/cm	74.7 µS/cm	51.3 µS/cm
Total Soluble Salt (TSS)	144.21 ppm	159.39 ppm	148.17 ppm	161.7 ppm	168 ppm	134.64 ppm	141.9 µS/cm	127.38 ppm	116.16 ppm	246.51 ppm	169.29 ppm
Available Calcium (Ca)	1194 ppm	848 ppm	970 ppm	1080 ppm	816 ppm	802 ppm	1144 ppm	1252 ppm	850 ppm	1496 ppm	1404 ppm
Available Magnesium (Mg)	303.6 ppm	228 ppm	246 ppm	265.2 ppm	283.2 ppm	220.8 ppm	265.2 ppm	266.4 ppm	235.2 ppm	318 ppm	349.2 ppm
Available Sodium (Na)	61.41 ppm	53.59 ppm	58.42 ppm	51.29 ppm	62.56 ppm	38.18 ppm	40.25 ppm	39.56 ppm	56.81 ppm	46.46 ppm	48.53 ppm
Available Nitrogen (N)	2.1 ppm	8.6 ppm	5.51 ppm	7.32 ppm	5.4 ppm	5.24 ppm	5.7 ppm	5.06 ppm	4 ppm	31 ppm	3.76 ppm
Available Phosphorus (P)	5.97 ppm	5.97 ppm	3.55 ppm	3.39 ppm	2.47 ppm	5.11 ppm	5.43 ppm	4.83 ppm	4.47 ppm	10.1 ppm	8.26 ppm
Available Potassium (K)	85.8 ppm	62.01 ppm	60.84 ppm	56.94 ppm	75.27 ppm	48.75 ppm	53.04 ppm	51.48 ppm	70.98 ppm	93.6 ppm	104.13 ppm
Available Sulphur (S)	6.66 ppm	3.94 ppm	4.92 ppm	5.56 ppm	6.1 ppm	5.11 ppm	4.9 ppm	5.29 ppm	5.15 ppm	5.71 ppm	7.68 ppm
Available Copper (Cu)	12 ppm	11.3 ppm	20 ppm	15.5 ppm	13.2 ppm	12.5 ppm	10.1 ppm	11.2 ppm	8.27 ppm	11.1 ppm	11.9 ppm
Available Zinc (Zn)	39 ppm	48.6 ppm	62.1 ppm	52.4 ppm	45.2 ppm	58.7 ppm	63.2 ppm	81.8 ppm	48.3 ppm	54.7 ppm	41 ppm
Available Iron (Fe)	12 ppm	9 ppm	11 ppm	7 ppm	6 ppm	7 ppm	14 ppm	9 ppm	10 ppm	10 ppm	15 ppm
Available Manganese (Mn)	3 ppm	7 ppm	6 ppm	7 ppm	4 ppm	5 ppm	4 ppm	3 ppm	3 ppm	7 ppm	7 ppm
Available Cobalt (Co)	0.49 ppm	0.44 ppm	0.66 ppm	0.44 ppm	0.44 ppm	0.48 ppm	0.41 ppm	0.42 ppm	0.37 ppm	0.51 ppm	0.47 ppm
Available Molybdenum (Mo)	0.09 ppm	0.16 ppm	0.17 ppm	0.17 ppm	0.12 ppm	0.18 ppm	0.12 ppm	0.10 ppm	0.08 ppm	0.11 ppm	0.15 ppm
Available Boron	0.25 ppm	0.29 ppm	0.27 ppm	0.26 ppm	0.28 ppm	0.23 ppm	0.29 ppm	0.24 ppm	0.23 ppm	0.34 ppm	0.43 ppm
Total Organic Matter (OM)	6%	5%	5%	4%	5%	4%	4%	6%	4%	5%	4%
Total Organic Carbon (OC)	2.77%	2.26%	2.43%	2.18%	2.25%	1.95%	2.25%	2.87%	2.13%	2.49%	2.23%
Exchangeable Calcium (meq/100g of soil)	5.73	3.97	4.6	5.09	3.82	3.78	5.45	6.02	4.06	6.99	6.71
Exchangeable Magnesium (meq/100g of soil)	2.43	1.78	1.94	2.08	2.21	1.74	2.1	2.14	1.87	2.48	2.78
Exchangeable Sodium (meq/100g of soil)	0.256	0.218	0.241	0.21	0.254	0.157	0.167	0.165	0.236	0.189	0.202
Exchangeable Potassium (meq/100g of soil)	0.211	0.149	0.148	0.138	0.181	0.118	0.13	0.127	0.174	0.224	0.255
Exchangeable Hydrogen (meq/100g of soil)	2.76	1.85	2.11	1.49	1.8	1.82	1.7	2.33	1.88	2.08	2.29
Cation Exchange Capacity (CEC)	11.4	7.97	9.04	9.01	8.26	7.62	9.55	10.8	8.22	12	12.2
Adjusted CEC	8.63	6.12	6.93	7.52	6.46	5.8	7.85	8.45	6.34	9.88	10
Exchangeable Sodium Percentage (ESP)	2.25%	2.74%	2.67%	2.33%	3.07%	2.06%	1.75%	1.53%	2.87%	1.58%	1.65%
Calcium / Magnesium Ratio (Ca/Mg)	2.36	2.23	2.37	2.44	1.73	2.18	2.59	2.82	2.17	2.82	2.41
Base Saturation Percentage (BSP)	77%	78%	78%	84%	79%	77%	83%	79%	78%	84%	82%

