Applied Use of Functional Diversity to Assess Soil Microbial Communities in Agricultural Landscapes

A thesis submitted in total fulfilment of the requirements for the degree of Master of Science

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STATEMENT OF AUTHORSHIP

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Statement of impact of the COVID-19 pandemic

This statement details the specific circumstances and impact on this thesis for this masters project due to the restrictions experienced during the 2020-2021 COVID-19 pandemic.

Examiners/assessors of this thesis are requested to take the following circumstances into consideration when assessing the quality of this thesis.

Access to the laboratory and/or field work was limited to 0 weeks in 2020.

The introduction/background section of this thesis constitutes the majority of this thesis (due to reduced capacity to undertake research) and should be marked accordingly ☐

The materials and methods section includes outlines of experiments that were planned but not able to be executed. ☐

Provenance of the results analysed:

All data analysed for this masters project was generated by the student in 2021 ☑

Data analysed for this masters project were generated partially by the student and additional data were provided to the student ☐

The percentage of student-generated data = 100%

If these data were not the student's own data, acknowledge the source/s of these data:

All data analyses were conducted by the student ☑

The discussion includes/consists of likely outcomes of the planned experiments, as well as future experiments that could be done and implications of the work that was planned.☐

I certify that this is a true and accurate record of the impact of COVID-19 on the masters project

Signed by student *Signed by supervisor*

Date: 15/12/2021 *Date: 15/12/2021*

Synopsis of thesis

The original sampling proposal was extensively designed to answer how tilling and burning affected soil microbial communities. COVID19 significantly impacted the original sampling protocol that was to be undertaken in this thesis. In particular, a two-year sampling design had been planned which would have involved sampling before and after tilling to see direct effects of tilling on a cropping paddock, instead of using pasture paddocks as un-tilled controls), and before and after burning to see direct effects of burning, over a two-year period. Additionally, a more rigorous sampling procedure would have been implemented, as per the BASE guidelines [1], including more paddocks and an extra location (farm willing to participate if COVID19 did not impact the sampling to be done by the student). Sampling within fields and between fields for each treatment would have been conducted, and this would have accounted for site variability and regional diversity. If this two-year experiment could have proceeded, it would have explicitly determined the effect of the two agricultural management practices (tilling and burning) on soil health and the microbial communities. Furthermore, the pandemic caused delays in primers resulting in some lab experiments unable to be completed, and the constant, mandatory isolations resulted in a loss of lab time. Bioinformatic analysis of next generation sequencing data (16S and ITS meta-barcoding survey) was done solely in 2021. This thesis also incorporated a meta-analysis of the wider literature on the use of bioindicators to identify soil health.

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LIST OF ABBREVIATIONS

°C Degrees Celsius

ASV Amplicon Sequence Variant

bp Base pairs

cm Centimeter

g Gram(s)

Ha Hectares

Hrs Hour(s)

L Litre(s)

M Molar

m Meter(s)

min Minute(s)

mL Millilitre(s)

mm Millimeter(s)

mM Micromolar

nm Nanometer(s)

nM Nanomolar

PERMANOVA Permutational multivariate analysis of variance

PCR Polymerase chain reaction

PICRUSt Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

pM Picomolar

µM Micromolar

µL Microliter(s)

µg Microgram(s)

rRNA Ribosomal ribonucleic acid

sec Second(s)

yr Years

SUMMARY

Agricultural management practices impact the biodiversity and functionality of soil microbial communities [2]. Microbes in soil are imperative for soil health. Therefore, it is essential to evaluate the impact of agricultural management practices on the soil microbial biodiversity and functionality. Soil health is hard to define and measure, especially in agricultural landscapes where the land is being constantly manipulated by the landholders. Aspects of the chemical or physical properties of the soil are common indicators of soil health; however, the use of microbes to assess soil health has become prevalent in the last decade [3-5]. Microbial communities living within soils make up the soil microbiota. This living component of the soil contributes significantly to nutrient cycling and other processes that can have both negative and positive effects on the soil health [6]. A meta-analysis of the ability of four existing soil microbial community parameters (Microbial Biomass Carbon (MBC), microbial activity, taxonomic diversity and functional diversity) to behave as bioindicators were assessed for their ability to respond repeatably to agricultural management practices. Studies conducted prior to 2010 have suggested that using MBC was an appropriate bioindicator of soil health however, newer methods that use microbial DNA to identify the microbial communities and their functions have arisen [7-10]. The findings of the present meta-analysis partially support the hypothesis that the four microbial community parameters could be used as bioindicators, as they appear to respond repeatedly to the different management practices.

This study also investigated the effect that tilling the soil and burning of crop residues have on the soil microbiota. Soil cores were collected from tilled and untilled pasture paddocks, and burned and unburnt cropping paddocks in Victoria, Australia. The analysis of bacterial 16S rRNA and fungal ITS regions produced taxonomic diversity data, and the PICRUSt program produced predicted bacterial functional pathways. It was hypothesised that the two management practices would both have effects on the soil microbial communities. This was hypothesised because both tilling and burning of crop residues are shown to have negative effects on the soil microbiota [11-15]. The results revealed that the microbial communities between the tilling treatments were taxonomically and functionally changing. The burning treatments caused taxonomic changes, but no functional changes. The data obtained from this research will provide a greater understanding of the bacterial and fungal communities within an agricultural landscape. This research will assist the Australian agricultural sector in identifying microbial bioindicators associated with soil health.

1.0 INTRODUCTION

1.1 Microbial communities play an important role in soil health

Land used for agricultural purposes exists as a unique environment due to it being primarily maintained and manipulated by the landholders, often referred to as an 'agroecosystem' [16]. Agroecosystems account for 50 percent of Australian land use as Australia is a large producer and exporter of wheat, barley, oats and canola [17]. The Australian agroecological weather consists of heavy seasonal rainfall and extended periods of drought, contributing to the soils' unique environment [18]. Banu, Singh and Copeland [19], have described Australian soils to be highly weathered, with a low pH, low organic carbon (C) content, and a high content of iron oxides. In addition to its chemical components, the soil consists of a living component which includes an abundance of microorganisms such as bacteria and fungi as well as archaea and small invertebrates. The definition of soil health varies however, it can be considered in this thesis as having the capacity to provide or a fitness for purpose [20]. Microorganisms contribute to a healthy soil, and in agroecosystems, a healthy soil is one that produces high yielding crops [20].

Microbial communities (also referred to as the microbiota) and their related activities assist soil health through a variety of mechanisms, such as: improved soil structure, carbon sequestration and nutrient cycling. These are important processes which are essential for agricultural production [19, 21]. In addition, microorganisms that live in the plant rhizosphere, defined as the soil directly influenced by root secretions, include many plant growth-promoting rhizobacteria (PGPR) which can stimulate plant growth [22]. In particular, PGPR assist in the mobilisation of phosphorus (P) as well as other nutrients such as nitrogen (N), among other processes highlighted in Table 1.1 [23].

Table 1.1. Examples of molecular mechanisms of bacterial and fungal species that assist with soil health and plant growth.

Process	Molecular mechanism	Example of microbial species involved	Reference
Improved soil structure and water infiltration/retention by aggregate formation	Production of mycorrhizal hyphae, mucilages, extracellular compounds, polysaccharides and soil proteins (hydrophobins and glomalin)	Rhizophagus irregularis Paraglomus occultum	[24, 25]
		Actinomycetes (class)	[26]
	Production of hyphae and polysaccharides		
Increased nitrogen supply to the plant through nitrogen fixation	Symbiotic bacteria form plant root nodules	Rhizobium spp. (Rhizobium leguminosarum)	[27]
		Majority of the species in the family Rhodospirillaceae	[28]
Production of nitrates through nitrification for plant uptake	Oxidation of ammonium to nitrite and then to nitrate	Nitrosomonas spp. (Nitrosospira multiformis, Nitrosospira briensis)	[29]
Reduction of nitrogen in soil by denitrification*	Microbes oxidise nitrogen compounds in the absence of oxygen	Micrococcus denitrificans	[30]
		Thiobacillus denitrificans	[31]
Increased phosphorus (P) solubilisation by Phosphate Solubilising Microorganisms (PSMs) for plants	Transform insoluble phosphorus to soluble forms	Pseudomonas aeruginosa BS8	[32]
		Pseudomonas alcaligenes	[33]
		Glomus mosseae	[34]
C sequestration increases C in soil	Certain microbial communities assist with the storage of C	Community level attribute	[35]

*a buildup of nitrogen in the soil has detrimental effects for plant growth [36].

In Australia, agricultural production is the primary motivator for land clearing [37]. The conversion of naturally occurring ecosystems to agricultural land reduces essential nutrients and impacts microorganisms in the soil [38]. Land clearing destroys ecosystems and is devastating to biodiversity and as a result, sustainable management practices need to be incorporated to avoid further destruction [39, 40]. With increasing land exploitation, it is important to understand the exact microbial mechanisms that facilitate healthy soils, and the roles the microbiota play in promoting resilient, functioning, sustainable agricultural systems. The soil microbial community influences the biogeochemical cycles with synergistic interactions occurring between the PGPRs and therefore, the maintenance of soil health through the microbial communities is critical in supporting the Australian agricultural industry.

1.2 Microbial communities have potential as bioindicators for assessing soil health in agricultural landscapes

There is growing interest in the use of soil microbes as biological indicators, also called bioindicators, of soil health [41]. The criteria used to identify an indicator of soil health includes; responsiveness to management practices, easy to measure and interpret, associated with major ecological soil processes and able to reflect field conditions under a given management [20]. Indicators of soil health have traditionally included soil chemical or physical properties, or the presence of invertebrates like nematodes as biological indicators. The soil microbiota inhabit specific niches that are susceptible to environmental changes and therefore, changes in microbial activities or their abundances may be used to determine the effect of different management practices on soil health [13]. When exposed to aspects of their environment some bacteria and fungi are fragile, while others are able to withstand environmental factors such as drought and severe heat [42]. Since microbes are susceptible to environmental conditions, they are highly influenced by the management practices they are exposed to [13]. There have been numerous attempts to identify an appropriate microbial bioindicator for the measurement of soil health in agricultural landscapes [3, 43-47]. However, to date, there is no clear consensus in the literature as to whether soil microbes can be used as accurate bioindicators of soil health.

Biological tests that seek to utilise soil communities as bioindicators for soil health typically target one of four community properties: Microbial Biomass Carbon (MBC), microbial activity, taxonomic diversity, or functional diversity (see Table 1.2 for advantages and disadvantages). One of the most common measurements used as a parameter of soil health is MBC. This is the measurement of C contained within the living component of the soil organic matter [7]. Microbial activity can be measured through substrate consumption on Biolog Ecoplates or by the activity of certain enzymes such as dehydrogenase, β-glucosidase, phosphatase, urease and arylsulphatase [4]. Biolog Ecoplates provide important community analysis information and provide a reliable and sensitive index of environmental change through analysing microbial substrate consumption [10, 48]. Specific enzyme activities in the soil are associated with microbial C and P cycling and the breakdown of nutrients therefore, can be an indicator of microbial activity [4, 49]. The potential bioindicator of taxonomic diversity is most frequently assessed via sequencing of the 16S rRNA gene. Although more advanced techniques are available that target the soil metagenome, RNA or metabolites, due to their associated costs, 16S rRNA profiling is the

most widely used method for taxonomic profiling of agricultural soils [9]. The functional diversity of soil can be measured as metabolic diversity via Biolog Ecoplates. As Biolog plates contain 31 different carbon substrates, the diversity of substrates a community can utilise can be used as a measure of community functional diversity. Importantly, this technique only measures the functional activity of the culturable proportion of the soil community, which has been estimated to be less then 1 % of the total diversity [50]. Functional profiling can also be achieved via molecular techniques. Metagenomic and transcriptomics can directly measure functional gene content and gene expression profiles, respectively. Taxonomic profiling, via 16S rRNA sequencing, can also be leveraged to generate functional profiles using programs such as PICRUSt and other emerging techniques which predict functional content from the community taxonomic profiles [7, 9, 51, 52]. While all four approaches are available to soil researchers, all have advantages and disadvantages (Table 1.2). An identified gap in the literature is that few studies have explored the functional diversity of the soil microbiota and how it can be used as a bioindicator of soil health in agricultural landscapes.

Table 1.2. The advantages and disadvantages of the four categorised microbial community properties utilised in bioindicator analyses.

Name of bioindicator		Advantages	Disadvantages	References
Microbial biomass carbon (MBC)		Indicates the presence of microbes Known indicator of soil quality Low cost Low complexity of data	Does not identify individual taxa Does not assess community function	[13, 53]
Microbial activity	Biolog Ecoplate	Sensitive and rapid method Assesses microbial metabolic potential Low cost	Culture-based – bias against slow growing species Does not identify individual taxa Sensitive to contamination	[10, 48, 54]
	Enzyme activity	Indication of specific microbial processes Various enzymes Relatively low cost	Answers specific questions about microbial processes	[49, 55]
Taxonomic diversity (Using DNA based molecular techniques)		Identifies individual taxa Culture-independent Detects rare taxa Requires relatively small amounts of data	May need available reference genomes Limited ability for functional data High cost Cannot differentiate between alive/dead microbes Moderate-high cost	[54, 56]
Functional diversity	Functional profile predicted from DNA metabarcoding	Identifies community functional potential Culture independent	Lack of reference data Standard procedure not developed Emerging technique High cost Does not identify the active microbial functions	[7, 9, 51, 52, 54, 56]
	Biolog Ecoplate	Identifies community functional potential	(As per microbial activity)	

1.3 Management practices that affect soil microbiota

As mentioned above, agricultural management practices influence the taxonomic structure and functional diversity of the soil microbiota [21, 57]. The microbial communities present within soil contribute to important processes and their abundances are constantly changing in response to management practices. This includes tilling of the soil, which disrupts the soil structure and microbial community, has been shown to facilitate the proliferation of plant pathogens which could be used as potential indicators of poor microbial soil health [41]. Management practices, such as crop rotation, have been shown to enhance the activity and diversity of the microbial communities [49, 58]. As such, increased soil community diversity is often considered indicative of a good soil management practice. Some management practices, such as the burning of crop residues or the application of fertiliser, have few studies investigating the functional diversity, making it difficult to determine how these practices impact the soil microbial communities.

Crop rotation and tilling of the soil have opposing effects on the soil, where crop rotation must be incorporated to avoid depletion of soil nutrients, tilling is typically considered to have degradative effects [21, 59] (Table 1.3). Tilling of the soil is the mechanised ploughing and inversion of soil, incorporated to disrupt the growth of weeds, reincorporate the organic matter back into soil and allow seedlings to germinate in soft soil [14, 60]. Tilling changes a soil's microenvironment by incorporating oxygen and breaking up essential soil aggregates that create a habitat for microorganisms [59]. Tilling has been shown to cause a loss of organic matter, and a decrease in MBC and microbial activity [55, 61, 62]. Incorporating the practice of no-till, where no tillage occurs is recommended as a good, regenerative management practice [35, 60, 63]. Crop rotation can be defined as growing different crops in succession on the same land, to avoid depleting specific nutrients available in the soil, and to control for pests, diseases and weeds [64]. Crop rotation supports a higher diversity of soil microorganisms, and hence, their input of nutrients into the soil contributes to improving plant growth [21]. Crop rotations help enhance the nutrient and organic material cycling strategies that microbes perform in soils [21]. To help rehabilitate and rebuild a cropping paddock's lost carbon, these paddocks are often turned into pasture, where plants are grown with the intent of animal grazing [65]. Although tilling the soil and crop rotations have clear effects on the soil microbiota, other management practices have varying effects.

Table 1.3. Positive and negative effects of each management practice on the soil microbiota.

Management practice	Positive effects on the soil microbiota	Negative effects on the soil microbiota	Reference
Burning of crop residues**	Reduces weeds Increase bacterial diversity	Lower microbial biomass Removal of organic matter	[11, 12]
Fertiliser ***	Increases microbial biomass C	Temporarily inhibit microorganism growth Alters microbial community composition	[10, 66]
Crop rotation	Increases MBC and microbial activity Higher in taxonomic and functional diversity	(No known negative effects)	[21, 43, 49, 57]
Tilling of soil	Reduces weeds Releases soil nutrients	Extreme erosion Lower level of organic matter Lower microbial biomass C Disruption of soil aggregates	[13, 14, 60]

**there is no consensus in the literature on whether burning of crop residues has positive or negative effects [61, 67].

***short term versus long term effects of fertiliser use vary [66].

The literature has opposing views on whether burning crop residues and the application of fertiliser have positive or negative effects on the soil microbiota [11, 66] (Table 1.3). Agricultural burning is where the crop residues remaining from the previous harvest are burnt [61]. From the growers' perspective, removing remaining crop residues through burning assists the upcoming plant growth, as well as controlling common pest populations e.g. slugs and snails [61]. Shen [12] reported that burning increased bacterial diversity and abundance in *Eucalyptus* ecosystems however, this has not been studied extensively in agroecosystems. Fertiliser has been incorporated in agricultural soils routinely since the 1950s due to its remarkable increases in crop yield [68]. The most common fertiliser used in agroecosystems is NPK (Nitrogen, Phosphorus and Potassium) and is most often applied yearly, at the time of seeding [10]. Zhong *et al.*, [10] found that the presence of fungi was higher under NPK treated soils. Furthermore, Geisseler *et al.*, [66] looked at the wider

literature and confirmed that fertiliser altered MBC in the short term, whereas microbial community composition was affected in both the short and long term.

1.4 Aims and hypothesis

This thesis aimed to investigate the utility of soil microbial communities in evaluating soil health in an agricultural landscape. Firstly, the current usage of microbial soil bioindicators in the wider literature was investigated. To address current knowledge gaps regarding the accuracy of microbial bioindicators in measuring soil health, a meta-analysis was performed. Specifically, the aim of this meta-analysis was to appropriately sample the literature to determine whether the four microbial community parameters (MBC, microbial activity, taxonomic diversity and functional diversity) had the ability to behave as bioindicators. The hypothesis of this meta-analysis was that the four microbial community parameters would respond in a repeatable way to the four agricultural management practices: tilling of soil, burning of crop residues, application of fertiliser and crop rotation.

This thesis also aimed to determine the effect of tilling the soil and burning of crop residues on the soil microbiota on properties in regional Victoria, Australia. This was investigated through analysis of bacterial 16S rRNA and fungal ITS regions for taxonomic diversity, and the PICRUSt program where bacterial functional pathways were examined. It was hypothesised that tilling of pasture paddocks and burning of crop residues would influence the bacterial and fungal taxonomic diversity, and bacterial functional diversity.

2.0 MATERIALS AND METHODS

2.1. Meta-analysis examining use of bioindicators in agricultural landscapes

The aim of this meta-analysis was to appropriately sample the literature to determine whether the four soil community parameters (MBC, microbial activity, taxonomic diversity and functional diversity) were accurate bioindicators of soil health.

2.1.1. Literature compiling

Literature was compiled for this meta-analysis using the database SCOPUS (Elsevier). Initial search terms were “Biological indicator” (subsequently refined to “Bioindicator”), “Management practices” and “Soil”. This initial search resulted in 5587 articles, from which only those that included agricultural/cultivated soils, and reported the effects of tillage, stubble management (through burning of crop residues), fertiliser or crop rotation on Microbial Biomass Carbon (MBC), microbial activity, taxonomic diversity, functional diversity or a combination of these variables were selected. The initial search was expanded by checking reference lists and citations of the studies that met the initial search criteria. The studies were not limited to a specific country. The final list of studies included in this meta-analysis comprises of 44 research articles ranging in publication dates from 1980 to 2020 (see Table 6.7, Appendix III for each reference used in the meta-analysis). The methodology for this meta-analysis is as presented in Figure 2.1.

