

Horticulture Innovation Australia

Final Report

Scoping herbicide impacts on banana production and soil health

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The Department of Agriculture and Fisheries (DAF)

Project Number: BA13002

BA13002

This project has been funded by Horticulture Innovation Australia Limited with co-investment from the Department of Agriculture and Fisheries (DAF), Banana (R&D Levy), The University of Queensland and funds from the Australian Government.

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ISBN 978 0 7341 3956 6

Published and distributed by:
Horticulture Innovation Australia Limited
Level 8, 1 Chifley Square
Sydney NSW 2000
Tel: (02) 8295 2300
Fax: (02) 8295 2399

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Summary

Herbicides are applied in most banana plantations to reduce competition with weeds. There are currently seven registered herbicides used in the Australian banana industry, with different modes of action, e.g. pre-emergence (pendimethalin), selective post emergence (haloxyfop or fluazifop), broad-spectrum systemic activity (glyphosate) and broad-spectrum knock-down activity (glufosinate, paraquat and diquat). The broad-spectrum knockdown herbicides are most commonly used once banana plantations are established. There is speculation that herbicides reduce soil functions, which potentially undermines the productivity and resilience of Australian banana plantations. Furthermore, in the wet-tropics region of Queensland, where the majority of bananas are grown, there is a need to demonstrate that agrochemicals are not impacting the Great Barrier Reef. To validate and improve the environmental credibility of the banana industry it is important to demonstrate the impacts of herbicides on soil functions through their influence on soil organisms. However, there are few and often conflicting reports about herbicides impacts on soil organisms and biological functions. Therefore, it is necessary to quantify changes in soil biological communities following the application of herbicides and to determine if biological remediation is a viable option.

Investigations concluded that a single application of all registered herbicides applied at recommended registered rates had minimal impact on soil microbial communities. However, there are still questions about the impacts of herbicides following multiple applications, which is the common scenario in banana plantations. Furthermore, if herbicides are applied above recommended rates, there was greater reduction in soil biological functions. The investigations found the herbicides had a temporary impact, one week after application on soil organisms that utilize organic acids. The knockdown herbicides like glyphosate and glufosinate tended to increase bacterial activity during their decomposition following application to the soil at recommended rates. However, glufosinate, paraquat and diquat could also reduce the capacity of soil organisms to utilize organic acids, such as malic, oxalic and citric acids. The chemical degradation pathway of the water soluble herbicides (glyphosate, glufosinate, paraquat and diquat) proved to be difficult to determine. However, it was possible to isolate soil bacteria that were chemically attracted (chemotaxis) to these compounds that could potentially move through soil water. When presented with a choice of carbon compounds only a population of *Gemmatimonadaceae* bacteria moved toward diquat in preference to glucose, indicating its potential as a bioremediation organism.

The chemical degradation of the water insoluble herbicides (pendimethalin, haloxyfop, and fluazifop) could be determined and was measured. They were compared with two environmentally problematic herbicides not used in the banana industry, atrazine and diuron. Haloxyfop and fluazifop exhibited typical degradation patterns over 60 day incubation. However, pendimethalin did not demonstrate a typical degradation pattern, indicating a potential to accumulate in soils. Unlike the water soluble herbicides, the water insoluble compounds were not found to significantly alter the microbial community compared to a control. The production of a draft herbicide risk tool and dissemination to banana grower groups will allow banana growers to assess the risks of herbicide application on soil organisms and their potential functions in the soil. Furthermore, the knowledge and research capacity to understand herbicide interactions with soil biology in banana plantation were outcomes from this project through two student theses and two prepared scientific manuscripts.

Keywords

Chemotaxis, chemical degradations, tropical agriculture, environmental degradation, agrochemicals, microbial diversity, soil biology,

Introduction

Bananas (*Musa* spp.) are a high-value commodity in Australia worth \$565 million (gross value) to the domestic economy in 2015 (<http://horticulture.com.au/wp-content/uploads/2016/10/Australian-Horticulture-Statistics-Handbook-Fruit.pdf>). Competition from weeds is one of the major constraints that can potentially limit banana production. Therefore, herbicides are applied in most banana plantations to reduce weed competition, with the broad-spectrum knockdown herbicides used once the banana plantations are established. However, herbicides have been insinuated to reduce soil functions, which potentially impacts on the productivity and resilience of banana production. There are currently seven registered herbicides used in the Australian banana industry, with different modes of action on weeds, such as pre-emergence (pendimethalin), selective post emergence (haloxyfop or fluazifop), broad-spectrum systemic activity (glyphosate) and broad-spectrum knock-down activity (glufosinate, paraquat and diquat), with examples of the common products and their registered use pattern given in Table 1.

Table 1. Registered herbicides used in the Australian banana industry, and their corresponding active ingredients, rates and frequency of application (Davis, 2014)

Active ingredient	Nature of herbicide	Commercial product example	Concentration of a.i. (g/L)	Rate of application (L/ha)	Frequency of application (no./year)
Pendimethalin	Pre-emergent (broadleaf and grass)	Stomp®	330	9–12	Used at establishment only
Fluazifop.p.butyl	Selective (grass)	Fusilade®	128	1.65	0–1
Haloxifop-methyl	Selective (grass)	Verdict™	520	0.4–0.8	0–1
Glyphosate	Post-emergent (Broadleaf and Grass)	Roundup®	360	3–9	Selectively used in crop
Glufosinate-ammonium	Post-emergent (broadleaf and grass)	Basta®	200	1–5	4–6
Paraquat dichloride hydrate Diquat dibromide monohydrate	Post-emergent (broadleaf and grass)	Sprayseed®	135 115	2.4–3.2	Up to 8
Paraquat dichloride hydrate	Post-emergent (broadleaf and grass)	Gramoxone®	250	1.6–3.2	Up to 8

Over the last 30 years there has been an increase in the amount of herbicide detected in the catchment waterways of some north Queensland rivers, and in seagrass and water surrounding inshore coral reefs (Ham, 2007; Shaw and Müller, 2005; Haynes *et al.*, 2000). The herbicides registered for use in banana production are not currently prioritised as being of concern to the quality of the Great Barrier (Reef Water Quality Protection Plan Secretariat, 2013). However, there has been increasing emphasis on the reduction of chemical and nutrient inputs and to demonstrate environmental best practice (King 2013).

To determine the impacts herbicides have on soil functions it is necessary to quantify impacts on soil organisms. Soil organisms play vital roles in the decomposition of organic matter, recycling of nutrients, assisting plants to obtain nutrients, suppression of pests and diseases, and maintenance of

soil structure (Coleman et al. 2004). Soil organisms include microscopic bacteria, archaea, fungi and protists, mesoscopic protists and animals (e.g. nematodes, mites and springtails) and macroscopic insects, millipedes and earthworms (Stirling et al. 2016) (Appendix 1). There are few reports on how herbicides impact soil organisms and the functions that they mediate (Rose et al. 2016). Furthermore, most reports focus on “persistent” chemicals such as atrazine and diuron (which are not used in the Australian banana industry) or glyphosate when applied to genetically modified crops.

To make an informed assessment of the impacts of herbicides on banana growth and soil functions it is necessary to determine the impacts of herbicides on soil organisms. Furthermore, there is a need to develop management tools to overcome any negative impacts that herbicides application have on soil ecosystem functioning.

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Methodology

Soil biology methodology

Soil organisms exist in vast numbers, with many different techniques for their assessment (Appendix 1). The approach taken in this project was to focus on bacteria, archaea and nematodes, due to the existence of techniques that not only give abundance of the various organisms, but are also linked with the functionality of the soil.

Nematode community

Soil nematodes were determined using a modified Baermann funnel technique (Whitehead and Hemming 1965) from a 200 g sample of field soil. Individual nematodes identified to genus level for plant-parasites and to the family level for free-living nematodes. Soil nematode community analysis was made on soil nematode trophic groups (parasites, fungivores (F), bacterivores (B), omnivores and predators). Indices of the nematode community composition were calculated from the number of nematode taxa, using the diversity index and bacterivore to fungivore ratio (B/(B+F)) (Yeates and Bongers 1999). Additionally, the weighted functional guilds concept was applied. Nematode families were assigned a colonizer-persister (c-p) score from 1–5, (colonizer c-p = 1; persister c-p = 5) (Bongers and Bongers 1998). The nematode functional guilds were used to calculate the basal, enrichment index (EI), structure index (SI) and channel index (CI) of the soil food web (Ferris et al. 2001). Plant-parasitic nematodes were identified to species level where possible and the abundance of each individual classification of plant-parasitic nematode was kept separate.

MicroResp™

Whole-soil sample Community Level Physiological Profiles (CLPPs) were assessed in duplicate using the MicroResp™ system as described by Campbell et al. (2003), incubated for 7 days, and sealed to a colorimetric CO₂-trap for a further 6 h. The respiratory response to 15 carbon sources was tested and selected according to the recommendations of Campbell et al. (2003) including; 5 carbohydrates (L-arabinose, D-fructose, D-galactose, D-glucose and N-acetyl-D-glucosamine), 3 carboxylic acids (citric acid, oxalic acid and L-malic acid), 5 amino acids (L-alanine, DL-aspartic acid, γ -aminobutyric acid, L-lysine hydrochloride and L-arginine) and one phenolic acid (protocatechuic acid). Fumaric acid was also selected due to its role as a signaling molecule in banana roots (Yuan et al. 2015). CO₂-trap absorbance was measured at 590nm (ThermoFisher Multiscan Spectrophotometer). A respiration index (RI) was calculated as the difference in absorbance between zero hours and 6 h of incubation in each well containing each C substrate divided by the difference in absorbance between zero hours and 6 h recorded in wells containing deionized water (Fernández-Gómez et al. 2011).

Soil enzyme assays

β -glucosidase activity was determined on whole soil samples in duplicate using the method of Eivazi and Tabatabai (1988) with the modification of using 0.1% Tween solution instead of toluene and using McIlvaine buffer (pH 6.0) instead of the modified universal buffer (Hayano 1973). β -glucosidase activity was expressed as μg p-nitrophenol released kg^{-1} soil h^{-1} measured by absorbance at 410 nm using a ThermoFisher Multiscan Spectrophotometer.

Fluorescein diacetate (FDA) hydrolysis rate was determined from duplicate 5 g sub-samples using a modified version of the method initially proposed by Schnürer and Rosswall (1982). The hydrolysis reaction was stopped after 30 minutes using acetone and following centrifugation and a 200 μL supernatant sample added to a 96 well microtiter plate. The absorbance was measured using ThermoFisher Multiscan Spectrophotometer at 490 nm and FDA hydrolysis was expressed as mg of FDA hydrolysed kg^{-1} soil h^{-1} .

Duplicate 5 g sub-samples of soil were oxidised using 33mM KMnO₄ following the methodology of Moody and Cong (2008). The KMnO₄ soil solution was centrifuged and a 200 µL supernatant sample added to a 96 well microtiter plate. The change in KMnO₄ was measured by absorbance at 550nm using a ThermoFisher Multiscan Spectrometer and used to estimate the amount of oxidized C (assuming that 1mM of KMnO₄ was consumed in the oxidation of 9mg of C). The results are expressed as g C kg⁻¹ air dried soil.

Characterisation of bacterial and archaeal communities

DNA extraction was from 0.25 g of soil from each treatment, which was suspended by vortexing, then DNA extraction using a PowerSoil[®] DNA isolation kit (MOBIO Laboratories Inc., USA) according to the manufacturer's instructions. Universal 16S rRNA genes were amplified by PCR using 926F and 1392R modified on the 5' end to contain the Illumina linker sequences 1 and 2, respectively. Amplicons were purified using Agencourt AMPure XP beads (Beckman Coulter, Inc.) and subjected to dual indexing using the Nextera XT Index Kit (Illumina) as per the manufacturer's instructions. Indexed amplicons were purified using Agencourt AMPure XP beads and then quantified using a PicoGreen dsDNA Quantification Kit (Invitrogen). Equal concentrations of each sample were pooled and sequenced on an Illumina MiSeq at The University of Queensland's Institute for Molecular Biosciences (UQ, IMB) using 25% PhiX Control v3 (Illumina) and a MiSeq Reagent Kit v3 (600 cycle; Illumina) according the manufacturer's instructions.

Experiments

Soils

Soil used in all laboratory and glasshouse experiments were sourced from a banana field in East Palmerston, Queensland, Australia (S17° 35'32" E 145° 49' 58"), with a clay loam texture (38% sand, 30% silt, 33% clay) and a pH in water of 6.7. The soil was passed through a 4 mm stainless steel sieve prior to use.

Laboratory incubation experiment

Soil was adjusted to 50% water holding capacity and then 1.6 kg of soil was loaded into 44, 2 L plastic containers (surface area = 407.75 cm²). These minicosms were incubated at 27°C for 14 days to allow prior to treatments application. Four herbicides (glyphosate, glufosinate, paraquat, paraquat/diquat) were solubilised in water while the other five herbicides (fluzifop, pendimethalin, haloxyfop, atrazine and diuron) were dissolved in methanol. Two control treatments were included; water-only and methanol-only. The herbicides were sprayed over the surface of the soil with a fine mist sprayer and care not to cross contaminate chemicals (Fig 1). A total of 11 treatments (9 herbicides and 2 controls) were included and, each treatment was replicated four times arranged in a randomised block design in a controlled temperature room at 27°C. Minicosms were then incubated at 27 °C for 60 days. Soil samples were collected from each minicosm after 1, 3, 7, 14, 30 and 60 days incubation using a 20 mm diameter soil core. 50 g samples were collected for soil enzyme assays and MicroResp™ CLPP, a further 30 g sample was immediately frozen at -20°C. A 200 g sample was taken for nematode extraction 3, 30 and 60 days after treatment application (Fig 1).

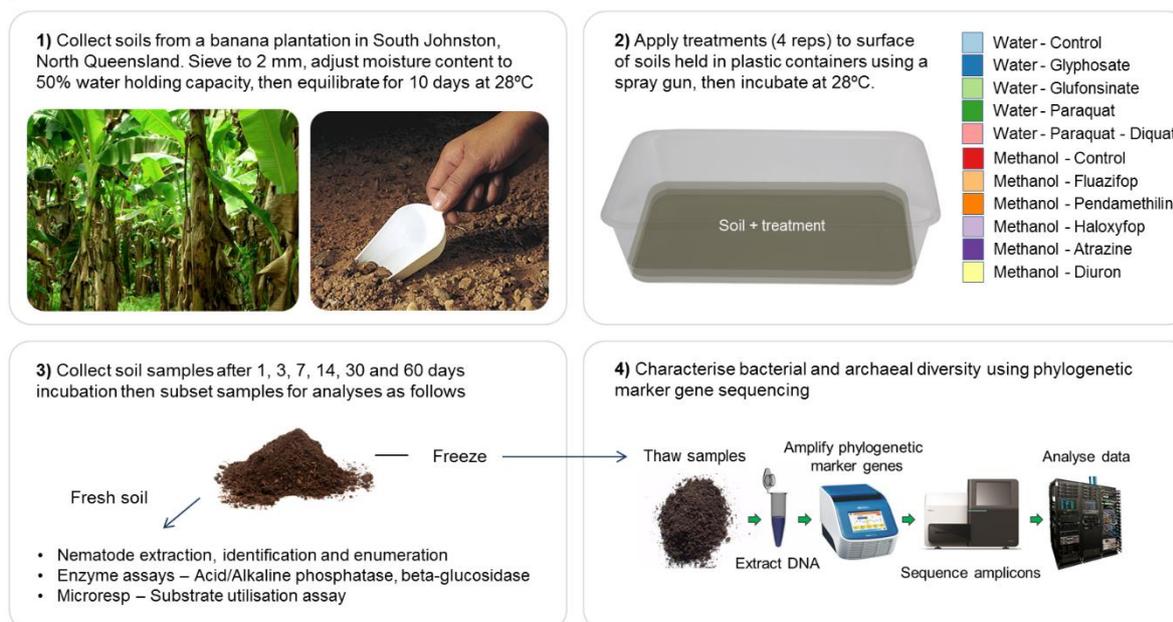


Fig. 1 Summary of experimental design and methodology used to assess impacts of herbicides on soil microbial diversity and function

Herbicide degradation

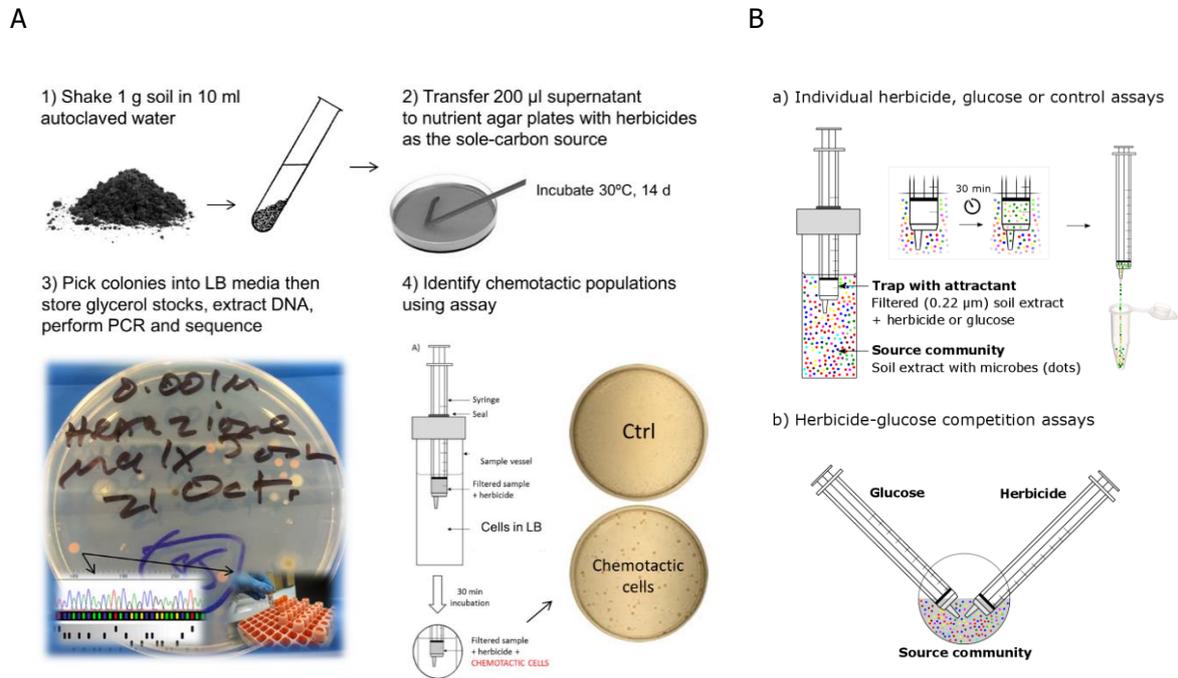
Extraction of soil samples at different time points allowed for quantification of herbicide concentration. For the organic herbicides (fluzifop, pendimethalin, haloxyfop, atrazine and diuron) a QuEChERS method was chosen due to its simplicity and robustness. The analysis of the extracts was done using an LC-MSMS method (Entox). Individual herbicide or internal standard stock solutions were prepared at 1 mg/mL in water (MilliQ) or methanol. Working solutions were prepared at 1 and 5 mg/mL for each herbicide, and at 2 and 40 µg/mL for internal standards. Herbicides were determined by HPLC-MS/MS using an AB/Sciex API5500Q mass spectrometer (AB/Sciex, Concord, Ontario, Canada) equipped with an electrospray (TurboV) interface coupled to a Shimadzu Nexera HPLC system (Shimadzu Corp., Kyoto, Japan).