4.0 Discussion

4.1.0 Chemical alternatives

A desktop study was completed to assess a number of feasible alternative options to glyphosate for controlling weeds. This enabled identification of suitable substitute or replacements herbicide strategies that could be used in place of glyphosate (Appendix 1). From over 50 weed management strategies, a shortlist of 9 alternatives were selected for further study based on key drivers. These drivers included cost, hazard (perceived, exposure, storage and handling), target specificity (residual, non-residual, non-specific to plants and grasses or specific to types of plants and grasses) and environmental impact (known toxicity to humans, flora, fauna, aquatic life, bacteria and/or fungi). From the list of the chemical alternatives, those selected by the project steering committee for further trialling were: glufosinate, pine oil, glyphosate, nonanoic acid, MCPA + dicamba, acetic acid + hydrochloric acid, prodiamine, clove oil, imazapyr and steam. Each of these trial options were chosen based on meeting the drivers; cost efficiency, reduced environmental impact, and minimal known risks towards humans.

4.2.0 Effect of the weed management strategies on weed species

The effects of the weed management strategies on knock-back of weeds (based on ability to reduce % plant coverage) was assessed at the two trial sites (Vermont South and Aspendale) over four seasons; winter, spring, summer and autumn. Across the two sites, there was a difference in both density and diversity of weed species. This translated to an obvious difference in the efficacy of contact-based weed management strategies (nonanoic acid, pine oil, clove oil and acetic acid+ hydrochloric acid); where generally a greater effect on weed reduction was observed at the Aspendale site. This was largely attributed to the lower weed density, meaning the contact acting herbicides were more easily applied to cover the majority of the plant material, resulting in effective destruction of the leaves, shoots and stems. Despite initial reductions to weed coverage, after 12 weeks post application these contact-based products had not had a significant effect on reducing or suppressing

weeds at either site. Due to the thick dense weed coverage at the Vermont South site, no obvious reduction in weed coverage was observed at 4 weeks or 12 weeks post application of the organic weed management strategies. The efficacy of the contact-based herbicides may also have been reduced due to the higher than average rainfall that occurred during the testing period increasing plant germination and growth rates. The average annual rainfall for Melbourne is approx. 650 mm. However, the annual rainfall in 2020 was 1074.1 mm and for 2021 to date the rainfall has been ~233.4 mm.

Both trial sites were observed as having a strong seed bank. This may also have reduced the impact of the contact-based herbicides pine oil, nonanoic acid, acetic acid + hydrochloric acid and pine oil (Fig. 2-5A, C), as well as the selective herbicides MCPA + dicamba and prodiamine (Fig. 2A). These products may have reduced weed coverage 4 weeks post first round of applications (Fig. 2A, C), however, long term (12 weeks and beyond) the seed bank would regenerate, and weed coverage recover to control levels. In the case of the selective herbicides MCPA + dicamba and prodiamine, the reductions to overall weed coverage was negligible with no observed impact on weed coverage beyond the one-off significant knockback observed at Vermont South for the first round of treatment (Fig. 2A). It was also observed that the selective herbicides did not reduce emergence of seasonal broadleaf weeds (marshmallow, dandelion, cat's ear, lamb's tongue and dock) beyond 4 weeks post treatment. The combined MCPA + dicamba treatment has a systemic mode of action via foliar application (Herbiguide, 2020). It is absorbed by plant roots, stems and leaves and translocated through the plant, systemically killing it off (Herbiguide, 2020). For prodiamine, the main mode of action once it has been absorbed through the roots, is disruption to cell division by inhibiting tubulin formation, an essential component for successful cell division (Herbiguide, 2020). Previous studies have shown that prodiamine is persistent but immobile in the soil. With the application rates used on the weeds at the two trial sites, no off-target impacts were observed (Herbiguide, 2020).

It was apparent after 4- and 12-weeks post application, both glyphosate and glufosinate effectively reduced weed coverage at both sites. Glufosinate was sprayed directly onto the leaves and stems. Glufosinate targets post emergence weeds and it is the most potent inhibitor of the glutamine synthetase (Krieger, 2010). This is critical to the assimilation of nitrogen by plants and by consequence photosynthesis (Krieger, 2010). Whilst glufosinate significantly reduced weed coverage for the autumn treatment, it did not reduce weed coverage as effectively as glyphosate (Fig. 5B-D). This may have been due to insufficient coverage of the herbicide on plants, as it has previously been reported that glufosinate efficacy can be limited if coverage is not sufficient (Krieger, 2010).

Interestingly, imazapyr did not have any obvious impact on weed coverage 4 weeks post first application. This was attributed to the time for imazapyr to be metabolised and having more of a longer-term effect on weed coverage due to its pre-emergent effects. After 12 weeks imazapyr showed to significantly reduce weed coverage compared to all other treatments including glyphosate and glufosinate at both sites. There were obvious signs of movement of imazapyr through the soil profile, particularly at the Aspendale site, where it could easily diffuse through the highly permeable sandy loam soil. Imazapyr is typically readily absorbed in soils with high organic and/or clay contents, with a half-life of 14-28 days in soils. After the four seasonal treatments of imazapyr at both sites, the off target die back was approx. 0.5 m beyond the 1 m^2 quadrat boundaries. The minimal regeneration observed was attributed to aerial seeds establishing. Imazapyr was observed to be very motile, expanding beyond the 1 m^2 border and into the buffer zone. The motility of imazapyr could have dire negative off target effects on sensitive native vegetation. Careful consideration of the potential off target affects should be taken into account before use.