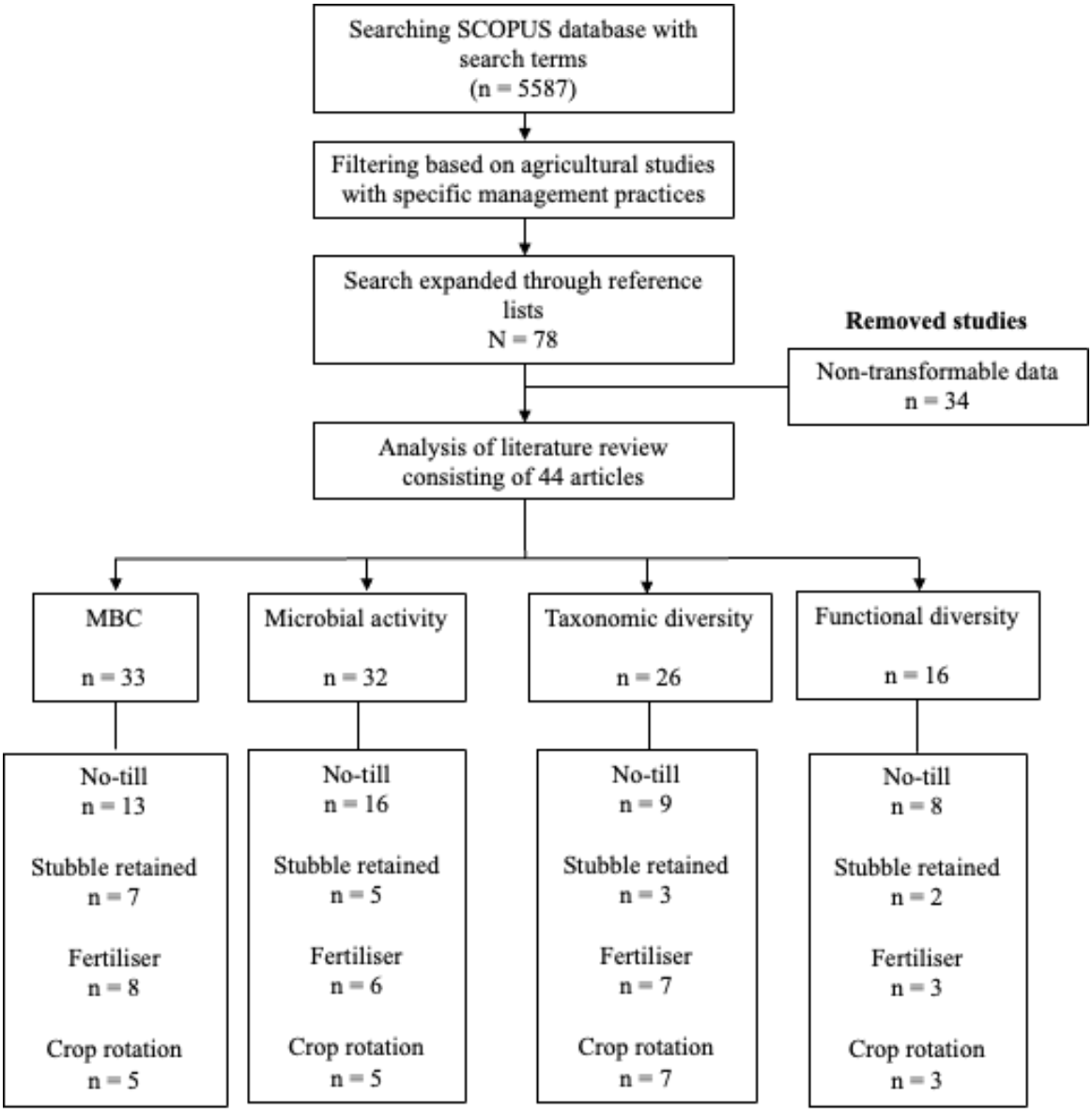

Figure 2.1. The flowchart of methodology for the present meta-analysis.

2.1.2. Data collection

Data was taken from both tables and figures in the publications that met our search criteria. The response variables used in this meta-analysis are MBC, microbial activity, taxonomic diversity and functional diversity. MBC is a direct measurement of the carbon contained from the living component of the soil via the fumigation and extraction method, first described by Jenkinson *et al.,* [69]. Microbial activity measurements included data obtained from Biolog Ecoplates or direct assays of the activity of different extracellular enzymes (such as dehydrogenase, β-glucosidase, phosphatase, urease and arylsulphatase). Biolog Ecoplates use a 96-well plate format to assay carbon substrate utilisation of 31 different carbon sources via a colorimetric reaction. Microbial activity was specifically measured as the rate of colour change [70]. Taxonomic diversity was consistently measured through DNA sequencing of the 16S rRNA gene to assess the microbial community structure. Functional diversity was measured either via Biolog Ecoplates, where richness of well response was taken for a proxy for functional diversity, or via predictive functional profiling whereby DNA sequencing of the 16S rRNA gene is used to predict the functional profile based on taxonomic relationships using programs like PICRUSt [9, 70].

When studies were conducted across a variable number of years, only the final timepoint of the data was taken. Where a study examined a range of sampling depths, only measurements from the top layer of soil were considered (usually 0 to 10 cm of the soil surface). Some studies used multiple variables or management practices.

2.1.3. Data Analysis

For each study, the impact of a given management practice on the four potential soil health indicators was recorded as ‘increased’, ‘decreased’ or ‘no significance difference’. To determine whether bioindicators exhibited repeatable responses to management practices across the literature, Chi-square tests were used to compare the observed outcomes to the null hypothesis of expected no trend in outcomes (Appendix III).

2.2. Effect of agricultural management practices on microbial communities

This field study was conducted in collaboration with the Goulburn Broken Catchment Management Authority (GBCMA). This study aimed to examine the impact of two common agricultural management practices on soil bacterial and fungal community structure and function. Two different management practices were examined: mechanical soil tilling and burning of crop residues. Tilling is described as the mechanised breaking up of the soil. In agriculture, specialised equipment attached to a tractor is used to break up the soil and reincorporate organic matter, approximately 20 cm deep. Agricultural burning of crop residues occurs when the previous harvests' crop residues are burnt. A drip torch is used, so flaming fuel can be slowly deposited for controlled burning of the paddock. Controlled burning of crop residues results in some patches of the paddock left unburnt due to the large scale that it occurs.

2.2.1. Soil sampling location in rural Victoria

This study conducted sampling across four different private properties in northern regional Victoria (Figure 2.2). Soil sampling was conducted in mid-April of 2020. The annual precipitation for this area is approximately 400 to 600 mm [71]. The average temperature during the month of April ranges from 8°C to 22°C [71].

The land is used primarily for agricultural purposes. Sampling was conducted approximately two to three weeks post-tilling to capture the effects of tillage on soil communities. All cropping paddocks had comparable NPKS fertiliser applications during seeding, pasture paddocks had no application of fertiliser.

All samples for tilled experiments were obtained from pasture paddocks, an area of land covered in grass and other ground cover plants for grazing animals. Each paddock was in pasture for more than five years with livestock grazing by sheep for the duration it was in pasture. Pasture crops on paddocks 2A, 3B and 4B was predominantly grass (*Phalaris* sp.), paddock 2B had a mixed pasture of rye (*Secale cereale*) and clover (*Trifolium* sp.). The reason for tilling 2A was not added because it was not discussed, but it can be assumed it was to reincorporate organic matter back into the soil.

The crop residue existing on the burnt treatments was wheat (*Triticum aestivum*). Burnt paddocks were sampled between post-harvest and pre-sowing where there were no active

crops. Sampling took place approximately one-week post burn. In previous years, there had been a yearly crop rotation procedure on the paddocks, where other crops such as oat (*Avena sativa)*, barley *(Hordeum vulgare)* and canola *(Brassica napus)* had been planted.

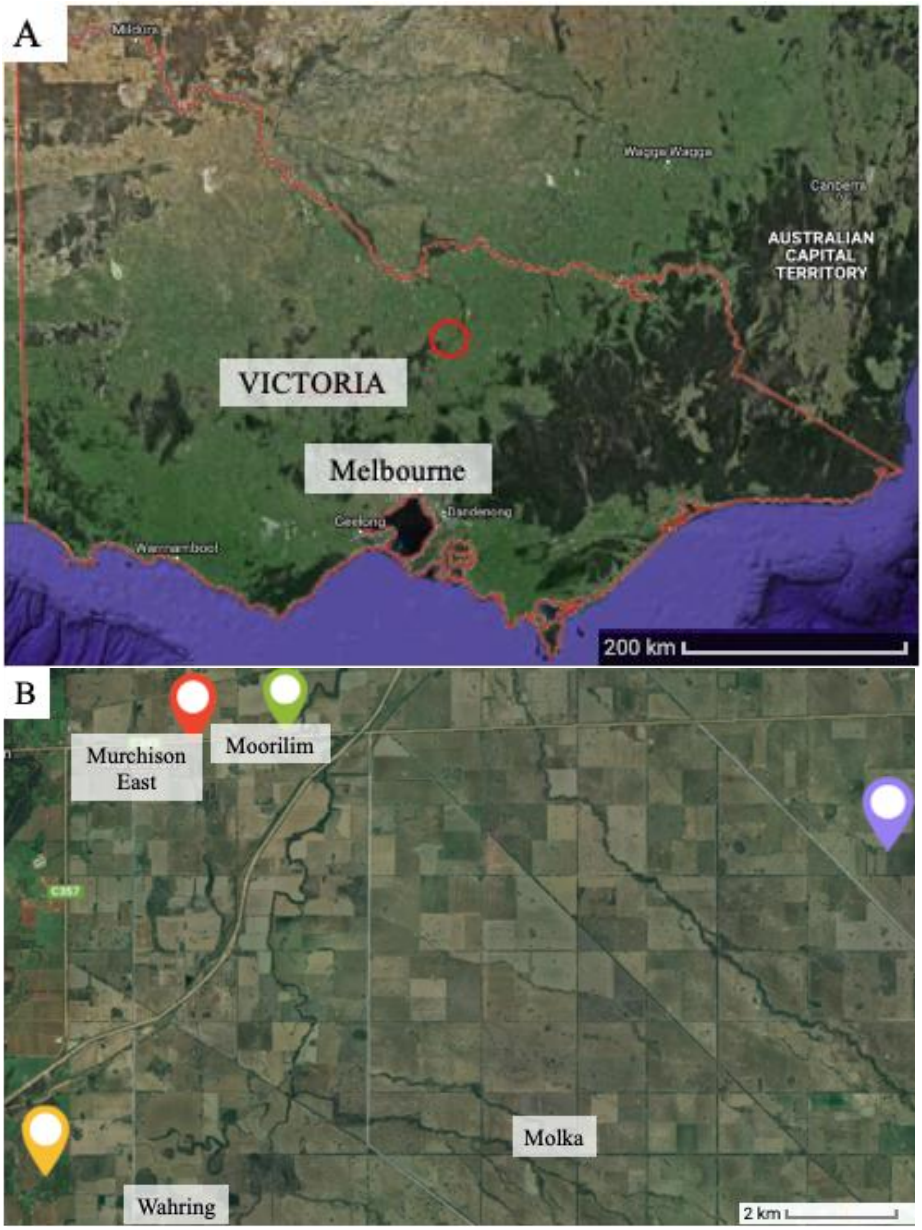

Figure 2.2. Satellite images showing A) State of Victoria with the region of sampling circled in red and B) Goulburn region and the location of the four collection sites in northern regional Victoria. Colour is indicative of location: site 1, site 2, site 3 and site 4. Coordinates of sites in Appendix I, Table 6.1.

2.2.2. Experimental design of tilled treatments

The effect of tillage on the bacterial and fungal communities was investigated by sampling four different paddocks across three different sites (Table 2.1). One tilled and three untilled paddocks were sampled. One of the tilled treatments were paired, where a tilled and un-tilled paddock were sampled from the same site (site two). An additional planned sampling site did not proceed due to farmer management choice.

Table 2.1. The different property sites and management practice for each tilled treatment.Unique property and paddock identifiers in brackets.

	Tilled paddock (A)	**Un-tilled pasture paddock (B)**
Site 1 (1)		
Site 2 (2)	✓	✓
Site 3 (3)		✓
Site 4 (4)		✓

2.2.3. Experimental design of burnt treatments

Five paddocks across four sites were sampled to investigate the effect that burning crop residues has on bacterial and fungal communities. Both the burnt and unburnt patches across two burnt paddocks were sampled (Table 2.2). Two paddocks on two separate sites were used as no-burn controls. Due to the 2020 COVID19 Victorian lockdown restrictions, the task of soil sampling was completed by the property owners. Therefore, replication and randomisation did not represent the original experimental design of this project.

Table 2.2. The different property sites and management practice for each burnt treatment.Unique property and paddock identifiers in brackets.

	Burnt paddock		**Unburnt crop-residue attached paddock (E)**
	Unburnt patch (C)	**Burnt patch (D)**	
Site 1	✓	✓	✓
Site 2			
Site 3			✓
Site 4 (paddock_i)	✓	✓	
Site 4_(paddock_ii)	✓	✓	

2.2.4. Soil core collection

For each paddock sampled, a total of five replicates were collected. For tilled paddocks, un-tilled pasture paddocks and un-burnt paddocks, five representative soil cores were collected from across the entire paddock (Figure 2.3A). For burnt paddocks, five replicate soil cores were collected from burnt areas and a second set of five replicate cores were collected from unburnt patches within the burnt paddock (Figure 2.3B). The average size of a paddock was between 40 to 60 ha.

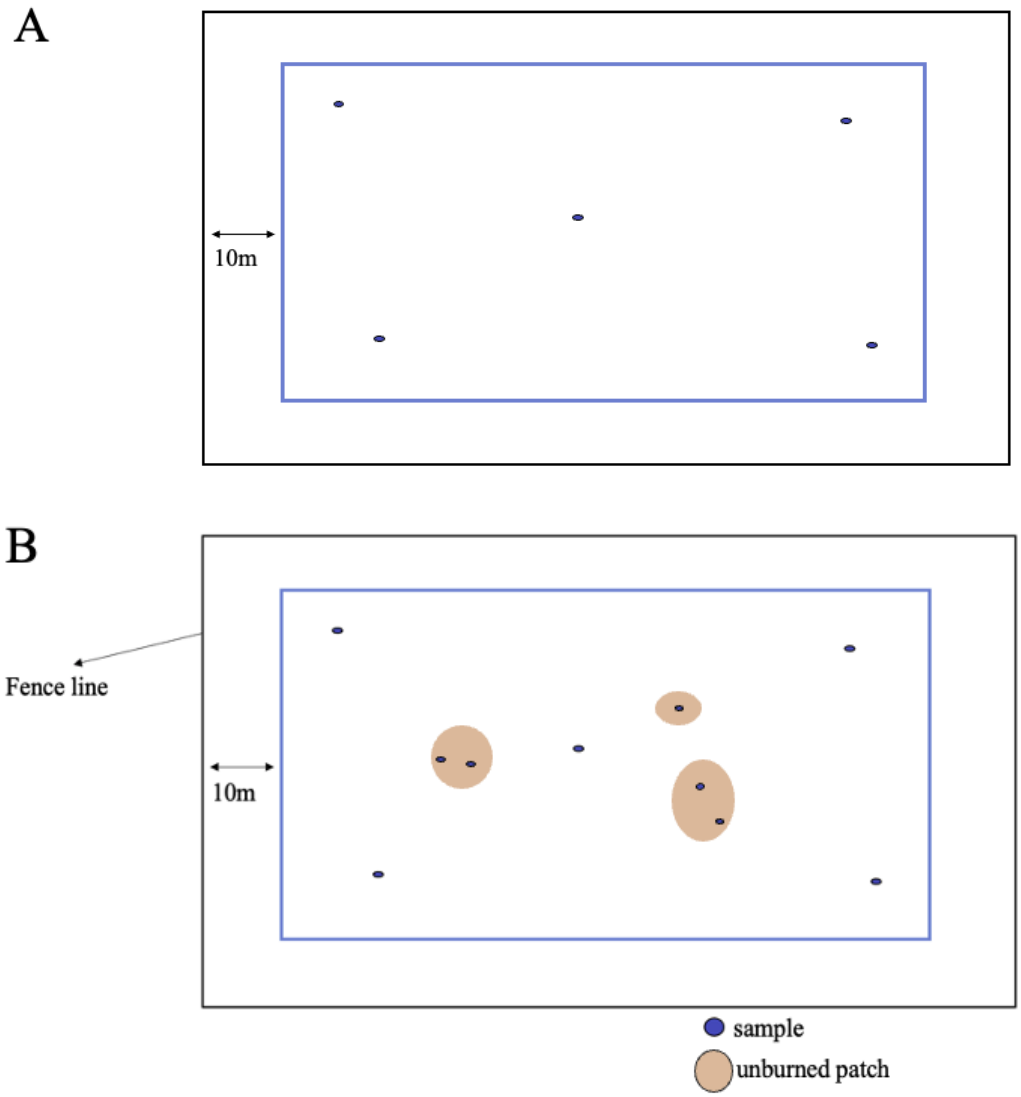

Figure 2.3. The experimental design for sampling the treatments: A) where tilled paddocks, un-tilled pasture paddocks, and paddocks with crop residues attached had 5 representative samples taken and B) burnt paddocks had 5 samples from the surrounding paddock, and 5 samples from unburnt patches (brown circle).

Cores were collected approximately 10 m away from the fence line to avoid soil conditions different to the rest of the paddock. Loose, organic matter was removed prior to where the soil sample was taken. The top layer of soil (approximately 10 cm) was collected as a core using 50 ml falcon tubes to avoid handling and disturbance (Figure 2.4). This resulted in a 2.5 cm in diametre soil core sample. A total of 60 falcon tubes were collected of soil, with nine paddocks sampled from four different properties. Once the soil samples were collected, they were stored in a freezer at approximately -20°C until they were posted via Express Australia Post and kept in a deep freezer at -30°C at La Trobe University until analysis.

Figure 2.4. Falcon tube method of surface soil sampling.Resultant soil core depth is 10 cm, core diameter is 2.5 cm. Falcon tube was placed on the surface of soil prior to being pushed into the ground.

2.2.5. Community DNA extraction from soil samples

DNA was extracted from 0.40 g of soil utilising the Qiagen DNeasy Powersoil® kit as per the manufacturer's protocol. The final yield of DNA concentrations for each sample was measured using the QUBIT® 3.0 Fluorometre broad-range and high-sensitivity assays. Once the concentration of DNA was determined, DNA samples were normalised to 5 ng/µL in 10 mM Tris-buffer 8.5 pH. Normalised DNA solutions were stored at 4°C prior to Illumina amplicon sequencing.

2.2.6. Bacterial 16S rRNA and fungal ITS amplicon sequencing

Soil bacterial and fungal communities were sequenced via 16S ribosomal RNA (rRNA) and Internal Transcribed Spacer (ITS) region metabarcoding on an Illumina MiSeq platform. All 60 DNA soil samples representing the tilled and burnt treatments were utilised in the procedure.

Amplicon libraries were prepared following the Illumina metagenomic sequencing protocol with modifications to the PCR conditions due to the protocol targeting the bacterial 16S rRNA V3 and V4 region [72]. Utilising the Nextera XT Index Kit, dual indices and Illumina sequencing adapters were attached following the Illumina protocol [72]. The bacterial metabarcoding primers 515F (5'-GTGYCAGCMGCCGCGGTAA -3') and 806R (5'-GGACTACNVGGGTWTCTAAT -3') which target the V4 hypervariable region of the 16S rRNA gene using primers were used [73]. PCR conditions involving an initial denaturation step at 94°C for 3 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 5 min.

The fungal ITS metabarcoding primers fITS7 (5'-AGGTGARTCATCGAATCTTTG -3') and ITS4 (5'- TCCTCCGCTTATTGATATGC -3') which target the ITS4 region, were used as per Egidi *et al.,* [67] protocol. PCR conditions involved an initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s, with a final extension at 72°C for 10 min.