Chemotaxis

A chemotaxis assay developed by Dennis et al. (2013) was modified to facilitate investigation of soil communities and competition experiments. To detach microbial cells from soil particles, 5 g soil was vortexed for 10 secs in 45 ml of autoclaved deionised water. Five millilitres of soil slurry was then transferred to a tube containing 45 ml of autoclaved deionised water and vortexed for 10 secs. This diluted soil extract was used as the source of microorganisms for subsequent chemotaxis assays (Fig 2A).

Microbial cells and soil particles were removed from a subsample of the diluted soil extract by passing the solution through a 0.22 µm syringe filter (Fig 2A). This filtered soil extract was then used to prepare 0.1 M solutions of diquat, paraquat and glucose. All assays were replicated 12 times, providing three samples for cell counting and nine samples for 16S rRNA gene amplicon sequencing. Bait-trap assays were performed by submerging the tips of 1 ml syringes containing 200 µl of a trap solution (i.e. filtered soil extract with 0.1 M herbicide or glucose) into 15 ml non-filtered soil extract (i.e. the source community) for 30 min (Fig. 2B). Filtered soil extract control assays with no added chemoattractant were performed in parallel and facilitated the measurement of cells into our assays. To characterise the responses of microorganisms to herbicide when offered a potentially preferential

attractant the positioning of the syringe tips was modified as shown in Fig. 2B. Post-incubation, the contents of each syringe was diluted 1:100 with filtered soil extract. Cell counts were measured using an Accuri C6 flow cytometer and expressed as cell counts per ml. Bacteria and archaea identification were determined using the methods described above.



Glasshouse experiment

The experiment was conducted in 19.3 cm diameter pots filled with 4 mm sieved soil (described previously) resulting in 3.0 kg of soil in each pot. The soil was allowed to equilibrate in the pots for 14 days before Cavendish (*Musa AAA*) tissue culture banana plants were planted. Seven different herbicides pendimethalin, glyphosate, glufosinate, paraquat, paraquat/diquat, haloxyfop and fluazifop were applied at field equivalent rates two weeks after the plants were transplanted into the pots (Table 2). The herbicides were applied to the soil surface with a fine mist sprayer, ensuring to avoid direct contact with the herbicides (Fig 3B). Pendimethalin, is a pre-emergent herbicide active and was applied to the soil prior to planting bananas. Including the water control, the eight treatments were replicated 5 times and arranged in the glasshouse in a randomised block design (Fig 3).



Fig. 3 Herbicide glasshouse experiment (A) – Allowing soil to equilibrate with space allowed for plant. (B) – Shielding plant pseudostem from herbicide application. (C) – Banana plants growing in glasshouse.

Table 2 Rates of herbicide active ingredients applied to soil surface in the glasshouse experiment

Commercial Product	Active ingredient	Active ingredient in product	Recommended Rate	Rate a.i. applied (g pot ⁻¹ = 3kg soil)
Basta	glufosinate	200 g/L	1-5 L/ha	0.00292
Sprayseed	paraquat	135 g/L (paraquat)	2.4-3.2 L/ha	0.00126
	diquat	115g/L (diquat)		0.00108
Fusilade	fluazifop	128 g/L	1.65 L/ha	0.00062
Stomp	pendimethalin	330 g/L	9-12 L/ha	0.0116
Verdict	haloxyfop	520 g/L	800 mL/ha	0.00122
Roundup	glyphosate	360 g/L	3-9 L/ha	0.00947
Gramoxone	paraquat	250 g/L	1.6-3.2 L/ha	0.00234

Plant growth was determined by weekly measurements of height (from the soil surface to the base of the most recently emerged leaf), emergence rate of leaves, and the leaf area of the most recent fully emerged leaf. The leaf area of all the leaves which emerged during the trial period were determined by multiplying the length and the width together and multiplying by 0.83 (Turner, 1972). Using a SPAD meter (Minolta) the chlorophyll contents were also estimated on all the leaves on a weekly basis. On completion of the trial the dry matter production of each plant was determined by weighing the shoots (leaves and pseudostem) of the banana plants after drying them in an oven at 75 °C for 3 days. Nematodes were extracted along with soil enzyme assays and MicroResp™ were also conducted on the soil at the completion of the experiment as described previously.

Field experiment

A field experiment was established at the South Johnstone Research Station, Queensland, to investigate the influence of Basta (glufosinate) on soil microbial community structure and function. The herbicide has been applied at 1X and 2X the recommended rate to vegetated plots that had not received herbicide in the previous 12 months and non-vegetated soil, which had five previous applications of Basta over the prior 12 months. Soil samples were collected at 1, 3, 5, 7, 14, 21, 28 and 60 days post-application and the enzyme activities, CLPP patterns (MicroResp™) determined.

Outputs

1. Herbicide risk tool

		Glyphosate	Gluphosinate	Paraquat	Paraquat - Diquat	Fluazifop	Pendamethilin	Haloxifop
Bananas	Plant height	Green	Green	Green	Green	Green	Green	Green
	Dry weight	Yellow	Yellow	Green	Green	Green	Green	Green
	Leaf area	Green	Green	Green	Green	Green	Green	Green
	Leaf emergence	Green	Yellow	Green	Green	Green	Green	Green
	Chlorophyll content	Green	Green	Green	Green	Green	Green	Green
Soil enzymes	FDA (total activity)	Green	Green	Green	Green	Green	Green	Green
	Betaglucosidase	Green	Red	Green	Green	Green	Green	Green
Nematodes	Bacterial feeders	Red	Red	Green	Green	Green	Green	Green
	Fungal feeders	Green	Green	Green	Green	Green	Green	Green
Bacteria	Community composition	Green	Green	Green	Green	Green	Green	Green
	Number of taxa	Yellow	Green	Green	Green	Yellow	Green	Green
	Evenness	Yellow	Green	Green	Green	Yellow	Green	Green
Archaea	Community composition	Green	Green	Green	Green	Green	Green	Green
	Number of taxa	Yellow	Green	Green	Green	Yellow	Green	Green
	Evenness	Yellow	Green	Green	Green	Yellow	Green	Green

Recommendations

Apply standard procedure	Green	No effects
Apply caution	Yellow	Marginal effect
Use a different herbicide	Red	Significant effect

Fig. 4 Herbicide risk assessment tool for the banana industry.

A herbicide risk tool has been produced which assess the risk of the different herbicides used in bananas on different soil microbial communities. This is a draft risk tool which can help banana growers make decisions on which chemicals can be used, given circumstances with soil management.

2. Grower articles

Two articles were published in Australian Bananas about activities undertaken in BA13002. The first in Issue 45, spring 2015, highlighted the project, outlining the activities and some preliminary results from glasshouse experiments. The second was published in Issue 48, spring 2016 highlighting the need for banana growers to contribute soil samples before and after herbicide application to determine the impacts on soil biology through changes in carbon utilization profile changes.

FARM PRACTICES 45

A look at herbicides and soil biology

Initial research has found that some of the seven active ingredients in herbicides may affect soil biology and suppress beneficial organisms.

By Tegan Kukulies, Tony Pattison and Paul Dennis*

The Australian banana industry has a growing focus on improving soil biology, in order to suppress diseases and maintain productivity. In the pursuit of improved soil health, there is a recurring question being asked by banana growers - do herbicides affect soil biology?

Until recently, there has been very little research conducted on herbicide use in bananas and the impact on soil ecology and plant productivity.

However, preliminary research being conducted by the Queensland Department of Agriculture and Fisheries in partnership with the University of Queensland to determine the potential impacts of herbicides has found that some of the seven active ingredients registered for bananas (Table 1) can affect the growth of soil organisms.

A screening experiment using a *Trichoderma viride*, considered to be "beneficial" fungal species capable of suppressing soil borne diseases, found reduced growth of the fungus when placed on growth media containing paraquat (e.g. Gramoxone®), diquat (e.g. Spraysed®) or glufosinate (Basta®), nearly completely inhibiting the growth of the fungi (Figure 1).

This implies that vital soil functions such as nutrient cycling and disease suppression may be suppressed when herbicides are applied to the soil, even at spot spray rates, due to the suppression of the soil organisms that perform these roles.

No growth impact

The second screening experiment used bananas grown in pots and was conducted to determine if the seven herbicides affected the growth of plants over a six-week period.

When the herbicides were applied around the base of the plants at recommended rates there was no reduction in plant growth, leaf-emergence rates or leaf chlorophyll content.

There was a minor trend for plants treated with glufosinate to produce fewer leaves, but overall this experiment showed that a single application of herbicides at the recommended field rates do not affect the growth of banana plants.

Nematode increase

Soil nematode communities can be used as indicator species to determine shifts in the composition of feeding groups and were used as indicators when the soil was treated with the seven different herbicides.

The application of glufosinate increased the percentage of bacterial feeding nematodes in the soil community (from 39 per cent to 63 per cent), which implies that glufosinate has caused a disturbance in the soil ecosystem leading to a bacterial-dominated biological system.

DNA sequencing

A further experiment which is currently being conducted to measure a larger range of soil biological indicators when treated with the different herbicides will use the latest molecular technology to sequence soil DNA.

Initial results indicate that glufosinate, paraquat, and paraquat/diquat are producing small changes to the soil microbial populations.

Overall, the initial results indicate that herbicides registered in bananas, if applied at recommended rates, do not have any harmful effects on banana plant growth. However, long term impacts of repeated applications and indirect impacts to the potential disruption of the soil ecosystem are yet to be completely understood.

**Tegan Kukulies is a Development Horticulturist and Tony Pattison is a Principal Nematologist with the Queensland Department of Agriculture and Fisheries and Paul Dennis is from the University of Queensland.*

Figure 1: Mean colony diameters (mm) of *Trichoderma viride* 44, 54, 68 and 102 hours after inoculation on plates amended with herbicides at recommended spot spray rates.

Active Ingredient/s	Commercial Examples	Weeds	Frequency of Application
Glufosinate	Basta®	Post-emergent (Broadleaf & grass)	Commonly used (up to 4-6 x per year)
Paraquat dichloride & Diquat dibromide	Spraysed®	Post-emergent (Broadleaf & grass)	Commonly used (up to 8x per year)
Fluazifop-p-pentyl ester	Fusticide®	Selective (Grass)	Occasionally used (0-1 x per year)
Pendimethalin	Stomp®	Pre-emergent (Broadleaf & grass)	Commonly used at establishment only
Haloxyfop	Herdick™	Selective (Grass)	Occasionally used (0-1 x per year)
Glyphosate	Roundup®	Post-emergent (Broadleaf & grass)	Selectively used in crop
Paraquat	Gramoxone®	Post-emergent (Broadleaf & grass)	Commonly used (up to 8x per year)

Spring 2015 | Australian Bananas MAGAZINE

Article in Australian Bananas Issue 45, Spring 2015

SOIL BIOLOGY 19

Understanding soil biology

Does the application of herbicides reduce the activity of soil microorganism around banana plants? This is the question that is being answered as a result of a two-year research project, funded by Horticulture Innovations Australia, Queensland Government and University of Queensland.

The outcomes so far from the project indicate that herbicides used in the banana industry, if applied correctly at the recommended rates, pose little risk to soil biology. However, if herbicides are applied at rates higher than recommended, there are subtle changes in the soil microbial community around banana plants.

Results from laboratory and field experiments indicated that some commonly used herbicides (glufosinate, paraquat, diquat, and glyphosate) can alter the way specific groups of microorganism interact with plants.

Some microorganisms can be attracted to the chemicals (a process known as chemotaxis) using them as a food source as they break down the chemicals. To other soil organisms the herbicides can be toxic, suppressing their ability to function.

These soil organisms may be important for plant defence mechanisms whereby the plant excretes carbon substrates through its roots (root exudates) to attract certain bacteria. These bacteria help defend the plant by building biofilms around the roots which help protect them from soil borne diseases.

Using these root exudates as indicators, laboratory trials have shown that some herbicides suppress these bacteria and therefore may play a role in reducing the soils capacity to suppress soil borne diseases.

To confirm these observation banana growers are being asked to participate in a survey of their soil by collecting and submitting samples before and after 1:1:1 apply herbicides on their paddocks.

This would involve taking a soil sample from three separate locations immediately before and after herbicides are applied.

Story by Tony Pattison. To obtain a soil sampling and collection kit, with instructions on how collect the samples please contact Tony at the Department of Agriculture and Fisheries, South Johnston on (07) 4220 4127.

Results from laboratory and field experiments indicated that some commonly used herbicides (glufosinate, paraquat, diquat, and glyphosate) can alter the way specific groups of microorganism interact with plants.

Spring 2016 | Australian Bananas MAGAZINE

Article in Australian Bananas Issue 48, Spring 2015

Fig. 5 Articles published in Australian Bananas from BA13002, Scoping herbicide impacts on banana production and soil health.

3. Student thesis

Two student thesis have resulted from collaborative work undertaken through BA13002:

1. Joshua Shields - Isolation and characterisation of herbicide-degrading soil bacteria, School of Agriculture and Food Sciences, The University of Queensland, Brisbane, QLD 4072, Australia. (Appendix 2).
2. Chelsea Engel - Chemotaxis of soil bacteria and archaea to diquat and paraquat with and without glucose as a competing attractant, School of Agriculture and Food Sciences, The University of Queensland, Brisbane, QLD 4072, Australia. (Not attached due to copyright permissions which may prevent further publication).

4. Grower presentations

Presentations containing the content developed within BA13002 were disseminated to banana growers at two separate multi-location forums;

1. Two presentations at regional meetings co-organised by the Soil Science Society Australian and natural resource management groups Terrain, Northern Gulf and Cape York. Approximately 80 people attended the presentations over the two days, with agreement that the meetings should be an annual event for north Queensland.



Participants at the Soil Science Australia symposium in Mareeba December 10, 2015.



Tony Pattison presents information on soil biology and initial findings on the impacts of herbicides at the Soil Science Australia symposium in Mareeba December 10, 2015.



Participants at the Soil Science Australia symposium in Lakeland December 9, 2015.



Tony Pattison (Queensland Department of Agriculture & Fisheries), John Armour (Tropical Landscapes) answer questions on soil management at the Soil Science Australia symposium in Lakeland December 9, 2015.

Fig. 6 Attendees at the north Queensland regional soil science forums where results from BA13002 were disseminated to banana growers and industry service personnel.

2. Banana roadshow series presentation were conducted in;
 - a. Mareeba, June 9, 2016,
 - b. Innisfail, June 10, 2016,
 - c. Tully, June 16, 2016,
 - d. Coffs Harbour July 5, 2016 and
 - e. Lismore July 7, 2016.

Included in the presentation was the influence of herbicides on soil biology and the draft risk assessment tool for growers. The draft risk assessment tool was also available as a handout to banana growers. Approximately 160 banana growers and industry service personnel attended the workshop series.

5. Draft scientific manuscripts

Two scientific manuscripts have been drafted and are awaiting finalization before submission to peer reviewed journals. These have not been included in the final report due to potential copyright issues which may prevent their publication in scientific journals (see page 29).

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Outcomes

The herbicides registered and recommended for use in bananas have been observed to have only small effects on soil organisms when applied as a single application at the recommended rate. As the banana industry does not regularly use persistent products such as atrazine and diuron, the potential impact on the Great Barrier Reef and soil environment is significantly reduced. Comparisons of the degradation of fluazifop and haloxyfop used in the banana industry showed a degradation over a 60 days in an incubation experiment, whereas pendimethalin tended not to degrade and the breakdown products of diuron increased. Therefore, overuse of pendimethalin in the banana industry could be problematic. However, it is used as a pre-plant herbicide once every five or more years reducing the environmental risk, but continued monitoring of pendimethalin use and movement from banana farms is recommended.

The banana industry is reliant on repeated applications of knockdown herbicides, glufosinate, paraquat, diquat and to a lesser extent glyphosate, to control weeds. The repeated application increase the potential for greater impacts on soil biology. For example, using twice the recommended rate of Basta® (glufosinate) in a field experiment significantly ($p < 0.05$) reduced the ability of soil microorganisms to utilise a variety of carbon substrates.

The risk that herbicides could be impeding biological functions in the soil and hence impacting on long-term banana production can be managed by knowing how the chemicals effect different soil organisms. The repeated application of the same product is likely to cause a shift in soil biological activity. Bacteria are the most likely organisms to respond to herbicide applications as many are able to degrade the ingredients to extract carbon as a food source. A preliminary result supporting the hypothesis of enhanced bacterial degradation occurred in the soil incubation study when glyphosate increased the number of bacterial Operational Taxonomical Units (OTUs) and Pangrolaimidae nematodes, which are fast growing nematodes that respond to bacterial enrichment of the soil.

Rotation of herbicide active ingredients, with a sufficient spell between applications, is likely to alter impacts on soil organisms as well as reduce the risk of weed resistance to herbicides and may be one strategy to manage herbicide risks to soil biological functions. Although, there was no evidence from the field experiment that previous exposure to a herbicide, like glufosinate, altered the impact of the herbicide on soil biological function. The application rate of the herbicide had a more profound effect on soil biological function than previous exposure to the herbicide.

The work conducted in this project focused on the active ingredients, their impacts on organisms and degradation. Commercial products have other chemicals such as carriers, solvents and adjuvants to enhance the efficacy of the active ingredient. Therefore, the focus on active ingredients may not necessarily provide all of the information on the impacts that herbicides have on soil biological function in commercial situations.

The use of MicroResp™ to determine the Community Level Physiological Profile (CLPP) of soil microorganisms, demonstrated a reduction in the utilization of the organic acids when glufosinate, paraquat, diquat and glyphosate were applied to the soil. These were all water soluble herbicides that are repeatedly applied to banana soils to reduce weed competition. Therefore, a screening method to determine if herbicides are impacting on soil biological functions could focus on the change in the utilization of the organic acids using the MicroResp™ test. This has been undertaken with a grower survey, but quarantine restrictions on soil movement and submissions of samples have made the task difficult to report on within the timeframe of the project.

Bacterial chemotaxis towards herbicide active ingredients was demonstrated in laboratory studies. Bacterial isolates were found to degrade specific herbicides and therefore demonstrated the potential

to alleviate negative effects of herbicides on soil biological function by enhancing their degradation in the soil. However, it was also demonstrated that chemotactic bacteria are distracted by other easily degraded carbon sources in the soil such as glucose. This implies that many of the soil bacteria that are chemotactic toward herbicides would only degrade herbicides if preferred sources of organic carbon were replete. Potential chemotactic bacteria in the soil are more likely to respond to plants treated with herbicides, particularly if they exude sugars and organic acids during their decay and then decompose the herbicides once easily degraded carbon compounds have been exhausted.

The glasshouse study provided evidence that herbicides applied at the recommended rates and application methods do not have a significant negative impact on plant growth. Under controlled conditions banana plantlets had similar chlorophyll, plant growth and dry weight relative to untreated plants. Leaf emergence rate was lower in the glufosinate treated plants for four week post application, but by the end of the experiment was the same as the control. The application of glufosinate increased the proportion of bacterial feeding nematodes in the soil but not the abundance. These results indicate that glufosinate may have very minor effects on soil biology and plant growth when applied at the recommended rate, but if applied at higher rates impacts on soil functions are more likely. This was observed in the field experiment when double the recommended rate was used and there was a decrease in the overall carbon utilization determined using MicroResp™.