Treatment of weeds with steam achieved instant death of weeds, with obvious significant knock back of weed coverage after 4 weeks post treatment. After 12 weeks for the first treatment round in winter the effect of steam was negligible compared to the untreated control, indicating it may have required more frequent application to treat weeds long term (Fig. 2B, D). However, for subsequent treatments steam showed to significantly reduce weed coverage at 12 weeks post treatment, indicating increased efficacy with repetition. This could be due to the steam treatment gradually reducing the seed bank. The coverage observed at 12 weeks was largely from weed runners moving in from the periphery covering the cleared area and aerial seeds establishing.

4.3.0 Impact of weed management strategies on soil properties

The cumulative impact of the different weed treatment on soil chemical and physical properties was assessed. After the four seasonally administered treatments, the only obvious alterations to soil nutrient profiles was the nitrogen (N) content in imazapyr treated soils at both sites (Table 11 and 12). This increased level of N could be due to the prolonged reduced plant coverage; where no plants are growing and actively taking mineralised N out of the soil and the decaying plant biomass and soil microorganisms are still generating N (Sarathchandra and Upsdell 1981, Korolkova et al 2013). The accumulation of N at the levels measured would probably be of benefit to areas where weeds are being cleared for revegetation. The initial boosted level of available N could help enhance plant growth. At Vermont South, higher levels of cobalt (Co) were measured in soils treated with steam (3.02 ppm) (Table 11). This is most likely an anomaly in the background Co level occurring in the soil at the steam treatment site.

4.4.0 Impact of weed management strategies on soil bacterial, fungal and arthropod communities

Soil conditions directly impact microbial populations, where dry conditions, acidity, salinity, soil compaction and lack of organic matter cause fluctuations in diversity and abundance (Reid, and Wong,2005). Based on colony count data, results showed that none of the various management

strategies significantly altered the abundance (CFU) of bacteria in soil. A seasonal increase was observed for summer and autumn where CFU per gram of soil increased by 10-fold at both sites.

The sequencing of total bacterial 16S rRNA and fungal ITS revealed that the steam treatment generally reduced the relative abundance of bacteria and fungi alike. Glyphosate, glufosinate and imazapyr were observed to significantly reduce weed coverage, without any obvious effect of bacterial or fungal diversity, apart from the spring glyphosate treatment that showed to have increased relative abundance of cyanobacteria. For steam-treated soils, reduced diversity of bacterial and fungal phyla was observed. Generally, seasonal variation impacted the bacterial and fungal community composition more than any treatment; where summer relative abundance was typically lower.

Assessment of arthropod diversity showed no discernible link between a particular weed management strategy and relative abundance (Fig. 6-7). On average Hymenoptera was the most abundant order at both sites across all seasons, with the order mostly represented by ants. Relative abundance of Hemiptera was higher at Aspendale compared to Vermont South, particularly for prodiamine, clove oil, imazapyr and steam treatments. This increased relative abundance of Hemiptera associated with these treatments at Aspendale was attributed to a cluster of *Lycium ferocissimum* (African Boxthorn) that were next to these transects and attracted the stink bugs.

4.5.0 Cost implications of the different weed management strategies

Glyphosate was the cheapest product, with the cost of a 1 L preparation at 10 mL/L being AU$0.10. The other alternative weed management strategies ranged from AU$0.21/L (glufosinate) to AU$22.61/L (clove oil) (Table 13). The organic acid, plant oil, MCPA + dicamba and prodiamine based products were all at least six times the cost of glyphosate and failed to demonstrate reproducible reductions to weed coverage. Glufosinate was the cheapest alternative to glyphosate at $0.21/L and significantly reduced weed coverage for at least 12 weeks post application. Whilst imazapyr ($2.02/L)

was 20 times more expensive than glyphosate ($0.10 per L), long term cost may be reduced depending on time required between application and efficacy of more dilute solutions (1.3 g/L opposed to the 13 g/L used). The long residual time and pre-emergent effects of imazapyr could reduce the need to reapply. Due to the high risk of off-target effects due to the mobility of imazapyr, its use would need to be subject to careful assessment to avoid killing desired plants and avoid accountability for inadvertently killing plants on private properties adjoining council-managed areas.

The general PPE and engineering requirements for the chemical weed management strategies are similar given protection against skin irritation, eye irritation, vapour control, spill containment and application systems are the same. There was some increased rate of wear on spray applicators for some products. This was mainly the organic acid and plant oil based products (acetic acid + hydrochloric acid, nonanoic acid and clove oil). This may have been due to improper cleaning of equipment after use or the particular type of sprayers used.

