Final report

P.PSH.1222- Impact of bushfires on soil, pasture, and the microbiome

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Abstract

Bushfires are a natural agent of disturbance often resulting in multifactorial damaging impacts. On farms, along with the health and wellbeing of farmers and stock being affected, the health of the soil that forms the foundation of farming is also influenced. The heat from unplanned fires has the capacity to sterilise soil, kill pasture seeds, remove soil nutrients, and temporarily decimate the soil microbiome. Prior to this project, comparatively little was known about the long-term recovery of soil, or growth of nutritionally rich vegetation tolerant to post-fire conditions.

Here we submit a detailed review of literature summarising possible direct and indirect consequences of fire on the recovery of soils and pastures. Also delivered are the results from varied analysis of soil and pasture following exposure to the bushfires experienced in rural NSW in 2019-2020, and a planned burn event done in January 2022. A toolbox of strategies and recommended courses of action have been developed for producers to manage post fire recovery of soil and vegetation.

Executive summary

Background

In 2007, CSIRO and the Bureau of Meteorology projected changes to temperature and annual rainfall over the next 30 years and predicted changes in frequency, intensity and duration of extreme weather and related events. It was predicted that 2020 would see an increased risk of bushfire, and this prediction has sadly been borne out by the catastrophic fire season in 2019-2020, which is estimated to have impacted 8.6 million sheep and 2.3 million cattle in NSW and Victoria alone. In the wake of the recent bushfires, the Australian landscape continues to change in terms of climate, and this will have significant impacts on capacity for ongoing livestock production. As the global population continues to grow, the requirement for food for human nutrition is predicted to grow by 70% by 2050 (FAO 2006). Australia is ably placed to meet the growing demand for meat but only if any negative impacts of climate and related events on livestock production processes are managed. Thus, there is a requirement for adaptation and mitigation strategies to counter and manage the consequences of future adverse events.

Several reports discuss the potential impacts of climate change to Australian livestock production and economic vulnerability [1, 2]. There is a range of suggested solutions dependent upon the livestock species and the desired production purpose (e.g., beef, wool, milk). Factors that may be relevant in the wake of the 2019-2020 bushfires include heat stress due to loss of shade, the need to breed resilient livestock [3, 4], the requirement for introduction of vegetation that will provide optimal nutrition to stock while growing under challenging conditions, and adaptations to manage parasite/disease risk [5]. One factor not taken into consideration is the effect of drought and bushfire on soil fecundity, even though soils are the foundation of every farming enterprise.

The biology of soils has long been recognised as central to the capacity of managed ecosystems to support production and, in recent years, the importance of the microbiome of the soil for seed germination, plant maturation, and suppression of plant disease has been recognised [6]. Soil biota comprises of living microorganisms interacting with plants to provide nutrition and other benefits, such as the capacity to increase plant-available nitrogen and phosphorus [7, 8].

Recent soil management approaches have sought to understand and optimise soil microbiomes for improved plant growth and production in an Australian setting [9, 10]. The microbiota of soil can confer tolerance [11] and potentially enhance nutritional value to native and introduced plants. In addition, the soil microbiome impacts upon the rumen microbiome and animal productivity both directly (through ingestion of soil) and indirectly (through vegetation growing in the soil). These factors are important in bushfire affected pastures where soil temperatures of up to 600°C have been reported for very hot fires, resulting in the potential sterilisation of soil and significant changes to the soil microbiota, organic matter, moisture content and nutrients, and potentially creating an environment inhospitable to plant growth. Wildfire in the USA resulted in persistence of soil damage for up to 25 months [12] and other studies report significant loss of nutrient quality of grasses post-fire in the USA [13] and Africa [14]. Soil conditions will impact on plant growth, which then translates into livestock feed and nutrition.

This project sought to gain insight of soil and pasture regrowth status post-fire in relation to pastures supporting livestock relevant to the Australian setting. A clearer understanding of the consequence of unplanned fire to livestock pastures in terms of soil health and vegetation recovery may identify factors impacting the capacity of producers to provide adequate nutritional support to their livestock. The goal of the research was to address knowledge gaps and provide a basis to inform the Australian livestock industry how to support future meaningful adaptations for pasture management, and how to optimise pasture recovery following bushfires which lead to improved livestock health and welfare.

Objectives

- A literature review is delivered summarising the possible direct and indirect consequences of fire on the recovery of soils, pastures, and other vegetation, and options for their management from similarly affected areas in Australia, North and South America, Africa, Russia, and Europe.

- Results and summary regarding the consequences of bushfire on soil quality as measured by assessment of soil microbiota (Section 4.6) and biogeochemistry (Section 4.4) is delivered.

- Insight is reported on the effect of bushfires and planned burns on nutrient values of collected pastures (Section 4.3) the growth patterns of seed banks of pasture vegetation (Section 4.5), and the effect of fire on pasture growth, normalised difference vegetation index and biomass accumulation as determined by remote sensing (Section 4.2).

- Insights to any interrelationships between nutrient content and the soil microbiome, vegetation and grazing livestock in pasture systems are discussed within individual summaries.

- An interdisciplinary toolkit for post-fire impacts and recovery to inform future post-fire pasture recovery and management was planned as an objective. Over the intervening years several excellent manuals, factsheets and web-based information portals have been generated. We have collated these resources and sought to add value through added content with regards to soil and pasture recovery post fire to complement the existing Bushfire recovery manual generated by the MLA.

Methodology

- A review of literature was done as follows. Scientific literature was systematically searched using the Scopus database to find online articles about post-fire effect of fire on agricultural land soil and vegetation. The keyword search parameters (grassland OR pasture OR wetland OR meadow OR steppe OR prairie OR savannah OR sward OR rangeland) AND (fire OR wildfire OR bushfire) AND (soil OR soil AND properties OR plant) AND postfire AND (regrowth OR germination OR seed OR grassland AND restoration OR management) AND (LIMIT-TO (LANGUAGE, "English")). This resulted in a list of 717 articles. Titles and abstracts of the articles were screened, and only those that focused on post-fire effects on soil and pasture regrowth in an agricultural context and, where appropriate, the management thereof were considered. Additional research articles were sourced by snowballing (examining reference lists of relevant literature for articles of interest to this review that were not picked up through formal literature searches). Keyword search structure was informed by consultation with a librarian. We concentrated on articles describing studies done in fire-prone regions with a biome like that of Australia as classified by Aguilera et al. [15] i.e., South and North America, Africa, Europe, Russia and Australia. These regions align as experiencing significant climate change-induced adaptation to increasing incidences of bushfire.