The QUBIT® 3.0 Fluorometre broad-range assay was used to quantify indexed amplicons. Prior to being pooled into a single microcentrifuge tube, all indexed samples were normalised to 4 nM/μL using 10 mM Tris-buffer 8.5 pH. The indexed amplicon library was denatured using freshly prepared 0.2 M NaOH and diluted to 20 pM with HT1 buffer. A PhiX control was also denatured with 20 pM diluted with 0.2 M NaOH and spiked into the sequencing run to act as a positive control. The pooled PhiX and amplicon library was diluted to 7 pM and 600 μL of pooled library was loaded into the MiSeq Reagent Kit V3 and run for 600 cycles.

2.2.7. Bioinformatic analysis of bacterial 16S rRNA and fungal ITS sequencing output

Bacterial 16S rRNA and fungal ITS FASTQ sequences were downloaded from the Illumina Basespace Sequence Hub (https://basespace.illumina.com). QIIME2 (2020.8) software was utilised for all bioinformatic analysis [74]. Both the forward and reverse primers were trimmed from the paired sequences utilising the Cutadapt plugin [75]. Denoising occurred as the bacterial paired sequences were truncated from 200 bp in forward reads and 180 bp in reverse reads prior to joining. The fungal paired sequences were truncated from 220 bp in forward reads and 200 bp in reverse reads prior to joining. The *DADA2* plugin was utilised to produce Amplicon Sequence Variants (ASVs), by implementing joining, quality filtering and chimera detection [76].

Following denoising, sequences were screened against a classifier to assign the *Silva* taxonomic classifier (version 138) trained against the V4 region for bacteria and the *UNITE* fungal classifier (version 8) trained against the ITS4 region [77, 78].

Separate bacterial and fungal ASV tables were produced utilising Excel to be imported into R for statistical analysis [79]. A total of 3,284,105 sequence reads were identified from 17,577 unique bacterial ASVs, with an average of 54,735 sequences per sample. A total of 2,978,783 sequence reads were identified from 5,631 unique fungal ASVs, with an average of 49,646 per sample.

One bacterial sample, soil sample DB7, was removed from downstream analysis due to an insufficient number of sequence reads (< 3000 sequences).

2.2.8. Statistical analysis of the bacterial 16S rRNA and fungal ITS sequencing data

Community structure analysis of the bacterial 16S rRNA and fungal ITS sequencing data was completed using R (version 3.6.3) using the packages *phyloseq, ggplot2, microbiome, vegan* [79-83]. From the bacterial and fungal data, mitochondria and chloroplasts were removed using the package *magrittr* [84].

For all statistical procedures, a *p*-value of less than 0.05 was considered statistically significant.

2.2.8.1. Redundancy Analysis (RDA) ordination

Redundancy Analysis (RDA) ordinations were produced to investigate the effect of pH, Soil Moisture (SM) and location on the soil microbial communities. The Z-score standardisation method was used to compare between the pH, SM and location data. Variance Inflation Factors (VIFs) measured how much the variance of a variable correlated with another. The VIF for pH, SM and location measured how easily they are predicted from a linear regression using each other. An ANOVA-like permutational test for the RDAs was ran to determine whether there was a significant influence of the three constraints on the bacterial and fungal communities [85]. Supplementary analyses within Appendix IV.

2.2.8.2. Taxonomic NMDS β-diversity ordinations

Prior to β-diversity analyses, Total-Sum Scaling (TSS) normalisation of the data via the package *metagMisc* was performed [86]. β-diversity data was visualised using non-metric multidimensional scaling (NMDS) ordinations. The NMDS ordinations describe the similarity between the microbial communities: the ordinates that share the smallest distance between them are most alike in community structure. β-diversity is a measure of the similarity between pairs of communities. β-diversity was calculated using abundance-weighted and unweighted (binary-transformed) Unifrac metrics which downweigh the importance of dominant ASVs [87].

For tilled experiments, due to the effect of location on the data, only site two (where a direct comparison between a no-till and tilled paddock exists) was statistically analysed. Analysis of Similarity (ANOSIM) was used to examine the strength of grouping between tilled treatments [88]. For burn treatments, due to influence of site on the microbial communities,

only the burnt paddocks and the unburnt patches within the same paddock were statistically reported. Permutational multivariate analysis of variance (PERMANOVA) was used to determine if the soil management practices caused significant changes to total community structure. Ellipses with a 95 % confidence interval were used to show the smallest area where the samples overlap [89]. Supplementary PERMANOVA analyses are presented within Appendix V & VI.

2.2.9. Bioinformatic analysis of predicted bacterial functional pathways using PICRUSt

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) produced predicted bacterial functional pathways based on the previous 16S rRNA sequencing [90]. PICRUSt uses the manually curated Metacyc database to assign predicted pathways. Singletons, sequences that are observed < 2 times, were removed. A low depth sample (DB7) was removed after rarefying to 35,000. PICRUSt pathway tables were produced in Excel. A total of 425 different Metacyc functional pathways were identified.

2.2.10. Statistical analysis of the PICRUSt predicted bacterial functional pathways

2.2.10.1. Functional pathways NMDS β-diversity ordination

β-diversity was calculated using abundance-weighted and unweighted Bray-Curtis metrics, where the unweighted data was binary-transformed which downweigh the importance of dominant ASVs [91]. β-diversity statistics followed the same methods as per the taxonomic data. Supplementary PERMANOVA analyses are presented within Appendix VII.

2.2.10.2. Differential abundance tests for functional pathways

Using the package *DESeq2*, a differential abundance analysis was performed on the PICRUSt predicted bacterial functional pathways to determine whether the tilled and burnt treatments were altering the functionality of the communities [92]. The differential abundance analysis detects differences in abundance of functional pathways using negative binomial distribution. The p-value was adjusted by the Benjamini-Hochberg (BH) method, to account for familywise error. Only pathways with a baseMean > 250 were reported, these

were pathways with a higher prevalence in the community (supplementary abundance testing information are presented within Appendix VIII).

2.2.11. Cumulative ASV curves

Cumulative ASV curve graphs were produced in Excel (v16.54) using the bacteria and fungi ASV tables. The curves were produced by treatment (i.e., tilled paddock and no-till paddock), where each new sample added the number of species not present in the previous samples.

2.2.12. Sampling pH and soil moisture content

Soil pH affects the chemical, physical and, most importantly, the biological properties. The pH was sampled as per Rayment [93] protocol where 5 g of soil from each treatment and 25 ml of 0.01 M $CaCl_2$ was added to 50 ml falcon tubes. This suspension was mechanically shaken for 1 hr and then centrifuged at 2000 rpm for 5 mins. For the most accurate pH measurements, the pH electrode was held vertically and gently agitated in the suspension. After calibration, pH measurements were read using a HANNA Instruments Ltd H1 2211 pH meter and recorded to two decimal places (see Table 6.2 Appendix II).

Soil moisture was measured from 10 g of soil in metal soil moisture content tins and the weight of the wet soil in the tin was recorded prior to being placed in a 105°C oven for 24 hrs. The weight of the dry soil in the tin was then recorded and used to calculate the percent (%) of soil moisture in each sample (see Table 6.2, Appendix II).

3.0 RESULTS

3.1. Meta-analysis: Microbial indicator techniques

In the literature, there are four soil community parameters commonly studied and used for interpreting soil health through microbes in agricultural systems. These parameters are microbial biomass carbon (MBC), microbial activity (as measured through the activity of certain enzymes or Biolog Ecoplates), taxonomic diversity, and functional diversity. Each of these microbial community properties uses different analytical techniques for measurement.

3.1.1. Usage of microbial techniques in literature

A meta-analysis was performed with a focus on the strategies for assessing soil health, in which analytical techniques were grouped into four categories based on which aspect of the microbial community they use to interpret soil health in agricultural-based studies. Figure 3.1A represents the frequency of the analytical techniques used in microbial soil health studies. Upon grouping the analytical techniques into four different categories of microbial community properties of soil health it was observed that MBC, microbial activity, taxonomic diversity, and functional diversity were all used in almost equal proportions (Figure 3.1B). The data also revealed that only 9.09 % of studies used all four of the microbial community parameters whereas, 38.63 % of studies used only one (Figure 3.1C).

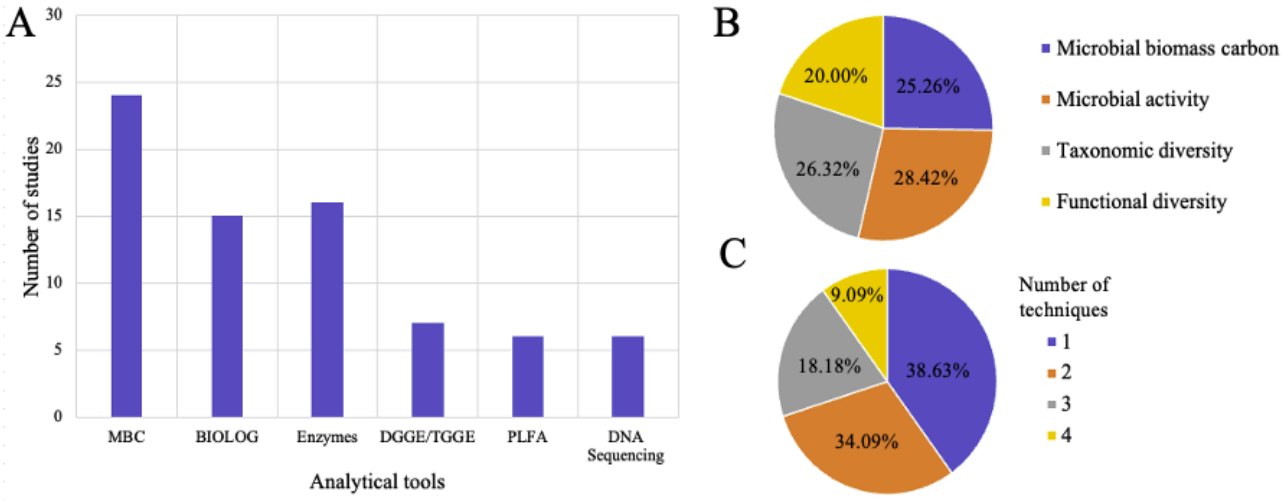

Figure 3.1. The usage of microbial techniques in literature.A) Frequency of different analytical techniques used to measure microbial community parameters of soil health in 44 studies. B) Relative percentage of soil community parameters used to investigate soil health. C) Representation of the number of techniques (from B) used in each study for the characterisation of soil health.

3.1.2. Examining the prevalence of the four microbial community parameters over time

This graph shows the relative prevalence of each of soil community parameters in the field over time. This figure represents 44 individual studies, where some may have used more than one microbial community property to assess soil health. The four different microbial community parameters used as bioindicators of soil health have varied over time (Figure 3.2). Microbial biomass carbon (MBC) was prominently used between 2006 to 2010 however, there is a notable increase in the use of taxonomic diversity and functional diversity of the microbial communities from 2015. The rise in these techniques corresponds to a decline in the use of MBC.

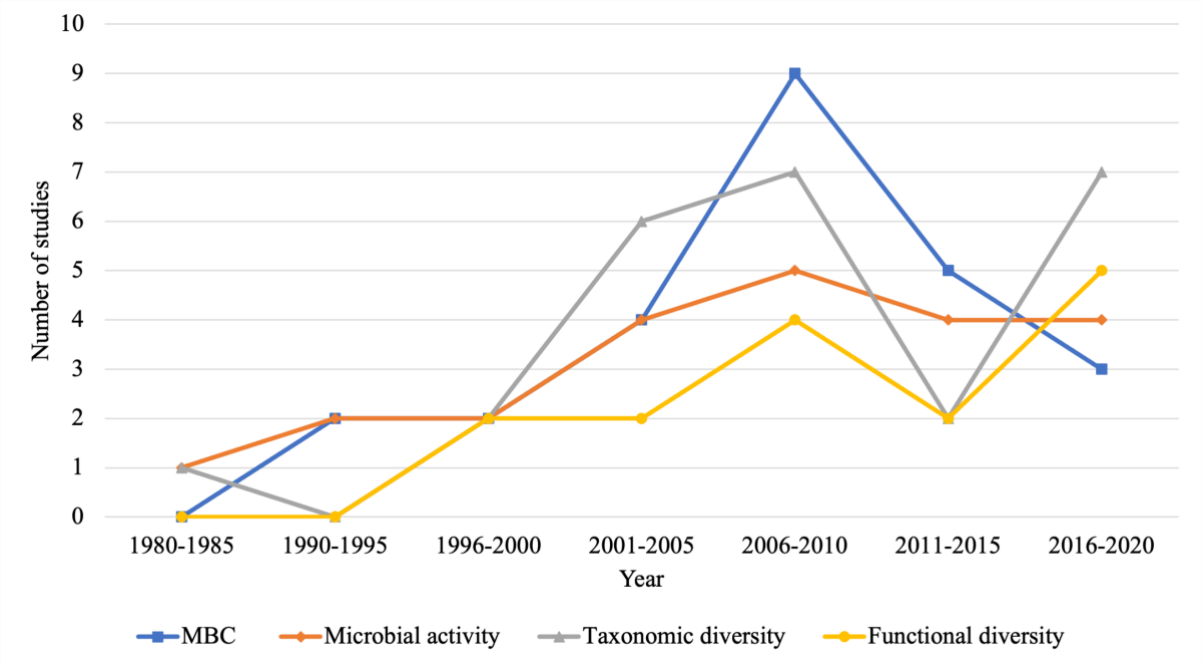

Figure 3.2. The number of studies using the four different microbial community parameters from 1980 to 2020. Time intervals in groups of five years. Data collated from total of 44 research articles.

3.1.3. Outcome of studies for the four microbial community parameters and management practices

To determine if the microbial community properties (MBC, microbial activity, taxonomic diversity and functional diversity) had the ability to behave as bioindicators of soil health, the results of the literature were assessed for repeatability. The percentage of studies that recorded either an increase, decrease or no significant change in soil microbial community parameters due to four different management practices was collated and a Chi-squared (χ^2) test was run to test how likely the distribution of the outcomes was due to chance (Figure 3.3). Chosen management practices included no-tilling, stubble retained, application of fertiliser and crop rotation.

In general, the four soil community parameters tended to increase under each of the four management practices. The main exception being that the response of MBC to fertiliser application and tilling was highly variable despite >8 studies for each comparison. Significantly repeatable outcomes were detected for each community parameter for 2 out of 4 management practices.

For MBC, there were significantly more studies reporting that stubble retention ($\chi^2_{(2, N = 7)} = 8.857$, $p < 0.05$) and crop rotation ($\chi^2_{(2, N = 5)} = 5.2$, $p < 0.05$) increased due to management (Fig 3.3A). For both microbial activity and taxonomic diversity, there were significantly more studies reporting that no-till ($\chi^2_{(2, N = 16)} = 12.5$, $p < 0.05$; $\chi^2_{(2, N = 8)} = 10.75$, $p < 0.05$) and crop rotation ($\chi^2_{(2, N = 5)} = 10$, $p < 0.05$; $\chi^2_{(2, N = 3)} = 6$, $p < 0.05$) increased community parameters (Fig 3.3B & 3.3D). For taxonomic diversity, significantly more studies reported that no-till ($\chi^2_{(2, N = 9)} = 12.666$, $p < 0.05$) and fertiliser application ($\chi^2_{(2, N = 7)} = 14$, $p < 0.05$) increased diversity (Fig 3.3C). See Appendix III for full chi-square results.

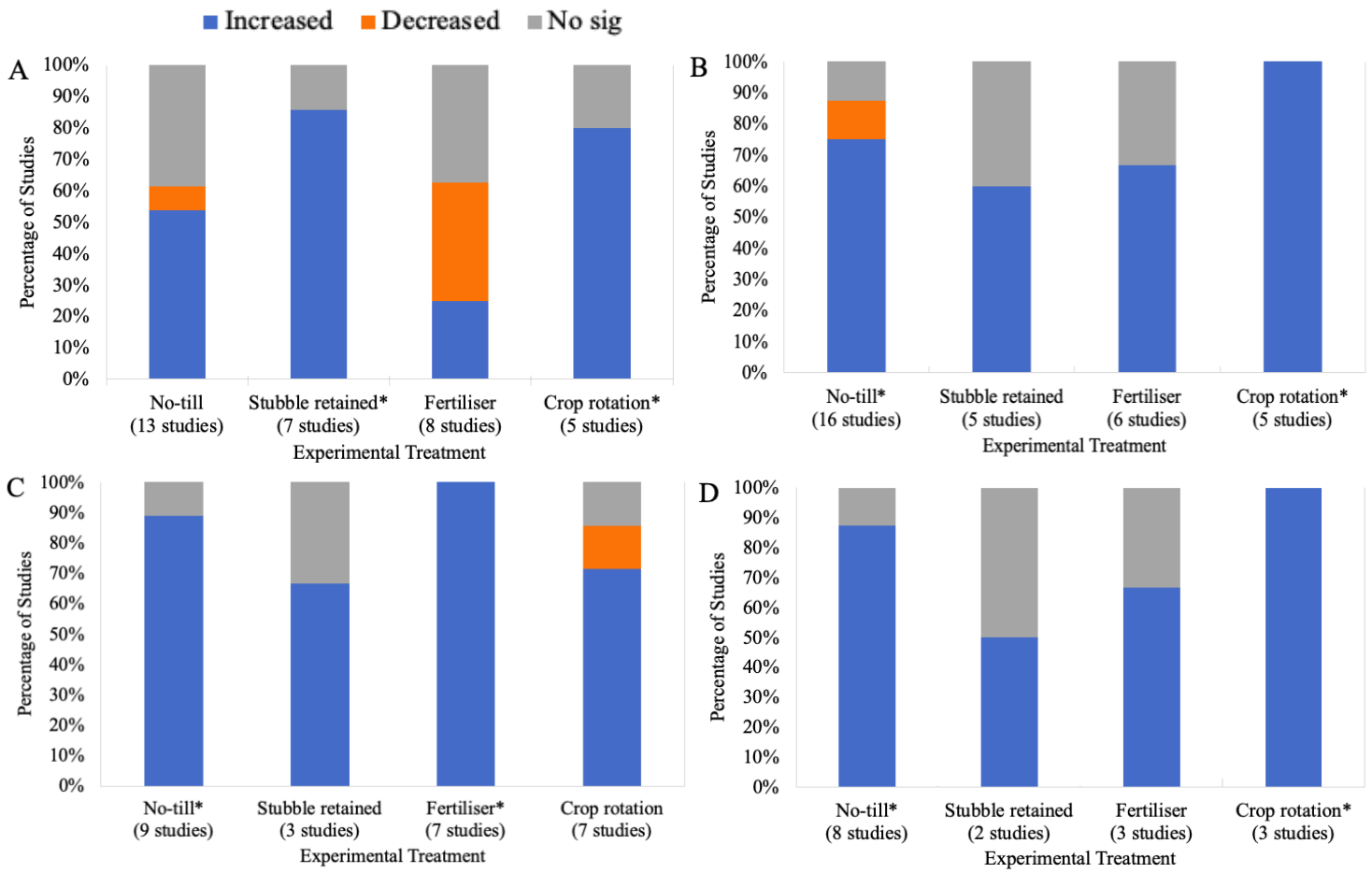

Figure 3.3. The percentage of studies that reported an increase, decrease, or no significant change in A) Microbial biomass carbon (MBC) B) Microbial activity (measured through Biolog or activity of certain enzymes), C) taxonomic diversity and D) functional diversity with the application of different agricultural management practices. The number of studies for each comparison are denoted in parenthesis below each bar. Where '*' denotes significance ($p < 0.05$).