Management to reduce the impacts of herbicides on soil biological functions may include a number options. Vegetated groundcover with selection of low growing species can reduce the need for repeated applications of herbicides. The use of mulches to suppress weeds when plants are being established may also reduce the need for herbicide application. Similarly, the use of "companion crops", where a selective herbicide is used, such as fluazifop, to kill the companion crop and form a surface mulch may reduce the need for repeated applications of the knockdown herbicides. The retention of banana crop residue, leaf and pseudostem material around the base of banana plants can provide some weed suppression in the ratoon banana crops, reducing the need for herbicide application. Finally the application of herbicide chemotactic bacteria has the potential to reduce the time that herbicide products are in the soil, and the impact they have on soil biological functions.

The evidence from this project indicated that single applications of herbicides applied at the recommended rate had minor and only temporary effects on soil biological functions. Therefore, manufacturers label directions provide a good indication of use of the product to have minimal impact on the soil environment. However, once application rates exceed those that are recommended by the manufacturer, herbicides can alter soil organisms and hence soil biological functions. The Australian banana industry uses herbicides that are less likely to have off-farm impacts on the environment, but caution needs to be exercised to ensure their applications do not vary from label recommendations.

Evaluation and Discussion

Laboratory incubation

The laboratory incubation studies demonstrated that the seven registered active ingredients impacted on soil biological functions, although effects were small and not always consistent, with results being further elaborated in Dennis et al (in preparation). Several techniques for measuring soil biological activity or function are required over at least 30 day times periods to ensure observations are not temporary fluctuations but changes caused by the treatments. The Community Level Physiological Profiling (CLPP) using the MircoResp™ system demonstrated sensitivity to changes caused by herbicides, was relatively inexpensive and produced rapid results. For example, relative to the water-amended control soils, glufosinate, glyphosate, paraquat and paraquat/diquat significantly influenced CLPPs, but only in soils incubated for seven days. Out of the 15 carbon substrates tested, significant effects of herbicides were only observed for three organic acids and one amino acid (Fig. 7). Relative to the methanol-amended control soils, atrazine, diuron, fluazifop, haloxyfop and pendimethalin did not influence CLPPs in any of the time points ($P > 0.05$).

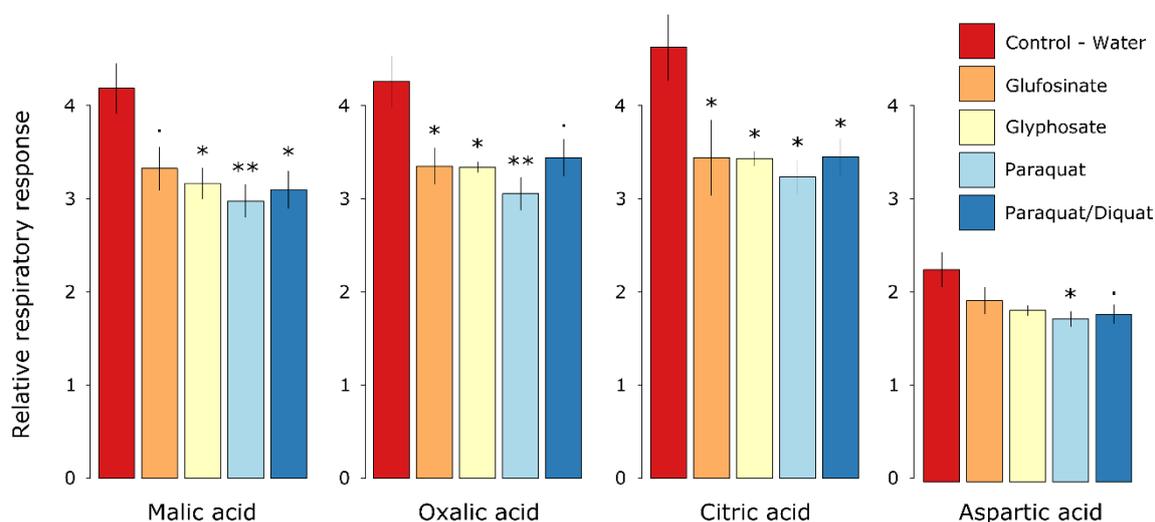


Fig. 7 Inhibitory effects of herbicides on the respiratory responses of soil microbial communities to added substrates after seven days incubation. Asterisks indicate significant differences between control and herbicide treated soils ($P < 0.10$, $P < 0.05^*$, $P < 0.05^{**}$).

Nematode community analysis demonstrated some sensitivity to application of herbicides, but were more variable than CLPP. In the laboratory incubation experiment nematode communities were dominated by representatives of the nematode families Tripylidae, Tylenchidae, Mononchidae, Pangrolaimidae, Monhysteridae, Cephalobidae and Dorylaimidae (Fig. 8). Relative to controls, glyphosate after 60 days incubation was the only herbicide observed to significantly influence nematode community structure ($P < 0.03$). From a taxonomic perspective, glyphosate addition led to a significant increase in the frequencies of Pangrolaimidae nematodes ($P = 0.032$, Fig. 9). From a functional perspective, glyphosate addition led to a significant increase in bacterial-feeding nematodes ($P = 0.018$) and members of the functional guild Ba1, which include Pangrolaimidae and Rhabditidae ($P = 0.028$). The Ba1 nematodes are enrichment opportunists that feed on increased bacterial abundance in the soil. It is hypothesised that glyphosate application stimulated bacterial degradation, which was detected as an increase in the Ba1 nematode guild 60 days after application.

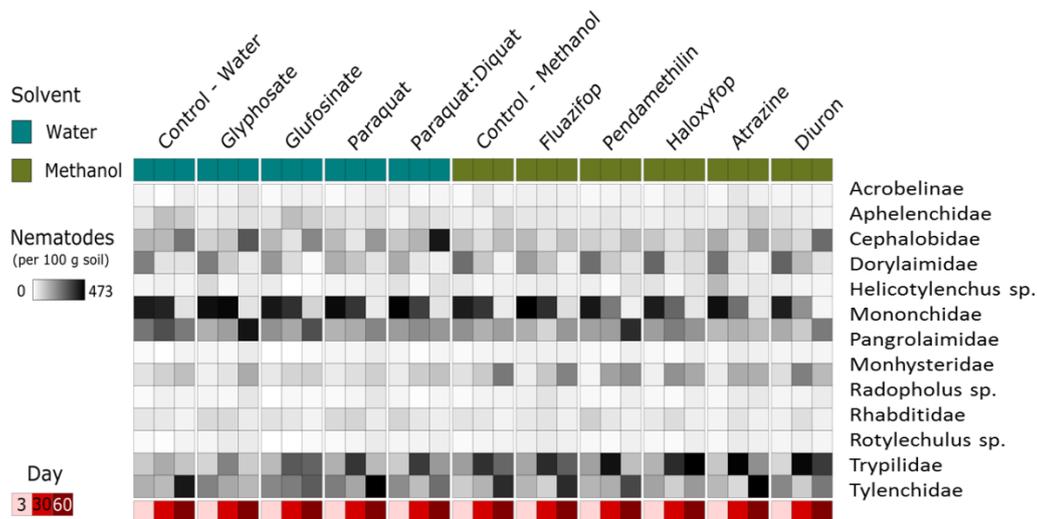


Fig. 8 Heatmap summarising the abundances of nematode groups in each treatment. Each cell represents the average number of nematodes per 100 g of soil and the darker the square, the more abundant that group was in that treatment.

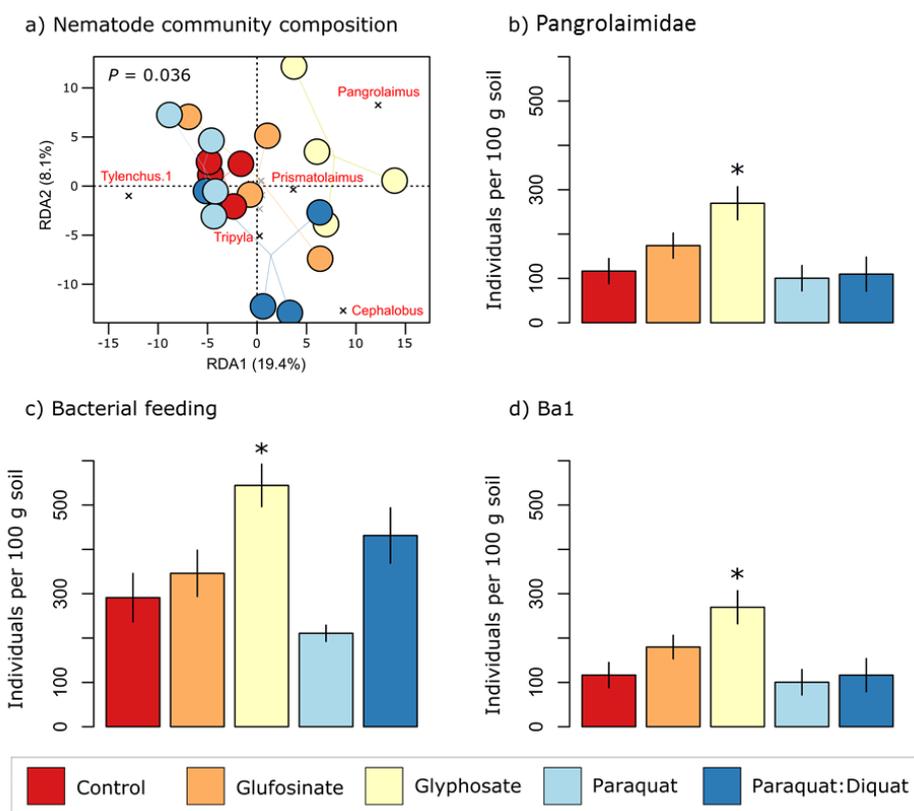


Fig. 9 Effects of herbicides on the structure of soil nematode communities. Asterisks indicate significant differences between control and herbicide treated soils ($P < 0.05^*$).

Next Generation Sequencing (NGS) revealed that soil microbial communities were dominated by members of the bacterial phyla Proteobacteria, Firmicutes and Bacteroidetes and the archaeal phylum Chrenarchaeota, with representatives of the Acidobacteria, Actinobacteria and Nitrospirae populations also constituting a significant fraction of each community (Fig. 10).

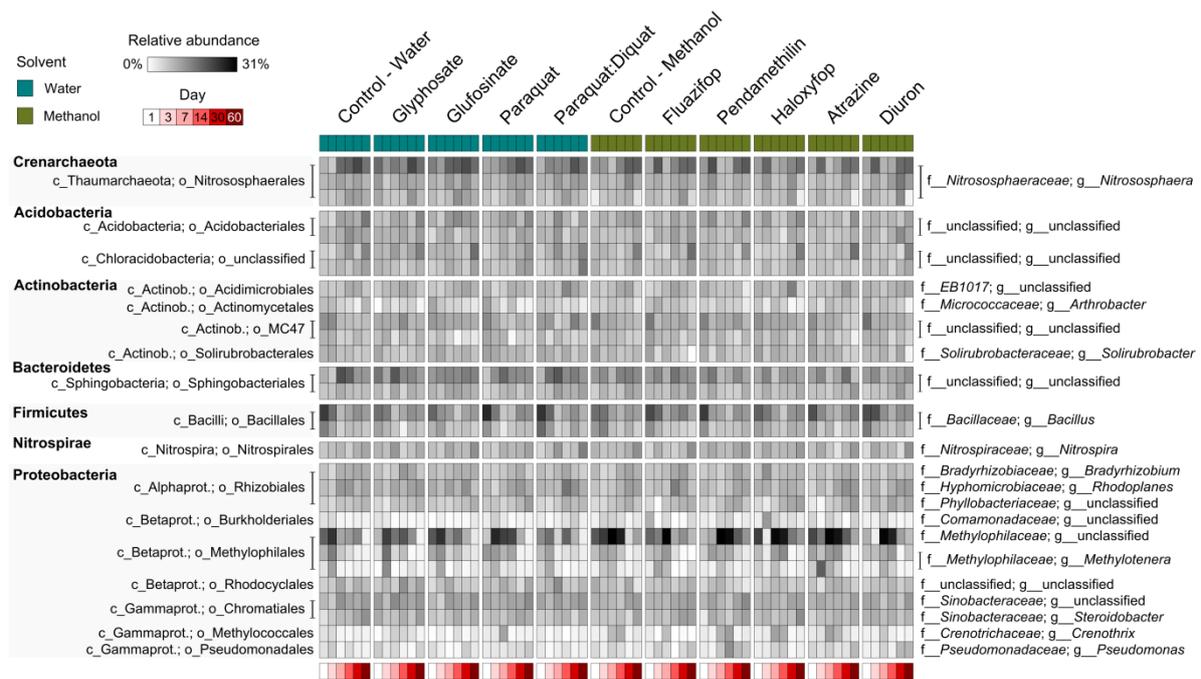


Fig. 10 Heatmap summarising the composition of soil microbial communities overtime within treatments. The darker the square, the more abundant that group was in that treatment. The OTUs listed are those present at $\geq 1\%$ average relative abundance in any treatment.

Relative to the controls, the application of herbicides did not significantly change the composition of soil microbial communities. Likewise, the addition of methanol applied as a control for the non-water soluble herbicides, did not influence microbial community composition except on day 7 ($R^2 = 26.3\%$, $P = 0.025$), when a dominant operational taxonomic unit (OTU) related to an unclassified genus within the *Methylophilaceae*. Throughout the incubations, however, this population was on average more abundant in the methanol-only controls than in the water-only controls ($P = 0.002$). An enrichment of *Methylophilaceae* in response to methanol addition is plausible as all genera within this family are known to be decompose methanol.

Relative to the controls, all herbicides, except for glyphosate and fluzifop, had no effect on the number of OTUs or their evenness in the microbial community (Fig. 10). Relative to the water-only controls, glyphosate addition was associated with a small but significant increase in the number of observed OTUs ($P = 0.018$; Fig. 10) and their equitability ($P = 0.007$). A small increase in the number of predicted OTUs was also observed upon glyphosate addition; however, this effect was only marginally significant ($P = 0.091$). The increase in OTUs supports the observation that an increase in the Ba1 nematodes was due to an increase in bacteria following the addition of glyphosate.

Relative to the methanol-only control, fluzifop addition did not influence the number of OTUs but a marginally significant increase in the evenness of microbial communities was detected ($P = 0.058$). The addition of methanol led to a significant reduction in the evenness of soil microbial communities ($P < 0.001$) and a marginally significant reduction in the observed number of OTUs ($P = 0.088$).

Analysis of the degradation of herbicides indicated that most herbicides measured were degraded over time at different rates (Fig. 11). The exception was pendimethalin, which did not degrade over the 60-day period. This could potentially be problematic for the banana industry as pendimethalin is used as a pre-emergent chemical when planting bananas and if it adheres to soil particles has the potential to be lost from land with eroded soil sediments into waterways. For atrazine and diuron,

which are not used in the banana industry, we could detect the degradation by-products as their parent compounds degraded. For diuron, the concentration of by-products (DCPMU, DCPU) increased over time and appear to be more recalcitrant than the parent compound. In contrast, by-products atrazine-desisopropyl and atrazine-desethyl peak in concentration before rapidly declining.

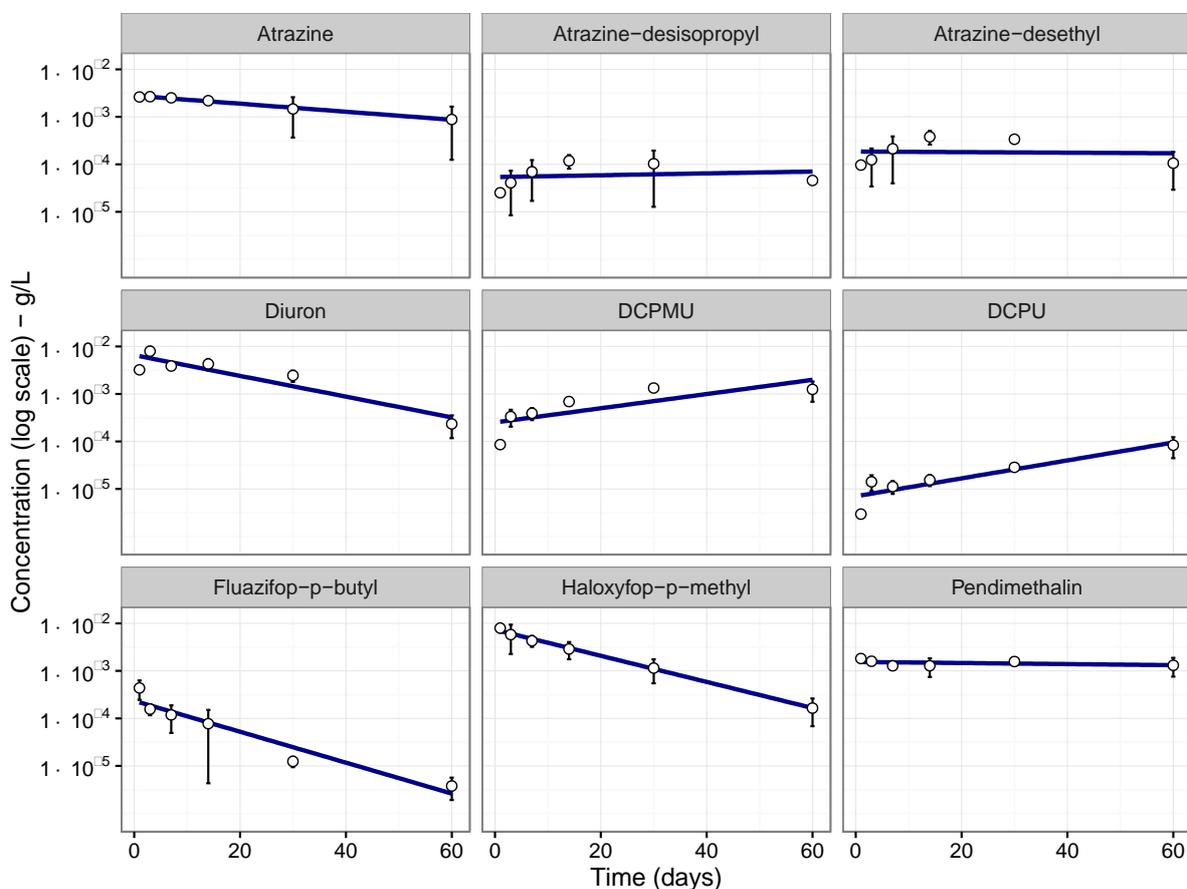


Fig. 11 Degradation analysis of soil samples spiked with five herbicides. DCPMU: 3-(3,4-dichlorophenyl)-1-methylurea and DCPU: 1-(3,4-dichlorophenyl)urea.

Chemotaxis

The novel chemotaxis assays was used to isolate chemoattraction by microorganisms to herbicides (Fig. 12). Further isolation of the organisms attracted to herbicides using plating techniques demonstrated that microbial growth of potential herbicide-degraders was greater at lower herbicide concentrations after 18 days. *Pseudomonas aeruginosa* (strains: BL43 and W45909) and *Acinetobacter calcoaceticus* (strain: PUCM1006) were identified as attractants to herbicides and were grown on minimal nutrient plates amended with the different herbicides. *P. aeruginosa* has previously been shown to degrade the active component of atrazine and similarly strains of *A. calcoaceticus* have been shown to degrade structures found within fluazifop-p-butyl. More details are given in Appendix 2.