- Soil and pasture biomatter were collected from five properties exposed to bushfire located from Nowra in Northern NSW to Cobargo in Southern NSW representing a range of soil and geographic locations. Samples of soil and pasture from burnt and unburnt paddocks on each property were collected from the locations at two timepoints (June 2021 and June 2022).
    - Further planned sampling was not possible due to NSW government and the University of Sydney response measures to the Covid 19 pandemic and repeated flooding events.

- A site located in Victoria was treated with a planned burn in January 2022. Samples of soil and was sourced by the producer in January 2022 (University of Sydney staff were unable to conduct field trials at this time) and soil, seedbanks and pasture samples were collected by research and field staff in July 2022. These samples served to compare early responses to low to medium fire intensity/burn exposure to those of bushfire affected properties.

- Remote sensing comparison analysis was done on all properties through use of a combination of remotely sensed data (primarily Sentinel-2 satellite imagery which collects data across south-eastern Australia on 5-day basis) and modelled data.

- Soil analysis included comparisons among samples from burnt and unburnt pastures in relation to biogeochemistry, microbiota (16S bacterial rRNA and ITS fungal gene community diversity and taxonomical identity), and phospholipid fatty acid soil composition.

- Pasture growth potential in burnt and unburnt sites, and nutritional viability of pasture re-growth was assessed through seed bank analysis and in-depth measures of dry matter (pasture) chemical composition.

Results/key findings

Within three months of the suppression of bushfires in 2020, the northern and eastern seaboards of Australia were exposed to El Niño-Southern Oscillation which then swung to La Niña conditions as the year proceeded and the alert remained in place throughout the remainder of the project since (http://www.bom.gov.au/climate/enso/wrap-up/archive.shtml). The consequences of this have been above average rainfall during spring and summer seasons resulting in multiple flood events particularly in the regions within which the sampling properties were located. This has been a potential driver in the recovery of pastureland following the 2019 and 2020 bushfires. Reports released by the Australian Bureau of Meteorology warn of the continuing influence of climate change on both the Australian and the global climate. Australia's climate has warmed by around 1.47°C since 1910 and there is a trend to a greater proportion of rainfall from high intensity short duration rainfall events, especially across northern and eastern Australia (http://www.bom.gov.au/climate).

At the culmination of this study, it must be acknowledged there is limited capacity to comprehensively report on the effects of bushfires on soil, pasture, and the microbiome due to several issues; primarily, collection of biological samples (soil, plant) did not commence until 18 months after the 2019/2020 bushfire event due to delays in sourcing funding and obtaining contract approvals. This was swiftly followed by lack of accessibility to properties and laboratories limiting planned repeat sampling and downstream processing and analysis due to the declaration of the global Covid 19 pandemic in Early 2021 and associated NSW government mandated state and regional lockdowns and the University mandated severe limitation in access to laboratory space and a moratorium on work related travel beyond 100 km from the University. This resulted in only two major sampling events at bushfire exposed properties (June 2021 and June 2022). Similarly, a comprehensive analysis of the effects of bushfire on the measured variables has been hindered by the effect of the El Niño/ La Niña making it impossible to separate the consequences of bushfire from the consequences of extreme rainfall and floods. Any conclusions drawn from this study regarding the long-term recovery of soil and pasture following the 2019/2020 bushfire must be accompanied by acknowledgment that future bushfire events may not be followed by multi-year heavy rainfall events over the spring-summer seasons. Thus, it is very difficult to accurately report on the integrated effect of bushfire on soils, nutrients, and plant matter and, as such, the various elements of the study are reported individually.

Bearing these unavoidable limitations in mind, the key findings from analysis of soil and plant matter collected from bushfire exposed properties at 18- and 30-months post-fire or following a planned burn are as follows:

- There is limited capacity to report on the immediate and interrelated effects of bushfires on soil, pasture, and the microbiome due to significant issues:
    - Collection of biological samples (soil, plant) was not commenced until 18 months after bushfires due to delays in finance and contract approvals.
    - Post-fire but pre-sampling there was repeat exposure of all properties to abnormally high rainfall events.
    - Lack of accessibility limited planned repeat sampling due to persistent flood events and a global pandemic. This resulted in only two major sampling events at bushfire exposed properties (June 2021 and June 2022).

- Use of satellite imagery and comparison with long range data shows:
    - The effects of the bushfire are relatively minor in relation to biomass following multi-year drought however there is evidence of a rebound in biomass across all properties.
    - The results must be considered in the context of three sets of (ab)normal natural conditions, i.e., multi-year drought, megafire and flood events. Despite this, results are indicative of relatively low level of productivity from pasture – a concern for livestock industries.

- Use of satellite imagery is an important and useful method for gaining a broader overview of property status and puts into context a range of factors including biomass estimations however satellite imagery needs to be matched with biological sampling as it is susceptible to geographic conditions (cloud cover, tree cover).

- Biochemical analysis of soil samples collected in bushfire exposed and unexposed paddocks as well as soils exposed to a planned burn has revealed persistent variation in extractable phosphorus (P), nitrate (NO_3^+), and available nitrogen (N) indicative of a soil with improved nutrient content for pasture growth in severely burnt pastures in comparison to unburnt sites. This finding suggests a positive aspect to bushfire exposure in the context of grassland fires however it must be stressed that this finding may be confounded by properties closely adjoined with forests since the predicted temperatures of these soils may be higher and this present study was not able to compare the effects of shade cover/tree proximity to fire affected soil.

- Nutritional analyses of plant tissue indicate that for most sites, pastures were of relatively low nutritive value for livestock. The effects of fire were small and not unequivocal, with very large variability observed within and among sampling sites (farms).