Steam was selected as a manual form of weed management. Steam is becoming an increasingly popular alternative weed management strategy, as it is instantly effective at inhibiting weed growth, is chemical-free and may require less people and time than manually hand-pulling weeds. Steam has a high capital cost and was observed to alter the relative abundance of both bacterial and fungal phyla compared to all other treatments. The cost of the steam unit used for the trials was approximately $35,000 and to cover an area of 1 m^2 took around 12 minutes. It was noted that to cover an area of 9 m^2 took around 3h, used approximately 600-700 litres of water, consumed approximately $15 in fuel (combined diesel consumption for the generator and petrol consumption for the water pump), and would require a minimum of 2 people to operate. The ongoing operational and servicing costs of the steam units is hard to determine but are likely to be high given the strain on the water pump and performance issues experienced during the trials, particularly for the summer treatments when the pump became inoperable. Wear to the lances, connectors and hose fittings was observed, posing a

burning hazard and indicate regular ongoing maintenance requirements. Generally, the steam units would perform best with a degree of preventative maintenance to pumps, pipes, hoses, connectors and fittings prior to each use. A cost consideration for the steamers is also the requirement of a vehicle with a 3-tonne towing capacity. Ideally, the steaming units requires two people at a minimum to effectively operate the unit, as the water temperature and unit performance needed to be constantly assessed and managed to achieve effective weed killing. This adds a labour cost element that needs to be factored in when considering weed steaming.

Table 13. Costs of products trialled. Costs shown are based on full retail price for one-off purchases of low volumes and may differ for repeated bulk orders.

	Cost ($)	Volume (L)	Volume (mL)	Dilution Factor(mL/L)	Cost per L ($)	Cost ($) per m^2
Glufosinate	41.5	1	1000	5	0.21	0.04
Acetic acid	111.5	5	5000	90	2.01	0.40
MCPA + Dicamba	24.5	1	1000	27	0.66	0.13
Prodiamine	190	1	1000	40	7.60	1.52
Imazapyr	155	1	1000	13	2.02	0.40
Glyphosate	199	20	20000	10	0.10	0.02
Clove oil	16.96	1	750	N/A	22.61	4.52
Nonanoic acid	12.98	1	1000	N/A	12.98	2.60
Pine oil	220	10	10000	200	4.40	0.88

[#]For steam treatment: Capital costs for steamer were estimated to be approx. $35,000 for the 1,000L dual lance unit used, Operating costs = approx. $6-8 per hour (excluding personnel), water usage = approx. 250 L per hour and other requirements for steamer to consider include size of unit: width = 2.52 m and weight = 2 - 3 tonnes.

4.6.0 Human health-related risks with weed treatment strategies

For the chemical-based weed management strategies, all were classified as either schedule 5 or 6 poisons and were specified as needing to be used with caution. Of the selected alternatives, those identified as schedule 5 at the time of this study were glyphosate, glufosinate, imazapyr, nonanoic acid and clove oil. Acetic acid + hydrochloric acid (organic acid), pine oil (organic plant based oil) and MCPA + dicamba (chemical) weed killers tested in the studies presented here were classified as schedule 6 poisons. At the time of this study, prodiamine was classified as "exempt". This classification schedule is important, as all the alternatives selected are not highly toxic to the public or suspected to cause major environmental impact due to runoff, and they pose a relatively low-risk hazard to the workers administering the weed killer.

Hereafter, the focus on safety implications is on glufosinate, imazapyr and steam. The acetic acid and plant oil based products were not shown to be effective weed control alternatives compared to glyphosate, glufosinate, imazapyr or steam based on weed coverage results (Fig. 2-5), cost. In addition, both pine oil and the acetic acid + hydrochloric acid products trialled were classified as Schedule 6 "poison", whereas as glyphosate, glufosinate and imazapyr are classified as a Schedule 5 "caution". This indicates the pine oil and the acetic acid + hydrochloric acid pose a potentially higher health risk. Acetic acid is an organic compound (contains carbon and hydrogen bonds) with a low or "acidic" pH. As such, acetic acid (and hydrochloric acid) is highly corrosive to skin and eyes, causing burns. Acetic acid and hydrochloric acid both produce strong vapours that can irritate eyes, skin, and the respiratory system and at high concentrations it may cause irreversible damage to eyes, skin, or the respiratory system. The pine oil and clove oil products tested were listed as having similar health risks as the acetic acid + hydrochloric acid based product (may cause severe eye and skin damage, and is a respiratory system irritant). In the case of the clove oil product this could be due to the acetic acid content of the product (40 g/L) in addition to the concentrated plant-based oil.

Glyphosate health precautions are detailed in the introduction including its Group 2A (probable carcinogen) classification.

Glufosinate and imazapyr are both Schedule 5 (caution) poisons, classified as hazardous, are not classified as dangerous goods and have the signal word “warning”. Glufosinate and imazapyr are not classified as carcinogens or probable carcinogens by the IARC. This may be due to glufosinate and imazapyr not being reviewed for classification due to low or no reported incidences, no evidence presented, or only low-risk evidence obtained from toxicity testing.

To date, chronic toxicity tests for imazapyr indicate that it is not carcinogenic, mutagenic, or neurotoxic. It also not known to cause reproductive or developmental toxicity and is not a suspected endocrine disrupter. Available information suggests imazapyr has low acute toxicity on the skin or if ingested but is harmful if inhaled and may cause irreversible damage if it gets in the eyes. Applicators must follow the protective control measures outlined in the safety data sheet (SDS) as detailed in the example information below. The break-down products from imazapyr are not suspected as being any more toxic than imazapyr itself and are likely to be excreted faster than imazapyr when ingested.