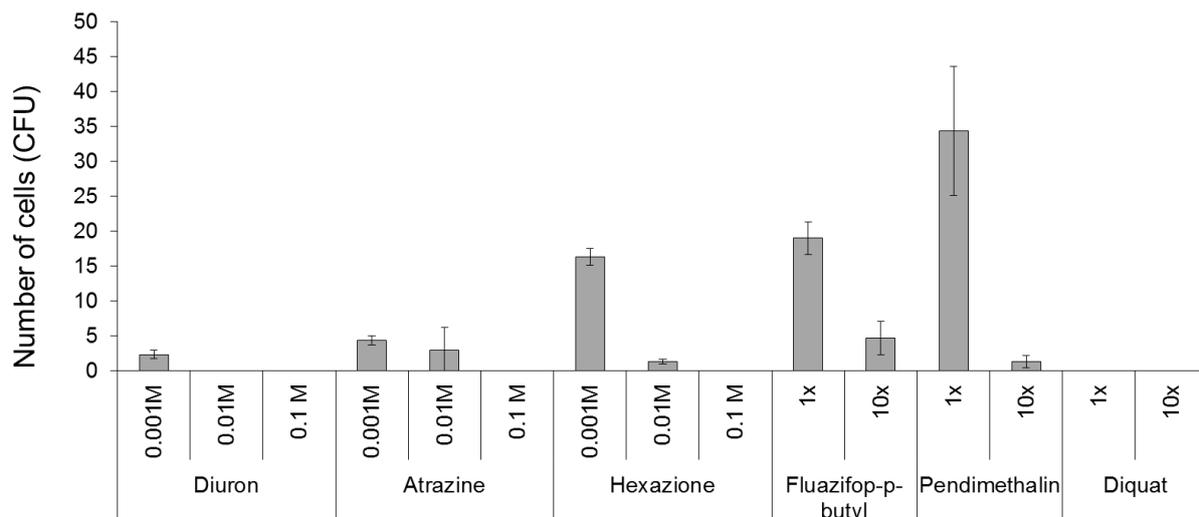


Fig. 12 Number of colonies grown on herbicide-amended agar plates

The second chemotaxis study found that diquat, paraquat and glucose on their own elicited positive chemotaxis to soil organisms. However, the majority of microbes either did not appear to have a preference or they moved toward glucose over the herbicides (Fig. 13). Only one population moved toward diquat in preference to glucose, indicating the potential for bioremediation. It is recommended that further research be performed in regards to this *Gemmatimonadaceae* bacterium (Fig. 14). The use of NGS with the chemotaxis assay has the potential to identify all microorganisms that have the ability to perform chemotaxis to herbicides, rather than only culturable organisms. Further results are included in Engel et al (in preparation).

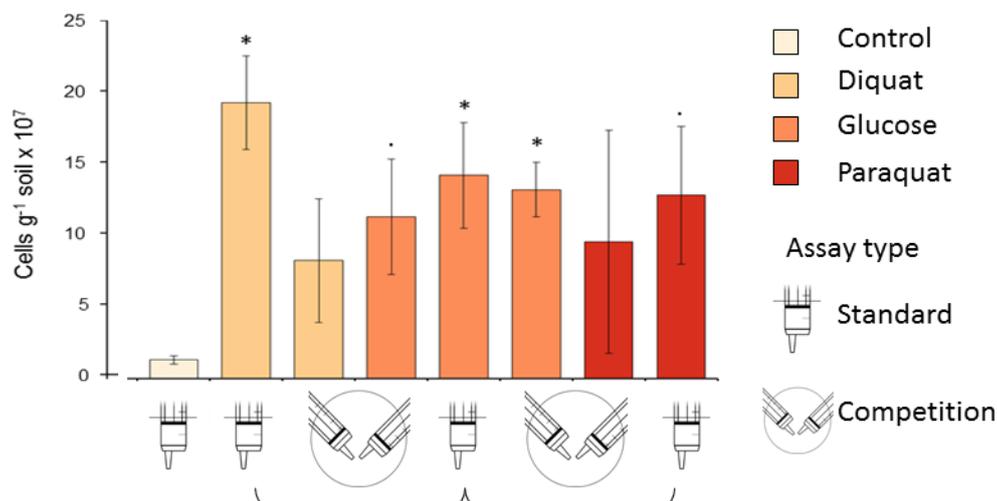


Fig. 13 Cell counts generated using flow cytometry. The standard bait-trap assays are filtered soil extract with and without added attractants, incubated for 30 min in non-filtered soil extract. The competition assays are filtered soil extract with added attractants that were competing in adjacent syringes, also incubated for 30 min in the non-filtered soil extract. Error bars are standard errors and differences relative to the bait-trap control assay are shown as $P < 0.1$ (.) and $P < 0.05$ (*). (From Engel et al (in preparation)).

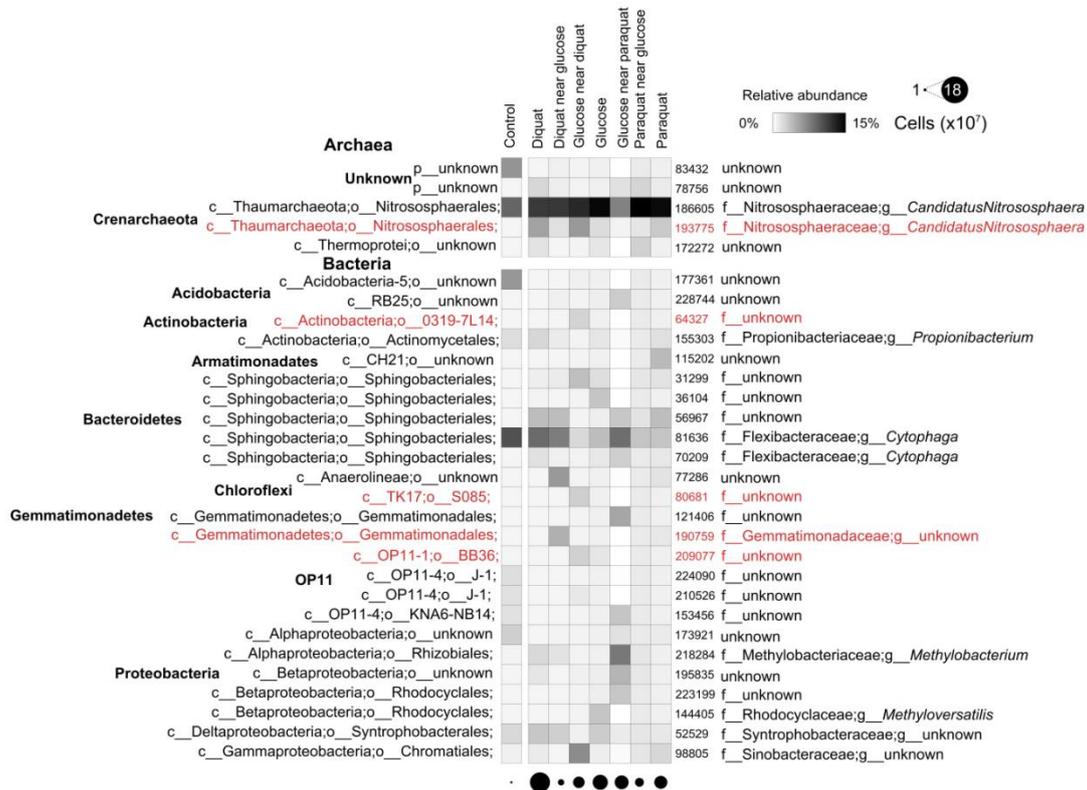


Fig. 14 Heatmap generated from sequencing and flow cytometry data. The darker the square, the more abundant that group was in that treatment. The size of the black circle indicates the mean total cell counts for that treatment (10^{-7}) (From Engel et al (in preparation)).

Glasshouse experiment

Assessment of the plant growth measurements found that a single application of eight herbicide active ingredients did not significantly reduce ($P > 0.05$) plant growth rates, leaf area or chlorophyll measurements, within the first 6 weeks (Fig. 14). Leaf emergence was slightly reduced following application of glufosinate, although there was no significant difference relative to the control treatment at the end of the experiment (Fig. 14).

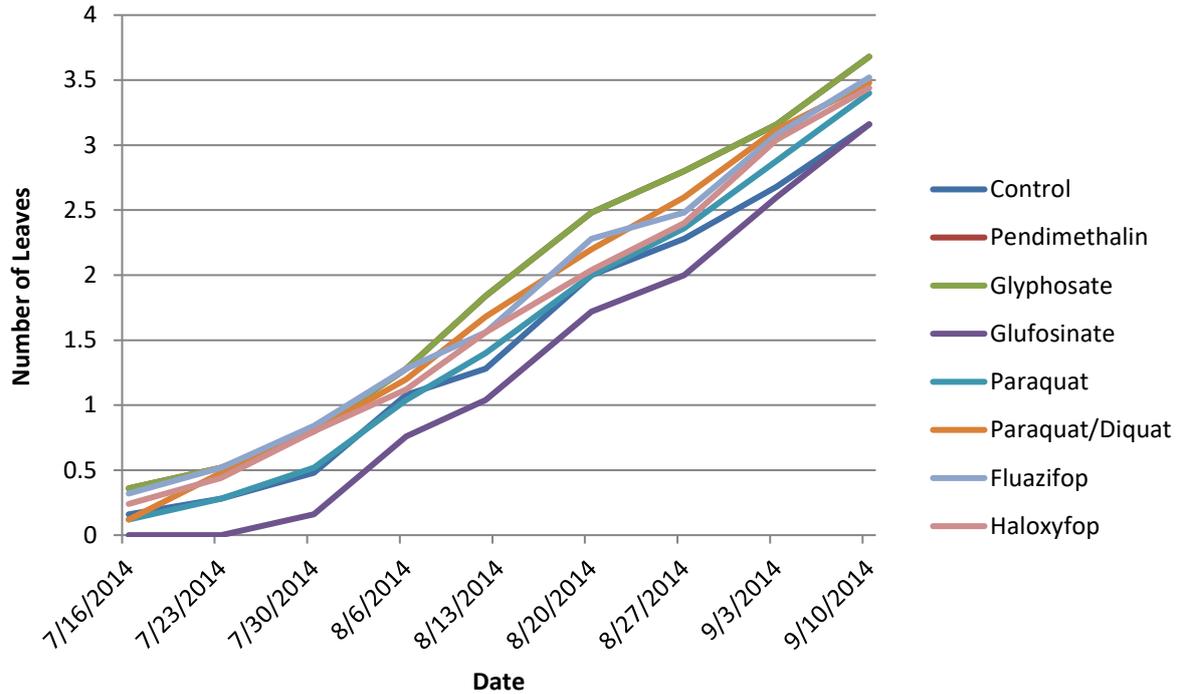


Fig. 14 Cumulative leaf emergence in the presence of herbicide active ingredients registered for use in bananas. All herbicides (excluding pendimethalin) were applied on the 30/07/2014. Pendimethalin (pre-emergent) was applied a month prior on the 30/06/2014).

Evaluation of the soil biological characteristics also revealed very few significant changes ($P > 0.05$) attributed to the application of herbicides. Two differences that were observed included an increase in the percentage of bacterial feeding nematodes found in the soils treated with glufosinate and a change in the relative respiration of carbon substrates using the MicroResp™ system (Table 3). Compared to the control, the soil treated with glufosinate and paraquat/ diquat significantly increased carbon utilization six week after application (Table 3).

Table 3 Soil nematode and enzyme activities of soil applied with herbicide active ingredients registered for use in bananas. Herbicides were applied to soil six weeks prior to measurement with the exception of pendimethalin (pre-emergent herbicide), which was applied 10 weeks prior to soil sampling.

	Control	glyphosate	glufosinate	paraquat	paraquat/ diquat	fluzifop	haloxyfop	pendimethalin
Total Nematodes	185	181	139	160	191	198	216	256
Total Plant-parasites/100g soil	14	10	5	9	14	23	10	25
Total Fungal Feeding/100g	48	49	27	37	55	39	68	59
Total Bacterial feeding/100g soil	73	72	84	52	59	82	94	74
Total Predatory/100g soil	33.7	37.7	14.9	41.4	43.4	46.0	29.1	70.2
Total Omnivores/100g soil	15.3	13.0	8.2	20.5	20.7	8.6	14.8	27.2
Plant parasites (%)	7.2	3.6	4.2	5.6	8.1	9.3	4.9	10.0
Bacterial (%)*	39.0 _a	42.2 _a	62.7 _b	33.1 _a	31.9 _a	40.5 _a	40.9 _a	30.1 _a
Fungal feeding (%)	27.0	24.8	18.5	22.7	25.6	20.4	32.8	24.1
Predatory (%)	26.8	29.5	14.6	38.6	34.5	29.8	21.4	35.8
Diversity H'	1.79	1.73	1.34	1.81	1.84	1.80	1.77	1.87
Enrichment index	69	73	84	73	73	75	67	75
Structure index	66	72	50	77	75	71	56	76
Channel index	25	26	12	24	26	18	31	25
B/(B+F) ratio	0.61	0.62	0.75	0.61	0.58	0.68	0.56	0.57
Detritus (%)	55.68	56.87	76	45	47	54	63	46
Predation (%)	41.15	41.31	22	52	49	41	34	49
Roots (%)	3.18	1.82	2	2	3	4	3	4
Taxa number	9	8	6	9	9	8	9	9
β-Glucosidase	30.05	27.60	25.60	26.51	27.04	29.45	28.82	24.25
FDA	45.85	45.47	50.67	43.73	45.32	45.20	43.98	45.17
MicroResp™ diversity *	1.62 _{bc}	1.75 _{ab}	1.79 _a	1.74 _{ab}	1.86 _a	1.73 _{ab}	1.76 _{ab}	1.50 _c

Different letters indicate significant differences between treatments (P < 0.05)

Field experiment

There were no significant differences between soil that had vegetated groundcover and no herbicide for 12 months, compared to soil that had repeated glufosinate application, other than β-glucosidase activity. The mean activity for β-glucosidase was significantly higher (P<0.05) for vegetated groundcover 19.9 μg p-nitrophenol kg soil⁻¹ h⁻¹ compared to bare soil 13.4 μg p-nitrophenol kg soil⁻¹ h⁻¹. There was no interaction with the rate of chemical application, which indicated that the soil with vegetated groundcover tended to have greater β-glucosidase activity than bare soil, which remained unchanged following herbicide application.

Examination of the utilization of carbon substrates using the MicroResp™ system found that organic acids tended to be utilized to a greater extent than other carbon compounds. The resulting dendrogram from the relative respiration index (RI) at a similarity of 0.85 (85%) found there were four groups (Fig 15), one contains only L-arginine, which had very low mean RI levels, mostly below 1 indicating inhibition of microbial activity. Another group contains citric acid, oxalic acid and malic acid, a third group includes L-arabinose, D-fructose, D-galactose and D-glucose. The remaining seven carbon substrates form the fourth group (Fig 15). Citric acid, oxalic acid and L-malic acid are all carbon substrates which had a high mean RI.

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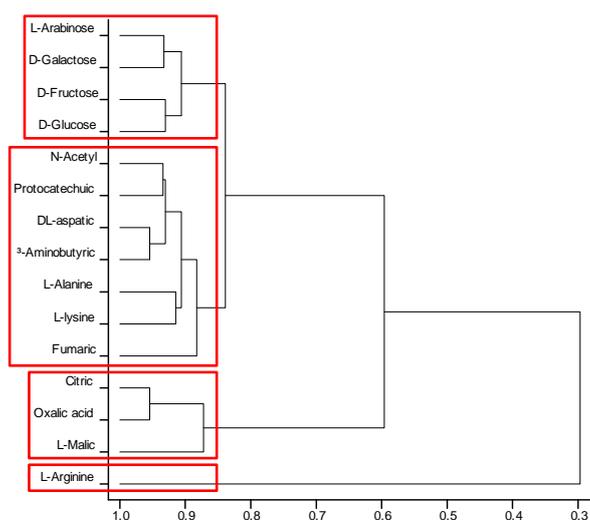


Fig. 15 Dendrogram of utilisation of carbon substrates in a field experiment comparing application of Basta® at 5 and 10 L ha⁻¹ with untreated soil over 60 days, where similarity between groups was determined at 0.85.

Analysis on the Average Well Colour Development (AWCD) from the MircoResp™ carbon utilization results found the mean AWCD for herbicide rate 5 L ha⁻¹ was significantly higher than rate 10 L ha⁻¹, but the samples with no herbicide were not significantly different to either rate 5 or 10 L ha⁻¹ (Table 4). The increase in AWCD at 5 L ha⁻¹ indicated that microbial activity could be stimulated with small amounts of herbicides added to the soil, which corresponded to findings in the laboratory incubation experiments. However, at higher rates, twice the recommended application rate, microbial activity and consequently biological functions could be impaired.

Table 4 MicroResp™ Average Well Colour Development of soil treated with two rates of Basta® relative to the untreated soil

	Rate of application of Basta® (200g L ⁻¹ glufosinate)		
	0 L ha ⁻¹	5 L ha ⁻¹	10 L ha ⁻¹
Average Well Colour Development	0.472 ab	0.480 b	0.468 a

Recommendations

The project aimed to determine whether herbicides impact soil microbial diversity and functioning and to consider the implications of such changes for the banana industry. The project has led to a number of recommendations which could improve the knowledge of the ultimate impacts of herbicides on soil biological functions.

- **Herbicide impacts on soil functions** - the MicroResp™ system of carbon substrate utilization provided a rapid test for determining herbicide impacts on soil biological activity. The utilisation of organic acids, malic, oxalic and citric acids by soil microbes was particularly sensitive to herbicide. Therefore, testing banana soils before and after herbicide application for utilization of organic acids provides a rapid and reliable indicator to changes in biological function.
- **Plant signaling** – herbicide application potentially make plants more susceptible to soil borne disease by impairing the organisms that respond to plant chemical signals. Organic acids such as, malic, oxalic and citric acids are common root exudates, exuded to attract beneficial soil organisms. This is an important function in disease suppression. Herbicides reduce the number organisms that can utilize organic acids and therefore and leaves the plants prone to attack from plant pathogens. This observation requires further investigation.
- **Problematic herbicides** – pendimethalin is used as a pre-plant herbicide in the banana industry and did not to degrade during the 60 days incubation. Further work on understanding the degradation and potential accumulation of pendimethalin in banana soil is warranted. If pendimethalin persists in the soil there is potential for movement into water ways and accumulation in the Great Barrier Reef lagoon. Therefore, greater knowledge on the environmental risk potential of pendimethalin is required and how it compares to diuron and atrazine, which are problematic herbicides used in the sugar cane industry.
- **Chemotactic organisms** – the application of chemotactic soil organism that are attracted to the active ingredients of herbicides can aid bioremediation of contaminated soils. The selection and development of chemotactic soil organisms can reduce the impacts that herbicides have on soil functions as well as reducing herbicide movement off-farm is required.
- **Commercial products versus active ingredients** –commercial herbicide products have addition chemicals in their formulation as well as the active ingredients. The additional products such as solvents and adjuvants may also impair soil biological functions. Further comparisons to test the active ingredients alongside the commercial products is required to clarify the difference between the two. It is also critical to determine which commercial herbicides are the least damaging on soil biological activity.
- **Field studies** – more samples are required from different environmental conditions and soil types to establish the impact of herbicides on soil biological functions in the banana industry. A proposed farm survey was problematic due to restrictions on soil movement within the banana industry and having access to farms at the time of herbicide application, but these could be overcome with more time
- **Alternative weed management options** – alternatives to the applications of herbicides need to be explored. Maintaining low growing vegetation around banana plants would reduce the need for herbicides and is being explored as a management option for soil borne diseases. Alternatives to herbicides used by banana growers such as citric acid, acetic acid, potassium permanganate and steam need to be evaluated as they potentially have greater impacts on soil biological functions than herbicides.