- Exposure to fire has no apparent detrimental effect on seed germination in the short term (six months post burn) as measured in the planned burn site and the long term (eighteen and thirty-months post bushfire event).

- Verbal reports from all the producers in this study and biological evidence in the plant matter growth in the seed bank analysis from soils collected 18 months post bushfire suggest that an unexpected issue faced by producers post bushfire was rapid and vigorous overgrowth of previously unseen weed species. Producer feedback and consultation with experts suggests the reasons may be multifactorial but include biosafety issues (transferal of seeds) brought about by e.g. high traffic of emergency and support vehicles post fire event, high winds because of the fire event transferring native and other seeds with greater resilience and lower nutrient requirements in comparison to the desired livestock fodder plant species. Tools/links for monitoring and addressing this issue are suggested in Section 5.

- Analysis of the microbiota (bacteria) and mycobiota (fungi) in soil collected from planned burn at six months post fire event, and bushfire exposed/unexposed soils at 18- and 30-months post fire exposure, found no consistent differences between burnt and unburnt soils at the community-level; however, there was evidence of a difference between proportions of lesser-known taxa that were more abundant in unburnt paddocks, which may have implications for soil quality and plant growth. For example, several taxa known to support plant growth were less abundant in the burnt paddocks than in unburnt paddocks. However, there was also evidence of a greater abundance of fire-resilient (pyrophilous) taxa within burn paddocks. These taxa thrive in a post-fire environment and increase nutrient availability, enhancing plant regeneration.

- Phospholipid fatty acid soil analysis of samples sourced from bushfire exposed properties eighteen months post the fire event show variation between properties for the total volume of fungi and bacteria measured in the soils. Analysis of the volumes of more specific microbial groups (Gram-positive and Gram-negative bacteria), show variation in response correlated to fire intensity with a greater volume of bacteria groups present in the unburnt soils. The data suggests that soil type may play a role in bacterial and fungal responses to fire exposure.

- In the years since the 2019/2020 bushfire event, Australian National and Local Government agencies and external organisations have developed frameworks, pathways, and sources of information for multiple aspects of support and recovery for agriculture after bushfire however consultation with primary producers revealed gaps in on-line resources currently available and several common queries and comments emerged. Based upon this feedback, the toolkit/recommendations outlined in section 5 addresses these queries in a Q&A format and provides links to appropriate tools and services including a glossary of key terms, evaluation of the effects of fire, soil condition after bushfire, and plant recovery after bushfire. It is the recommendation of this group that a web-based toolkit be developed providing easy access to updated national and regional information.

Benefits to industry

Reported insights to pasture and soil viability following the 2019/2020 bushfires and subsequent rainfall and flood events in livestock producing properties located in NSW provides a unique understanding to producers of the measured consequences of bushfire relevant to the Australian setting. The review of literature revealed a paucity in similar analysis of the consequences of bushfires to Australian livestock producers. These findings will serve as reference for producers and feedback from producers has been utilised to generate an informative factsheet containing links to tool for recovery post-fire (Section 5).

Future research and recommendations

- Ensure funds and procedures are in place in case of future bushfires to allow for accurate and rapid assessment of biological variables as soon as possible after the event. This might be achieved by putting in place a repository of collection kits that could be sent to producers soon after the bushfire event with easy-to-follow instructions. Rapid collection of soil and plant matter is essential to accurately gain an understanding of the consequence of bushfire to agricultural processes.
- While previous research has shown that satellite imagery is an important and useful method for gaining a broader overview of property status, further extensive research is required to assess value and specificity of the method in relation to agricultural land and as a complement of biological sampling. Future research should include:
    - Expansion of sampling conditions
    - Establishing a baseline/database of pasture quality across the varied geographic areas/conditions within Australia
    - Collate database of all past trials e.g., estimates of biomass in disparate regions. There is much unpublished evidence that is held within government and other reports that would be vital to build a comprehensive database for future referral and will allow for the development of modelling algorithms/programmes.
- There is a paucity in published data relevant to soil biochemistry after bushfire in Australian agriculture settings. Development of a repository of such data that included variables such as soil type, and variations in microbial and nutrient content across the Australia and across varying times of the year would allow for improved insight.
- After bushfire, producers should put in place weed management systems and maintain vigilance to ensure that new invasive and possibly damaging (poisonous) species do not gain a foothold in fire-affected paddocks.
- A comprehensive and easily accessible compendium of weeds should be developed showing images of weeds at multiple stages of growth and highlighting at-risk plants (poisonous to livestock) and suggesting management methods to enable producers to rapidly identify and eradicate weed matter.
- While the results of this study have provided insights to the long-term (18 to 30 months) consequence of bushfire to microbiota in soil, there remains a paucity in data related to the

immediate effects of bushfire on soil microbiota. It is recommended that for improved understanding of the potential benefits of adaptation of soil microbiota to enhance nutrient availability for support of pasture growth, future studies should:

- Conduct meta-analysis of past data and establish a comprehensive collection of soil across the Australian agricultural range with varying productivity/soil types and other important variables to establish baselines.
- Introduce annual microbiota testing alongside soil nutrient testing and create a comprehensive accessible database to allow for future modelling efforts.

- The literature review identified key knowledge gaps in the efficacy and feasibility of mulching in the agricultural context and in particular, the economic feasibility of mulching applications in the Australian agricultural context are not readily available and may limit functionality. A comprehensive study of the efficacy of mulching in bushfire recovery through use of planned burn simulations may provide producers with increased confidence.