3.2. Effect of agricultural management practices on the soil microbial communities

To further investigate how agricultural management practices affect soil health and whether the microbial parameter of functional diversity is an appropriate bioindicator of soil health due to the lack of studies investigating it, analysis of the two management practices (tilling the soil and burning of crop residues) on the bacterial and fungal communities was investigated.

3.2.1. RDA showing the effect of pH, soil moisture content and location on the soil microbial communities

A Redundancy Analysis (RDA) was used to elucidate the role played by two different environmental variables (pH and soil moisture) and location effects in modulating the structure of microbial communities. No factors were removed from analysis from either ordination due to all Variation Inflation Factors (VIFs) being < 10 (Appendix IV, Table 6.8). An ANOVA-like permutation test was used to assess the significance of the three constraints: SM, pH and site. There was a significant influence of the three constraints for bacterial data ($F_{5,54} = 1.691$, $p < 0.05$, Appendix IV, Table 6.9, Figure 3.4A). There was also a significant influence of the three constraints for fungal data ($F_{5,54} = 1.610$, $p < 0.05$, Appendix IV, Table 6.10, Figure 3.4B). Both the bacterial and fungal RDA ordination had a significant influence of pH and site (Table 6.11 & Table 6.12).

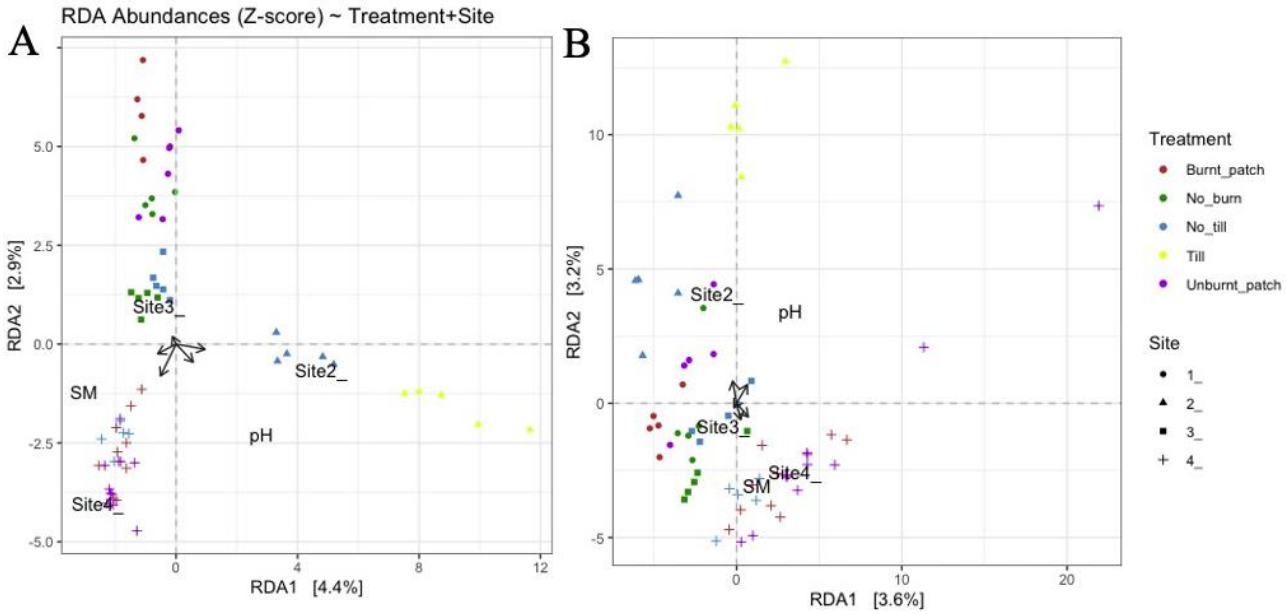

Figure 3.4. Redundancy analysis (RDA) using the Z-score transformed proportions of pH and soil moisture (SM) for A) bacteria and B) fungi. Symbols represent the individual sites, and their proximity to each other indicates compositional similarity. The colour of symbols represent the treatment of samples.

3.2.2. Cumulative ASV curves

Cumulative Amplicon Sequence Variant (ASV) curves were produced to determine whether sampling was sufficient to capture the diversity of the bacterial and fungal communities in each treatment (Figure 3.5 & Figure 3.6). ASVs are the unique DNA sequences that can be used to identify specific species, and a read is any given individual sequence. In the case of the data analysed, ASV's refer to different fungi or bacteria present.

3.2.2.1. Tilled treatments had continuous ASV accumulation

All treatments, including both tilled and no-till paddocks, did not plateau with further sampling indicating that the treatments were not sampled broadly enough, and that more replicates are needed (Figure 3.5).

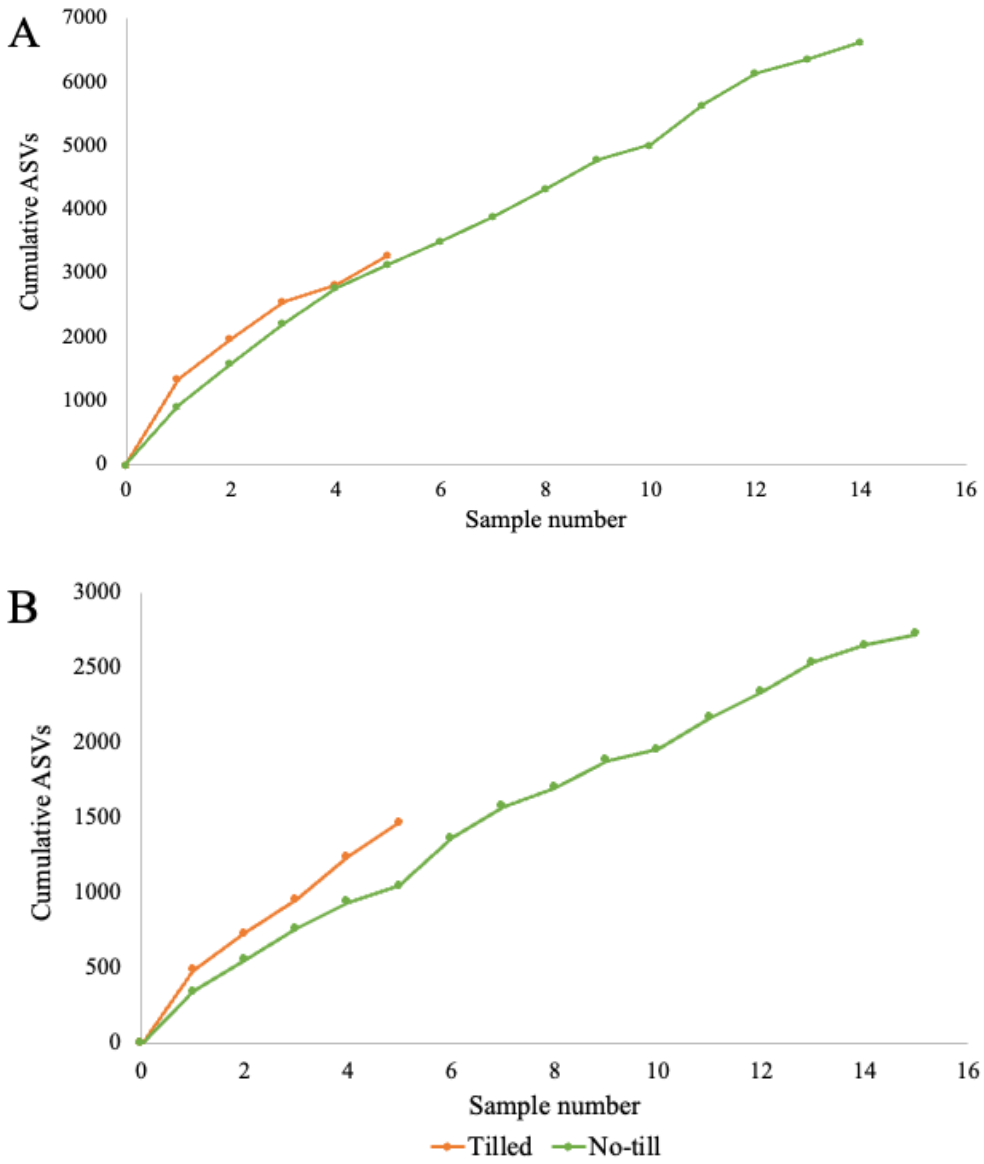

Figure 3.5. ASV accumulation curves for tilled treatments showing ASVs accumulating per sample for A) bacteria and B) fungi. Colour indicative of sampling location: tilled and no-till.

3.2.2.2. All burn treatments continuously accumulated ASVs

All treatments, including the burnt and unburnt patches in a paddock and no-burn paddocks, did not plateau with further sampling indicating that the treatments were not sampled broadly enough, and that more replicates are needed (Figure 3.6). Interestingly, unburnt patches tended to accumulate more ASVs than unburnt and no-burn treatments.

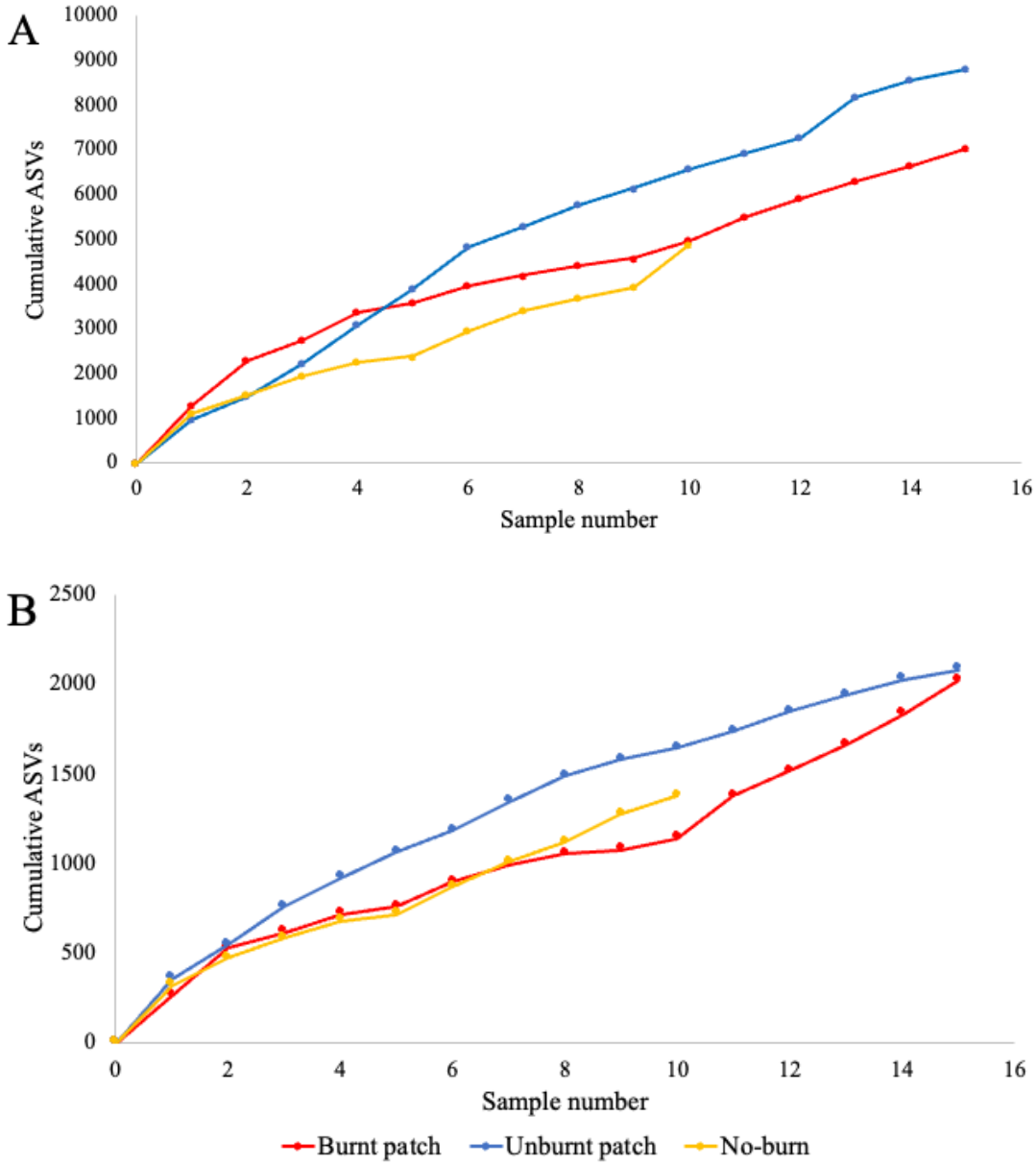

Figure 3.6. ASV accumulation curves for burn treatments showing ASVs accumulating per sample for A) bacteria and B) fungi. Colour indicative of sampling location: unburnt patch, burnt patch and no-burn paddock.

3.2.3. COMMUNITY ANALYSIS: Tilled treatments β-diversity ordination

To examine the effect of tillage on the bacterial and fungal communities, NMDS ordinations were produced. Due to influence of site on the microbial communities, only site two (where a direct comparison between a no-till and tilled paddock exists) was statistically analysed (see Appendix V, Figure 6.1 & Figure 6.2 for ordinations with all sites).

3.2.3.1. Tilling altered the bacterial communities

To investigate the similarities in bacterial community structure between tilled and no-tilled treatments, community β–diversity was analysed using Permutational Multivariate Analysis of Variance (PERMANOVA) on Unifrac distance matrices. Non-metric multidimensional scaling (NMDS) ordinations were used to visualise community relationships. The tilled treatments NMDS ordinations had stress values of 0.029 (Figure 3.7A) and 0.055 (Figure 3.7B) and thus, can be considered an accurate representation of community relationships. Analysis of Similarity (ANOSIM) was used to examine the strength of grouping between treatments and there was significant low-level grouping between treatments for weighted bacterial community data ($R = 0.03$, $p < 0.05$). Grouping due to treatment increased ($R = 0.7$, $p < 0.05$) when data was binary transformed, suggesting treatment differences are due to a turnover in the suites of ASVs as well as changes in the dominant species present.

PERMANOVAs on abundance-weighted data (Figure 3.7A) compares community similarity in the context of shared ASVs and the similarity of their respective abundances. PERMANOVAs on unweighted data (Figure 3.7B), in which ASV abundances were binary transformed (i.e., were recorded as either present or absent) compares community similarity in the context of shared ASVs only. There were significant differences between tilled and no-tilled communities for abundance weighted data (pseudo-$F_{(1, 9)} = 2.258$, $p < 0.05$), where the R^2 value explained 22 % of the variation. The strength of this grouping increased when data was presence-absence transformed (pseudo-$F_{(1, 9)} = 1.773$, $p < 0.05$), where the R^2 value explained 18 % of this variation. The abundance-weighted data (Figure 3.7A) and presence-absence transformed data (Figure 3.7B) both indicated that the tilled soil was distinct from the no-till soil (see Appendix V, Table 6.13 & Table 6.14).

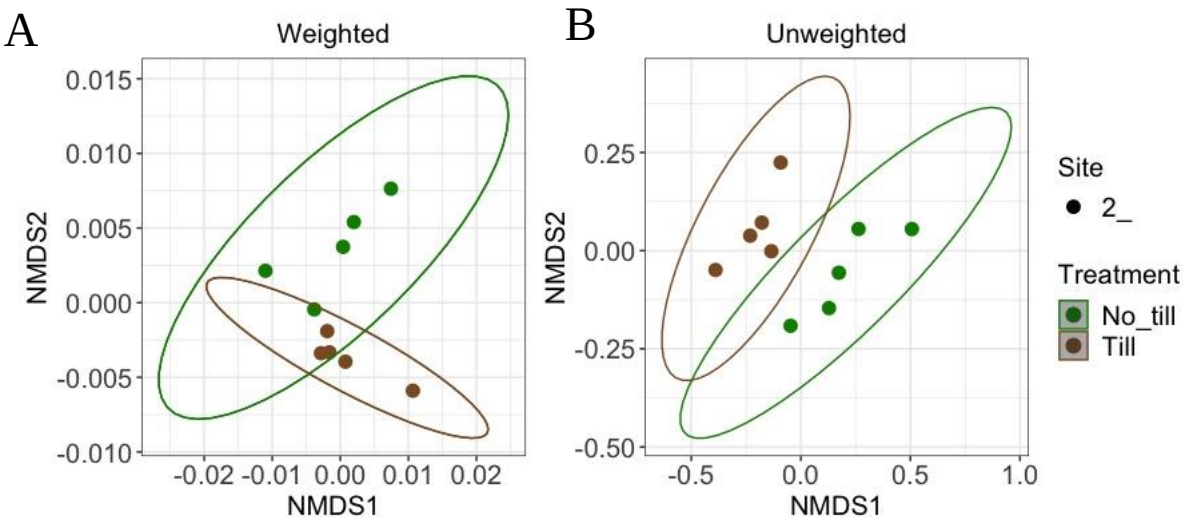

Figure 3.7. Non-metric multidimensional scaling (NMDS) ordinations of bacterial communities using the Unifrac dissimilarity metric under tilled treatments.Ordinations are of A) abundance-weighted community data and B) presence-absence transformed community data. 2-dimensional stress 0.029 (A) and 0.055 (B) (< 0.2). Ellipses with a 95% confidence interval.

3.2.3.2. Tilling altered the fungal communities

To investigate the similarities in fungal community structure between the tilled and no-tilled treatments, community β–diversity was analysed using PERMANOVA on Unifrac distance matrices. The tilled treatments NMDS ordinations had stress values of 0.105 (Figure 3.8A) and 0.042 (Figure 3.8B) and thus, can be considered an accurate representation of community relationships. Using ANOSIM showed a significant level of grouping between the treatments for weighted fungal community data ($R = 0.64$, $p < 0.05$). When the data was binary transformed, the grouping due to treatment increased ($R = 0.72$, $p < 0.05$), indicating dissimilarity of fungal ASVs between the treatments.

The differences of community structure between the two treatments were statistically tested via PERMANOVA. There was significant grouping between the habitats for abundance weighted data (pseudo-$F_{(1, 9)} = 2.861$, $p < 0.05$), where the R^2 value (0.263) explained 26 % of the variation. The strength of this grouping decreased when data was presence-absence transformed (pseudo-$F_{(1,9)} = 2.006$, $p < 0.01$), where the R^2 value (0.20) explained 20 % of this variation. The abundance-weighted data (Figure 3.8A) and presence-absence transformed data (Figure 3.8B) both indicated that the tilled soil was distinct from the no-till soil (see Appendix V, Table 6.15 & Table 6.16).

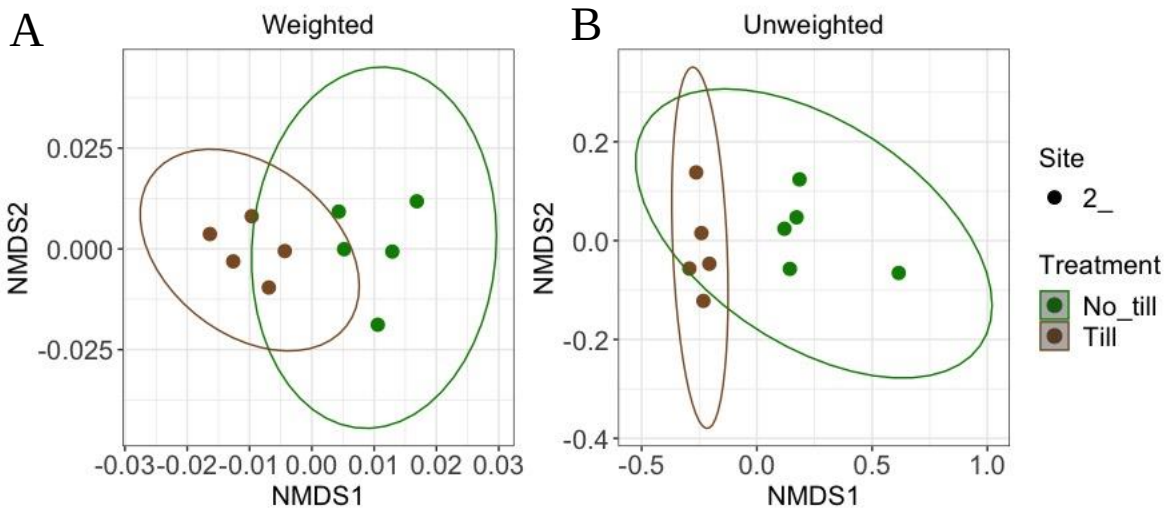

Figure 3.8. Non-metric multidimensional scaling (NMDS) ordinations of fungal communities using the Unifrac dissimilarity metric under tilled treatments.Ordinations are of A) abundance-weighted community data and B) presence-absence transformed community data. 2-dimensional stress 0.105 (A) and 0.042 (B) (< 0.2). Ellipses with a 95% confidence interval.