Scientific Refereed Publications

Engel, C.R., Pattison, A.B., Dennis, P.G. (In preparation). Chemotaxis of soil bacteria and archaea to diquat and paraquat with and without glucose as a competing attractant.

Dennis, P.G., Kukulies, T.L., Forstner, C., Lindahl, F., Plisson, F, Eaglesham, G., Pattison, A.B. (In preparation). Effects of one-off applications of nine active herbicide compounds on the microbiome of a tropical soil used for banana production

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Intellectual Property/Commercialisation

None to report

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Acknowledgements

Tony Pattison gratefully acknowledges the work conducted by Dr Paul Dennis and his team Christian Forstner, Fredrick Lindahl, Fabien Plisson, Joshua Shields and Chelsea Engel at the School of Agriculture and Food Science, University of Queensland, St Lucia for contributing their expertise and skills to this project. Geoff Eaglesham, Maria Jose Gomez Ramos and Jochen Mueller from the National Research Centre for Environmental Toxicology are also gratefully acknowledged for their help with the herbicide degradation analyses.

The work conducted by the team at the Department of Agriculture and Fisheries, South Johnstone by Tegan Kukulies, Anna McBeath and David East is also gratefully acknowledged.

Thanks also to the banana growers who contributed soil to the project, Matt Abbott, Craig Buchanan and Paul Inderbitzin.

This project has been funded by Horticulture Innovation Australia Limited using the research and development banana industry levy and funds from the Australian Government.

Appendix 1: Literature Review

Effect of herbicides on soil biology – Scoping the impact of herbicides on soil biological functions for the Australian banana industry

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Abstract

Herbicides used in agriculture have the potential to negatively affect soil biology and impede vital soil function. Australian banana growers have shown interest in determining the potential impact that herbicides have on soil biology and functional capacity. This review scrutinises the methodology used to assess soil biological communities. It also examines the approaches that have been employed or that show promise to determine the effects of herbicides on soil biology. Traditional methods of measuring soil biology such as microbial biomass and dilution plating are often not sensitive enough to detect changes in biological activity following the applications of herbicides. Soil enzymes, soil respiration and earthworm evaluations were techniques that emerged from the review as being sensitive to herbicide application and suitable for inclusion in future studies. From the literature an analysis of the soil microorganism's lipids and DNA appear to be more sensitive measures. However, these have not been widely used in herbicide studies. These methods may provide a better understanding of the changes in soil communities following the application of herbicides. To date, no studies have been conducted on the effects of herbicides on the biology of soils under banana cultivation. In this review the effects of seven active ingredients of currently registered herbicides on soil biology and functional capacity is examined. The effects of the seven registered herbicides on soil biology used in bananas were often inconclusive and contradictory. This was attributed to the type of trial, the biological indicators measured, the rate of herbicide application or soil type. While it is acknowledged that all herbicides registered for use in the Australian banana industry go through a rigorous registration process there are knowledge gaps with regard to the indirect effect that herbicides have on crop productivity, the impact herbicides have on beneficial organisms and on the suppression of soil borne diseases. The conclusions from the review recommend that laboratory and glasshouse assays be conducted on the effect of herbicides on soil biology and soil functions to gain a better understanding of the potential impacts on soil biological process and functions before developing more rigorous management options for the banana industry.

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1. Banana production in Australia

Bananas (*Musa* spp) are a high-value commodity in Australia worth \$467 million (gross value) in 2012–13 to the domestic economy (ABS, 2014a). Bananas are Australia's second largest horticulture industry being preceded only by citrus (ABS, 2014a). Production of bananas in Australia is estimated to be from 15 348 ha, with Queensland being the largest producer (13 886 ha), followed by northern New South Wales (1 252 ha), Northern Territory (121 ha), and north-west Western Australia (45 ha). In 2012–13 Queensland produced 93% of all bananas produced in Australia (ABS, 2014b). The majority of these were produced in the Wet Tropics region of north Queensland. The Wet Tropics is characterised by high rainfall, high humidity and warm temperatures for most of the year. It is located in close proximity to the World Heritage listed rainforests and is in the catchment areas of rivers that flow into the Great Barrier Reef lagoon. To maintain and preserve the integrity of these environmentally sensitive areas there is a need to manage soil quality, and the use of nutrients and pesticides both on-farm and off-farm. To reduce the potential impact that banana production may have on the surrounding environment best management practice guidelines for reducing inputs of pesticides and fertilisers for the banana industry have been developed (King, 2013).

A large range of pesticides, insecticides and herbicides are used in conventional banana production to manage pests and weeds in order to improve crop productivity and quality. Recently there has been conflicting research regarding the use of chemicals in agriculture, with some studies suggesting that with the correct applications, agricultural chemicals degrade and they are not a risk to human health or the environment (Ratcliff *et al.*, 2006; Cooper and Dobson, 2007). Conversely, other studies indicate that agricultural chemicals do pose a risk to human health and the environment (Relyea, 2005; Pingali *et al.*, 1994). To maintain high yields and banana quality, the Australian banana industry uses a range of fungicides, insecticides and herbicides to reduce competition from pests and diseases. Fungicides are the most commonly used agrochemicals for the control of yellow sigatoka (*Mycosphaerella musicola*). There have been limited studies on the potential negative effects of pesticides in banana plantations. Henriques *et al.*, (1997) reviewed the use of agrochemicals in banana plantations (including paraquat, 2 4-D and chlorothalonil) in Latin America and found that there were concerns about the risk to tropical terrestrial and aquatic environments.

2. Importance of soil health

In recent years banana growers have introduced best environmental management practices, with the aim of reducing chemical and nutrient inputs. This approach requires increased reliance on the biological functioning of soils for nutrient recycling and disease suppression. For example, an integrated nematode management plan resulted in a 50% reduction in chemically applied nematicides used within the banana industry, with suppression achieved through cultural practices and improved soil biology (Stirling and Pattison, 2008). Furthermore, a reduction in the quantity of nitrogen applied to bananas over the last 10 years means there is greater reliance biological nitrogen recycling processes. The increased reliance on soil biological functioning to suppress soil borne diseases and supply nutrients for banana production means that impairments to the biological process could have a large impact on banana productivity and the goal of reducing chemical inputs. The possible non-target effects of herbicides to soil biology and consequently the functioning of the soil are not well understood.

Over the last 30 years there has been an increase in the amount of herbicide used in the Wet Tropics; herbicides having been detected in the catchment waterways of some north Queensland rivers, in seagrass and water surrounding inshore coral reefs (Ham, 2007; Shaw and Müller, 2005; Haynes *et al.*, 2000). The herbicides registered for use on banana farms are not currently prioritised as being of concern to the quality of the Great Barrier (Reef Water Quality Protection Plan Secretariat,

2013). However, with the desire for the Australian banana industry to improve best practice environmental management and the potential for regulators to focus on off-farm impacts of other pesticides, including herbicides used in the banana industry, there is a need to gain a better understanding of the herbicides used and their impacts on soil biology.

3. Registration of chemicals for Australian agriculture: environmental requirements

Registration of chemicals for use in agriculture is governed by the Australian Pesticides and Veterinary Medicines Authority (APVMA), which includes the herbicides used in banana production. As well as the efficacy of the products, their impacts on the environment must also be determined. The Department of the Environment, Water, Heritage and the Arts (DEWHA) undertakes the environmental risk assessments of chemicals for the APVMA. DEWHA have strict environmental requirements that need to be met before a chemical can be registered for agricultural use and the environmental risk assessments are similar in structure to the Organisation for Economic Co-operation and Development (OECD) recommendations (Lee-Steere, 2009). Application volumes, expected exposure and behaviour of active ingredients, the location of where the chemical is to be applied, the potential harmful effects on birds, mammals, aquatic life (fish, invertebrates, algae and higher plants), terrestrial invertebrates (honeybees and other non-target arthropods, earthworms), soil microbial processes and non-target terrestrial plants are factors which are assessed prior to registration (Lee-Steere, 2009).

Focusing on the soil requirements to determine environmental impacts of potentially new agrochemicals, there are a number of tests which may be conducted as part of the environmental risk assessment. These include laboratory tests which determine the degradation of active ingredients in soil, including aerobic and anaerobic degradation and soil photolysis. Also, field testing of the products which includes; soil dissipation testing, soil residue testing and soil accumulation testing in representative soils. Testing the environmental effects on soil ecosystems will vary based on the nature of the chemical under scrutiny, but may include testing the acute toxicity to earthworms, the sub-lethal effects on earthworms and other soil macroorganisms (e.g. collembola), nitrogen transformation, carbon mineralisation and recovery rates following treatment. With respect to soil organisms, if there is less than a 25% deviation in respiration or nitrogen transformation due to the application of a chemical at the highest rate on the label, no further microbial analysis is required (Lee-Steere, 2009). The nature of requirement for some of these tests is established on expert judgment based on the persistence of the active ingredient, number of applications and risk to soil ecosystems.

The registration process offers consistent testing across all pesticides in Australia and allows the environmental risks to be identified and minimised. This may include changes to product use and environmental warnings to be included on labels. All the herbicide products included in this review have been approved by the APVMA. Therefore, the products have been deemed to have minimal impact on the environment and safe for use in agriculture following label recommendations. However, herbicides may have temporary effects on soil ecological parameters, which may impact the soils capacity to perform biological functions required to support environmental best practice banana production.

4. Soil biology

The soil ecosystem is notorious for its complexity (Whitman *et al.*, 1998). In order to understand how farm management inputs, such as herbicides, alter soil ecosystem interactions, an understanding of soil organisms and their role in soil functioning is imperative. Soil organisms can be divided into five groups based on their size. Protists, which include bacteria and fungi, are the smallest, followed by microfauna that are organisms typically less than 100 microns in length and includes tardigrades and rotifers. The next largest organisms are classified as mesofauna; these include nematodes, mites and the pauropods. Slugs, snails, millipedes and beetles are organisms that are grouped as macrofauna. The largest soil organisms such as earthworms fall into the megafauna category.

Each of these groups of soil organisms has been used to determine the biological condition of soil and ecosystem functioning. However, because they form the foundation of soil ecosystems, emphasis has been placed on the lower order organisms (protists). Several approaches are taken when it comes to defining and detecting changes to the soil biological community.

The following section of this review is divided into two main sections: community structure and diversity and the functioning of the soil ecosystem.

4.1 Determining soil biological community structure and diversity

4.1.1 Protists – biomass

Soil microbial biomass is a measure of the living component of soil organic matter and is one of the most widely used soil biological indicators. The living soil component, includes bacteria, fungi and protozoa (soil organisms smaller than $5 \times 10^3 \mu\text{m}^3$) and excludes larger soil organisms and plant roots (Jenkinson and Ladd, 1981). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) are measured using chloroform fumigation-incubation style methods (Jenkinson and Powlson, 1976; Voroney and Paul, 1984). Soil microbial biomass is a good indication of total activity; however, it does not detect the diversity of a soil community and therefore it should be used in conjunction with other methods which include diversity assessments.

4.1.2 Protists – culturable counts and observations

Soil bacterial and fungal communities can be evaluated using different culturing techniques on a variety of media that are targeted to recover select species. Traditional culturing methods previously used to assess the effects of herbicides on soil biology include dilution plating, most probable number assessments, and radial growth observations of specific species on herbicide amended agar (Westerhuis *et al.*, 2007; Ahmad and Malloch, 1995; Ahmad *et al.*, 1995; Wardle and Parkinson, 1990). In a study involving bananas a radial growth assay demonstrated that glyphosate was shown to reduce the growth of the fungal pathogen *Sclerotium rolsii*, which affected the emergence of young banana plants from corm pieces (Westerhuis *et al.*, 2007). These radial growth assays have the potential to provide a quick assessment of the impacts that herbicides have on culturable organisms considered to be beneficial in banana production.

The development of culture-independent methods of assessing soil microbial communities has estimated that less than 0.1% of soil organisms are able to be recovered using traditional culture based techniques (Torsvik *et al.*, 1996; Amann *et al.*, 1995; Torsvik *et al.*, 1990). Although culture dependant techniques have limitations due to the non-cultivable nature of soil biology, investigations that couple these traditional methods with culture independent approaches will allow a more complete understanding of the changes to soil biology that are due to perturbations such as herbicide application.

4.1.3 Soil respiration

Aerobic respiration of living organisms results in the production of CO₂. When this occurs in the soil it is termed "soil respiration" and the amount of CO₂ released provides an estimate of the microbial activity within the soil. CO₂ from the soil can be captured in airtight chambers and measured either by a gas chromatograph, an infrared gas analyser or colorimetrically— by a reaction with an alkali solution (Raich *et al.*, 1990; Heinemeyer *et al.*, 1989; Rowell, 1995). Soil respiration can be either measured in the field or on soil samples in controlled laboratory conditions.

4.1.4 Protists – community level physiological profiling

Community level characterisation and classification of protist microbial communities can be measured using commercially available BiOLOG EcoPlates™ (<http://www.biolog.com/products/?product=Microbial%20Community%20Analysis%20with%20EcoPlates%26%23x2122%3B>). These microplates rely on a tetrazolium dye reduction to indicate utilisation of the different carbon substrates (Garland and Mills, 1991). The EcoPlates™, which are manufactured for ecological applications, contain 31 environmentally specific carbon substrates (Campbell *et al.*, 1997). The carbon utilisation patterns of the substrates are determined and comparisons can be made by calculating the average well colour development (AWCD), richness and the Shannon-Weaver index. Further cluster and principal component analysis are popular methods of comparing the profiles as well as investigations of specific carbon sources (Hackett and Griffiths, 1997).

The use of EcoPlates™ to assess bacterial populations is often debated with the benefits and weaknesses of this method heavily scrutinised (Preston *et al.*, 2002). The main downfall of this method is the bias towards fast-growing bacteria in aqueous environments (Preston *et al.*, 2002). However, the EcoPlates™ method has proved to be sensitive enough to detect changes in soil communities either after organic matter amendment, (Gomez *et al.*, 2006), plant diversity scenarios (Stephan *et al.*, 2000), or tillage management systems (Govaerts *et al.*, 2007). It has also been employed to analyse the effects of herbicides on soil bacterial communities (El Fantroussi *et al.*, 1999).

Using a similar concept to that employed by BiOLOG EcoPlates™, a more recently available system commercially marketed as MicroResp™ measures CO₂ evolution from soil samples after the addition of different carbon substrates (Campbell *et al.*, 2003). The absorption of CO₂ is detected by a colour change in the microplate that contains a pH indicator dye gel. As different microbial species have varying abilities to utilise different carbon substrates, resulting in respiration changes, a unique community physiological profile can be determined for each soil. The MicroResp™ method has several advantages over the BiOLOG EcoPlates™ method, in that it does not favour readily extractable and fast-growing organisms in nutrient-rich aqueous conditions (Campbell *et al.*, 2003). It also facilitates the measurement of substrate induced respiration by monitoring CO₂ before and after the addition of a carbon source (Campbell *et al.*, 2003). The MicroResp™ method has been adapted to quantify the ecotoxicological impact of contaminants on soil (Wakelin *et al.*, 2013). The method has not been widely used to assess the specific impacts of herbicides. However, MicroResp™ was used by (Fernández-Gómez *et al.*, 2011) in a study to determine the changes in the respiration of soil microbes in different vermicomposts after the application of a pesticide. They found that respiration following pesticide application was reduced in a vermicompost with low biological activity, but was unaffected in vermicompost with a higher level of biological activity.

4.1.5 Soil enzymes

Soil enzymes acting within and outside living cells are the driving force behind all biochemical transformations taking place in the soil (Gianfreda *et al.*, 2012). They are recognised as useful

indicators of soil quality because of their relationship to soil biology, ease of measurement and their relatively rapid response to changes in soil management regimes (Dick, 1994; Dick *et al.*, 1996; Bandick and Dick, 1999). Soil enzymes are sensitive to changes in management regimes and environmental conditions, which suggests that they are useful in identifying changes to environmental quality. Since soil enzymes are interconnected to nutrient cycling and other essential biological transformations, they may be sensitive to changes in anthropogenic effects such as the addition of herbicides. The use of enzymes as indicators of pesticide contamination was shown to be more sensitive than BiOLOG EcoPlates™ (Floch *et al.*, 2011). Several investigations have been undertaken on the effects of pesticides on soil enzymes; however, no clear conclusions can be drawn (Sannino and Gianfreda, 2001; Gianfreda and Rao, 2011; Gianfreda *et al.*, 2012). The soil enzymes that are most frequently measured include; fluorescein diacetate, dehydrogenase, β -glucosidase, urease, and phosphatase.

Fluorescein diacetate activity (FDA) and dehydrogenase activity are two enzyme assays that are routinely used to assess disturbances to soil biology. Fluorescein diacetate is a colourless compound that can be hydrolysed by esterases, proteases and lipases. It produces a yellow end-product that can be measured by spectrophotometry (Schnürer and Rosswall, 1982). With more than 90% of the energy in the soil being transformed by microbial decomposers FDA provides a good estimate of the total microbial activity within a soil.

The activity of dehydrogenase in soils is a measure of microbial oxidative activity (Skujinš, 1973; Le Casida *et al.*, 1964) that has been shown to closely correlate with other soil microbial measurements, especially carbon dioxide evolution (Von Mersi and Schinner, 1991; Garcia *et al.*, 1997; Frankenberger and Dick, 1983). Due to their relative ease of measurement FDA and dehydrogenase are the two enzyme activity assays that are mostly used to give an indication of soil microbial activity. Even though FDA is sensitive to both free and membrane-bound enzymes and that the dehydrogenase enzyme is intracellular a study comparing the two methods indicated that both correlate closely with adenosine triphosphate content of cells and cell density (Stubberfield and Shaw, 1990). However, FDA was shown to give better estimates of decomposer activity and respiration rate (Stubberfield and Shaw, 1990). Both of these enzymes have been used to determine the impact of pesticides on soil biology and are discussed further in this review.

The reaction mediated by β -glucosidase is the rate limiting step in the degradation of cellulose—a major component of soil organic matter and plant residues (Stott *et al.*, 2010). β -glucosidase activity in the soil as an indicator of soil health was first used in 1973 (Hayano, 1973), but was more recently evaluated for soil health assessments by developing a scoring equation from published data sets representing different soils and management regimes, (Stott *et al.*, 2010). β -glucosidase activity has not widely been used in herbicide assessments; however, it has been used to assess the effects of fungicides on soil biology. Ridomil Gold Plus® and methyl bromide have all been shown to significantly ($P \leq 0.05$) reduce β -glucosidase activity (Demanou *et al.*, 2004; Klose *et al.*, 2006).

Other soil enzymes involved in nutrient cycling which are of particular interest to determine the effects of herbicides on soil functioning include urease and phosphatase that are involved in the soil nitrogen and phosphorus cycles respectively. Urease is the enzyme that catalyses the hydrolysis of urea to CO₂ and NH₃ (Tabatabai and Bremner, 1972) and phosphatase enzymes is the general term used to characterise a broad group of enzymes that catalyse the hydrolysis of esters and anhydrides of phosphoric acid (Schmidt and Laskowski, 1961). Acid and alkaline phosphatase activities are measured in soils; however, acid phosphatase activity is more frequently measured (Eivazi and Tabatabai, 1977). Both urease and acid phosphatase have proven to be sensitive indicators in herbicide studies (Sannino and Gianfreda, 2001).