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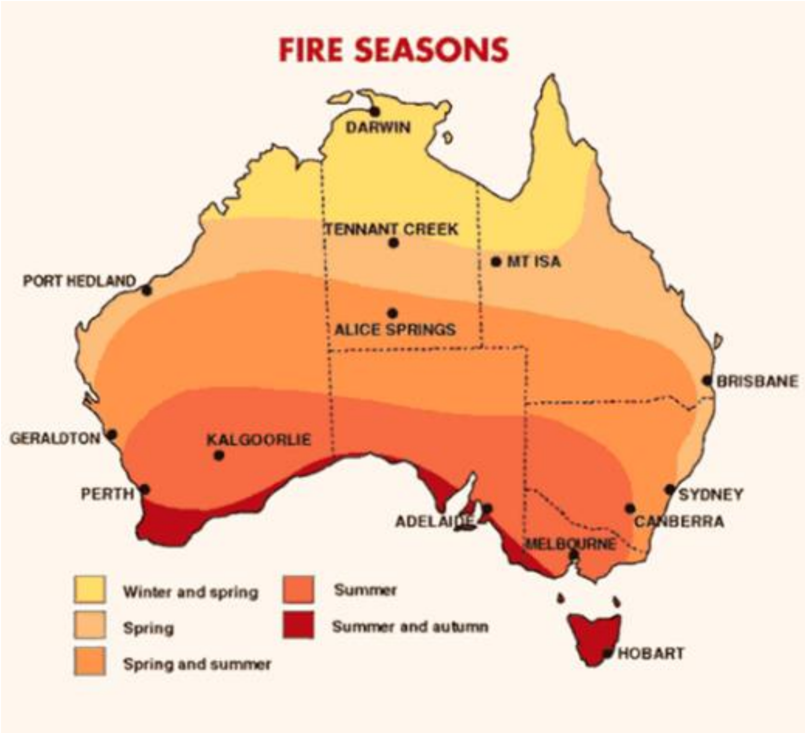
1. Background literature review

1.1 Post-fire consequences for soil fertility and vegetation regrowth in agricultural grasslands – Introduction

Fire activity is a strong evolutionary force that has contributed to shaping present biomes [16-19]. It has influenced human evolution and society across geographic settlement, food procurement and the development of agriculture and technology [20-22]. In Australia, the indigenous peoples have long utilised the tradition of anthropogenic grassland burning to support and shape the habitat [23-26]. Many ecosystems and plant species are well adapted to local fire regimes, and the germination of several plant species (pyrophytes) has evolved to be contingent upon exposure to fire [27, 28]. Low severity fires may exert beneficial impacts on soil properties and, improve vegetation density [29]. In a low severity, controlled fire the temperatures reached are generally not high and the loss of nutrients is generally minimal. Examples of beneficial fires are prescribed fires carried out during the autumn and winter seasons for landscape management [30]. Beneficial consequences of controlled fires to soil and plant productivity may include production of ash rich in carbon, an increase of soil organic matter, pH, electrical conductivity, and extractable cations such as calcium, magnesium, sodium, and some forms of nitrogen such as ammonia, important for vegetation recuperation [30, 31].

Shifts in fire regimes can test the resilience of plant species and the ecosystem, becoming a destructive force responsible for loss of life and accumulating high socio-economic losses. Australia is one of the most fire prone regions in the world with annual variations in fire seasons across the continent. The traditional fire season in Australia ranges from the dry winter season in northern Australia, to spring and summer for the sub-tropics, and middle to late summer for the southern regions of Australia (Fig. 1).

Figure 1. The fire season in Australia moves south as the year progresses. In northern Australia the peak fire season is in the winter and spring while in the south, the peak fire season occurs in the summer [32].

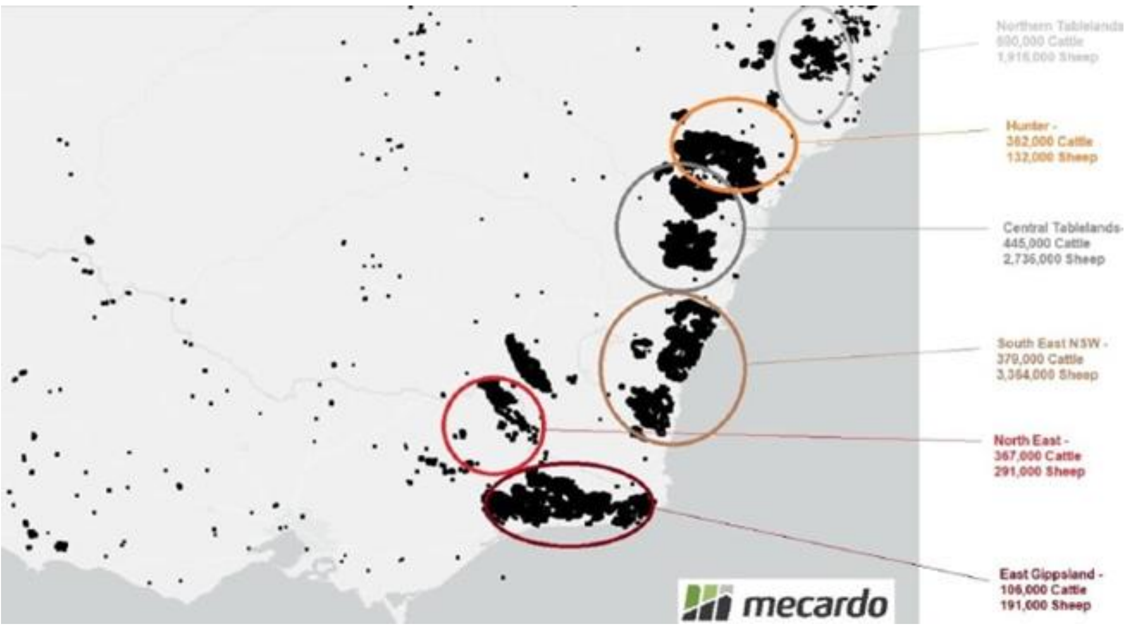


In 2007, the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the Australian Bureau of Meteorology projected changes to temperature, evaporation and annual rainfall over the next 30 years predicting changes in frequency, intensity and duration of extreme weather and related events including the potential for an extended and more severe fire season [33]. In 2019-2020, this prediction was borne out by a catastrophic fire season across regions of Eastern Australia following multiple years of drought and triggered by the arrival of hot dry windy weather conditions and ignitions in early September 2019 in the north and spreading south as extreme 'mega-fire' weather conditions continued throughout spring and early summer [34, 35]. Between September 2019 and January 2020, an estimated 7.38 million hectares were burned, including 0.53 million hectares of agricultural land. Exposure to the fires is estimated to have killed 1.25 billion animals (included wildlife and livestock) and although it is difficult to estimate the exact numbers of livestock impacted, it is estimated to have significantly affected 8.6 million sheep and 2.3 million cattle in New South Wales and Victoria [36] (Fig. 2).