3.2.4. COMMUNITY ANALYSIS: Burning treatments β-diversity ordination

To examine the effect of burning of crop residues on the bacterial and fungal communities, β–diversity ordinations were produced. Due to influence of site on the microbial communities, only the burnt paddocks and the unburnt patches within the same paddock were statistically reported. This means the completely no-burn paddocks were excluded from analysis (see Appendix VII, Figure 6.3 & Figure 6.4 for ordinations with all sites).

3.2.4.1. Burning altered the bacterial communities

The similarity in bacterial community structure between the burn treatments was investigated through β–diversity using PERMANOVA tests on NMDS ordinations. Stress values of 0.1 (Figure 3.9A) and 0.14 (Figure 3.9B) are considered a fair representation.

The differences of community structure between the two treatments were statistically tested via PERMANOVA. There was a significant effect of site and treatment on the community composition between the treatments for abundance weighted data (pseudo-$F_{(1, 29)} = 3.1$, $p < 0.05$). Location remained a strong significant factor influence on community structure when data was binary transformed. However, the amount a variance explained, and the significance of burning effect on bacterial communities, was reduced when data was presence-absence transformed (pseudo-$F_{(1,29)} = 1.35$, $p < 0.05$). This data highlights the major impact of location on bacterial community composition and indicates that burning had an impact on both the abundances of ASVs and the suites of ASVs present (see Appendix VI, Table 6.17 & Table 6.18).

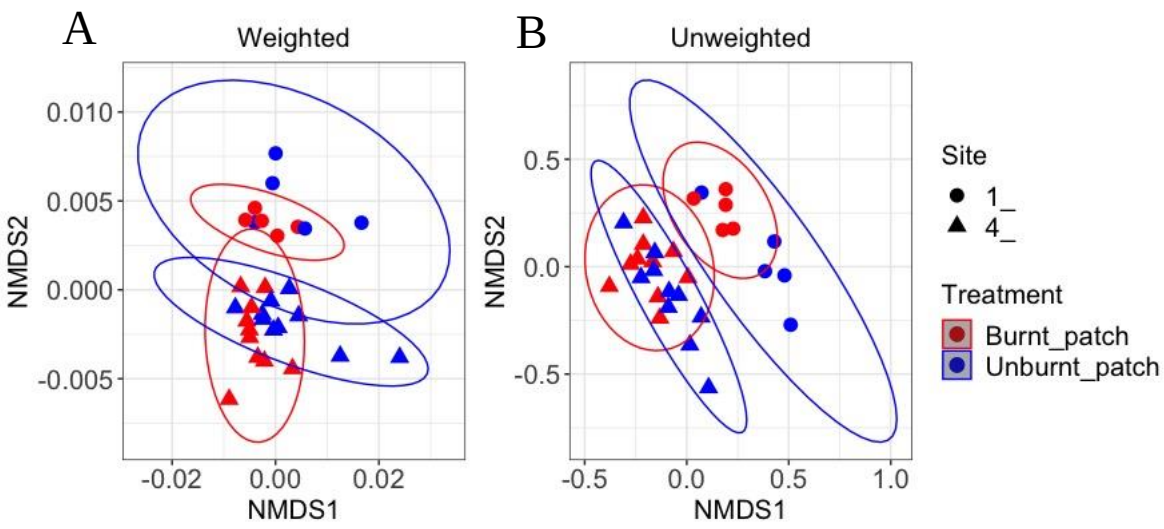

Figure 3.9. Non-metric multidimensional scaling (NMDS) ordinations of bacterial communities using the Unifrac dissimilarity metric under burn treatments.Ordinations are of A) abundance-weighted community data and B) presence-absence transformed community data. 2-dimensional stress 0.1(A) and 0.14(B) (< 0.2). Ellipses with a 95% confidence interval.

3.2.4.2. Burning did not alter the fungal communities

The similarity in fungal community structure between the burn treatments was investigated through β-diversity using PERMANOVA tests on non-metric multidimensional scaling (NMDS) ordinations. Stress values of 0.167 (Figure 3.10A) and 0.155 (Figure 3.10B) are considered a fair representation. The differences of community structure between the two treatments were statistically tested via PERMANOVA. In contrast to bacterial communities, PERMANOVA reveal no significant impact of burning on fungal community structure. However, as with bacterial communities', location was a significant main effect for both weighted and unweighted community data (see Appendix VI, Table 6.19 & Table 6.20).

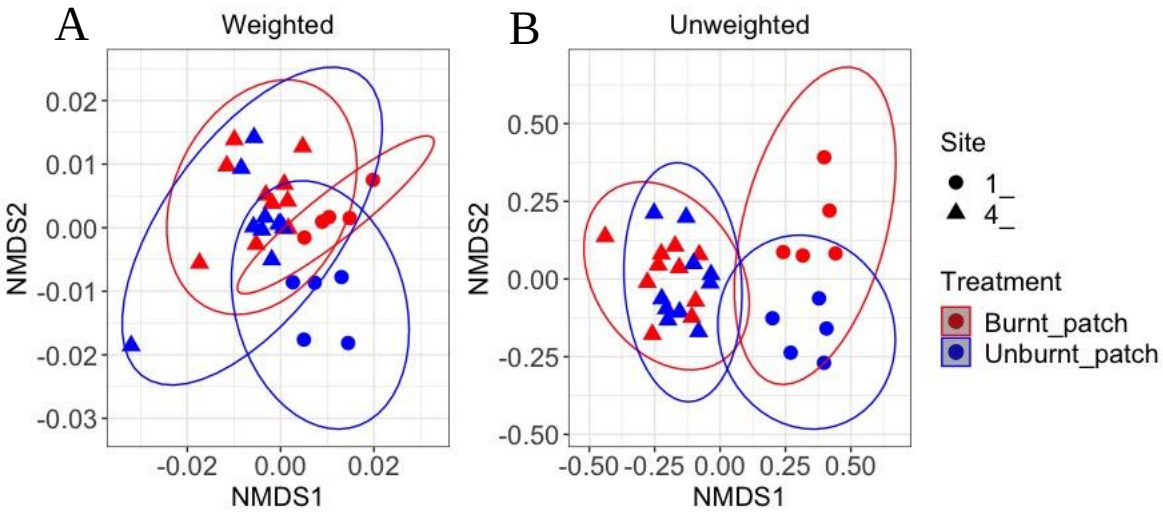

Figure 3.10. Non-metric multidimensional scaling (NMDS) ordinations of fungal communities using the Unifrac dissimilarity metric under burn treatments.Ordinations are of A) abundance-weighted community data and B) presence-absence transformed community data. 2-dimensional stress 0.167 (A) and 0.155 (B) (< 0.2). Ellipses with a 95% confidence interval.

3.2.5. BACTERIAL FUNCTIONAL ANALYSES: Predicted bacterial functional pathways β-diversity ordination

To investigate the similarities in predicted bacterial functional pathways between the treatments, β-diversity analysis was carried out using PERMANOVA tests on Bray Curtis distance matrices. PERMANOVA on abundance-weighted data compares community similarity in the context of shared pathways and the similarity of their respective abundances. PERMANOVA on unweighted data, in which pathway abundances were binary transformed (i.e., were either recorded present or absent) compares community similarity in the context of shared pathways only.

3.2.5.1. Tilling altered the functional profiles of bacterial communities

The tilled treatments NMDS ordinations had stress values of 0.021 (Figure 3.11A) and 0.063 (Figure 3.11B) and thus, can be considered an accurate representation of functional relationships between bacterial communities. The ordination indicated that there was a clear separation due to treatment and this was supported by the ANOSIM, where there was a significant level of grouping between the treatments for weighted pathways data ($R = 0.684$, $p < 0.05$). When the data was binary transformed, the grouping due to treatment increased ($R = 0.742$, $p < 0.05$), indicating dissimilarity of bacterial functional pathways between the treatments.

The differences of functional relationships between the two treatments were statistically tested via PERMANOVA. There was significant grouping between the habitats for abundance weighted data (pseudo-$F_{(1, 9)} = 6.147$, $p < 0.05$), where the R^2 value (0.435) explained 44 % of the variation. The strength of this grouping increased when data was presence-absence transformed (pseudo-$F_{(1,9)} = 8.107$, $p < 0.05$), where the R^2 value (0.503) explained 50 % of this variation. The abundance-weighted data (Figure 3.11A) and presence-absence transformed data (Figure 3.11B) both indicated that the tilled soil had distinct predicted functional pathways from the no-till soil (see Appendix VII, Table 6.21 & Table 6.22).

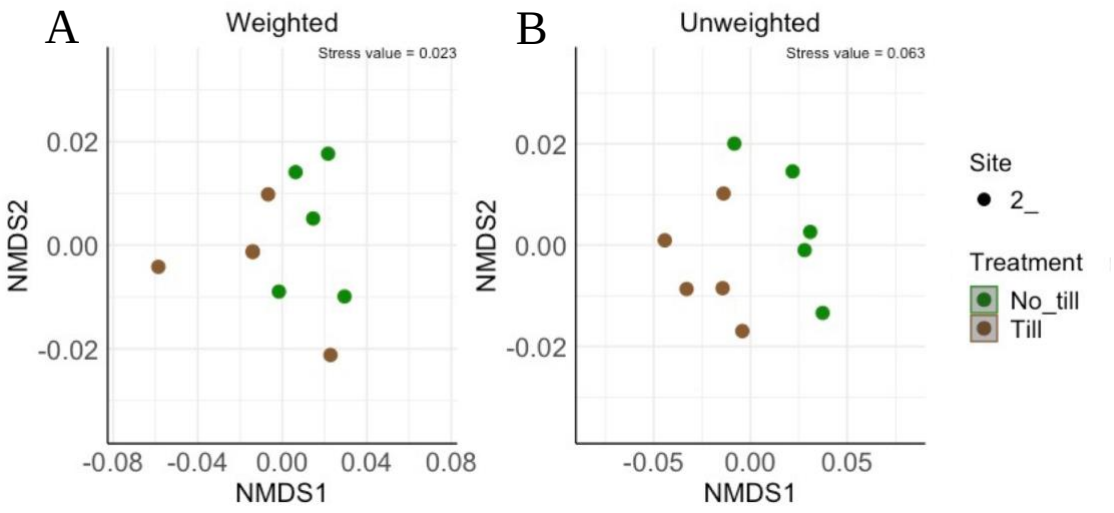

Figure 3.11. Non-metric multidimensional scaling (NMDS) ordinations of predicted functional potential of bacterial communities under tilled treatments using the Bray Curtis metric. Ordinations are of A) abundance-weighted pathways and B) presence-absence transformed pathways data. 2-dimensional stress 0.021 (A) and 0.063 (B) (< 0.2).

3.2.5.2. Burning did not alter the functional profiles of bacterial communities

Only Figure 3.12A can be considered an accurate representation of functional relationships between bacterial communities for burn treatments as the NMDS ordination had a stress value of 0.128. Whereas Figure 3.12B had a stress value of 0.2 and therefore, cannot be considered an accurate representation. The differences of community structure between the two treatments were statistically tested via PERMANOVA. The PERMANOVA revealed no significant impact of burning on the bacterial functional pathways however, location was a significant main effect for both weighted and unweighted data (see Appendix VII, Table 6.23 & Table 6.24). There is a separation due to site on the x-axis, and there is separation due to treatment on the y-axis however, as the PERMANOVA indicated, while the site effect was significant the impact of burning was not.

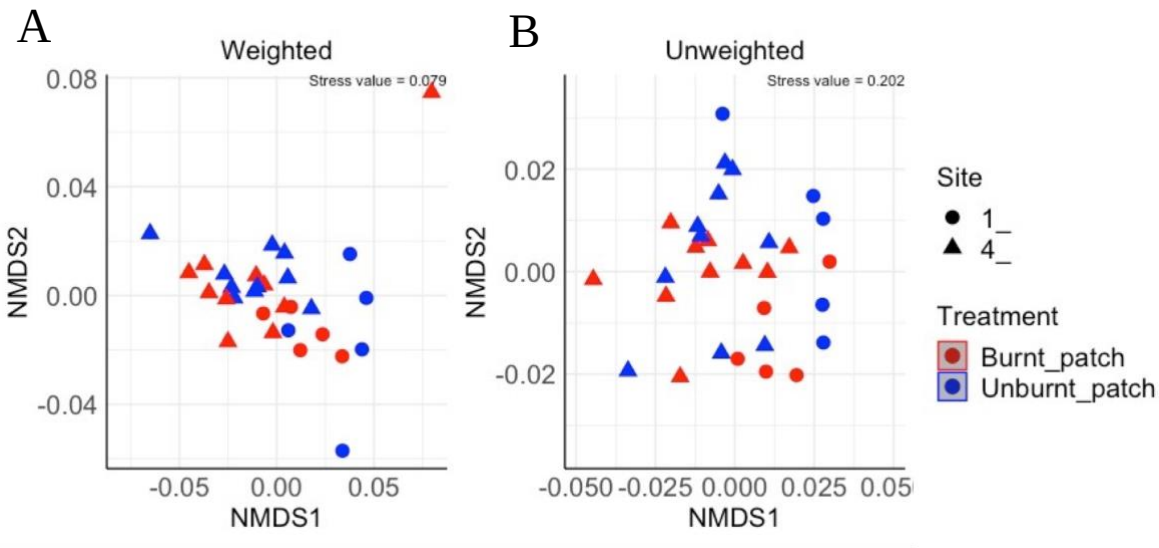

Figure 3.12. Non-metric multidimensional scaling (NMDS) ordinations of predicted functional potential of bacterial communities under burn treatments using the Bray Curtis metric. Ordinations are of A) abundance-weighted community pathways and B) presence-absence transformed pathways data. 2-dimensional stress 0.128 (A) and 0.2 (B) (< 0.2).

3.2.6. DIFFERENTIAL ABUNDANCE TEST: Bacterial functional pathways

A differential abundance test was performed on bacterial functional pathways data to compare two different treatments. Where one treatment is the control, then the other is being compared to this control. As per previous analysis, only site two (where a direct comparison between a no-till and tilled paddock exists) was statistically analysed for tilled data. Additionally, only the burnt paddocks and the unburnt patches within the same paddock were statistically reported. This means the completely no-burn paddocks were excluded from analysis.

3.2.6.1. Bacterial functional pathways significantly changed from tillage

The bacterial functional pathways with a significant level of change between the no-tilled and tilled paddocks were graphically represented in Figure 3.13. There were 25 different pathways that have a significantly (adj $p < 0.05$) increased (blue) or decreased (red) abundance when comparing the completely untilled paddock to a tilled paddock (see Appendix VIII, Table 6.25).

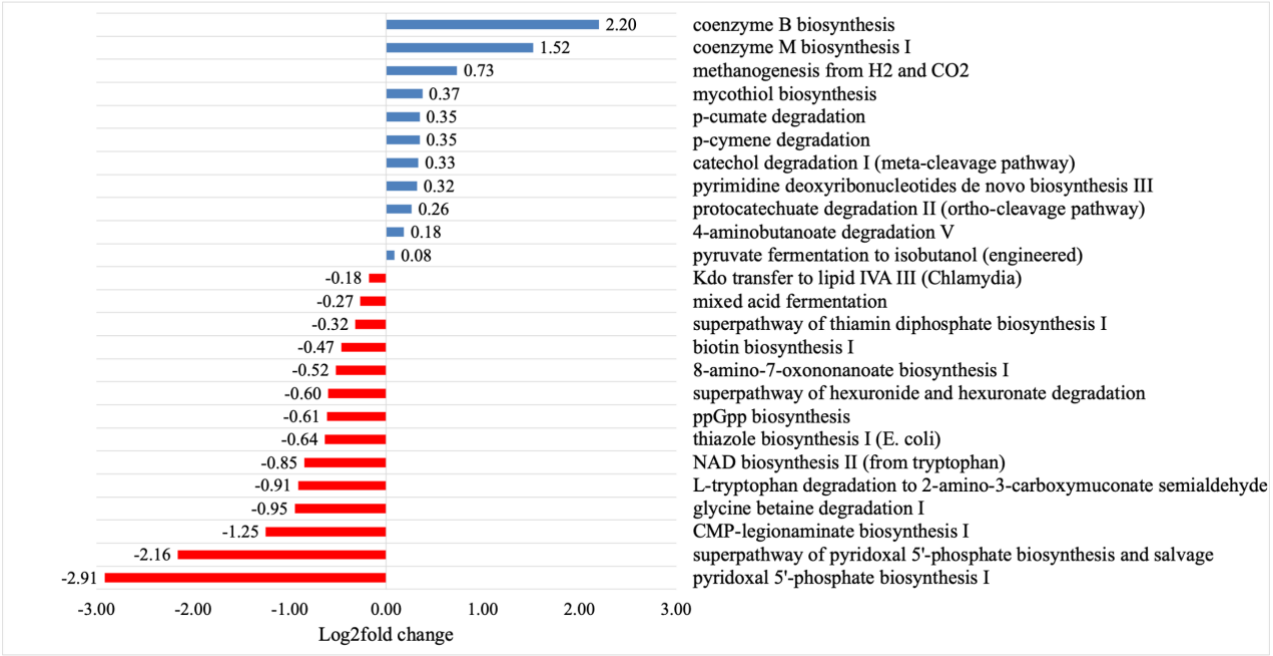

Figure 3.13. Log_2foldchange for site two: no-till and tilled paddock for bacterial functional pathways data.Only pathways with a significant ($p < 0.05$) change shown. Blue= increased with tillage. Red= decreased in tillage. Pathways with a baseMean < 250 removed.

3.2.6.2. No bacterial functional pathways were significantly changed from burning

There were no bacterial functional pathways that significantly changed in abundance from the unburnt patches to the burnt patches.

4.0 DISCUSSION

Fungi and bacteria play many important roles for soil health, including organic matter deposition and enhancing soil structure [13, 94]. Due to an increasing number of microbial focused studies in the last decade, it is understood that an imbalance in the microbial soil community results in detrimental effects on plant growth, especially for growers using the land for agricultural purposes.

Whilst the use of soil microbiota as bioindicators of soil health has been widely discussed, there is no consensus in the literature as to which microbial parameter is best, or how management practices affect soil health as measured by microbial parameters. In this study, the ability of four parameters of the microbial community (microbial biomass carbon (MBC), microbial activity, taxonomic and functional diversity) to behave as bioindicators were assessed. A successful bioindicator was considered one that exhibited a repeatable and predictable response to agricultural management practices. As will be discussed, the findings of the present meta-analysis partially support the hypothesis that the four microbial community parameters would respond in a repeatable way to four agricultural management practices (tilling of soil, burning of crop residues, application of fertiliser and crop rotation). It is suggested that more research needs to be conducted on agricultural management practices and how they affect the four soil community parameters used as potential bioindicators of soil health.