4.1.6 Lipid based community analysis

Soil bacterial communities can be characterised by extracting and analysing their lipids directly from soil samples. Bacterial lipids are energy storage and cell membrane compounds that include free fatty acids and membrane bound fatty acids such as phospholipids (Kennedy, 1994). Different bacteria taxa possess different fatty acid profiles that can be detected by high-resolution fused-silica capillary gas chromatography, allowing changes in the soil bacterial community to be monitored.

Fatty acid methyl ester (FAME) analysis and phospholipid fatty acid (PLFA) analysis, which measure all fatty acids and specifically phospholipid fatty acids respectively, are two protocols that are commonly used to measure lipids in soil. Studies employing PLFA to evaluate the effects of herbicides on soil biology have been limited to herbicides including 2,4-D and imazethapyr (Zhang *et al.*, 2010a; Zhang *et al.*, 2010b). Of the herbicides used in bananas, PLFA analysis has been employed to determine the effects on soil biology after glyphosate and glufosinate application (Ratcliff *et al.*, 2006; Ernst *et al.*, 2008).

4.1.7 Nucleic acid-based analysis

All organisms have a unique genome comprised of nucleic acids. There are three types of nucleic acids that are important in soil microbial ecology: DNA, ribosomal RNA (rRNA) and messenger RNA (mRNA) (Topp and Y, 2008). Analysing genomes by sequencing different nucleic acids provides a pathway to identify and determine the abundance of organisms in the soil community. Specific target organisms or groups of organisms of interest can also be evaluated by sequencing known regions of nucleic acids. Microorganisms living in the soil in particular bacteria can be identified by sequencing a small subunit of rRNA known as 16S rRNA (Hugenholtz *et al.*, 1998). Since bacteria constitute the largest group of organisms in soil 16S rRNA gene characterisation of bacterial communities is the most widely used technique for soil analysis (Hugenholtz *et al.*, 1998).

Investigations identifying changes in microbial communities using 16S rRNA gene sequencing are limited. For example, a study that evaluated the long-term effects of atrazine and metachlor under maize monoculture, 16S rRNA was used to characterise the entire bacterial group as well as group specific primers to evaluate the actinomycetes (bacteria which form branching filaments) the ammonium oxidisers and the methanotrophic bacteria (Seghers *et al.*, 2003). This study found that the long-term use of these herbicides altered the soil community structure especially the methanotrophic bacteria (Seghers *et al.*, 2003). Of the herbicides used in banana production, soil microbial communities have only been briefly evaluated using 16S rRNA sequencing after the application glyphosate or glufosinate (Barriuso *et al.*, 2010; Gyamfi *et al.*, 2002).

Other groups of organisms in the soil can also be characterised using the same principles of amplifying specific sections of DNA that 'barcode' different kingdoms of taxa. Similar to the 16S rRNA molecule used to distinguish bacteria, the internal transcribed spacer (ITS) region of the ribosomal cistron is considered the barcode region for identifying taxa of fungi (Schoch *et al.*, 2012) and the ribosomal gene 18S rDNA is used to evaluate soil microfauna, mesofauna, macrofauna and megafauna (Zrzavý *et al.*, 1998). Studies that use ITS and 18S rDNA to evaluate changes in fungal communities and disturbances to larger soil organisms following herbicide application are even more scarce than those that evaluate bacterial communities using 16S rRNA. A literature search revealed that changes in soil fungal communities have not been evaluated using the ITS barcode. The community changes evaluating 18S rDNA following herbicide application is limited to just one study on glyphosate (Pesce *et al.*, 2009).

Metagenomics is another nucleic acid-based approach to analysing soil biological communities. The term "metagenomics" was first used by Handelsman *et al.*, (1998) and is defined as the *genomic analysis of the collective microbial assemblage found in an environmental sample*. A metagenome

represents the DNA of all the indigenous soil microorganisms isolated from a soil (Handelsman *et al.*, 1998). Unlike the nucleic acid based approaches previously discussed—which use 'barcodes' to evaluate bacterial, fungal and larger soil organisms—metagenomes are extracted directly from soil samples by direct lysis and separation of cells without the need for primers to amplify genes of interest. The metagenome is then sequenced and referenced against a library of soil DNA. Evaluation of the metagenome facilitates measurement of the genetic and functional diversity in a soil sample. Other similar emerging technological tools for evaluating microorganisms at the DNA level include metatranscriptomics and metaproteomics that evaluate the collective RNA and proteins respectively from environmental samples (Maron *et al.*, 2007). These approaches provide information about the functionality of microorganisms. As these whole-soil sample genome approaches are all relatively new to the field of soil ecology they have not been employed in any studies evaluating the possible non-target influence of herbicides on soil biology (Johnsen *et al.*, 2001).

Generally, the use of molecular based technology to assess soil biological changes due to herbicide addition has been underutilised. The genetic methods have been successfully used to identify shifts in communities under different farm management systems, such as tillage, organic matter management and fertiliser regimes (Jangid *et al.*, 2008; Ceja-Navarro *et al.*, 2010). In banana farm management systems these molecular methods have potential for detecting changes in soil biological communities, particularly following chemical inputs.

4.1.8 Mesofauna – soil nematodes

Within the soil food–web there is a large intermediate domain between protists and macrofauna. Soil nematodes are small (0.3–5 mm) worm-like animals that inhabit all soil types (Yeates, 1979). There are 20 000 known species of nematodes and an estimated 100 000 more unidentified species, found in virtually all soil types (Gobat *et al.*, 2004). Soil nematodes occupy different niches within the soil food–web as primary consumers, plant-parasitic nematodes, secondary consumers of fungi and bacteria (fungivores and bacterivores) and predators (Yeates *et al.*, 1993). Soil nematodes are also highly abundant, have a high biodiversity and play an important role in the soil food–web, particularly in the recycling of nutrients, by feeding on bacteria, fungi and other microfauna (Overstreet *et al.*, 2010). All of these characteristics along with their relative ease of extraction and identification make nematodes ideal indicators of soil quality (Neher, 2001; Schloter *et al.*, 2003). Indices of nematode community structure have been developed that integrate nematode feeding group classification and life strategy concepts. These are based on colonisation and persistence (c-p scale) to develop the concept of nematode 'guilds' (Bongers & Bongers, 1998, Yeates & Bongers, 1999, Ferris *et al.*, 2001). Several indices are derived through the weighting applied to these guilds that function as bioindicators for disturbance to the soil environment and condition of the soil food web. The structure index represents the number of trophic layers in the soil food–web and the potential regulation by predators (Ferris *et al.*, 2001). The enrichment index indicates the food–web response to available resources and the response of primary decomposers to the resources (Ferris *et al.*, 2001). The channel index provides an index of the nature of decomposition channels through the soil food–web (Ferris *et al.*, 2001).

Substrate, texture, climate, biogeography, organic inputs as well as both natural and anthropogenic disturbances are all factors that influence the nematode species composition (Neher, 2001; Ferris *et al.*, 2001; Yeates, 2003). Plant-parasitic nematodes are notorious for their destructive capabilities, which has meant most work on nematodes focused on their control rather than using them as indicator species. However, the effect of herbicides on the overall soil nematode community was reviewed, which found that herbicides do alter nematode community composition (Zhao *et al.*, 2013). More specifically, Zhao *et al.*, (2013) found that bacterivores were more tolerant while predators and fungivores were more sensitive to herbicide presence.

4.1.9 Megafauna – earthworm responses

Earthworms are important components of the soil ecosystem with regard to soil formation, structure and fertility. Earthworms are thought to move between two and 250 tonnes of soil per hectare per annum, equivalent to bringing a layer 1–5 mm thick to the soil surface per hectare per annum (Edwards, 2004). Because earthworms ingest such large quantities of soil and their skin is permeable being a route for contamination uptake, they are ideal candidates for use as bioindicators of toxicity (Reinecke *et al.*, 1997).

Earthworm mortality is used to evaluate the chemical toxicity; however, from an ecotoxicological point of view the progressive measurements of an earthworm's life (i.e. growth and reproduction) provides more information about the sub-lethal effects of chemicals (Van Gestel *et al.*, 1992; Springett and Gray, 1992). The OECD (OECD 2014) has prepared a draft revised test guidelines for the testing of chemicals which includes an assay that evaluated the impact on the reproduction earthworms. The majority of earthworm studies are carried out on the most common oligotroph species *Eisenia fetida* and *Eisenia andrei* (Lumbricidae). Due to their profound movement in the soil environment these studies are usually done in a laboratory. One study investigated the effects of nine chemicals on the earthworm *E. andrei*, finding that different reproductive and growth parameters had different sensitivities depending on the chemical under scrutiny (Van Gestel *et al.*, 1992). Earthworm reproduction has been evaluated at a lower level with sperm counts of *Lumbricus terrestris* proving to be a sensitive indicator (Cikutovic *et al.*, 1993). Similarly, earthworm avoidance assays have proved to be a sensitive indicator of the presence of herbicide. Verrell and Van Buskirk (2004) found that *E. fetida* preferred to move into soil that was free of a commercial glyphosate based herbicide. A review of the effects of pesticides on the growth and reproduction of earthworms concluded that high concentrations of a chemical pollutant can be quickly and easily determined by acute earthworm toxicity, contaminated soils with sub-lethal concentrations of pollutants require more sensitive assays, such as measurement of earthworm reproduction (Yasmin and D'Souza, 2010).

4.2 Soil ecosystem functions

Soil organisms are important components of the soil system, performing specific functions, which sustain the soil environment and support plant growth. The ability for organisms to perform their functions is dependent on their environment, ecosystem interactions and is a consequence of the physical and chemical impacts as well interactions with other organisms. To sustain crop production the soil must act as reservoir for water and nutrients and as a buffer to change, to support active and diverse soil communities. In return for the stable environment, the soil organisms degrade organic matter into basic components, prevent accumulation of toxins, suppress pests and pathogens, prevent dominance by a single organism or group of organisms and contribute to the physical stability of the soil structure. The degradation of organic matter by soil organism is their most basic ecological function, from which cascades a number of other soil functions such as the recycling of nutrients immobilised in the organic material. The decomposition of complex organic material means there is a diversity of organisms in the soil environment, which can help to maintain equilibrium in soil organisms to prevent the domination of plant pathogens. It is possible to apply the previously discussed methods to measure soil biology, to determine how herbicides may be impacting soil functions

4.2.1 Decomposition of organic matter

The decomposition of organic matter is the process by which plant residues are returned to the soil. This biological process is an important part of the carbon cycle that involves the physical breakdown and biochemical transformation of complex molecules into simpler organic and inorganic molecules (Juma, 1999). Organisms from bacteria to earthworms are either directly or indirectly involved at different stages of decomposition. The addition of organic matter stimulates soil biological activity as

it serves as a food source for soil microorganisms.

The decomposition of organic matter is often measured using the litter-bag method. This involves placing known weights of organic matter in a perforated bag and following the decomposition of the organic matter by mass (Falconer *et al.*, 1933). This method offers an economical option for field studies over long periods of time, but it doesn't provide sensitivity for laboratory assays or give any early indication of change. Of the previously described methods of determining biological activity, specific enzymes provide an indication of soil organisms ability to decompose organic matter, as enzymes are secreted by soil microorganisms, assist the chemical breakdown of organic material. In particular the measurement of β -glucosidase activity, which is involved in the degradation of cellulose, is a good indicator of organic matter decomposition (Stott *et al.*, 2010).

Since organic matter decomposition is an integrated process that involves a large number of soil dwelling organisms across all size groups, any change in the populations or diversity may alter a soil's capacity to decompose organic matter and make nutrients available to plants. There are many different methods that link soil biological measurements to organic matter decomposition. However, their sensitivity to measure the impacts of herbicides on organic matter decomposition requires further clarification. Monitoring changes in β -glucosidase activity along with other biological activity measurements following herbicide application is an important method of assessing the soil's ability to decompose organic matter as a precursor to nutrient recycling.

4.2.2 Nutrient cycling

Nitrogen cycle

The soil–nitrogen cycle consists of vital processes in which soil organisms transform mineral nitrogen compounds into organic forms and vice versa. These processes include nitrogen fixation ammonification, nitrification and denitrification. Nitrogen fixation occurs when nitrogen gas is converted to ammonium by bacteria often associated with the roots of leguminous plants. Ammonification is the process in which the organic forms of nitrogen tied up in the proteins of dead plants and soil animals are transformed to ammonium. Nitrification, which is the conversion of ammonium to nitrate is the next step. Denitrification is the process where nitrogen in the nitrites and nitrates are then lost from the soil system as gaseous forms, of nitrous oxide and nitrogen gas. The cycle is continued with the fixation of atmospheric nitrogen. Farm management disturbances such as herbicide application have the potential to affect nitrogen cycling. Therefore a close examination of one or multiple processes in the nitrogen cycle can indicate impacts on the capacity of the soil's function to supply nitrogen to bananas. The measurement of nitrification, denitrification and nitrogen fixation has been used in various pesticide related studies (Carlisle and Trevors, 1986); (Yeomans and Bremner, 1985; Pell *et al.*, 1998). Of the seven herbicide active ingredients used in the banana industry only the effects of glyphosate and glufosinate on the nitrogen cycling processes have been studied. These two studies reported that glyphosate and glufosinate did not inhibit or stimulate nitrification (Malkomes, 1988; Pell *et al.*, 1998). However, another study in a conifer forest observed a stimulation of ammonification activity after glyphosate application (Stratton and Stewart, 1991). Monitoring urease enzyme activity also provides an indication of a soils capacity to supply nitrogen, as it indicates the soils capacity to hydrolyse urea (Tabatabai and Bremner, 1972). Many soil organisms, particularly protists are involved in nitrogen cycling and therefore changes in their activity or diversity may impair a soils ability to make nitrogen available to banana plants.

Phosphorous cycle

Phosphorus enters the soil ecosystem through rock weathering, erosion, plant and animal residues, and inorganic and organic fertiliser inputs. Phosphorus is an important element for plant growth and

soil biological activity and is a fundamental element of DNA, RNA and adenosine triphosphate molecules in living organisms (Frossard *et al.*, 2012). When phosphorus enters the soil it is mineralised into forms available for uptake either directly by plants or soil fauna. Soil microorganisms have developed strategies to release phosphate from otherwise inaccessible forms of phosphorus. Some bacterial and fungal species secrete extracellular enzymes that hydrolyse esters of phosphate to elemental phosphate. When these soil microbes die the phosphorus is returned to the soil for plant uptake. The measuring of acid and alkaline phosphatase enzyme activity gives an indication of the soils capacity to breakdown complex phosphorus elements and making phosphorus available to banana plants. Earthworms also play a role in phosphorus cycling through the decomposition of organic residues. Their casts and burrow walls contain a high concentrations of plant-available phosphate (Chapuis-Lardy *et al.*, 2009; Tiunov *et al.*, 2001). Therefore, any possible non-target effects of herbicides on earthworms may also affect phosphorus availability.

Other nutrient cycles

Cycling and the availability of potassium, sulphur and other trace elements are also important elements required for banana growth plants and production. Different levels of available nutrients or specific enzymes for different cycles (e.g. sulphur – arylsulphatase) can be measured to determine changes in the cycling of these elements. Similar to nitrogen, phosphorus and carbon cycling, soil microorganisms play important roles in making these elements available for plant uptake. Since soil organisms are either directly or indirectly involved in maximising the availability of nutrients to plants, alterations of soil microbial communities may impact on the nutrient cycling and the availability of nutrients to plants. Additionally changes in any of the previously described soil biological indicators following farm management operations, such as herbicide application could indicate that cycling of these elements could be compromised.

4.2.3 Disease suppression

Soil borne diseases are a major limitation to agricultural production, particularly in horticultural industries such as bananas. Soil borne diseases that pose the greatest risk to the Australian banana industry include: nematodes, bacterial corm rot, bacterial heart rot and Panama disease. The management of Panama disease is the biggest challenge to Australia banana production, due to its widespread devastation of bananas around the world. It is caused by the fungus *Fusarium oxysporum* f.sp *cubense*. Panama disease of bananas in Australia is a significant production constraint for some varieties; Race 1 and Subtropical Race 4 strains of the disease are the main constraints to production in the subtropical regions of eastern Australia, while Race 1 limits the production of susceptible non-Cavendish varieties in the Wet Tropics. The highly virulent *Panama disease* strain Tropical Race 4 (TR4) is currently only present in the Northern Territory where it has reduced banana production from 261 ha in 1998–1999 (ABS 2000) to 121 ha in 2012–2013 (ABS 2014b).

The main focus for controlling Panama disease has relied on quarantine (restricting the entry of TR4) and selection of resistant varieties. However, the use of integrated management strategies that increase soil biological activity and suppression of the pathogen is developing as a viable option for growth of susceptible banana cultivars. This relies on either enhancement of indigenous antagonistic soil organisms to provide competition or suppression with the pathogen or inundation with specific known antagonistic organisms. *Trichoderma spp.* are considered to be beneficial, opportunistic, antagonistic species that are important in agricultural systems (Harman *et al.*, 2004). *Trichoderma spp.* may play a role in the suppression of Panama disease, since *Trichoderma harzianum* under in-vitro conditions has been shown to inhibit mycellial growth of *Fusarium oxysporum* (Thangavelu *et al.*, 2004).

From a herbicide perspective a review on the effects of glyphosate on plant diseases in agricultural

crops found that an increase in disease severity was associated with the application of glyphosate (Johal and Huber, 2009). This resulted from either the direct effects of the weakening of plant defences, an increase in the pathogen population or indirectly by predisposing plants to diseases by altering soil microbial communities. The application of glyphosate has been shown to result in an increase in diseases caused by *Fusarium* spp in other crops including: *Fusarium* crown and root rot of tomatoes (Brammall and Higgins, 1988), *Fusarium* root rot in sugar beet (Larson *et al.*, 2006) and *Fusarium* head blight of wheat (Fernandez *et al.*, 2005). In relation to the Australian banana industry there is currently no knowledge about the effects that herbicides have on the interaction with soil organisms, specific soil organisms and soil borne disease suppression.

5. Herbicides used in Australian banana production

In the Australian banana industry, herbicides are used to reduce weed competition, for farm staff comfort and safety and to maintain an aesthetic farm condition. However, over spray may reach the soil surface or herbicides may be tied up in the tissues of dying plants being released into the soil when the plant is degraded, which may impact on soil organisms. A review of the pesticides currently used in the banana industry found that there were seven active ingredients of herbicides registered and commonly used in the banana industry as pre-emergent (before planting), broad-spectrum post-emergent knock down herbicides or selective post-emergent herbicides (Table 1) (Davis, 2014). The different banana production regions in Australia, because of their differing climatic conditions, have differing requirements for herbicides (Table 2). The largest banana production area is in the Wet Tropics of North Queensland, which has the highest requirement of herbicides. This is due to the warm temperatures, high rainfall and high humidity. These environmental conditions not only favour banana production, but also are conducive to prolific weed growth. Banana production on the Tablelands in North Queensland requires fewer herbicide applications than the Wet Tropics area. In the smaller production areas (New South Wales, Western Australia and the Northern Territory) herbicides use is infrequent due to drier conditions and that result in less weed growth. In relation to the Australian banana industry no research has been conducted on the impacts that the use of registered herbicides have on soil ecology, soil functions or productivity. Therefore, this review focuses on the effects of the herbicide active ingredients, registered for use in Australian bananas, from other crops and scrutinises the methods used to assess their impacts on soil functions.