Globally there is increased focus on prediction of future fire risk under climatic change [37-39] since there is already evidence of a shift in the expected patterns of rainfall and global temperature, and frequency, size, and intensity of fires. Within the past four years there have been reports of other uncontrollable destructive 'mega-fires' around the world including Russia [40], Portugal [41] and California [42]. Total fire emissions in western United States in 2020 were approximately three times higher than the mean achieved between 2003 and 2010, and the Arctic experienced its most severe fires in terms of carbon emitted into the atmosphere, with most of the fires occurring in Arctic Asia. In the tropics, the Amazon saw its highest fire activity since 2012 [43].

The consensus regarding a global climate change trend supports an uncertain future where forecasting for reliable rainfall and temperatures is increasingly complex [44]. Overall, the modelling agrees that precipitation will decrease throughout the global Mediterranean-type climatic regions and low-to mid-latitude dry regions will expand due to increased evaporation. These regions are traditionally sites for livestock production with grasslands and pastures accounting for between 40 - 90% of total agricultural land use [45]. Grasslands are a major component of the global ecosystem, occupying over 30% of the biosphere land mass and forming the backbone of the agricultural food web thus performing a vital role in global food supply [46]. Furthermore, grasslands are a significant contributor to regulation of biospheric and atmospheric carbon concentration through sequestration of soil organic carbon and the incorporation of photosynthetic pathways (C3 and C4) in grass species to incorporate carbon dioxide into three- (C3 grasses) or four- (C4 grasses) carbon compounds [47]. The projected changes to climate such as change in rainfall patterns, increasing aridity, variations to local temperature, and relative air humidity will result in the enforced adaptation of grassland species and other vegetation distribution and their growth patterns. This, coupled with the ideal environment for high intensity bushfires will impact on the capacity for ongoing pasture productivity and livestock production [48, 49].

Figure 2. NSW and Victorian fires and potential livestock impacted. NASA mapping data of bushfires cross referenced with Meat & Livestock Australia livestock data (Figure prepared by Mercado bit.ly/324Ge0q).



Australian farmers are well accustomed to climate variability but climate change presents new challenges since agriculture intrinsically depends on reliable climate conditions [50]. While climate forecasting models predict large changes in future rainfall including lower rainfall in southern Australia and more severe droughts, longer and more intense fire seasons, and floods across the entire arable region [33, 51], over the past 20 years, instability in the Australian climate and the negative impact of global climate change has already been observed. Existing changes include reductions in average winter rainfall in southern Australia and a general increase in temperature. These longer-term shifts towards higher temperatures and lower winter rainfalls have had negative effects on average agricultural productivity [52-55]. The Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) estimates Australian farms have on average, lost almost $30,000 each a year in profits over the past 20 years due to climate change, relative to earnings in the latter part of last century [56]. Extensive research exists on the potential risks and impacts of climate change both globally and particular to the Australian ecosystem in relation to frequency of fires. Additionally, excellent research has explored the consequence of climate change on livestock production and economic vulnerability and there is a range of suggested solutions dependent upon the livestock species and the desired production purpose (i.e. beef, lamb, wool, milk) [1, 57, 58]. The potential for increased risk of longer and more intense fire seasons also requires a clearer understanding of the consequences of unplanned fire on livestock supporting grassland pastures in terms of post-fire soil health and vegetation recovery as a means to identify areas of targeted intervention to support rapid recovery.

This review focuses on the short- and long-term consequences to grassland soil function, fertility, and vegetation regrowth in the aftermath of bushfire.

1.2 Literature search

Scientific literature was systematically searched using the Scopus database to find online articles about post fire effect of fire on agricultural land soil and vegetation. The keyword search parameters (grassland OR pasture OR wetland OR meadow OR steppe OR prairie OR savannah OR sward OR rangeland) AND (fire OR wildfire OR bushfire) AND (soil OR soil AND properties OR plant) AND postfire AND (regrowth OR germination OR seed OR grassland AND restoration OR management) AND (LIMIT-TO (LANGUAGE, "English")) resulted in a list of 717 articles. These article titles and abstracts were screened, and only those that focused on post fire effects on soils and pasture regrowth in an agricultural context and, where appropriate the management thereof were considered. Additional research articles were sourced by snowballing (examining reference lists of relevant literature for articles of interest to this review that were not picked up through formal literature searches). Keyword search structure was informed by consultation with a librarian. We concentrated on articles describing studies carried out in fire-prone regions with a biome similar to that of Australia as classified by Aguilera et al. [59] i.e., South and North America, Africa, Europe, Russia and Australia. These regions align to those experiencing significant climate change induced adaptation to bushfire incidences.

1.3 Fire terminology and assessment of damage

Several terms are utilised to describe vegetation fires external to the urban environment. In Australia the term *bushfire* describes any vegetation fire while globally the term *wildfire* is commonly utilised to describe any unplanned vegetation fire and encompasses grass fire, forest fire and scrub fires [60].

The primary drivers that determine the potential for and the intensity of a bushfire are: *ignition*, either by human action or from natural sources such as lightning; *biomass/fuel abundance* or load; *fuel dryness*, and; appropriate *weather conditions* for fire spread (hot, dry and windy) [61].