Tilling the soil and burning of crop residues both have conflicting views in the literature as to whether they are beneficial for soil health. Moreover, there is a fundamental lack of research regarding the effect of these two management practices on the functional diversity of the soil microbial community. To investigate this, we examined whether tilling of pasture paddocks and burning of wheat stubble had an effect on soil community function using a predictive functional profiling approach. It was hypothesised that both tilling the soil and burning of crop residues would influence the bacterial and fungal taxonomic diversity, and bacterial functional diversity (an assessment of fungal diversity was beyond the scope of this study). This revealed changes in the microbial communities between the tilling and burning treatments.

Studying the functional and taxonomic diversity allowed the assessment of what functions the microbial communities are providing. For example, microbes with the ability to fix

nitrogen are important for plant life, it can be assumed that changes in the abundances of these microbes would affect the environment in which they are located. As highlighted in Table 1.1, the microbial communities, and specific microbes, play very important roles in the processes that underlie soil health. Due to functional redundancy, i.e., many taxa being able to carry out similar functions, a change in the microbial communities' taxonomic structure may or may not result in changes to the community functions performed [33, 95].

4.1. The implementation of bioindicators in assessing soil health (a meta-analysis)

A meta-analysis was performed to determine whether different soil microbial community parameters were accurate bioindicators of soil health. This meta-analysis examined the results of 44 individual studies and aimed to determine whether the four microbial community parameters had the ability to be an accurate bioindicator, by responding in a repeatable way to different agricultural management practices. A variety of analytical tools were used across the studies (Figure 3.1A), which were categorised into one of the four soil microbial community parameters: MBC, microbial activity, taxonomic diversity or functional diversity (Figure 3.1B). It is important to note that many of these studies used only one of these parameters (Figure 3.1C). For future studies, it would be recommended to use more than one parameter, to be able to accurately determine which of the four soil microbial parameters is best under the same experimental conditions, which is a limitation of this meta-analysis.

4.1.1. The potential bioindicators of soil health generally respond in a repeatable way to management

The results of whether a soil microbial parameter responded in a repeatable way to the four management practices (no-tillage, stubble retainment, application of fertiliser, and crop rotation) was assessed. These four management practices are typically known to increase soil health. In general, where a significant trend could be detected, bioindicators of soil health were increasing in response to management practices (Figure 3.3). It is possible that the four microbial parameters investigated do not make a suitable bioindicator however, in many cases there was not enough studies (statistical power) to determine significance.

Despite most indicators responding in a repeatable way, the study of MBC highlighted instances where statistical power was present but no trend in response to management could be detected (Figure 3.3). This was unexpected, as the general conclusion in the literature is that tillage causes a reduction in MBC. Interestingly, the response of MBC to tilling and fertiliser application was highly variable [46, 65, 96-99]. However, significantly ($p < 0.05$) more studies reported that no-tillage increased microbial activity, taxonomic diversity and functional diversity (Figure 3.3). These findings support the theory that tilling will negatively impact the soil community. The variable response of MBC to tilling may reflect differences in the soil type, or the microbial community's response to tilling. For example, in a community adapted to a temperate environment, such as a pasture paddock, the change

in nutrients from tilling could stimulate the community. It is also known that the turnover of soil from tilling breaks up aggregates and changes the microenvironments from anaerobic to aerobic. Strict anaerobic organisms consequently die, which is likely why MBC decreases. Due to tillage being the most disruptive management practice, incorporating no-till is important to enhance the soil health, as investigated through the four potential bioindicators.

Burning of crop residues does have opposing results in the literature where many studies reported it increased MBC, while another reported that it has no significant effect on MBC (Figure 3.3A). The meta-analysis found that, with the respect to the retention of crop residues (i.e., no burning), three of the microbial community parameters, microbial activity, taxonomic diversity or functional diversity, had no repeatable trends across the literature. Interestingly, studies either reported that stubble retention increased, or had no significance for each of the microbial community parameters, suggesting that this management practice does not have a negative effect on the soil health. Although these results highlight that there is no consensus in the literature on whether burning of crop residues affects the microbial communities, this result may be due to a low replication of studies, where three of the parameters did not have enough power for significance. This signifies stubble retention may increase the bioindicators, but more studies were needed. Retaining crop residues enhances the chemical and physical properties of the soil, such as soil basal respiration and water stability [11, 100]. Furthermore, a forest fire can cause a significant decrease and changes to the composition of microbial communities [101]. Hence, it is noteworthy, that no study investigated reported a decrease in any of the microbial parameters. However, forest fires have greater heat transfer to the soil than controlled grassland fires and thus, the heat from the fire may not contribute as much to altering the communities [101].

The application of fertiliser has been described as critical for plant growth and reproduction in agricultural landscapes [102]. However, previous studies have reported that fertiliser can temporarily inhibit microbial growth, subsequently altering the microbial community composition [66]. This can be seen in the results illustrated in Figure 3.3A, which shows 80 % of the studies reported either a decrease or no significant effect, indicating an inhibition of microbial growth. Figure 3.3C shows that all seven studies reported that fertiliser increased the taxonomic diversity, which mean the microbial community composition was altered. Allison *et al.*, [103] found that the composition of a microbial

community is directly influenced by chemical fertilisers (e.g. NPKS) and hence, the application of fertiliser is possibly altering the taxonomy of the microbes and their associated C sequestering functions [104]. Implementing management practices that improve MBC has been suggested previously because an increase in this microbial community parameter implies an increase in microorganisms and hence, an increase in soil health [105]. The different microbes within an existing microbial community will be affected differently by fertiliser application. It is clear that fertiliser enhances the taxonomic diversity of microbes (Figure 3.3C) however, many taxa perform the same functions, and this could explain the lack of significant increases in functional diversity (Figure 3.3D). More research needs to be conducted as to whether fertiliser is a sustainable management practice.

The scientific literature describes crop rotation as one of the best management practices as it consistently increases the different soil community parameters by suppling a diversity of nutrient and carbon sources to the soil [21, 43, 49, 57]. This was supported in this meta-analysis where significantly more studies reported that MBC, microbial activity and functional diversity increased due to crop rotation (Figure 3.3). In contrast to the hypothesised beneficial effect of crop rotation documented in the literature (Table 1.3), this meta-analysis found that 20 % of studies reporting that there was a decreased or no significant effect of crop rotation on taxonomic diversity. It is likely that the choice of crop species being rotated influences the effect that crop rotation has on taxonomic diversity [106]. Crucially, whether this change in the abundance of microbes' present is good or bad for soil health is yet to be determined and is a key area for future work.

An oversight to this study was that it was only considered that an increase in the soil microbial parameters improved soil health. Reese *et al.*, [107] reported that diversity of communities is not an inherit feature of microbial communities, implying that an increase in diversity is not always better. It needs to be considered that an increase in taxonomic and functional diversity cannot be inferred as an increase in soil health.

4.1.2. The lack of a healthy soil benchmark impedes the use of bioindicators soil of health

The discussion surrounding how soil health should be measured is complex. An identified gap in the literature is that although the soil microbial community parameters have been investigated, it is difficult to obtain a benchmark result of 'what is healthy?' from the four microbial community parameters used as bioindicators of soil health. Furthermore, there are many advantages and disadvantages of the four categorised microbial community properties utilised in bioindicator analyses, as illustrated in Table 1.2. As interpreted from Figure 3.2, the use of functional diversity as a parameter of soil health has increased in recent years. Functional diversity has been previously described to be a great potential bioindicator however, there are no benchmarks identified for a study to compare their results [108-110]. Due to the lack of a benchmark for 'healthy soils' many studies stated what their results were but could provide little explanation as to the outcomes for soil health and very few studies were confident enough to state whether the soil assessed was "healthy" [43, 47].

As functional diversity has increased in use, MBC is decreasing (Figure 3.2). This decline may be because the technique of MBC does not answer the question of the identity of the individual microbes (Table 1.2). The lack of detailed information and sensitivity that is offered by MBC and microbial activity could be contributing to this decline. Further studies are required as different soil types and the time of year sampling occurred are known variables to affect results [44, 111, 112]. In addition, these published studies were conducted at varying times after a management practice was performed, which would have affected the outcome. For instance, the management practice of tilling the soil, where the soil surface is initially completely disturbed, has different effects at different weeks post-tillage [113].

The results of the present meta-analysis led to the conclusion that further studies are required to confirm these findings, due to the limited number of studies available for taxonomic and functional diversity. Further studies need to be conducted to determine whether these four microbial community parameters are accurate bioindicators of soil health. More studies have used MBC as a potential bioindicator for soil health compared to taxonomic or functional diversity. Although many studies use MBC, both no-tillage and

application of fertiliser did not have repeatability in the literature, hence, may imply that MBC cannot be used as an accurate bioindicator.

4.2. The influence of agricultural management practices on soil microbial communities (a field study)

Analysis of bacterial and fungal community structure through 16S rRNA and ITS amplicon sequencing was performed to determine whether the two agricultural management practices, tilling the soil and burning of crop residues, had an effect on the soil microbial community. As well as this, PICRUSt was incorporated to analyse the predicted bacterial functional pathways. The overall question of the study was whether there was a functional diversity effect of the management practices, as well as a taxonomic influence.

4.2.1. Environment and location affect the soil microbial community structure

There are many ways to assess soil chemistry, such as using pH or oxygen availability [114, 115]. There was a significant influence of pH on the soil microbial communities and therefore, it is likely that the soil chemistry also had an effect on the results of the meta-analysis. Soil chemistry and substrate availability are known to affect the spatial variation of microbial communities [116, 117]. Microbial activity can be significantly affected by the availability of some substrates, particularly C [117].

The RDAs produced show the effect of pH, soil moisture (SM) content and location on the soil microbial communities. Both the bacterial (Figure 3.4A) and fungal (Figure 3.4B) communities were significantly influenced by the pH and site. The effect of location on microbial communities has been reported substantially in the literature. The reason being is that there are multiple environmental variables, including pH and SM, that change between different locations. pH has been reported to be the primary driver in modulating bacterial communities however, exhibiting little effects on fungal communities and hence, pH was expected to have a significant effect [118, 119]. Furthermore, the Goulburn region sampled from for this field study is classified as a medium rainfall area (400 to 600 mm per year), where dry and wet seasons occur. Because differences in SM can impact the microbial community, it is suggested that future studies consider this and samples are taken at more than one time point and various times of year [71]. For future research into the impact of stubble burning, samples should be taken from the same paddocks prior to and post burning to minimise the impact of location on microbial analyses. This would have allowed a direct comparison between the burn treatments. In this study, due to the observed

impact of different environmental conditions at locations, statistical analysis that only including treatments from the same site was justified.

4.2.2. The effect of tilling the soil on the soil microbial communities

Tillage has been reported to change the microbial community structure, but their associated functions have not been examined. More importantly, if certain species are changing, it is possible they are being replaced by microbes with similar functions [33, 95]. The differences in the composition of the soil microbial communities due to the effect of tillage was investigated through β-diversity. This revealed that the tilled paddock had different bacterial and fungal communities to the no-till pasture paddock. The significant differences observed for abundance weighted data highlights that the bacterial communities did not have shared dominant ASVs between the tilled treatments, and this trend can also be seen for the fungal data (Figure 3.7A & Figure 3.8A). This is likely a result of the physical destruction of soil from tilling by altering the microenvironments and subsequently the dominate ASVs within a community. The significant grouping for both treatments for the bacterial and fungal communities (Figure 3.7B & Figure 3.8B respectively) indicates that the genetic pool and taxonomies of microbes was different, possibly implying differences in functional potentials between the treatments. To elaborate on this, the PICRUSt data, which uses the 16S rRNA bacterial data, did in fact show the same significance, where the no-till and tilled paddock had distinct pathways (Figure 3.11). This implies that the functional potentials of the microbes between the tilled and no-till paddock was different. To understand the implications for soil health, we further investigated which functions were altered.

To further investigate the functional differences between the tilled treatments, differential abundance tests were performed. The effect of tillage resulted in an increased abundance of 11 different pathways (Figure 3.13). The top three pathways that increased due to tillage were related to the production of methane. This form of microbial metabolism was possibly increased due to the incorporation of manure into the soil [120]. Methanogens live within the gut of livestock and therefore, it is possible that the fecal matter deposited contained DNA from methanogens that was consequently detected [120]. Interestingly, many of the pathways that decreased due to tillage were in relation to the production of energy. The disruptive management practice of tilling, where the physical turnover of soil occurs, has potentially impeded the ability of the bacterial community to carry out essential functions

[6]. Smith *et al.,* [6] found that no-till soils had a higher microbial activity with distinct microbial communities. This study however, did not analyse the specific pathways associated with functional diversity, highlighting that a deeper understanding of the functions changing between microbial communities is needed.

Microbial communities not only have altered taxa between environments, but their functions also change. This suggests that specific functions are selected by the environment, and in relation to this study by management practice [121]. These analyses indicate that the microbial communities and their associated functions are changing with tillage.

4.2.3. The effect of burning of crop residues on the soil microbial communities

Burning of crop residues only had significance in the previous meta-analysis with decreasing MBC, outlining that there is no determined effect on microbial communities. In relation to functional diversity, only two studies had been reported (Figure 3.3D). The differences in the composition of the soil microbial communities due to the effect of burning crop residues was firstly investigated through β-diversity. The significance for Figure 3.9A highlights that the bacterial communities do not have shared dominant ASVs between the burnt paddock and unburnt patches within. Similar to the tilled data, the burn treatments bacterial communities (Figure 3.9B) indicate differences in the functional potentials. Neither of these trends are continued for the fungal data, where there was no significance due to treatment (Figure 3.10). Interestingly, there was no significance in the bacterial functional pathways changing between the burn treatments (Figure 3.12). To further investigate the functional differences between the burn treatments, differential abundance tests were performed. The effect of burning resulted in no bacterial functional pathways significantly changing in abundance. This is an important finding, known as functional redundancy, where the bacterial community has a taxonomic change but no functional change.

The significance for location as a main effect on burning data for both bacteria and fungi was expected, as suggested by the RDA ordination (Figure 3.4). The lack of a treatment effect could also be caused by the field design, where unburnt patches were taken from a burnt paddock. The dripping torch used to deposit the flaming fuel for burning would have been applied to the whole paddock evenly and hence, could have affected both the burnt

and unburnt patches. As well as this, the heat from the surrounding fire, despite being "low-burn", could have affected the unburnt patches.

4.3. Implications for agricultural management and research

The results of this meta-analysis highlighted the lack of studies investigating the impact of agricultural management practices on the microbial community. Majority of the studies that investigated no-tillage, reported that it significantly increased taxonomic and functional diversity (Figure 3.3). However, the studies that investigated the retention of stubble (i.e., no burning), reported non-significant results for both taxonomic and functional diversity. Further study on the effect of stubble retention would be suggested, as there were only three studies investigating taxonomic diversity and two studies investigating functional diversity to date. The lack of clarity surrounding the assessment of soil health in agricultural landscapes could lead to poorly interpreted data, with the limited studies available for reference.

Both taxonomic and functional diversity had a significant number of studies reporting increases in the measurements, as reported by the meta-analysis. The field-study concluded that both measurements responded to tilling however, this change in functional diversity resulted in a possible loss of some key soil functions. This further demonstrates that these measurements cannot be used and reported as "increases" or "decreases" of soil health. Due to the diversity of microbes, key functions within a microbial community, and not the overall community themselves, should be used as future measurements.

4.4. Conclusions and future perspectives

The cumulative ASV curves showed that for both the tilled and burn treatments (Figure 3.5 & Figure 3.6 respectively) sampling was not sufficient in capturing the diversity, and each treatment was a continuous community; meaning there was a gradual increase in diversity. These curves, if paddocks were sampled adequately, would have plateaued between each new paddock added (known as a discrete community). To improve the current field study, a larger sample size is suggested. The original sample design, as described by Biomes of Australian Soil Environments (BASE), included 25 samples per paddock [122]. This sample design would have allowed a larger replication and would have captured the diversity of the paddocks better. Furthermore, soil type, plant species and livestock were all fluctuating between the paddocks, and these would have been confounding variables. It can be suggested, that combining future field studies, with potted glasshouse trials could aid in fixing this issue.

Other aspects of soil chemistry, on top of pH and soil moisture, should have also been considered in the field study. Soil chemistry is suggested to be incorporated into all future research of the potential bioindicators to know whether it is affecting the results, as some studies were investigating it, and others were not.

Further investigation into whether an increase in taxonomic or functional diversity a sign for good soil health is suggested. Functional diversity needs to be considered with taxonomic diversity, as simply looking at what is in the soil is not adequate to say a microbe is important for soil health. A benchmark for soil health is difficult to achieve. The chemical, physical and biological aspects of soil health all need to be considered. Microbes occupy specific niches and therefore, are able to provide a sensitive measure of soil health. The emerging technique of functional diversity through techniques such as shotgun sequencing often comes with a high cost that makes it inaccessible for some studies.

More robust testing needs to be implemented. Instead of using amplicon sequencing and PICRUSt, a database that relies on predicting functional pathways from the 16S rRNA gene, a technique such as whole genome shotgun sequencing is recommended [90]. Whole genome shotgun sequencing allows all genes from all organisms to be comprehensively sampled however, it is more expensive [123]. Fungi have a prominent role in decomposition, as well as large roles in nitrogen fixation, see Table 1.1 for more

information. In this study, the functional diversity of fungi was not studied. Using shotgun sequencing to identify fungal functions has rarely been done and is a suggested area for future research [124]. For the current field study, it is suggested to incorporate FUNGuild, a tool that takes the taxonomic data and places them into functional guilds [125]. This database does not use metabolic pathways like PICRUSt, instead it is manually curated into trophic guilds. However, FUNGuild allows the assessment of fungi functions, with accuracy unattainable with the current bioinformatics pipeline [125]. Despite both resources being available, they do not allow the functional evaluation of the whole microbial community. It is possible for enzyme assays to assess the functional aspects of both bacterial and fungal communities at the same time and hence, is an area suggested for research. German *et al.*, [126] concluded that despite enzymes being used for many years, most researchers were not properly assessing the potential enzyme activity due to failing to optimise the protocol for their study site.

The findings of the meta-analysis led to the suggestion that more research needs to be conducted on the four soil community parameters (MBC, microbial activity, taxonomic diversity, and functional diversity). This will allow the knowledge of whether they have the ability to behave as bioindicators of soil health in agricultural landscapes. The data obtained from this research will provide a greater understanding of the bacterial and fungal communities within an agricultural landscape. This research will assist the Australian agricultural sector in identifying microbes associated with soil health.

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6.0 APPENDIX

APPENDIX I – Coordinates of locations

Table 6.1. Coordinates of the four different locations

Site	Coordinate
1	-36.61795498601183 S, 145.28756430307274 E
2	-36.64167901596458 S, 145.44937921070922 E
3	-36.612294223836834 S, 145.30373222312392 E
4	-36.707872188054935 S, 145.23377242639054 E

APPENDIX II – pH & Soil Moisture (SM) raw data

The raw measurements of the pH and soil moisture (SM) are as shown in Table 6.2.

Table 6.2. Raw pH & SM (%) measurements. W= wheat, P= pasture, BB= burnt patch, BU= unburnt patch, NB= non-burn, T= till and NT= no-till.