Table 1. Registered herbicides used in the Australian banana industry, and their corresponding active ingredients, rates and frequency of application (Davis, 2014)

Active ingredient	Nature of herbicide	Commercial product	Concentration of active ingredient in product (g/L)	Recommended Rate of product (L/ha)	Spot spray recommended rate (mL/100L)	Frequency of application
Pendimethalin	Pre-emergent (broadleaf and grass)	Stomp®	330	9–12	na	Used at establishment only
Fluazifop.p.butyl	Selective (grass)	Fusilade®	128	1.65	80–160	0–1 times per year
Haloxyfop-methyl	Selective (grass)	Verdict™	520	0.4–0.8	25–50	0–1 times per year
Glyphosate	Post-emergent (Broadleaf and Grass)	Roundup®	360	3–9	500–700	Selectively used in crop
Glufosinate-ammonium	Post-emergent (broadleaf and grass)	Basta®	200	1–5	500	4–6 times per year
Paraquat dichloride hydrate Diquat dibromide monohydrate	Post-emergent (broadleaf and grass)	Sprayseed®	135 115	2.4–3.2	240–320	Eight times per year
Paraquat dichloride hydrate	Post-emergent (broadleaf and grass)	Gramoxone®	250	1.6–3.2	160–320	Up to eight times per year

na=not applicable

Table 2. Estimates of potential of each active ingredient registered for banana use over a ten year period for each of the production regions in Australia

Banana growing region	Proportion of total production area (%)	Typical life of plantation (years)	10-year potential application of active ingredient use (kg/ha)						
			Pendimethalin (Stomp®)	Fluazifop (Fusilade®)	Haloxyfop (Verdict™)	Glyphosate (Roundup®)	Glufosinate (Basta®)	Paraquat/Diquat (Sprayseed®)	Paraquat (Gramoxone®)
NQ – Wet Tropics	87	7	4.7	0.3	0.6	4.2	60	34.6 / 29.4	64
NQ – Tablelands		10	3.6	0.2	0.4	3.2	40	25.9 / 22.1	48
NSW	10	15	2.4	0.1	0.3	2.2	40	17.3 / 14.7	32
WA	2	7	4.7	0.3	0.6	4.2	40	17.3 / 14.7	32
NT	1	7	4.7	0.3	0.6	4.2	40	17.3 / 14.7	32

5.1 Pre-emergent herbicides

5.1.1 Pendimethalin

Pendimethalin [N-(10ethypropyl)-2,6-dinitro-3,4-xylidine] is a dintroaniline herbicide commonly marketed as Stomp®. It functions as a pre-emergent herbicide because it inhibits the steps in plant cell division responsible for chromosome separation and cell wall formation (Strandberg and Scott-Fordsmand, 2004). In banana production pendimethalin is only used prior to planting, during the establishment of banana paddocks. Banana paddocks in the different growing regions in Australia have different life spans; therefore, growing regions with shorter life spans would apply pendimethalin more frequently (Table 2).

As most of the research on this chemical has been associated with its persistence and degradation in soils, research conducted on its effects on soil biology has received little attention (Zimdahl *et al.*, 1984; García-Valcárcel and Tadeo, 2003). Shetty and Magu (1998) conducted a laboratory experiment that assessed the evolution of carbon dioxide and dehydrogenase enzyme activity of a sandy loam soil after the application of pendimethalin. These parameters were assessed after four, eight, and 12 weeks incubation. Carbon dioxide evolution was reduced by 15 to 75% over the duration of the incubation, indicating that pendimethalin had greatly reduced the microbial population. Pendimethalin also affected dehydrogenase activity with reductions in the level of activity observed at all but the lowest concentration four weeks after application (Shetty and Magu, 1998). In a similar laboratory experiment dehydrogenase, phosphatase, total microbial biomass and carbon substrate utilisation (BiOLOG) were measured periodically for 97 days (Kumar Singh *et al.*, 2002). This study found no differences in any of the biological parameters measured and they concluded that pendimethalin had no impact on phosphatase, microbial biomass and BiOLOG assessments. There was also little difference in the dehydrogenase activity with the exception of the 90 day sampling, where there was a significant reduction. These two contrasting studies formed opposing conclusions about the effects of pendimethalin on soil biology (Kumar Singh *et al.*, 2002; Shetty and Magu, 1998).

A review of the impacts of pendimethalin on soil microorganisms revealed that there was a lack of investigations published that dealt with the effects on soil invertebrates (Strandberg and Scott-Fordsmand, 2004). Standberg and Schott-Fordsmand (2004) recommended that research should be focused on separating direct and indirect effects of pendimethalin, defining whether the observed effects are caused directly by pendimethalin or indirectly by the effects on the food chains. Since this active ingredient has received little attention and is extensively used for pre-emergent weed control in the preparation stage of banana plantations, knowledge about the possible negative effects on soil biology is necessary to determine if alternative weed management strategies at plant establishment should be evaluated.

5.2 Post Emergent – broad spectrum herbicides

5.2.1 Glyphosate

Glyphosate [N-(phosphonomethyl)-glycine] which is a non-selective systemic herbicide is the most widely used herbicide worldwide (Baylis, 2000).

As bananas are susceptible to glyphosate (e.g. Roundup®) it is used selectively for general weed control; predominantly on inter rows and headlands. Glyphosate can also be injected into the pseudostem to eradicate banana plants in old fields prior to the fallow period and replanting. Glyphosate

controls weeds by inhibiting the synthesis of aromatic amino acids necessary for protein formation. More specifically it inhibits 5-enolpyruvylshikimate 3-phosphate synthase, which is an intermediate enzyme in the synthesis of aromatic amino acids via the shikimic acid pathway (Tu *et al.*, 2001; Duke *et al.*, 2003). Higher order organisms tend to lack the ability to synthesise amino acids and are therefore, unaffected by glyphosate (Bentley, 1990). However, microorganisms require the shikimic acid pathway for aromatic amino acid synthesis, making them to be susceptible to glyphosate (Bentley, 1990).

Due to its versatility, effectiveness and the development of glyphosate tolerant crops, investigations on glyphosate are more extensive than the other active ingredients. Recently, there have been numerous studies conducted that investigated the possible non-target effects of glyphosate on soil microorganisms. There is conflicting research on the impact of glyphosate on soil organisms, with some studies demonstrating that at recommended field rates there were no negative impacts to soil biological activity (Busse *et al.*, 2001; Haney *et al.*, 2002; Ratcliff *et al.*, 2006), while other investigations produced results which indicated otherwise (Zabaloy *et al.*, 2008a; Sebiomo *et al.*, 2011).

The effect that glyphosate has on soil microbial biomass differs based on method of biomass determination, application rate and soil type. An incubation study on the impacts of glyphosate assessed four concentrations (0.48, 0.96, 1.92 and 3.84 L a.i./ha) four and 45 days after application (Gomez *et al.*, 2009). After four days the highest two concentrations significantly reduced the MBC, while after 45 days only the highest concentration suppressed MBC (Gomez *et al.*, 2009). Another study, which assessed the effect of glyphosate under different tillage systems, revealed that glyphosate stimulated MBC with the highest values determined 18–37 days after application (Panettieri *et al.*, 2013). Most investigations concluded that at recommended field rates glyphosate does not reduce soil microbial biomass; but acts as a carbon source and stimulates microbial biomass (Wardle and Parkinson, 1990; Haney *et al.*, 2002).

Soil respiration rates were not increased following glyphosate application in investigations by Busse *et al.* (2001) and Gomez *et al.*, (2009), but CO₂ evolution increased by 10–15% after glyphosate was applied at 2.16 mg a.i./kg of soil (Araújo *et al.*, 2003). Furthermore, at much higher concentrations (5000 mg a.i./kg) soil respiration was stimulated (Busse *et al.*, 2001).

Cultivable bacteria and fungi have also been used to evaluate the possible implications of glyphosate application to soil biology. Investigations using these traditional methods revealed no change to bacteria, fungi and actinomycete communities at recommended field rates (Roslycky, 1982; Ratcliff *et al.*, 2006). However, examinations at higher concentrations of glyphosate caused changes in the community composition (Ratcliff *et al.*, 2006; Zabaloy *et al.*, 2008b). Ratcliff *et al.* (2006) showed that at a high concentration (5000 mg a.i./kg) there was a shift in community composition from a fungal dominated community toward an equal ratio of bacteria to fungi.

Community level physiological profiles using BiOLOG systems have been used to assess soil microbial populations after glyphosate application. Two studies of forest soils using two variations on sample preparation produced contrasting results. When recommended field rates of the commercial formulation were added to the soil no differences were detected (Ratcliff *et al.*, 2006); however, when 25 mM and 50 mM of glyphosate was added to soil dilutions before inoculating the BiOLOG plates growth rates of bacterial populations were significantly ($P \leq 0.05$) reduced (Busse *et al.*, 2001). Both of these studies concluded that the effects of glyphosate applied at recommended rates in the field were not detrimental to soil health, but reported changes in the community profile when assessed under laboratory conditions.

Soil enzymes are popular indicators in measuring the effect of glyphosate on soil biology. In a study soil

where two different soil types, with and without history of glyphosate application were collected the FDA increased greatly in all four soils, 32 days after the application of 2.16 mg kg⁻¹ of glyphosate (Araújo *et al.*, 2003). The soils that had a long-term history of glyphosate application had higher FDA compared to soils that had no history of application. This suggested that soil microbes inhabiting soils that are routinely applied with glyphosate are more likely to use glyphosate as an available food source than soils that have not been exposed to glyphosate (Araújo *et al.*, 2003). In another study using glyphosate at 10 times the recommended field rate there was a significant (14%) reduction in FDA activity in the soil after 7 days, which continued for two weeks, although was no longer significant (Zabaloy *et al.*, 2008a). FDA in the soil in another laboratory assay again revealed a small but significant ($P \leq 0.05$) reduction three and fourteen days after application of glyphosate at three times the recommended rate (Weaver *et al.*, 2007). These three studies, while all similar in nature reveal contrasting results, possibly due to the variation in different soil types and application rates.

Dehydrogenase enzyme activity follows the same inconclusive trend as FDA. The study previously mentioned conducted by Zabaloy *et al.*, (2008a), which measured FDA, also measured dehydrogenase activity and found no consistent changes following glyphosate application. Contradictory research by Gomez *et al.* (2009) found that dehydrogenase activity was significantly ($P \leq 0.05$) greater in glyphosate treatments compared to the control in incubation experiments. Further conflicting research from cassava farms in Nigeria treated with atrazine, primeextra, paraquat and glyphosate found a significant decline in dehydrogenase activity following glyphosate application (Sebiomo *et al.*, 2011). Once again, different studies with variable control parameters produced very different outcomes when measuring the impacts of herbicides on dehydrogenase activity. Urease and phosphatase enzyme activities were assessed in 22 different soils following glyphosate application. The application of glyphosate increased urease activity, but markedly decreased phosphatase activity (Sannino and Gianfreda, 2001). However, another investigation on the effects of glyphosate found that the herbicide showed a trend of decreasing urease activity and suppression of phosphatase activity (Tejada, 2009). The activity of β -Glucosidase activity was also measured in this study, but the application of glyphosate did not alter the activity (Tejada, 2009). All of these studies show that soil enzymes are sensitive to applications of glyphosate, indicating changes in soil biological communities; however, results appear to be variable depending on soil type and cropping situation.

Determination of PLFAs and FAMES are among the tools employed in more recent evaluations of potential soil biological changes after glyphosate application. Once again some studies employing these methods have failed to detect any changes (Ratcliff *et al.*, 2006; Lane *et al.*, 2012; Zabaloy *et al.*, 2012). Using PLFA analysis Rosenbaum *et al.*, (2014) found no differences in microbial properties, including total PLFA, bacteria, protozoa, fungi or bacteria: fungi ration from glyphosate resistant and glyphosate susceptible common water hemp, treated with glyphosate grown in soil sourced from soybean fields, which had been sterilised or untreated . However, the analysis of *Fusarium sp.* colonisation of the roots of common water hemp found that sterile soil treated with glyphosate had a significantly ($P \leq 0.05$) more root infection than the non-sterile soil that did not receive an application of glyphosate, which suggested that glyphosate may predispose plants to soil-borne pathogens (Rosenbaum *et al.*, 2014). Banana production in Australia is faced with the challenges of soil borne diseases including *Fusarium oxysporum* f. sp *cubense*. Understanding if and how glyphosate and other herbicides influence banana soils capability to suppress has not been explored.

The use of nucleic acids to detect changes in biology after glyphosate application in particular the use of 16S rRNA to characterise bacterial communities is beginning to allow a more in depth understanding of changes. The application of gene sequencing to understand the effects of glyphosate is limited with only

disjointed results. Sequencing of full length 16S rRNA of soils under glyphosate-tolerant maize revealed that glyphosate significantly reduced the percentage of actinobacteria populations from 21% of the population to only 4% (Barriuso *et al.*, 2010). Another study found that the abundance of *Burkholderia* spp increased following the application of glyphosate (Lancaster *et al.*, 2010).

Analysis of earthworm growth and reproduction characteristics under laboratory conditions has proven to be a sensitive indicator of non-target glyphosate application. Glyphosate (1, 10, 100, 500, 1 000 mg/kg) has been shown to significantly reduce the weight of *Eisenia fetida* by 50% after 56 days incubation and prevented the development of cocoons or juveniles (Correia and Moreira, 2010). Similar laboratory studies also observed a significant ($P \leq 0.05$) reduction in growth and reproduction of earthworms following glyphosate application (Yasmin and D'Souza, 2007; Springett and Gray, 1992; Casabé *et al.*, 2007). In one study the effect of two different commercial formulations of glyphosate on earthworm (*Eisenia andrei*) were compared. The results found that one formulation was four and a half times more toxic (% weight change) than the other (Piola *et al.*, 2013). *E. fetida* has also shown avoidance behavior from soils applied with glyphosate (Casabé *et al.*, 2007; Verrell and Van Buskirk, 2004).

Following evaluation of the existing published results no clear conclusions could be drawn on how the application of glyphosate changes soil biology. Many of the studies suggested that after a single application at the recommended field rate for the cropping scenario, there were no adverse impacts to the soil biology. However, there are just as many studies that have detected changes in the composition and activity of soil biology. Even though this chemical appears to have less impact than other herbicide active ingredients discussed later in this review there was no current information available in a banana specific context.

5.2.2 Glufosinate

Glufosinate-ammonium (2-amino-4-[(hydroxymethyl) phosphinyl]-butanoic acid) is the active ingredient found in commercial chemicals such as Basta[®], a non-selective post emergent herbicide that is commonly used in banana plantations in Australia. The chemical is a microbial toxin, first isolated from *Streptomyces viridochromogenes* and *Streptomyces hygroscopicus* (Bayer *et al.*, 1972). Also known as phosphinothricin the chemical inhibits glutamine synthetase activity and as a result impedes the incorporation of ammonium into organic compounds. These reactions affect the organism's nitrogen metabolism. There has been an emphasis on this product due to the release of genetically modified crops that contain the *pat* gene from *Streptomyces* and making them glufosinate tolerant. The literature associated with this topic is mostly associated with genetically modified glufosinate tolerate canola, maize and wheat.

Using traditional culturing techniques soil fungal and bacterial populations were significantly reduced 30 and 40 days respectively after glufosinate application, whereas actinomycetes were significantly reduced after only six days from application (Pampulha *et al.*, 2007). This study also showed that glufosinate had a inhibitory effect on dehydrogenase activity (Pampulha *et al.*, 2007). A similar study that used traditional culturing methods to monitor changes in community composition also found that glufosinate reduced the number of fungi and bacterial by about 20% and 40% respectively (Ahmad and Malloch, 1995). An interesting outcome of this study was that mycoparasitic species *Trichoderma harzianum* was found to be among the most sensitive organism to glufosinate (Ahmad and Malloch, 1995). From a banana growing perspective this is interesting since *Trichoderma harzianum* under *in vitro* conditions has been shown to be effective at inhibiting mycelial growth of *Fusarium oxysporum*, the fungal plant pathogen that is responsible for Panama disease (Thangavelu *et al.*, 2004).

Griffiths *et al.*, 2008 compared the processes and organisms of genetically modified maize and Basta® (glufosinate) in a glasshouse pot trial. To determine the possible impacts of genetically modified maize and herbicide application a number of biological parameters were assessed including; respiration, nematode community, protozoa, ester linked fatty acids and community level physiological profile (BiOLOG plates). They found that glufosinate reduced respiration, altered the community level physiological profile of the microbial community and reduced the abundance of protozoa (Griffiths *et al.*, 2008). Even though this study detected negative impacts of glufosinate to soil microorganisms, the impacts varied between soil type and plant variety, and overall the study concluded that glufosinate had little effect on soil biology compared to other standard agricultural practices.

The effects of glufosinate on transgenic glufosinate-tolerant oilseed rape was compared to a wild type, with and without herbicide application in another glasshouse trial (Sessitsch *et al.*, 2005). In this trial 16S rRNA community fingerprints of active bacteria, microbial biomass and a series of enzyme assays including invertase, alkaline phosphatase, urease and arylsulfase were measured at early and late flowering and senescence (Sessitsch *et al.*, 2005). At senescence the application of glufosinate significantly reduced invertase, urease and phosphatase activity. However, analysis of the soil bacterial community by sequencing 16S rRNA revealed that the effect of glufosinate was insignificant compared to the plant development stage (Sessitsch *et al.*, 2005). A similar study of 16S rRNA amplified from DNA using eubacterial and pseudomonas specific primers, revealed only slightly altered microbial communities in the rhizosphere of transgenic oilseed rape after glufosinate application (Gyamfi *et al.*, 2002). Again, these changes were minimal compared to those detected during the plants developmental stages (Gyamfi *et al.*, 2002).

Like glyphosate, the studies which scrutinise glufosinate suggest that the chemical does not significantly impact soil biology. However, there are noted shifts in soil community compositions which raise the concern that soil functions like organic matter breakdown and nutrient cycling, may be compromised with repeated or over application.

5.2.3 Paraquat/Diquat

The herbicidal potential of paraquat (1,1'-dimethyl-4,4'-dipyridinium salt) and diquat (6,7-dihydrodipyridido (1,2-a:2',1-c) pyrazidinium salt) were first discovered in the mid-1950s (Akhavain and Linscott, 1968). These two active ingredients are commonly used in combination in commercial products (e.g. Sprayseed®) but paraquat is also used on its own as an active ingredient (e.g. Gramoxone®). Paraquat is the only one discussed in this review. There is one published article that reports on a study that was conducted in bananas. Although the authors did not assess changes in soil microbial indices, they did find that after 21 days indigenous soil microbes had mineralised 60% of the herbicide in comparison to only 10.3% when applied to sterile soil (Murray *et al.*, 1997). This study reiterates the importance of the involvement of soil microorganisms in the degradation of herbicides. Other than this study there are very few studies identifying the effects of paraquat and diquat on soil microorganisms.

Microbial enumeration of culturable bacteria, fungi and actinomycetes along with dehydrogenase activity was assessed over a six-week period after paraquat was applied to soil sourced from cassava farms with no history of pesticide application (Sebiomo *et al.*, 2011). The authors found that paraquat significantly reduced the bacterial, fungal and actinomycete populations and dehydrogenase activity, indicating that paraquat negatively affects soil microbiology (Sebiomo *et al.*, 2011). Other studies also report a reduction in dehydrogenase activity after of paraquat application (Smith and Pugh, 1979), but an increase in fungal populations and urease activity (Sannino and Gianfreda, 2001; Mekwatanakarn and Sivasithamparam, 1987). Different weed control treatments were assessed in another study comparing

the use of a paraquat/diquat product and different cultivation techniques, to mowing of weeds and found a reduction in respiration and dehydrogenase activity (Yeates *et al.*, 1976). Weed control with paraquat/diquat also significantly ($P \leq 0.05$) reduced total nematodes, number of nematode species, reduced aerobic bacteria and actinomycete populations (Yeates *et al.*, 1976). However, it should be considered that these differences were observed in comparison to a control that had a maintained weed population. The weeds in this control would have provided an environment that would have support a more biologically diverse soil ecosystem; therefore it is not a direct comparison of the impacts of the herbicides on soil microbial activity. This reiterates the importance of deciding the types of controls which should be used for comparison in these types of trials.