The metrics or terms that quantify fire effects on for example, soil, vegetation, and organic matter are diverse and, in some cases, there are variations in definition [62-64]:

Fire intensity represents the energy released over the duration of the fire measured in kilowatts per metre (kW/m) of fire front [65]. It may also be defined as the rate of heat energy released per unit time/per unit of the length of the fire line [66]. It may be understood as the rate by which fire produces thermal energy. Intensity is classified to three sub-categories, but it should be noted that the categorisation is largely reliant on data sourced from forest fires and the average temperature range for grassfires may generally be within the *mild* to *cool burn* range:

Mild or *cool-moderate burn*: may produce a fire intensity of up to ~350 kW/m with maximum peak atmospheric temperatures of 400°C and maximum soil temperatures of 250°C at the soil surface, 100°C at 2.5 cm soil depth and ≤50°C at 5 cm soil depth [67]

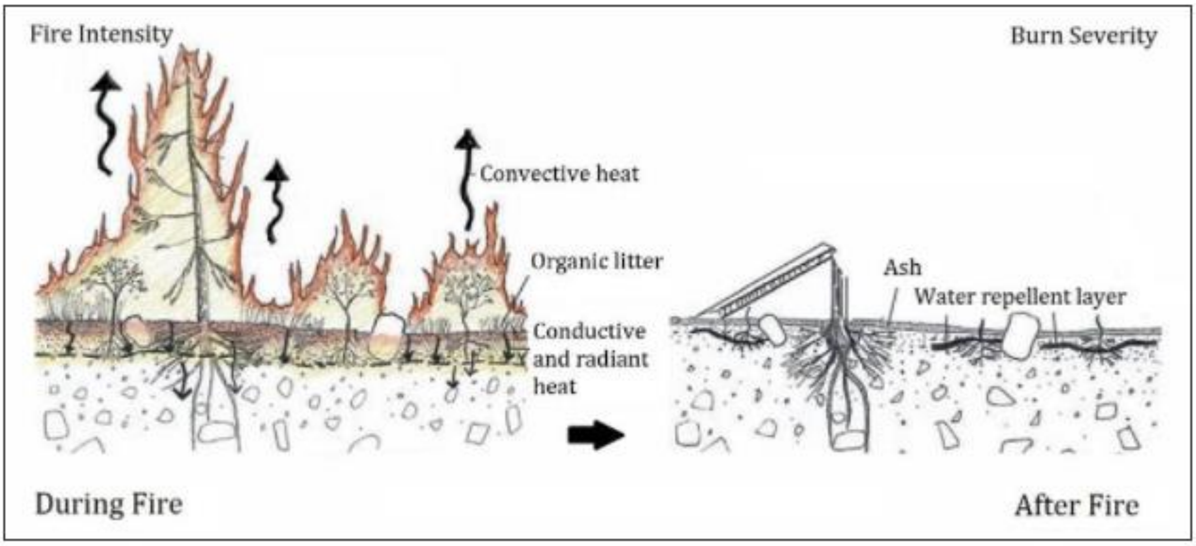
Medium intensity or *hot burns*: may produce a fire intensity of 1700-3500 kW/m and as a result, the maximum soil surface temperatures may reach 400°C, 175°C at 2.5 cm and ≤50°C at 5 cm soil depth [67].

Extreme intensity or *very hot burn*: this is an extreme fire and may generate fire intensity of 20,000-60,000+ kW/m, with maximum soil surface temperatures up to 900°C, and dependent on the vegetation type, the subsoil temperature may achieve 150°C [67].

Fire trials and other experimental research carried out in Australia by Soil Science Australia (www.soilscienceaustralia.org.au) and CSIRO [68] and researchers in Europe and the Americas [60], suggest that regardless of fire intensity, due to the short duration and the fact that much of the fuel is held above the ground, grassland temperatures peak rapidly and therefore soil heating into the range where biological damage is expected (usually considered to be >60°C) occurs only at the surface or to a depth of approximately 10 cm. The poor conductive nature of soil and especially dry soil, results in the formation of temperature gradients associated with fire intensity, time of exposure and soil depth [69].

Fire or Burn severity describes the heating-induced alteration of soil and surface properties caused by fire and although there is some lack of clarity regarding it definition, the term is increasingly being utilised to quantify the response of the ecosystem to fire (Fig. 3). It is recommended for use in quantification of the degree of soil burning and the loss of organic matter both on the surface of the soil and the below-ground effect of fire on biomass [70].

Figure 3. Illustration of fire intensity versus burn severity (Source: US Fire Service).



Bushfires often result in a mosaic of fire intensities resulting in variation in burn severities. Since the greatest proportion of heat is released to the air and not to the ground, fire intensity may not be a good indicator for changes to soil properties or fertility following exposure to fire. Rapid diagnosis of the extent of soil burn severity (depth and surface area) is essential for providing appropriate post-fire rehabilitation to facilitate rapid regrowth on agricultural grasslands. Several early empirical studies attempted to measure fire severity and formulate metrics. The studies largely relied on surface measures associated with forests and shrublands [71, 72] but have evolved to utilise visual indicators such as estimation of the quantity and quality of remaining organic surface litter and plant matter, the quantity, colour and structure of ash or soil, and the colour and depth of charcoal present above and below ground [60, 73, 74]. These metrics have been condensed to an index for early assessment of burn intensity (Table 1)

Table 1. Burn severity index relating to changes on surface vegetation and soil organic matter originally developed by Ryan and Noste [75] and modified from Keeley [64].

Burn severity	Description
Unburnt	Plant parts are green and undamaged, no direct effect from heat
Scorched	Unburnt but plants exhibit leaf loss from radiated heat
Light	Trees scorched but leaves remain; surface litter, mosses and grasses charred; soil organic layer mostly intact and charring limited to shallow depth (mm)
Moderate or severe surface burn	Canopy cover consumed, some charring but few leaves remain in trees; all understorey shrubs and larger plants charred or consumed; soil organic layer largely consumed
Deep burning	Canopy trees killed; surface litter and soil organic layer largely consumed; white ash deposition and charred organic matter to depth of several cm

This is useful for initial assessment of burn severity although these methods are unreliable in predicting post-burn soil fertility [76]. Other methods of burn severity quantification include estimation of changes to soil chemical and biological properties such as soil organic carbon, pH and acid phosphatase activity [76]; these methods are suitable for in-depth assessment but may be unfeasible for rapid assessment of large burnt areas resulting from mega-fires across grasslands.