Sample ID	Sample code	pH	SM (%)	Sample ID	Sample code	pH	SM (%)
DB1	W-NB	5.92	9.573	**MR11**	W-BU-1B	4.40	5.963
DB2	W-NB	4.60	6.932	**MR12**	W-BU-2B	5.38	7.763
DB3	W-NB	4.42	5.840	**MR13**	W-BU-3B	6.50	4.541
DB4	W-NB	4.63	6.588	**MR14**	W-BU-4B	5.20	7.162
DB5	W-NB	4.15	4.104	**MR15**	W-BU-5B	5.06	6.167
DB6	P-NT	5.32	5.673	**MR16**	W-BB-1B	4.40	7.087
DB7	P-NT	4.90	5.832	**MR17**	W-BB-2B	5.22	5.655
DB8	P-NT	4.35	3.946	**MR18**	W-BB-3B	4.99	6.849
DB9	P-NT	4.68	7.756	**MR19**	W-BB-4B	4.92	3.922
DB10	P-NT	5.91	6.175	**MR20**	W-BB-5B	5.29	6.193
PB1	P-T	6.28	3.276	**MR21**	P-NT	4.60	6.095
PB2	P-T	7.01	4.509	**MR22**	P-NT	4.21	7.209
PB3	P-T	5.94	4.555	**MR23**	P-NT	4.79	6.697
PB4	P-T	6.09	3.212	**MR24**	P-NT	4.73	7.326
PB5	P-T	6.28	3.664	**MR25**	P-NT	4.47	6.089
PB6	P-NT	4.80	3.446	**RB1**	W-BU	4.71	6.041
PB7	P-NT	4.85	5.077	**RB2**	W-BU	4.69	3.249
PB8	P-NT	4.69	2.738	**RB3**	W-BU	4.83	5.842
PB9	P-NT	5.15	3.200	**RB4**	W-BU	4.26	3.543
PB10	P-NT	4.72	3.841	**RB5**	W-BU	5.00	5.636
MR1	W-BU-1A	5.98	4.872	**RB6**	W-BB	4.07	3.700
MR2	W-BU-2A	4.86	7.369	**RB7**	W-BB	4.30	4.600
MR3	W-BU-3A	4.88	7.477	**RB8**	W-BB	4.21	5.148
MR4	W-BU-4A	5.55	7.850	**RB9**	W-BB	4.66	3.721
MR5	W-BU-5A	4.61	5.937	**RB10**	W-BB	4.34	7.346

MR6	W-BB-1A	5.23	7.776	**RB11**	W-NB	4.47	5.748
MR7	W-BB-2A	5.18	5.498	**RB12**	W-NB	4.47	4.793
MR8	W-BB-3A	4.46	7.188	**RB13**	W-NB	4.40	4.258
MR9	W-BB-4A	5.76	7.081	**RB14**	W-NB	4.57	6.673
MR10	W-BB-5A	5.03	7.756	**RB15**	W-NB	5.04	7.156

APPENDIX III – Meta-analysis

Statement of null hypothesis and alternative hypothesis

Null hypothesis (H_o)

There will be no significance difference between the management practice increasing, decreasing or no significant effect for the microbial bioindicator technique.

Alternative hypothesis (H_1)

There will be a significance difference between the management practice increasing, decreasing or no significant effect for the microbial bioindicator technique.

Raw data and calculation of chi-squared to compare the effect of the management practices on the four different microbial indicator techniques.

Table 6.3. Raw data. Observed and expected for microbial biomass carbon and chi squared.

No-till			Stubble retained		
	Observed	Expected		Observed	Expected
Increased	7	4.333	Increased	6	2.333
Decreased	1	4.333	Decreased	0	2.333
No significant effect	5	4.333	No significant effect	1	2.333
N= 13			N= 7		
Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected} = \frac{(7 - 4.333)^2}{4.333} + \frac{(1 - 4.333)^2}{4.333} + \frac{(5 - 4.333)^2}{4.333} = 4.308$ Therefore p>0.05. Accept H_O.			Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected} = \frac{(6 - 2.333)^2}{2.333} + \frac{(0 - 2.333)^2}{2.333} + \frac{(1 - 2.333)^2}{2.333} = 8.857$ Therefore p<0.05. Reject H_O.		
Fertiliser application			Crop rotation		
	Observed	Expected		Observed	Expected
Increased	2	2.666	Increased	4	1.666
Decreased	3	2.666	Decreased	0	1.666
No significant effect	3	2.666	No significant effect	1	1.666
N= 8			N= 5		

Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected} = \frac{(2 - 2.666)^2}{2.666} + \frac{(3 - 2.666)^2}{2.666} + \frac{(3 - 2.666)^2}{2.666}$ $= 0.25$ Therefore p>0.05. Accept H_O.	Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected} = \frac{(4 - 1.666)^2}{1.666} + \frac{(0 - 1.666)^2}{1.666} + \frac{(1 - 1.666)^2}{1.666}$ $= 5.2$ Therefore p<0.05. Reject H_O.

Table 6.4. Raw data. Observed and expected for microbial activity and chi squared.

No-till			Stubble retained		
	Observed	Expected		Observed	Expected
Increased	12	5.333	Increased	3	1.666
Decreased	2	5.333	Decreased	0	1.666
No significant effect	2	5.333	No significant effect	2	1.666
N= 16			N= 5		
Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected} = \frac{(12 - 5.333)^2}{5.333} + \frac{(2 - 5.333)^2}{5.333} + \frac{(2 - 5.333)^2}{5.333}$ $= 12.5$ Therefore p<0.05. Reject H_O.			Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected} = \frac{(3 - 1.666)^2}{1.666} + \frac{(0 - 1.666)^2}{1.666} + \frac{(2 - 1.666)^2}{1.666}$ $= 2.8$ Therefore p>0.05. Accept H_O.		
Fertiliser application			Crop rotation		
	Observed	Expected		Observed	Expected
Increased	4	2	Increased	5	1.666
Decreased	0	2	Decreased	0	1.666
No significant effect	2	2	No significant effect	0	1.666
N= 6			N= 5		
Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected}$			Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected}$		

$= \frac{(4-2)^2}{2} + \frac{(0-2)^2}{2} + \frac{(2-2)^2}{2}$ $= 4$ Therefore p>0.05. Accept H_O.	$= \frac{(5-1.666)^2}{1.666} + \frac{(0-1.666)^2}{1.666} + \frac{(0-1.666)^2}{1.666}$ $= 10$ Therefore p<0.05. Reject H_O.

Table 6.5. Raw data. Observed and expected for taxonomic diversity and chi squared.

No-till			Stubble retained		
	Observed	Expected		Observed	Expected
Increased	8	3	Increased	2	1
Decreased	0	3	Decreased	0	1
No significant effect	1	3	No significant effect	1	1
N= 9			N= 3		
Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected} = \frac{(8-3)^2}{3} + \frac{(0-3)^2}{3} + \frac{(1-3)^2}{3}$ $= 12.66$ Therefore p<0.05. Reject H_O.			Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected} = \frac{(2-1)^2}{1} + \frac{(0-1)^2}{1} + \frac{(1-1)^2}{1}$ $= 2$ Therefore p>0.05. Accept H_O.		
Fertiliser application			Crop rotation		
	Observed	Expected		Observed	Expected
Increased	7	2.333	Increased	5	2.333
Decreased	0	2.333	Decreased	1	2.333
No significant effect	0	2.333	No significant effect	1	2.333
N= 7			N= 7		
Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected} = \frac{(7-2.333)^2}{2.333} + \frac{(0-2.333)^2}{2.333} + \frac{(0-2.333)^2}{2.333}$ $= 14$ Therefore p<0.05. Reject H_O.			Chi-squared $= \frac{(Observed - Expected)^2}{Expected} + \frac{(Observed - Expected)^2}{Expected} = \frac{(5-2.333)^2}{2.333} + \frac{(1-2.333)^2}{2.333} + \frac{(1-2.333)^2}{2.333}$ $= 4.57$ Therefore p>0.05. Accept H_O.		

Table 6.6. Raw data. Observed and expected for functional diversity and chi squared.

<table>
<tr><td colspan="3">No-till</td><td colspan="3">Stubble retained</td></tr>
<tr><td></td><td>Observed</td><td>Expected</td><td></td><td>Observed</td><td>Expected</td></tr>
<tr><td>Increased</td><td>7</td><td>2.666</td><td>Increased</td><td>1</td><td>0.666</td></tr>
<tr><td>Decreased</td><td>0</td><td>2.666</td><td>Decreased</td><td>0</td><td>0.666</td></tr>
<tr><td>No significant effect</td><td>1</td><td>2.666</td><td>No significant effect</td><td>1</td><td>0.666</td></tr>
<tr><td colspan="3">N= 8</td><td colspan="3">N= 2</td></tr>
<tr><td colspan="3">Chi-squared
$= \frac{(Observed - Expected)^2}{Expected}$
$+ \frac{(Observed - Expected)^2}{Expected}$
$= \frac{(7 - 2.666)^2}{2.666} + \frac{(0 - 2.666)^2}{2.666}$
$+ \frac{(1 - 2.666)^2}{2.666}$
$= 10.75$

Therefore p<0.05. Reject H_O.</td><td colspan="3">Chi-squared
$= \frac{(Observed - Expected)^2}{Expected}$
$+ \frac{(Observed - Expected)^2}{Expected}$
$= \frac{(1 - 0.666)^2}{0.666} + \frac{(0 - 0.666)^2}{0.666}$
$+ \frac{(1 - 0.666)^2}{0.666}$
$= 1$

Therefore p>0.05. Accept H_O.</td></tr>
<tr><td colspan="3">Fertiliser application</td><td colspan="3">Crop rotation</td></tr>
<tr><td></td><td>Observed</td><td>Expected</td><td></td><td>Observed</td><td>Expected</td></tr>
<tr><td>Increased</td><td>2</td><td>1</td><td>Increased</td><td>3</td><td>1</td></tr>
<tr><td>Decreased</td><td>0</td><td>1</td><td>Decreased</td><td>0</td><td>1</td></tr>
<tr><td>No significant effect</td><td>1</td><td>1</td><td>No significant effect</td><td>0</td><td>1</td></tr>
<tr><td colspan="3">N= 3</td><td colspan="3">N= 3</td></tr>
<tr><td colspan="3">Chi-squared
$= \frac{(Observed - Expected)^2}{Expected}$
$+ \frac{(Observed - Expected)^2}{Expected}$
$= \frac{(2 - 1)^2}{1} + \frac{(0 - 1)^2}{1} + \frac{(1 - 1)^2}{1}$
$= 2$

Therefore p>0.05. Accept H_O.</td><td colspan="3">Chi-squared
$= \frac{(Observed - Expected)^2}{Expected}$
$+ \frac{(Observed - Expected)^2}{Expected}$
$= \frac{(3 - 1)^2}{1} + \frac{(0 - 1)^2}{1} + \frac{(0 - 1)^2}{1}$
$= 6$

Therefore p<0.05. Reject H_O.</td></tr>
</table>

Table of references for meta-analysis

A total of 44 references were used in the meta-analysis (Table 6.7).

Table 6.7. The 44 references included in meta-analysis and the associated bioindicators used.

Article title	Year of study	Bioindicator Used				Ref. no.
		MBC	Microbial activity	Taxonomic diversity	Functional diversity	
Soil microbial and biochemical changes associated with reduced tillage	1980	0	1	1	0	[127]
Microbial and biochemical changes induced by rotation and tillage in a soil under barley production	1993	1	1	0	0	[62]
Evaluation of soil biological properties as potential bioindicators of soil health	1995	1	1	0	0	[43]
Microbial biomass and activity in silt and sand loams after long-term shallow tillage in central Germany	1998	1	1	0	1	[128]
New quality of assessment of microbial diversity in Arable Soils using molecular and biochemical methods	1998	0	0	1	0	[129]
Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation	1998	0	0	1	1	[21]
Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization	1999	1	1	0	0	[112]
Performance of Soil Condition Indicators Across Taxonomic Groups and Land Uses	2000	1	1	0	0	[130]
Soil Enzymatic Factors Expressing the Influence of Land Use, Tillage System and Texture on Soil Biochemical Quality	2000	0	1	0	0	[47]
The structure of microbial communities in soil and the lasting impact of cultivation	2001	0	0	1	0	[131]
Variation of Microbial Communities in Soil, Rhizosphere, and Rhizoplane in Response to Crop Species, Soil Type, and Crop Development	2001	0	0	1	0	[132]
Effect of tillage and stubble management on chemical and microbiological properties and the development of suppression towards cereal root disease in soils from two sites in NSW, Australia	2002	1	0	1	0	[133]
Soil Type Is the Primary Determinant of the Composition of the Total and Active Bacterial Communities in Arable Soils	2003	0	1	1	1	[134]
DGGE-fingerprinting of arable soils shows differences in microbial	2004	0	0	1	0	[135]

community structure of conventional and organic farming systems						
Soil Microbial Community Response to Land Use Change in an Agricultural Landscape of Western Kenya.	2005	1	1	1	1	[136]
Crop productivity and soil fertility in a tropical dryland agroecosystem: impact of residue and tillage management	2005	1	0	0	0	[137]
Soil quality changes in land degradation as indicated by soil chemical, biochemical and microbiological properties in a karst area of southwest Guizhou, China.	2006	1	1	1	0	[138]
Seasonal changes in microbial function and diversity associated with stubble retention versus burning	2006	1	1	1	1	[139]
Impacts of management on soil biota in Vertosols supporting the broadacre grains industry in northern Australia	2006	1	1	0	0	[98]
The effects of stubble retention and nitrogen application on soil microbial community structure and functional gene abundance under irrigated maize	2007	1	0	1	1	[51]
Dryland plant biomass and soil carbon and nitrogen fractions on transient land as influenced by tillage and crop rotation	2007	1	0	0	0	[65]
Microbial communities and enzyme activities in soils under alternative crop rotations compared to wheat–fallow for the Central Great Plains.	2007	1	1	1	0	[49]
Soil microbial biomass, functional diversity and enzyme activity in glyphosate-resistant wheat–canola rotations under low-disturbance direct seeding and conventional tillage	2007	1	1	1	1	[99]
Microbial Indices Related to Soil Carbon as Affected by Management Practices in Arid Forest and Agricultural Ecosystems	2010	1	0	0	0	[140]
Members of soil bacterial communities sensitive to tillage and crop rotation	2010	0	0	1	0	[141]
The effects of mineral fertilizer and organic manure on soil microbial community and diversity	2010	1	0	1	1	[10]
Rhizosphere effects on soil nutrient dynamics and microbial activity in an Australian tropical lowland rainforest	2011	1	0	0	0	[142]
Tillage and manure effect on soil microbial biomass and respiration, and on enzyme activities	2012	1	1	0	0	[96]
Microbial indicators related to yield and disease and changes in soil microbial community structure with ginger farm management practices	2013	0	0	0	1	[45]
Interactive effect of nitrogen fertilizer and hydrocarbon pollution on soil biological indicators	2014	1	1	0	0	[143]

Characterisation of the soil microbial community of cultivated and uncultivated vertisol in Australia under several management regimes	2015	0	0	1	1	[144]
Impact of ecological and conventional farming systems on chemical and biological soil quality indices in a cold mountain climate in Slovakia	2015	1	1	0	0	[57]
Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: implications for soil quality.	2015	1	1	1	0	[97]
Soil physicochemical and microbiological indicators of short, medium and long term post-fire recovery in semi-arid ecosystem	2016	0	0	1	0	[44]
Strategic tillage increased the relative abundance of Acidobacteria but did not impact on overall soil microbial properties of a 19-year no-till Solonetz	2016	1	1	1	0	[46]
Microbial community responses to soil tillage and crop rotation in a corn/soybean agroecosystem	2016	0	0	0	1	[6]
Effects of long-term tillage practices on the quality of soil under winter wheat	2017	0	1	0	0	[145]
Microbial biodiversity in arable soils is affected by agricultural practices	2017	0	0	1	0	[146]
Microbial community diversity and the interaction of soil under maize growth in different cultivation techniques	2017	1	1	1	1	[147]
Microbial community structure is affected by cropping sequences and poultry litter under long-term no-tillage	2017	0	0	1	0	[111]
Variations in Soil Bacterial Community Diversity and Structures Among Different Revegetation Types in the Baishilazi Nature Reserve	2018	0	0	1	0	[148]
Fungal Genetics and Functional Diversity of Microbial Communities in the Soil under Long-Term Monoculture of Maize Using Different Cultivation Techniques	2018	0	0	1	1	[95]
Effects of different soil management practices on soil properties and microbial diversity	2018	0	1	0	1	[149]
Microbial biomass, metabolic functional diversity, and activity are affected differently by tillage disturbance and maize planting in a typical karst calcareous soil	2019	1	0	0	1	[7]

APPENDIX IV – Redundancy Analysis (RDA) Ordination

The VIFs for both bacteria and fungi RDA ordinations (Table 6.8). If a VIF is high (i.e., >10) for 1 predictor, it indicates that that predictor is highly correlated with the other predictors.

Table 6.8. Variation Inflation Factors (VIFs) for both bacterial and fungal ordinations.

	SM	pH	Site 2	Site 3	Site 4
Bacteria	1.805	1.402	2.205	1.547	2.034
Fungi	1.805	1.402	2.205	1.547	2.034

Permutational test results for bacteria and fungi (Table 6.9 & Table 6.10 respectively).

Table 6.9. Permutation test of RDA model: Bacteria ~ site × pH × SM

	Df	Variance	F	Pr(>F)	
Model	5	2379.2	1.691	0.001	***
Residual	54	15197.8			

Table 6.10. Permutation test of RDA model: Fungi ~ site × pH × SM

	Df	Inertia	F	Pr(>F)	
Model	5	730.6	1.610	0.01	**
Residual	54	4900.4			

A test similar to an ANOVA was performed to test the constraining variables for both bacteria and fungi (Table 6.11 & Table 6.12 respectively).

Table 6.11. Test of constraining variables for bacterial RDA

	Df	Variance	F	Pr(>F)	
SM	1	325.1	1.155	0.069	.
pH	1	571.5	2.030	0.001	***
Site	3	1399.3	1.657	0.001	***
Residual	54	15197.8			

Table 6.12. Test of constraining variables for fungal RDA

	Df	Variance	F	Pr(>F)	
SM	1	85.4	0.941	0.705	
pH	1	124.7	1.373	0.006	**
Site	3	416.5	1.53	0.001	***
Residual	54	4900.4			

APPENDIX V – Tilled treatments bacterial and fungal PERMANOVAs, including ordinations for all sites

Bacterial NMDS PERMANOVA tilled treatments site 2 only

The weighted and unweighted (Table 6.13 & Table 6.14 respectively) Unifrac PERMANOVAs for bacterial β-diversity for site two only.

Table 6.13. PERMANOVA for bacterial tilled treatments (only site 2) showing weighted Unifrac.Abundance-weighted community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1(Treatment)	1	0.0002	2.08E-04	2.258	0.220	0.012	*
Residuals	8	0.0007	9.21E-05	0.779			
Total	9	0.0009	1				

Table 6.14. PERMANOVA for bacterial tilled treatments (only site 2) showing unweighted Unifrac.Presence-absence community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1(Treatment)	1	0.518	0.518	1.773	0.181	0.012	*
Residuals	8	2.339	0.292	0.818			
Total	9	2.857	1				

Fungal NMDS PERMANOVA tilled treatments site 2 only

The weighted and unweighted (Table 6.15 & Table 6.16 respectively) Unifrac PERMANOVAs for fungal β-diversity for site two only.

Table 6.15. PERMANOVA for fungal tilled treatments (only site 2) showing weighted Unifrac.Abundance-weighted community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1 (Treatment)	1	0.001	9.65E-04	2.861	0.263	0.013	*
Residuals	8	0.002	3.37E-04	0.736			
Total	9	0.004	1				

Table 6.16. PERMANOVA for fungal tilled treatments (only site 2) showing unweighted Unifrac.Presence-absence community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1 (Treatment)	1	0.584	0.584	2.006	0.201	0.01	**
Residuals	8	2.330	0.291	0.799			
Total	9	2.914	1				

NMDS tilled treatments all sites ordination for both bacteria and fungi

The below ordinations show all sites associated with tilled treatments.

The bacterial tilled treatments NMDS ordinations had stress values of 0.029 (Figure 6.1A) and 0.055 (Figure 6.1B) and thus, can be considered an accurate representation of community relationships. There was no significance with either weighted ($R = 0.041$, $p = 0.304$) or unweighted ($R = 0.218$, $p = 0.06$) ANOSIMs. There was significant grouping between the treatments for abundance weighted data (pseudo-$F_{(1, 17)} = 2.58$, $p < 0.05$), where the R^2 value (0.04) explained 4% of the variation. The strength of this grouping increased when data was presence-absence transformed (pseudo-$F_{(1,17)} = 2.02$, $p < 0.01$), where the R^2 value (0.21) explained more of this variation.