Earthworm characteristics have also been evaluated in soil where paraquat had been applied. Paraquat did not affect the growth of *E. Andrei*, but significantly ($P \leq 0.05$) reduced cocoon and juvenile production at 1000 mg a.i./kg (Van Gestel *et al.*, 1992). Even though the available studies involving paraquat and diquat are very limited (especially for the paraquat/diquat combination) they imply that both active ingredients cause changes in the microbial community, which could be detrimental to the biological function of soil.

5.3 Post Emergent – selective herbicides

5.3.1 Fluazifop

Fluazifop, [butyl 2-(4-(6-trifluoromethyl-2-pyridyloxy) phenoxy) propionate] is a post-emergent herbicide used for the selective control of annual and perennial grass weeds (Plowman *et al.*, 1981). Fluazifop (present as fluazifop.p.butyl, e.g. Fusilade®) is typically applied to control weeds when bananas are being established. Research on this herbicide's potential impact to soil microorganisms is limited with only two published studies that are both limited in their approaches and techniques.

The non-target effects of fluazifop on soil fungal populations were investigated over a range of fluazifop-butyl concentrations (0, 0.6, 3 and 6 $\mu\text{g/g}$) for eight weeks (Abdel-Mallek *et al.*, 1996). Using plating techniques, the study showed that there was a temporary reduction in the fungal populations observed at the highest two concentrations after one and two weeks of incubation; however, no effects were recorded at the lowest concentration (Abdel-Mallek *et al.*, 1996). This suggested that at lower concentrations fluazifop does not impact on soil fungal populations; however, at higher concentrations it would have a negative effect.

The effects of fluazifop on microbial activity of soils under no-till and conventional systems found that after the application of fluazifop, MBC was significantly ($P \leq 0.05$) lower irrespective of the tillage system (Santos *et al.*, 2006). Overall the study concluded that the negative effects of fluazifop could be reduced by the adoption of no-till system. These two studies although limited in their complexity of measurement of soil microbial communities indicates that fluazifop has little impact on soil biology.

5.3.2 Haloxifop

Haloxifop (present as haloxifop-methyl, e.g. Verdict™) typically applied annually to specifically control grasses predominantly when banana plants are being established.

Haloxifop is the least studied herbicide active ingredient registered for use in bananas with very few

published results available. In a sandy soil haloxyfop reduced fungal activity after a one-week incubation period; however, fungal activity returned to normal levels after three weeks (Tu, 1992). Haloxyfop inhibited nitrification and sulphur oxidation, three weeks after application (Tu, 1992). Dehydrogenase activity was stimulated; however, phosphatase and urease remained unaffected in these short term measurements (Tu, 1992). The other study identifying the effects of haloxyfop on soil biology observed the radial growth of fungal species in the presence of haloxyfop (100, 500 and 1000 mg/g) (Abdel-Mallek *et al.*, 1994). Haloxyfop significantly ($P \leq 0.05$) reduced the radial growth rate of *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium funiculosum*, *Rhizopus stolonifer* and *Trichoderma harzianum* (Abdel-Mallek *et al.*, 1994). As previously reported, glufosinate also reduced the radial growth of *Trichoderma harzianum*, a fungal species which is identified as being beneficial in banana production and a potential antagonist to *Fusarium oxysporum* (Thangavelu *et al.*, 2004).

The available studies on this active ingredient suggest that haloxyfop impacts on soil microbiological community, but the extent to which it interferes with the soils capacity to maintain soil biological functions is unknown.

6. Strategies to reduce the impact of herbicides in bananas

6.1 Chemical reduction strategies

Australian banana growers are actively introducing best management environmental practices to reduce chemical and nutrient inputs, while maintaining banana production levels. Current management options and recommendations to reduce herbicide use in banana plantations are promoted in the banana industry's best management guidelines, which are based on maintaining vegetated groundcover and crop residue management (King, 2013).

Most of the Australian banana industry maintains vegetated headlands and inter-rows, which is managed through regular mowing. This not only eliminates the requirement for herbicides but also stabilises the soil, improves soil structure, reduces water erosion and promotes soil biological activity and diversity. Another practice that is beginning to be employed is the use of "side-throw slashers" that transfer material from the inter-row into the banana row also smothering weeds. The concept of inter-row vegetation can be extended closer to the plant as another weed management option, promoting companion planting groundcover species amongst the banana row (Johns, 1994). However, the suitability of different species in different growing regions and the logistics of maintaining them have not been evaluated. Furthermore, banana growers also place residual banana plant material over the row area between the plants to assist with weed suppression.

The establishment phase of banana plantations is the time when banana fields require the most weed control due to the absence of a canopy and to reduce competition as banana plants become established. The planting of fast growing grass species, such as Japanese millet (*Echinochloa esculenta*), which is sprayed with a single application of a knock-down herbicide (e.g. fluazifop) to create a thick surface mulch has been suggested as one method to protect the soil, reduce the total amount of herbicide applied and suppress weeds. The effectiveness of this proposed management showed promise and did not reduce plant productivity, but has not been comprehensively investigated and requires further development (Kukulies, 2010). All of these weed management options reduce the application frequency of herbicides in bananas.

6.2 Chemical degradation strategies

Chemotaxis, the directed movement of an organism away or towards a chemical, is an option for identifying the organisms that degrade herbicides. This could potentially be another management strategy to overcome the adverse impacts of herbicides on soil organisms. Soil microorganisms are not only responsible for the decomposition of organic matter to make nutrients available to plants, they are responsible for the breakdown of complex molecules and pesticides; a process termed *biodegradation*. Soil microorganisms gradually degrade the complex molecules of herbicide active ingredients into simpler molecules. The rate at which different pesticides biodegrade varies depending on their chemical composition, environmental factors and soil chemical, physical and biological attributes (Arias-Estévez *et al.*, 2008). If herbicides are not degraded in banana soils in north Queensland before they enter soil ground water or surface run-off they can pose a threat to waterways and the Great Barrier Reef. The identification of specific microorganisms involved in the degradation of herbicides and their application to soil could be potentially enhance the biodegradation of the herbicides, reduce the amount of time the herbicides are present in the soil and reduce the risk of movement into sensitive environmental areas. For this management option to be explored the organisms responsible for the degradation of the herbicides used in Australian banana plantations need to be identified. The concept of bacterial chemotaxis provides an approach to identifying bacterial species, which are likely to be involved in herbicide degradation.

The chemotactic behaviour of bacteria toward oxygen, minerals and organic matter has been observed since the 19th century. *Escherichia coli* was utilised to confirm that chemotaxis allows bacteria to locate the environment that has the most energy-rich resource (Adler, 1966). This ability to source optimum surroundings rich in the chemical resource they require, also means they can avoid unfavourable conditions or chemicals (Adler, 1966). Positive chemotaxis describes the movement of bacteria toward a chemical (chemoattractant), which acts as a carbon or energy source, whereas negative chemotaxis is the movement away as the chemical (chemorepellent) is toxic for bacteria. The chemotactic movement of bacteria is facilitated by the high speed rotation of the flagella (18 000 rpm), which is powered by the proton motive force (DeRosier, 1998). Their movement whether it is toward a light, oxygen or chemical source is detected by receptors, which activate signal protein interactions and direct the movement of the flagella. The protein-protein interactions and movement systems have been well studied in bacteria such as *Escherichia coli* and *Salmonella enterica* (Bren and Eisenbach, 2000).

The chemotaxis of bacteria is typically measured with variations of capillary style assays (Adler, 1973; Segel *et al.*, 1977; Adler and Dahl, 1967; Adler, 1966). Microcapillary tubes that contain the solution of chemoattractant are placed in a sample of motile bacteria. As the chemical diffuses from the tip of the capillary tube, bacteria, which exhibit chemotaxis, move toward the chemical gradient and into the capillary tube. The number of cells in the capillary tube determines the strength of the chemoattractant (Grimm and Harwood, 1997). Modified from these capillary style assays is a syringe-based assay, which has been used to characterise the chemotactic diverse bacterial populations in lake water toward nitrate, ammonium and phosphate (Dennis *et al.*, 2013). This method combines the use of flow cytometry and 16S rRNA to produce a direct culture-independent characterisation of organisms from environmental samples, which exhibit chemotactic behaviour (Dennis *et al.*, 2013). This method shows promise from an environmental pollution perspective with the ability to infer which bacterial families are involved in the degradation of individual chemical pollutants.

Bacterial chemotactic behaviour has been used to evaluate the degradation of 2,4-dichlorophenoxyacetate (2,4-D) and atrazine. Chemotaxis assays revealed that *Ralstonia eutropha*

JMP123, which contains genes responsible for 2, 4-D degradation (*tfd*), is attracted to 2, 4-D (Hawkins and Harwood, 2002). This *tfd* cluster of genes also encodes a nonessential permease *tfdK* for growth on 2,4-D and the *tfdK* mutant was found to be non-chemotactic. This defined the role of *tfdK* as a receptor in the chemotactic response to 2,4-D. (Hawkins and Harwood, 2002). The most studied atrazine degrading bacteria *Pseudomonas sp.* strain ADP (atrazine degrading pseudomonad) also showed positive chemotactic response to atrazine and the atrazine degradation intermediates N-isopropylammelide and cyanuric acid.

The concept of chemotaxis potentially provides a safe, efficient and cost effective mechanism for minimising the impacts of herbicides to soil biology, waterways and sensitive environmental areas.

7. Discussion

Australian banana growers are being encouraged to work toward more sustainable farming systems and reduce on-farm and off-farm environmental impacts. As growers maximise the use of their soil resources there is concern that herbicides reduce the ability for soil microbial communities to perform critical ecosystem services required for growth, productivity and disease suppression of their banana plants. This review found that, while there was rigorous process to determine environmental impacts of herbicides and other agri-chemicals on the environment through the registration process, there was a lack of specific information on how the herbicides used in the banana industry impact on soil biological functions. Furthermore, the majority of the Australian banana industry is located in tropics, where there is very limited information on the effects of herbicides on tropical crops and soils. Since there has been little research conducted on this topic, specifically in a banana farming context, information on the impacts of herbicides to soil biology was drawn from studies involving the seven active ingredients (pendimethalin, glyphosate, glufosinate, haloxyfop, fluazifop, diquat and paraquat) currently used in the Australian banana industry from other crops and laboratory assays.

Glyphosate followed by glufosinate were the two most studied active ingredients, due to their global application and use in genetically modified crops. These two active ingredients are considered to be less toxic than the other herbicides and some studies suggested that they had no significant effect on soil microbiology (Ratcliff *et al.*, 2006; Haney *et al.*, 2002; Busse *et al.*, 2001). However, the literature review found investigations which had observed changes in biological indicators (Sebiomo *et al.*, 2011; Zabaloy *et al.*, 2008a; Pampulha *et al.*, 2007). There were fewer investigations into the other five active ingredients used in bananas on soil biological functions they followed the same trend of contradictory research results. These contrasting results reiterate the need and opportunity to increase the knowledge of the possible impacts herbicides may have on soil biology and functioning under banana production systems.

The studies reviewed on herbicide active ingredient impacts on soil biology produced a range of outcomes based on a number of variables. Firstly, the herbicide in question: different herbicides have different modes of action, which effect soil biological communities differently. Second, the assay procedure used to determine the effects of herbicides to soil biology varied between studies, but the majority of studies were conducted in controlled laboratory conditions. Fewer experiments were conducted in glasshouse conditions, with interaction with the plant rhizosphere and even fewer field evaluations. Thirdly, the methods used to evaluate soil biology in these assays also differed between investigations. This attributed to the different conclusions about herbicide impacts on soil biology. More traditional methods such as microbial biomass and dilution plating were often not sensitive enough to

detect changes in soil biological activity following herbicide applications at field rates. Soil enzymes, respiration and earthworm evaluations were techniques that emerged as being sensitive and suitable to investigate herbicide impacts. Analysis, soil microorganism's lipids and DNA appear to be more sensitive measures, but had not been, widely used in herbicide studies in an agricultural context. The genetic methods have the potential to provide a better understanding of the shifts in soil communities following herbicide applications to banana soils and how these shifts may implicate the soils ability to function and sustain banana production.

Some investigations evaluated the pure active ingredient of herbicides and some assessed the commercial formulations. The chemical composition of the commercial formulations of herbicides was unknown, apart from the concentration of the active ingredient. The commercial formulations from one company or batch may also produce different effects to another as well as the active ingredient alone. This variable has received little attention, although two glyphosate-based formulations produced very different effects on earthworms (*Eisenia andrei*) (Piola *et al.*, 2013). Furthermore, the rate at which the chemical is applied has an impact on the results. Many of the studies reviewed found that at the recommended rate of field application there was no significant impact on the soil biological community, but at higher concentrations the effects were more pronounced. Most of the studies in the reviewed focused on detecting the impacts on soil biology of a given herbicide after a single application. In banana paddocks there are repeated applications of a herbicide or a series of herbicides may be applied. Unfortunately, there are limited studies on the use of herbicides in this manner. Even though it is important to observe the impacts to soil biology following a single application, repeated applications, or applications in sequence could be even more detrimental to the soil ecosystem and functional capacity and warrants further investigation and modelling.

A limiting factor of this review is that none of the studies scrutinised were conducted on banana soils. Different plant species support different biological communities in the rhizosphere (Grayston *et al.*, 1998); therefore, soil microbiology under different banana plant regimes may react differently to those in different cropping systems. Furthermore, there is a need to investigate the impact of herbicides not only on soil biology, but also on the possible flow-on effects of the soils capacity to carry out organic matter decomposition, nutrient cycling and disease suppression.

The application of herbicide may have indirect effects on biological soil functions, such as the potential to suppress soil borne diseases. Like many banana growing countries, Australia faces the challenges of Panama disease, the soil borne disease caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) that results in widespread devastation of bananas. Creating soils that suppress Panama disease by increasing biological diversity and activity was one suggested management approach (Pattison *et al.*, 2014). How herbicides interfere with the suppressive capacity of banana soils warrants further investigation. More specifically, several soil organisms have been identified as possible antagonists of *Foc* including *Trichoderma harzianum*, *T. veride* and *Pseudomonas fluorescens* (Thangavelu *et al.*, 2004; Saravanan *et al.*, 2003). Glufosinate and haloxyfop were found to reduce radial growth of *T. harzianum* in culture (Abdel-Mallek *et al.*, 1994; Ahmad and Malloch, 1995) and of the 18 fungal species isolated from soil by Ahmad and Malloch (1995), *T. harzianum* was found to be one of the most sensitive species in the presence of glufosinate. The DNA approach to measuring soil microbial communities may clarify the impacts of herbicides on specific beneficial organisms, such as *Trichoderma* spp. Changes in the DNA profiles of soil microbial communities in the soil may be one way of quantifying the effects of herbicides on soil quality. Glufosinate products are routinely applied in bananas, particularly in north Queensland. This may have implications for the management of Panama disease in susceptible varieties, as suppression in the antagonistic organism, such as *Trichoderma* spp. or *Pseudomonas* spp., may allow a

window of opportunity for the pathogen to colonise the roots. Further research on this topic may result in herbicide management being integrated into the management of *Foc*.

Most studies evaluated in this review concluded that herbicides do not negatively impact on soil biology applied at recommended rates. However, there were some studies that question these findings, detecting changes in biological activity, which may result in indirect effects on biological soil functions. Although there are many contradictory results further evidence is required to determine herbicide impacts on the soil ecosystem in a banana specific context. The determination of these non-target effects and extent of impacts will assist in the development of alternative weed management strategies, which reduce herbicide applications and chemical inputs. By developing an understanding of how herbicides impact on soil biological soil functions there is an opportunity to refine crop management practices to improve banana productivity without impacting on the environment.

8. Recommendations

From the literature review of herbicide impact on soil biology there are a number of recommendations that should be addressed:

- There is a lack of information on how herbicides impact on soil biology and functioning particularly in tropical conditions and specifically in bananas.
- Laboratory and glasshouse assays should be conducted to determine possible effects. If effects are found to be detrimental to soil functioning then alternative management options should be assessed in the field.
- The methods likely to yield the most information on the possible effects of herbicides on soil biology are culture independent protocols including: MicroResp™, soil respiration, soil enzymes, soil nematodes (glasshouse and field trials), pyrosequencing DNA and whole-soil genomic analysis.
- Herbicide active ingredients, rather than commercial products should be used in laboratory and glasshouse trials where possible to ensure results are comparable and applicable in the future.
- Soil functions most at risk are decomposition of organic matter, nutrient cycling, particularly of nitrogen and phosphorus and disease suppression.
- Management options to address the use of herbicides should be investigated when more information is known about how the herbicides impact on soil biological functions.
- Further exploration of the indirect involvement that herbicides and pesticides in general play in reducing soils capacity to suppress soil borne diseases such as Panama disease warrants future investigation.

9. Conclusion

Herbicides application is a routine management practice used in the banana industry in Australia. There are seven different active ingredients that are registered for application to banana soils. These can be classified as pre-emergent, selective post-emergent and broad-spectrum post-emergent herbicides. Herbicides belonging to the broad-spectrum post-emergent group, particularly glyphosate and glufosinate are the compounds that are most widely investigated. However, the impact of the herbicides on soil biology, particularly in a tropical banana context remains unknown.

Many different techniques of measuring soil biological changes have been used to clarify the impacts of herbicides on soil biology and soil functions, often with conflicting results. Culture independent techniques appear to offer the greatest potential for developing a greater understanding of how the herbicides impact on soil biology and functioning. Culture independent techniques can be employed at different levels firstly to determine impacts on overall soil biology by measuring soil respiration, soil enzyme activity, MicroResp™ profiles and soil nematode community analysis. However, genetic culture independent techniques have the potential to develop a greater understanding of how the herbicides impact on specific soil organisms and soil functions.

The soil functions most at risk from herbicide applications are organic matter decomposition, nutrient recycling and disease suppression. Within the banana context there appears to be potential for herbicides to reduce disease antagonistic organism such as *Trichoderma* sp., which have the potential to suppress soil borne diseases such as *Fusarium oxysporum* f. sp. *cubense*. This review has demonstrated the need for increased knowledge on the impacts that herbicides have in soils, techniques for measuring these impacts and soil functions that are most at risk.

Further work is required to develop farm management techniques that overcome the risks ensuring continued soil functions. However, in the process of developing a greater understanding of herbicides on soil biology it is also possible to develop a greater understanding of the organisms that degrade the chemicals, not only providing more information about the impacts that herbicide have on soil biology, but also providing a potential solution for reducing the risk of herbicides in the soil environment.

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Appendix 2: Honors Thesis – Joshua Shields
Isolation and characterization of herbicide-degrading soil bacteria

SAFS UNIVERSITY OF QUEENSLAND

Isolation and characterisation of
herbicide-degrading soil bacteria

ENVM4322

42613754

Joshua Shields

18/05/2016

Word Count (excluding reference list, figures, tables and appendices): 7296