Technological advances enable use of remote spectral sensing to determine changes in soil properties after fire. The method generally accepted for assessing and mapping burn severity utilising spectral indices is the normalised burn ratio (NBR). This is an index designed to highlight burnt areas in large fire zones utilising a formula combining near infrared (NIR) spectroscopy and shortwave infrared (SWIR) wavelengths. This and other spectral or remote sensing methodologies provide insights to soil fertility and have successfully been utilised in assessment of post-fire recovery and future fire risks both in Australia and other countries [77-81] but there is some lack of agreement regarding classification of conditions utilised to determine the indices [82].

1.4 Post fire effect on soil

Soils are the foundation of every farming enterprise and underpin the capacity of managed ecosystems to support pasture growth and ultimately livestock production. Soil health is a dynamic relationship encompassing minerals, organic matter, air, water and living macro- and microorganisms that interact with plants to provide nutrients, control pests, and convey other benefits, such as the capacity to increase plant nitrogen. Soil conditions impact on plant regeneration and growth, translating into variations in nutritive value of livestock feed. Recognition of the importance of soil health has led to a growing body of literature describing the effects of fire on soil properties [30, 65, 77, 83-86].

Bushfires may result in direct and indirect consequences on grassland soil properties and dynamics, depending on various factors such as the fire history and intensity, burn severity, soil type, post-fire weather, topography, vegetation recuperation [87], and post-fire management [88]. The effects themselves are usually temporary since soils have a reduced thermal conductivity. The most significant changes to soil are considered to occur within the immediate period following fire exposure, especially in the case of fires with extreme intensity and deep burning although the consequences of fire exposure may impact long term recovery. The following subsections report on the current understanding of the primary post-fire impacts of bushfire on soil. Stratification by soil type is beyond the scope of this review and where possible we describe effects on grassland.

1.4.1 Soil water repellence and erosion

Fire can create, strengthen, or destroy the capacity of soil to repel water, this has potential implications for soil hydrology, surface water runoff and erosion [89-91]. The extent of fire-induced change is influenced by fire intensity and the subsequent soil temperatures.

At low to mid fire intensity, changes to soil water repellency are not significant but increasing fire intensity may affect water repellency. Stavi et al. [92, 93] conducted a study on the soils of grazed rangeland and cropland in north-western semi-arid Negev in southern Israel. The soils had been exposed to an uncontrolled fire that broke out during a heat wave (temperatures exceeded 40°C). Fire severity within the rangeland area was deemed as moderate to high severity based on remote spectral indices and visual assessment of surface organic matter. The exposure to fire resulted in a significant increase in soil water repellency. Cawson et al. (Australia) [91] carried out prescribed burn experiments, exposing a variation of soil types/topographies to a range of fire temperatures. One of the sites comprised of 42% grass cover and the burn at this site resulted in an average surface peak temperature of 129°C and a sub-soil (20 mm) peak temperature of approximately 90°C. The soil repellency was measured 2-6 weeks post burn; there was evidence of stronger water repellency in the burnt soils and to a greater depth, compared with soils from unburnt areas. The data suggest that grasslands exposed to intense heat will have soils with greater water repellence and decreased capacity for moisture infiltration. This is supported by the findings of other reports in the Mediterranean [83] and other regions including that of Hubbert et al. [94] reporting on a long term assessment of the consequences of unplanned wildfire on soil water repellency, moisture content and erosion in samples collected twice a year for five years post the original fire event. The site comprised of grassland and chaparral in the San Dimas Experimental Forest region of California. These are not grazed agricultural lands, but the presence of grassland and the duration of the study allows for comparison. The wildfire burnt 90% of the selected test site and 100% of the vegetation, the fire intensity was classed as extreme with deep burn intensity. In the years following the fire event the average rainfall pattern was slightly below the regional mean, however one year after the fire there was an above average rainfall season. Soil repellency was measured in samples collected at the surface and depths of 2 and 4 cm and overall, soil water repellency increased with soil depth, decreased with time following the fire, and was inversely related to soil moisture content (the soil was least repellent during the winter after the annual rains and most repellent during the summer).

Burn severity has a primary role in soil erosion and deep burning (complete burn of all vegetation) in combination with increased soil repellence results in increased soil erosion. Vieira et al. [95] performed a meta-analysis of existing literature encompassing 109 individual observations that analysed a broad geographic range (including grass and shrubs in Australia, Spain, USA, Mexico and Israel), varying fire intensity and both bushfire and prescribed burns. The analysis determined the effect size of post-fire runoff and erosion response was determined by four key factors: soil burn severity, time elapsed since fire, rainfall intensity and bare soil cover. Burn severity was a key factor in soil erosion rates with a strong correlation between low burn severity and low erosion ranging to high burn severity and corresponding high long term soil erosion. The erosion was most severe soon after the fire event but there was clear evidence of ongoing post-fire related erosion up to 3 years post-event. The conclusions of the meta-analysis suggest that high rainfall intensity immediately post fire event was strongly associated with high risk of erosion. There is, however, variability in actualisation of such risk [96].

1.4.2 Soil nutrient content

To support plant growth, soils should contain a steady supply of organic carbon, and macro and micronutrients. Soil organic matter is a product of plant and animal decomposition and leads to the formation of soil organic carbon via mineralisation. The quantity of belowground organic matter varies widely dependent on factors such as vegetation type, temperature, and moisture. Grassland soil's organic carbon content ranges from 1.4 g C kg^{-1} in semiarid conditions to 13.5 g C kg^{-1} in dry sub-humid Mediterranean climate zones. The higher proportion of organic carbon is sequestered to the plant root layer within soil, an area particularly at risk in high intensity fire [97]. Macronutrients supplied by the soil (nitrogen, phosphorus, potassium, sulphur, magnesium, and calcium) are essential for the formation of crucial cellular components in the growth of plants such as proteins and nucleic acids. Micronutrients contribute as cofactors of plant enzyme activity and are also known as trace elements. Within soil the essential micronutrients and are molybdenum, copper, zinc, manganese, iron, nickel, boron and chlorine [98]. Several factors contribute to the capacity of plants to take up nutrients from soil and are therefore important for propagation of soil fertility: soil properties (pH, texture), microbiome (influence organic carbon and organic decomposition Section 3.3), soil organic matter (regenerative source of nutrients) and soil hydraulic properties. Exposure to fire leads to a change in the abundance , form and distribution of available and total nutrients present in soil [60, 67]. Nutrients may be lost in gaseous form (volatilization, gasification) or dispersed in particulate form in smoke [99].