For glufosinate, testing of foetuses during pregnancy in rats and rabbits indicated no teratogenic potential (birth abnormalities). Mutagenicity tests have also indicated glufosinate to be non-genotoxic. Chronic toxicity testing in rats and dogs yielded no-observable-effect levels of 2 and 5 mg/kg body weight/day, respectively. Oncogenicity studies in rats and mice revealed no carcinogenic potential. On the basis of this toxicity data, it was concluded that this herbicide is safe under conditions of recommended use (Ebert et al 1990).

Based on a consensus of information in multiple manufacturers’ SDS, the first aid and protective measures for glyphosate, glufosinate and imazapyr with details for exposure treatment are as follows:

- **Inhalation:** If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical attention if breathing problems develop.
- **Skin Contact:** In case of skin contact, brush away granules (imazapyr), immediately remove contaminated clothing and wash affected areas with water and soap. Seek medical attention if symptoms occur.
- **Eye Contact:** In case of eye contact, brush away granules (imazapyr), hold eyelids open and rinse with water for at least 15 minutes. Seek immediate medical attention.
- **Ingestion:** If swallowed, do not induce vomiting. Immediately rinse mouth with water. Give a glass of water. Never give anything by mouth to an unconscious person. Seek immediate medical attention.
- **Engineering Controls**: Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapour below occupational exposure standards.
- **Respiratory Protection**: Use an approved vapour respirator under conditions where exposure to the substance is apparent (e.g., generation of high concentrations of mist or vapour, inadequate ventilation, development of respiratory tract irritation) and engineering controls are not feasible.
- **Skin Protection**: Wear PVC or butyl rubber gloves. When selecting gloves for use against certain chemicals, the degradation resistance, permeation rate and permeation breakthrough time should be considered. Occupational protective clothing (depending on conditions in which it has to be used, in particular as regards the period for which it is worn, which shall be determined on the basis of the seriousness of the risk, the frequency of exposure to the risk, the characteristics of the workstation of each worker and the performance of the protective clothing).
- **Eye and Face Protection**: Eye and face protectors for protection against splashing materials or liquids.

For any herbicide or agent being used to control weeds, local area management plans need to be developed that details the appropriate PPE (gloves, protective clothing, eye protection and face protection), ventilation requirements and ways to minimise vapours and risk of exposure. All applicators need to be informed and aware of the risks of working with agents. This is best done by reviewing the information and operating in accordance with the information specified by the product manufacturers and in accordance with Safe Work Australia (SWA), Work Health and Safety (WHS) (Managing Risks of Hazardous Chemicals in the Workplace) Code of Practice, Global Harmonised System (GHS) and the Australian Dangerous Goods (ADG) code, which are detailed in the product SDSs. When applying herbicides workflow patterns need to be predetermined to eliminate the need for the applicators to revisit or come in contact treated areas.

In considering physical weed control strategies such as steam, risks assessments need to be performed and risks associated with hot water or steam, fuel (unleaded petrol and/or diesel) and exhaust fumes accounted for (operate up wind from steamer unit). Applicator exposure time increases risk (exposure time to agents); where environmental factors such as heat, fatigue, noise and sun need to be accounted for. Protective measures such as hearing protection, safety glasses, heat proof gloves, and clothing that covers bare skin worn. Even for hand pulling weeds (not reviewed here), exposure risks need to be assessed and managed, with repetitive straining activities, non-ergonomic positioning, sun exposure, exposure to plants that may be irritants, and sharp objects such as broken glass accounted for.

A considerable risk associated with operating the steamer unit is the use of fuels, unleaded petrol, and diesel. For the water pump, petrol (unleaded) is required, which is a Schedule 5 poison and the exhaust fumes generated considered as “possibly carcinogenic” (Group 2B) (IARC, 2012). The generator associated with the boiler that generates the steam uses diesel, which is a Schedule 5 poison. Diesel exhaust emissions are classified as carcinogenic to humans (Group 1). The IARC reviewed diesel

exhaust emission and concluded the following: "*The scientific evidence was reviewed thoroughly by the Working Group and overall it was concluded that there was sufficient evidence in humans for the carcinogenicity of diesel exhaust. The Working Group found that diesel exhaust is a cause of lung cancer (sufficient evidence) and also noted a positive association (limited evidence) with an increased risk of bladder cancer (Group 1)*". The risks associated with handling, transporting and burning of petrol and diesel need to be considered when using a steamer to eradicate weeds. All risks and risk reduction or avoidance measures should be detailed in a work safety risk assessment and control form and incorporated as part of the standard operating procedure. Operators must consider their and the public's risk of exposure to the exhaust emissions associated with using the weed steamer. This could be achieved by measures such as ensuring operators are up-wind of the steam unit, public access is restricted within a certain proximity to the unit when in operation, and the unit is only operated in wide open spaces or distanced far enough away from buildings and housing to enable dispersion of exhaust emissions.

4.7 Accessibility issues when using steam to manage weeds

Managing weeds requires time and often weeds are in areas with limited access due to motorways, infrastructure, or environmental conditions (hills, vegetation or waterways). Chemical treatments are largely able to be carried on person, with spray packs of up to 20 L capacity available as a "backpack". With regards to accessibility, steaming presents challenges that may render it inappropriate or not an option in some situations. The dimensions of the steam unit used for the trials conducted here was 2.52 m in width and weighed over 2.5 tonnes with a full 1,000 L water tank and 60 L of fuels. Logistically this means that a vehicle with sufficient weight, power, suspension and braking capacity is required, and ideally all-wheel or four-wheel drive if working on unsealed surfaces. In areas where there is no solid road surface, such as Aspendale where there was a sandy roadway or at Vermont South when there had been sufficient rain and the clay had softened to mud, a potential to become bogged was

experienced. The width also limits accessibility and despite the hose length being 40 m, narrow roads and laneways will be largely inaccessible.