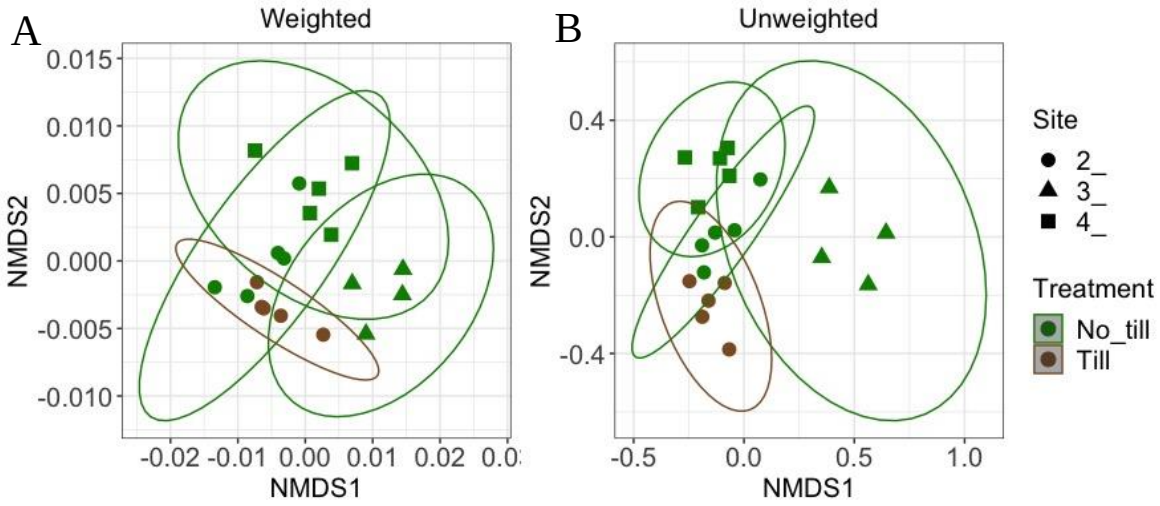

Figure 6.1. Non-metric multidimensional scaling (NMDS) ordinations of bacterial communities using the Unifrac dissimilarity metric under tilled treatments (all sites). Ordinations are of A) abundance-weighted community data and B) presence-absence transformed community data. 2-dimensional stress 0.029 (A) and 0.055(B) (< 0.2).

The fungi tilled treatments NMDS ordinations had stress values of 0.139 (Figure 6.2A) and 0.115 (Figure 6.2B) and thus, can be considered an accurate representation of community relationships. There was significance with both the weighted (R = 0.352, p = 0.009) and unweighted (R = 0.247, p = 0.003) ANOSIMs. There was significant grouping between the habitats for abundance weighted data (pseudo-$F_{(1, 17)} = 3.739$, $p < 0.05$), where the R^2 value (0.35) explained 35% of the variation. The strength of this grouping decreased when data was presence-absence transformed (pseudo-$F_{(1,17)} = 2.18$, $p < 0.01$), where the R^2 value (0.24) explained of this variation.

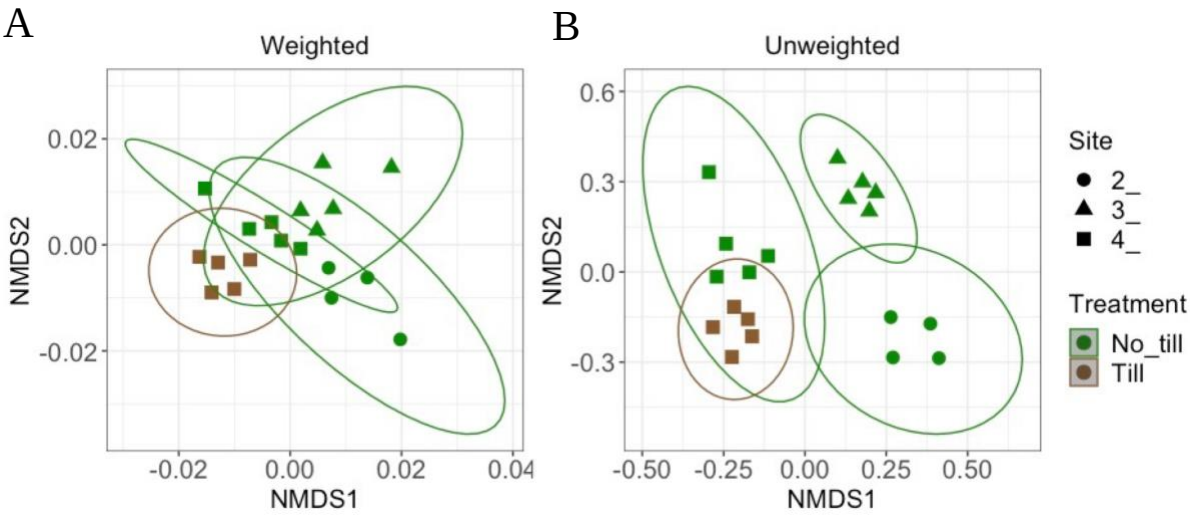

Figure 6.2. Non-metric multidimensional scaling (NMDS) ordinations of fungal communities using the Unifrac dissimilarity metric under tilled treatments (all sites). Ordinations are of A) abundance-weighted community data and B) presence-absence transformed community data. 2-dimensional stress 0.139 (A) and 0.115(B) (< 0.2).

APPENDIX VI - Burned treatment bacterial and fungal NMDS, including all sites

Bacterial NMDS PERMANOVA burn treatments, excluding no-burn

The weighted and unweighted (Table 6.17 & Table 6.18 respectively) Unifrac PERMANOVAs for bacterial β-diversity for burnt paddocks and unburnt patches within.

Table 6.17. PERMANOVA for bacterial burn treatments (excluding no-burn paddocks) showing weighted Unifrac.Abundance-weighted community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1 (Treatment)	1	0.0004	0.0004	3.101	0.09	0.002	**
Group2 (Site)	1	0.0005	0.0005	4.182	0.122	0.001	***
Group1:Group2	1	0.0001	0.0001	0.949	0.028	0.432	
Residuals	26	0.003	0.0001	0.76			
Total	29	0.004	1				

Table 6.18. PERMANOVA for bacterial burn treatments (excluding no-burn paddocks) showing unweighted Unifrac.Presence-absence community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1 (Treatment)	1	0.434	0.434	1.348	0.043	0.023	*
Group2 (Site)	1	0.992	0.992	3.083	0.097	0.001	***
Group1:Group2	1	0.392	0.392	1.218	0.038	0.094	.
Residuals	26	8.369	0.322	0.821			
Total	29	10.187	1				

Fungal NMDS PERMANOVA burn treatments, excluding no-burn

The weighted and unweighted (Table 6.19 & Table 6.20 respectively) Unifrac PERMANOVAs for fungal β-diversity for burnt paddocks and unburnt patches within.

Table 6.19. PERMANOVA for fungal burn treatments (excluding no-burn paddocks) showing weighted Unifrac.Abundance-weighted community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1 (Treatment)	1	0.0006	0.0006	1.464	0.043	0.072	.
Group2 (Site)	1	0.002	0.002	5.098	0.15	0.001	***
Group1:Group2	1	0.0006	0.0006	1.434	0.042	0.114	
Residuals	26	0.012	0.0004	0.765			
Total	29	0.016	1				

Table 6.20. PERMANOVA for fungal burn samples (excluding no-burn paddocks) showing unweighted Unifrac. Presence-absence community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1 (Treatment)	1	0.371	0.371	1.196	0.037	0.113	
Group2 (Site)	1	1.173	1.173	3.780	0.117	0.001	***
Group1:Group2	1	0.374	0.373	1.204	0.037	0.084	.
Residuals	26	8.067	0.310	0.808			
Total	29	9.984	1				

NMDS burn treatments all sites ordination for both bacteria and fungi

The below ordinations show all sites associated with burn treatments.

Stress values of 0.126 (Figure 6.3A) and 0.149 (Figure 6.3B) are considered a fair representation. The differences of community structure between the three treatments were statistically tested via PERMANOVA. There was significant grouping between the habitats for abundance weighted data (pseudo-$F_{(2, 38)} = 2.68$, $p < 0.001$), where the R^2 value (0.123) explained 12% of the variation. The strength of this grouping increased when data was presence-absence transformed (pseudo-$F_{(2,38)} = 1.73$, $p < 0.001$), where the R^2 value (0.177) explained more of this variation.

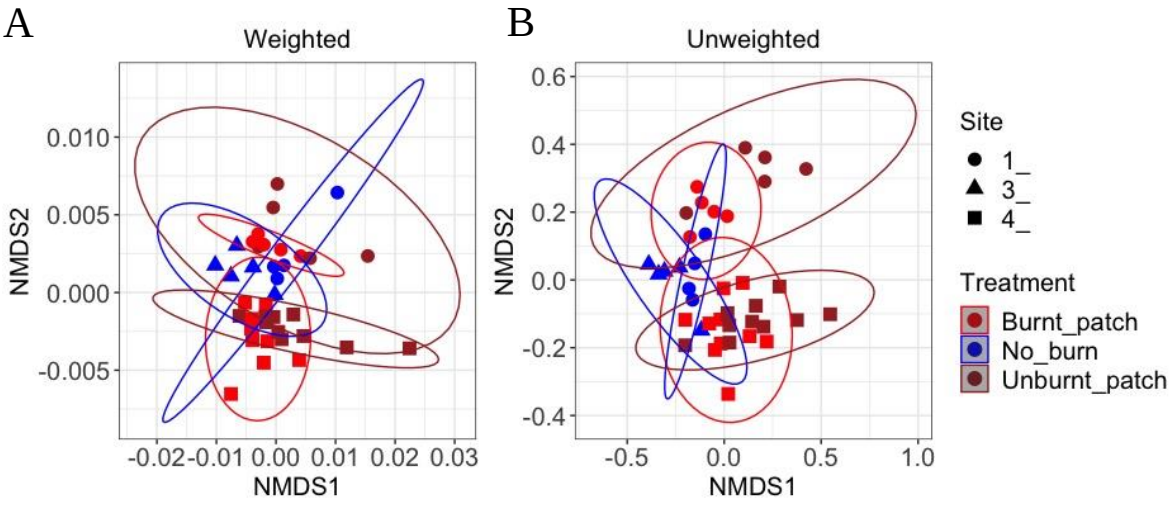

Figure 6.3. Non-metric multidimensional scaling (NMDS) ordinations of bacterial communities using the Unifrac dissimilarity metric under burn treatments (all sites).Ordinations are of A) abundance-weighted community data and B) presence-absence transformed community data. 2-dimensional stress 0.126 (A) and 0.149 (B) (< 0.2).

Stress values of 0.161 (Figure 6.4A) and 0.189 (Figure 6.4B) are considered a fair representation. The differences of community structure between the five sampling locations were statistically tested via pairwise PERMANOVAs. There was significant grouping between the habitats for abundance weighted (pseudo-$F_{(2, 38)} = 1.95$, $p < 0.01$), where the R^2 value (0.172) explained 17% of the variation. The strength of this grouping increases when data was presence-absence transformed (pseudo-$F_{(2,38)} = 1.52$, $p < 0.001$), where the R^2 value (0.196) explained more of this variation. These analyses suggest a turnover in the suites of ASVs as well as dominant species present.

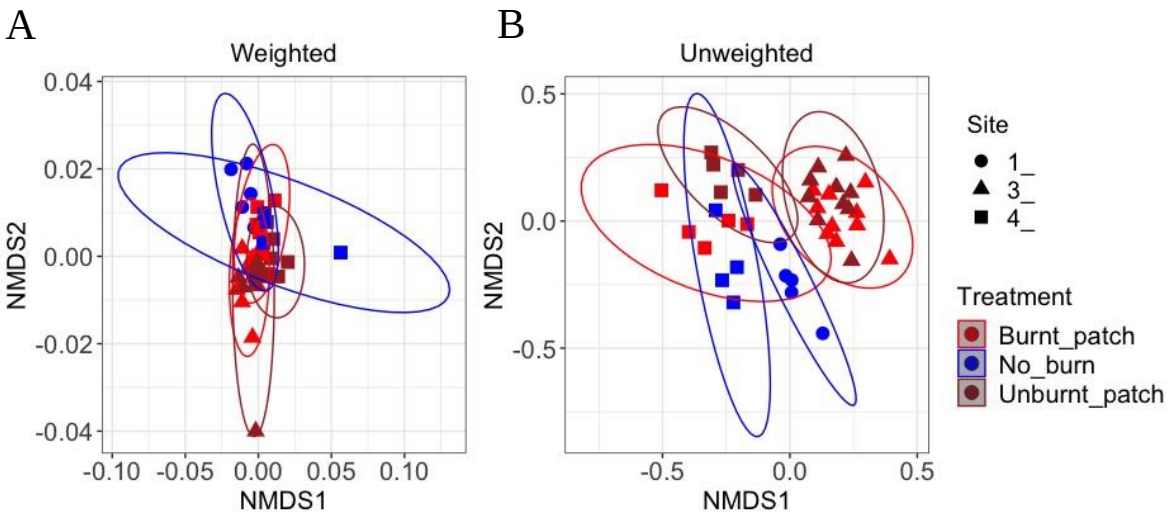

Figure 6.4. Non-metric multidimensional scaling (NMDS) ordinations of fungal communities using the Unifrac dissimilarity metric under burn treatments (all sites).Ordinations are of A) abundance-weighted community data and B) presence-absence transformed community data. 2-dimensional stress 0.161 (A) and 0.189 (B) (< 0.2).

APPENDIX VII - Predicted functional pathways β-diversity

Functional NMDS PERMANOVA tilled treatments site 2 only

The weighted and unweighted (Table 6.21 & Table 6.22 respectively) Bray Curtis PERMANOVAs for bacterial functional pathways β-diversity for tilled paddocks (site 2 only).

Table 6.21. PERMANOVA for functional pathways tilled treatments showing weighted Bray Curtis.Abundance-weighted community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method. (Site 2 only).

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1 (Treatment)	1	0.001	0.001	6.147	0.435	0.024	*
Residuals	8	0.002	0.0002	0.565			
Total	9	0.003	1				

Table 6.22. PERMANOVA for functional pathways tilled treatments showing unweighted Bray Curtis.Presence-absence transformed community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method. (Site 2 only).

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1 (Treatment)	1	0.002	0.002	8.107	0.503	0.02	*
Residuals	8	0.002	0.0002	0.497			
Total	9	0.004	1				

Functional NMDS PERMANOVA burn treatments (excluding no-burn)

The weighted and unweighted (Table 6.23 & Table 6.24 respectively) Bray Curtis PERMANOVAs for bacterial functional pathways β-diversity for burnt paddocks and unburnt patches within.

Table 6.23. PERMANOVA for functional pathway burn treatments showing weighted Bray Curtis.Abundance-weighted community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method. (No-burn removed)

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1 (Treatment)	1	0.0006	0.0006	1.464	0.040	0.159	
Group2 (Site)	1	0.004	0.004	8.023	0.220	0.001	***
Group1:Group2	1	0.0004	0.0004	0.942	0.026	0.434	
Residuals	26	0.011	0.0004	0.714			
Total	29	0.016	1				

Table 6.24. PERMANOVA for functional pathway burn treatments showing unweighted Bray Curtis.Presence-absence transformed community data. The p-value was adjusted by the Benjamini-Hochberg (BH) method. (No-burn removed)

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Group1 (Treatment)	1	0.0005	0.0005	1.362	0.037	0.253	
Group2 (Site)	1	0.003	0.003	7.636	0.210	0.001	***
Group1:Group2	1	0.0005	0.0005	1.397	0.038	0.207	
Residuals	26	0.009	0.0003	0.714			
Total	29	0.012	1				

APPENNDIX VIII - Tilled differential abundance tests

Table 6.25. Bacterial functional pathways differential abundance test comparing no-till paddock to tilled paddock.Only significant pathways shown and pathways with no baseMean cutoff.

baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Pathway
114.25	-10.26	1.45	-7.08	1.41E-12	5.87E-10	benzoyl-CoA degradation I (aerobic)
3318.88	-2.16	0.39	-5.55	2.88E-08	5.97E-06	superpathway of pyridoxal 5'-phosphate biosynthesis and salvage
447.06	2.20	0.41	5.40	6.75E-08	9.34E-06	coenzyme B biosynthesis
27769.54	0.32	0.06	5.31	1.09E-07	1.13E-05	pyrimidine deoxyribonucleotides de novo biosynthesis III
219.50	-11.20	2.20	-5.10	3.33E-07	2.76E-05	sucrose degradation II (sucrose synthase)
2468.10	-2.91	0.66	-4.43	9.42E-06	6.52E-04	pyridoxal 5'-phosphate biosynthesis I
175.17	-5.44	1.24	-4.38	1.21E-05	7.20E-04	polymyxin resistance
6666.97	0.37	0.09	4.28	1.85E-05	9.59E-04	mycothiol biosynthesis
18775.90	0.26	0.06	4.21	2.54E-05	1.17E-03	protocatechuate degradation II (ortho-cleavage pathway)
46.47	-6.99	1.67	-4.19	2.83E-05	1.18E-03	vitamin E biosynthesis (tocopherols)
1132.78	1.52	0.38	4.04	5.34E-05	2.02E-03	coenzyme M biosynthesis I
1312.02	-0.95	0.25	-3.85	1.21E-04	4.17E-03	glycine betaine degradation I
18840.79	0.18	0.05	3.72	1.96E-04	6.26E-03	4-aminobutanoate degradation V
2092.80	-1.25	0.34	-3.70	2.17E-04	6.43E-03	CMP-legionaminate biosynthesis I
4814.69	-0.61	0.18	-3.41	6.51E-04	1.72E-02	ppGpp biosynthesis
82025.75	0.08	0.02	3.40	6.63E-04	1.72E-02	pyruvate fermentation to isobutanol (engineered)
12.00	-5.81	1.80	-3.24	1.21E-03	2.79E-02	vitamin B6 degradation
26567.76	-0.18	0.06	-3.25	1.15E-03	2.79E-02	Kdo transfer to lipid IVA III (Chlamydia)
13178.25	-0.64	0.20	-3.21	1.33E-03	2.91E-02	thiazole biosynthesis I (E. coli)
22547.97	-0.27	0.08	-3.18	1.45E-03	2.92E-02	mixed acid fermentation

7708.83	-0.60	0.19	-3.18	1.48E-03	2.92E-02	superpathway of hexuronide and hexuronate degradation
16008.99	-0.47	0.15	-3.16	1.57E-03	2.97E-02	biotin biosynthesis I
14591.14	-0.52	0.17	-3.13	1.76E-03	3.18E-02	8-amino-7-oxononanoate biosynthesis I
4314.82	0.33	0.11	3.09	1.99E-03	3.44E-02	catechol degradation I (meta-cleavage pathway)
3647.65	0.35	0.11	3.03	2.41E-03	3.74E-02	p-cymene degradation
3647.65	0.35	0.11	3.03	2.41E-03	3.74E-02	p-cumate degradation
30350.80	-0.32	0.11	-3.03	2.43E-03	3.74E-02	superpathway of thiamin diphosphate biosynthesis I
664.63	0.73	0.24	3.01	2.57E-03	3.82E-02	methanogenesis from H2 and CO2
4751.54	-0.85	0.28	-2.99	2.83E-03	4.04E-02	NAD biosynthesis II (from tryptophan)
3154.61	-0.91	0.31	-2.93	3.39E-03	4.69E-02	L-tryptophan degradation to 2-amino-3-carboxymuconate semialdehyde