4.8 Potential impacts of glyphosate, glufosinate and imazapyr on aquatic environments

This study did not investigate the impacts of the weed management strategies on aquatic organisms or aquatic environments. Generally, for glyphosate, glufosinate and imazapyr, beyond aquatic plants, photosynthetic green algae are reported as being among the most sensitive aquatic organisms to herbicides. This is largely due to the mode of action for these herbicides disrupting conserved metabolic pathways. It has been proposed that when developing a risk assessment for using pesticides, both the toxicity of pesticides and the expected exposure to organisms should be considered (Tsui and Chu 2003). Here a brief review of the impact glyphosate, glufosinate and imazapyr is described.

The general information provided by manufacturers' SDS documents details the impacts of glyphosate-based herbicides as being mostly towards aquatic plants and algae, with minimal impact on fish, frogs and other aquatic invertebrates. It has been proposed that in aquatic environments, the acute toxicity of glyphosate is reported as being highly species dependant across all taxa, with toxicity depending on the timing, magnitude, and route of exposure (Annett et al 2014). Annett et al 2014 summarised that much of the toxicity data for glyphosate has focused on amphibians due to their increased sensitivity compared with other vertebrates. The lethal concentration 50 (LC_{50}) levels for frogs have been reported as ranging from 27 to 911 mg/L depending on the formulation (Mann and Bidwell 1999). This is a large range, with the type of glyphosate formulation altering the LC_{50} (Mann and Bidwell 1999). It has previously been suggested that the surfactants used to increase the efficacy of glyphosate as being more likely to have toxic impacts on non-targeted species; where the surfactants in the different formulations could also dictate the large difference in toxicity (LC_{50}) towards frogs (Annett et al 2014). In marine environments, data obtained from short-term acute

toxicity tests has indicated that glyphosate is generally lethal at high levels. However, long-term exposure data suggests that glyphosate can markedly affect biological responses of marine invertebrates (Matozzo et al 2020). The further information available on glyphosate impacts on aquatic organisms suggest that levels typical in run-off waters can alter microbial populations (Piola et al 2013).

The manufacturers' SDS lists the LC_{50} for glufosinate towards a number of aquatic invertebrates as being >710 mg/L. Previously the lethal concentration (LC_{50}) of a glufosinate based herbicide for the marine medaka *Oryzias dancena* characterised as 8.76 mg/L (Kang et al 2014). For fish the LC_{50} values reported range from 13-65 mg/L, which like the LC_{50} of the marine medaka could be present in runoff water if a sufficient application rate had been used. Based on a general consensus of manufacturers product information, the application rate for glufosinate is around 1 g/L, with 100 L sufficient for covering up to 2 hectares. At the intended application rate across a >1 ha area, the risk of glufosinate toxicity from runoff waters carrying glufosinate into creeks, rivers and ponds is really low. Despite this, the appropriate application times, rates and buffer zones from waterways need to be considered to avoid the risk of runoff water carrying glufosinate into water ways, creeks and rivers.

Exposure of aquatic plants to imazapyr can results in their death. Imazapyr has been proposed as a means for reducing weeds in irrigation water ways and canals to remove unwanted plant growth, however, the application time and rate need to be carefully managed to avoid negative impacts on cropped plants due to the presence of residual imazapyr (Dugdale et al 2020). The half-life of imazapyr in pond and river sediment has been reported as ranging from 180 up to 240 days, with a potentially very slow hydrolysis and biodegradation rate in water. In the context of this study, the use of imazapyr near waterways where it could be washed into waterways in run-off water or enter waterways from spray drift, needs to be considered. Particularly in instances where levels could be sufficient enough to kill off aquatic plants or algae unintentionally. This could be managed by leaving a substantial buffer

between water ways and application area (>1 m buffer zone), only applying in drier months where there is a low risk of rainfall runoff carrying residual imazapyr into the waterway, using appropriate application rates to avoid excess imazapyr and reduced residual time, and not spraying in windy conditions.

In situ analyses of the impacts of imazapyr on fish and aquatic invertebrates have suggested that there are negligible impacts or any impacts are relatively short lived (Breckels and Kilgour 2018). Imazapyr product safety sheets (SDS) list the environmental toxicity as: moderate toxicity to birds, moderately toxic to fish and non-toxic to bees.

4.9 Summary

For this project, a comprehensive desktop study was performed to identify possible weed management alternatives that could replace glyphosate use on land managed by councils. Based on a multifaceted selection criterion of cost, availability, ease of use, any known off-target toxic effects and known hazards for use, storage and negative environmental impacts, the following shortlist of 10 strategies were selected for trialling: glyphosate, glufosinate, imazapyr, nonanoic acid, acetic acid and hydrochloric acid, clove oil, MCPA + dicamba, pine oil, prodiamine and steam. Beyond the desktop review, the efficacy of these alternative weed control strategies were compared to untreated (negative) and glyphosate treated (positive) controls at two sites with different soil types. The Vermont South trial site represented a heavy clay profile and the Aspendale site a sandy loam profile.

At both sites, 4 weeks post application of winter and spring treatments, mixed results were observed for changes to weed coverage by the different weed management strategies; where glyphosate, glufosinate and steam were the only treatments to significantly reduce weed coverage at both sites for both seasons. After 12 weeks, glyphosate, glufosinate and imazapyr significantly reduced weed coverage at both sites, with this outcome consistent across all four seasonal treatments. For steam,

spring, summer and autumn treatments showed a longer -term efficacy, where after 12 weeks post treatment weed coverage remained significantly reduced. Assessment of the bacterial and fungal communities in soils exposed to the different weed management strategies revealed that only the steam treatment reduced the diversity of bacterial and fungal phyla present in the soil. Glufosinate ($0.21/L) was estimated to be twice the cost of glyphosate ($0.10/L), whilst imazapyr cost approx. $2.02/L. Steam has a high capitol cost (>$20,000), and potentially high operating costs (such as equipment maintenance and fuel). Based on the results of field trials, and taking into consideration cost, safety information and off-target impacts, glyphosate is considered to be the most effective weed management strategy of the different approaches scrutinised by this study.

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Appendices

Appendix 1. Table of chemical weed management strategies established by a desktop review.

Chemical Name	Products containing chemical as active constituent	working concentration	Group	Chemical Structure	Chemical formula	Generic name	CAS Number	GHS Symbols	Appearance	Storage	Shelf life/ stability
Glyphosate (N-(phosphonomethyl)glycine)	Weedmaster Duo, Roundup, Zero	0.06M	Group M		$C_3H_8NO_5P$	roundup	1071-83-6		white odourless powder	stable under recommended storage conditions	and it's salts are non-volatile, do not photochemically degrade and are stable in air. It is also
Diquat	Reglone	3.76e-04 M	Group L		$C_{12}H_{12}Br_2N_2$	Aquacide	Dibromide: 85-00-7; Dichloride: 4032-26-2; Dication: 2764-72-9		white/yellow crystals		binds to organic particles where is remains strongly for years without degrading
Dicamba	Dicamba 500	0.03M	Group I		$C_8H_6Cl_2O_3$	Mediben	1918-00-9		white crystal solid	easily vaporises, store without humidity	1-6 weeks
Atrazine	Primextra	1.30e-04 M	Group C		$C_8H_{14}ClN_5$	Gesaprim	1912-24-9		colourless solid	store in an area without drain or sewer access. Keep in tightly closed container in well ventilated place	stable but can become flammable exposed to the right conditions
Sulfentrazone	EucMix GR, EucMix WDH	110 mg/l (pH6) at 25 °C; 780 mg/l (pH7) at 25 °C; 10000 mg/l (pH 7.5) at 25 °C	Group B		$C_{11}H_{10}Cl_2F_2N_4O_3S$		122836-35-5				an aerobic half life of 1.5 years and an anaerobic half life of 9 years.
Clomazone	Clomazone	0.00M	Group Q (inhibitor of carotenoid synthesis)		$C_{12}H_{14}ClNO_2$	Dimethazone	81777-89-1		white solid	without sunlight, in aqueous solution and it is stable at 50 oC	2 years
Metribuzin	Sencor	0.01M	Group C		$C_8H_{14}N_4OS$	Sencor	21087-64-9		colourless crystal solid	store in areas without drain or sewer access. Store in cool dry place	shelf life of 2 years under normal conditions
Linuron	Linuron DF, Linuron MCPA	3.01e-04 M	Group C		$C_9H_{10}Cl_2N_2O_2$	Afalon	330-55-2		colourless crystal solid	store in a dry location under normal condition	stable at its melting point and in neutral aqueous media.
Chloramben	Ornamental Weeder, Vegiben, Amiben	0.00M	Group H		$C_7H_5Cl_2NO_2$	Amiben	1076-46-6 (ammonium salt); 1954-81-0 (hydrochloride salt); 28283-88-7 (aluminum salt)		colourless crystal solid	away from light with ventilation	rapidly decomposes when exposed to light
Alachlor	Lasso, Chimiclor	8.30e-04 M	Group C		$C_{14}H_{20}ClNO_2$	Metachlor	15972-60-8		crystal solid	not sensitive to light or heat.	highly stable
Trifluralin	Bolta Duo	7.01e-07 M	Group LD		$C_{13}H_{16}F_3N_3O_4$	Treflan	1582-09-8		yellow crystals	in a dry place away from ultraviolet light	more than 2 years
Pendimethalin	Stomp Xtra	1.07e-06 M	Group D		$C_{13}H_{19}N_3O_4$	Penoxaline	40487-42-1		orange-yellow crystals	store away from light	stable in acids and alkalis. Very stable in storage
Oryzalin	Surflan, Rout	2.5 mg/l	Group D		$C_{12}H_{18}N_4O_6S$	surflan	19044-88-3		orange-yellow crystals	away from UV irradiation	stable under normal condition, susceptible to decomposition. Shelf life is 3 years

Chemical Name	Organic acid	Inorganic acid	Oxidising agent	Flammable liquid	Flammable solids	Exposure Risks	boiling point	melting point	Solubility	common form	metabolic pathway
Glyphosate (N-(phosphonomethyl)glycine)	yes	no	yes	no	no	causes serious eye damage, can cause skin irritation, gastrointestinal tract and respiratory tract. toxic to aquatic life.	decomposes before boiling	189.5oC	insoluble in common organic solvents, soluble in water at room temperature	salt	enzyme inhibitor
Diquat	yes	no	yes			fatal if swallowed, inhaled, or absorbed through the skin in large quantities	decomposes before boiling	decomposes before melting	71.80%	salt	
Dicamba	yes	no	yes	no	no	moderately toxic if ingested or dermal contact	199 °C	114 to 116 °C	in water	salt	
Atrazine	yes	no	yes	non combustible	yes	lethal if high dose is ingested	200 oC	175 oC	7% (low)	solid	Metabolic pathway
Sulfentrazone	yes	no	yes			harmful if inhaled. Damage to organs through prolonged exposure.		121-123 oC			
Clomazone	yes	no	yes				275 oC	25 oC	readily soluble in Hexane	brown liquid	
Metribuzin	yes	no	yes	not highly flammable	not highly flammable, not likely to self ignite	reproduction problems	decomposes before boiling	125 oC	0.1% at 20 oC	liquid	
Linuron	no	yes	yes	no	no	harmful if swallowed, carcinogenic may damage fertility, toxic to aquatic life.	stable when boiling	93 oC	partially soluble in Toluene. Really soluble in chloroform.	solid	
Chloramben	no	yes	yes	decomposes when heated		releases toxic fumes when heated, dangerous when in contact with acids	200 oC	194-197 oC	7% in water	purplish white powder or purple light solid	
Alachlor	yes	no	yes	no	no	harmful if swallowed, allergic skin reaction, carcinogenic, very toxic to aquatic life	100 oC	104-106 oC	2% (very poor)	white-greyish crystal	inhibits biosynthesis of fatty acids
Trifluralin	no	yes	yes	stable in liquid state	no	allergic skin reactions, suspected of carcinogenicity, toxic to aquatic life	139 - 140oC	46 - 47oC	not soluble in water	orange/ yellow crystals odourless	
Pendimethalin	no	yes	no	no	yes	risk of causing pancreatic cancer and other forms of cancer	330 oC	47 - 58 oC	soluble in most organic solvents	liquid	
Oryzalin	yes	no	yes	stable in liquid state	no	skin irritating	265 oC	141.0 oC	soluble in ethanol, low solubility in water	orange-yellow crystals	

Appendix 2. Table of organic acid based weed management strategies.

organic chemcial name	Products containing chemical as active constituent	working concentration	Chemical Structure	Chemical formula	Generic name	CAS Number	GHS Symbols	Appearance	Storage	Shelf life/stability	Organic acid	Inorganic acid	Oxidising agent	Flammable liquid	Flammabe solids	Exposure Risks	boiling point	melting point	Solubility	common form	organic approval
Nonanoic acid	slasher	0.00M		$C_9H_{18}O_2$	Pelergonic acid	112-05-0		colourless oily liquid at room temperature	do not contaminate with water, store in tightly sealed contained in a dry place	combustable, and deactivated when in contact with water.	yes	no	yes	yes	no	causes skin irriation, eye irritation and harmful to aquatic life.	254.5 °C	12.3 °C	soluble in organic solvents	coconut smelling liquid	yes
acetic acid	vinegar, Green Gobbler Vinegar Weed & Grass Killer, Doctor Kirchner Natural Weed Killer, ECO GARDEN PRO – 100% White Vinegar Organic Weed Killer, Yates natures way organic weed killer, HomeSafe5	16.65M		$C_2H_4O_2$	ethanoic acid, Galacial acetic acid	64-19-7		colourles clear liquid	storage in dry place away from open flame.	stable under normal laboratroy condations. Decomposes when heated and emits fumes	yes	no	yes	yes	no	flammable liquid and vapour, causes severe skin and eye burns and damage	117.9 °C	16.6 °C	Miscible in water	liquid	
citric acid	Bonide Fast Acting Weed and Grass Killer	3.08M		$C_6H_8O_7$	anhydrious citric acid	77-92-9		white crystalline solid, dry powder, liquid	avvoid high temperatures and allow for open ventilation areas	not stable when exposed to air.	yes	no	no	no	no	causes skin and eye irritation	decomposes befo	153 °C	soluble in organic solvents	crystalline solid	

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Appendix 3. Organic weed management strategies identified

organic weed killer with no acidic component	type of herbicide	leave harmful residue	constituent	less effective in weather condictions other than sun	organic certification	time taken to kill weeds	glyphosate free	dilution necessary	biodegradable
natural armour	non selective			yes		within hours	yes	no	yes
BioSafe Weed and grass killer	non-selective	no	soap based	yes		within 1-2 hours of application	yes	yes to specified concentration	
yates natures way organic wee killer	non selective	no	clove oil based	yes	yes	1 hour after application	yes		
bioweed	non selective		pine oil	no		within hours of application	yes	yes 10% to 20%	yes
Agro gold WS weed slayer	non selective		clove oil based					yes	
corn gluten meal	pre emerging weed control		corn by product		yes				yes

Appendix 4. Manual weed management strategies.

physical	method
solarization	covering weeds with clear plastic
hand pulling	hand pulling weeds out
flame weeder	burning weeds with a propane gas cylinder
Boiling water	hot water is applied to weeds
goats	goats eat the weeds
brush cutting	cutting the weeds with brush cutter/ line trimmer
steam	steam boiler diesel fuel operated machine

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