While use of low intensity anthropogenic fires is considered beneficial to soil fertility (improved pH, soil organic compounds, transferrable nitrogen and exchangeable carbon) [100], increasingly it is recognised that uncontrolled fire of mid to high fire intensity or burn severity may result in a decline in soil organic matter and change in soil nutrients leading to impacts on soil fertility [101]. Fire intensity and severity are key factors in changes to soil nutrient content [102] for example, specific key nutrients such as organic carbon and nitrogen are highly susceptible to volatilisation at high temperatures (200°C-500°C [69]) while others are more resilient, which may result in an unequal abundance of essential nutrients. In particular, loss of adequate nitrogen and organic carbon from the soil may impair function and soil fertility [99, 103].

1.4.2 Macronutrients and micronutrients

Low intensity burns equivalent to a prescribed burn do result in changes to some source macro and micronutrients. Pereira and colleagues [104] analysed nutrients in soils immediately after and again at regular intervals (2, 5, 7 and 9 months) post exposure to a low intensity grassland fire in Lithuania. The results showed that a low severity fire results in significant variations of soil nutrients, but these impacts were mainly limited to the immediate seven-month period post fire event (decrease in soil pH, aluminium, and manganese but increased calcium, magnesium, and potassium). Ubeda et al. [105] did a similar longitudinal (one year) study on soils obtained from grassland exposed to low intensity fires from a region in north-east Spain. In contrast to the Lithuanian study, immediately post fire pH was increased but a year later, it had returned to pre-burn levels. Similarly, potassium increased immediately post-burn and a year later it decreased to below pre-burn levels. In contrast, phosphorus levels were elevated after fire and remained elevated a year later.

Higher intensity and severity fires result in a more varied changes in soil bound nutrients. Ekua Amoako and Gambiza [100] report on the results of analysis of the effect of a gradient of fire intensity on grassland savanna in Ghana, West Africa. Soils were collected from six districts and a range of land use types. The fire intensity and severity at the collection sites ranged from low to mid and results show a clear correlation between increasing fire intensity and loss of total nitrogen and calcium.

1.4.3 Soil organic carbon

The soil organic carbon concentration in post-burn soils sourced from grazed or ungrazed grassland is influenced by the sampling depth, burn intensity, moisture content, plant species and wind and rain conditions post burn. High burn intensity will result in significant reduction of soil organic carbon content in the short term due to mineralisation of carbon from organic matter followed by volatilization and then, if wind is a factor, the removal of carbon rich ash. If wind and erosion is not a factor, then the soil organic carbon content can increase following fire due to incorporation of carbon-rich ash into the soil [84, 106]. Studies report increased soil organic carbon following moderate to high intensity fires in varied Mediterranean ecosystems [84], short term (2 weeks post uncontrolled wildfire in grazed savanna in Israel [93]) and long term analysis over a ten year period of ungrazed grassland comprising of both C3 and C4 grass species, and exposed to annual prescribed fire and periodic wildfire events in Texas, USA [107]. Carbon and nitrogen concentrations were generally greatest in soils growing C3 grasses but decreased exponentially with soil depth. Exposure to more intense burns increased soil organic carbon in the top 20cm of soil in comparison to soils exposed to low intensity fires.

These findings suggest long term carbon sequestration within grassland soils is not at risk due to the action of bushfires and although there may be short term alterations of available organic carbon immediately post fire-event, the long-term impact is not damaging to soil fertility and plant regrowth.

1.4.4 Soil microbial community (microbiome) diversity

Soils represent an important habitat for microbial populations that exert significant impacts from the microscale to ecosystem wide processes and they play a major role in post-fire recovery [108-111]. Soil microorganisms are key factors in maintaining long term sustainability of soil ecosystems as they control the decomposition of organic matter and the amounts of soil carbon and nutrients via nitrification, denitrification or mineralization of nitrogen and carbon, and immobilization processes [112]. The term microbial population commonly describes a collection of microorganisms living together. Specifically, microbial communities are defined as multi-species groupings, in which (micro) organisms interact with each other in a contiguous environment [113]. The term microbiome has emerged to consolidate a combination of terms associated with microbial communities: “micro” and “biome”, naming a “characteristic microbial community” in a “reasonably well-defined habitat which has distinct physio-chemical properties” as their “theatre of activity” [114]. Living microorganisms such as bacteria, archaea, fungi, algae, and small protists should be considered as members of the microbiome [115].

Members of the soil microbiome are primary factors responsible for soil recovery post fire [116] and their rapid response to environmental conditions make them ideal for use as early indicators of changes in soil quality. Predominantly research exploring the effect of fire on the microbiome has been performed on arboreal landscapes but increasingly research is exploring the effect of fire on grassland or savanna. The results of numerous studies suggest several fire-induced mechanisms affect the soil microbiome: heat induced killing of the microbes and destruction of the microbial habitat; alteration of the soil chemistry (e.g., increased pH, adaptation of water repellence), altered biological environment (e.g., reduction of microbial taxon variability or loss of symbiont plants) [117, 118]. Fire impacts soil microbiome biomass and diversity in part by limiting soil resources and results in a change in the dominance of competing bacterial and fungal microbes that in turn, may have significant impacts on soil and plant recovery [119, 120].

Sáenz de Miera and colleagues [120] investigated the differences among bacterial communities of three different Mediterranean ecosystems, two shrubby and one arboreal, in samples sourced 2 months after a wildfire of high burn intensity. Core samples were collected from the top 10 cm of the collection sites and combined prior to analysis. Two levels of fire severity (high and low) were compared to control unburnt soil and the studies confirmed that greater fire severity results in a reduction in diversity of soil bacterial communities (reductions of 41% and 59% of the control values for richness and Shannon's diversity, respectively, whereby richness captures the number of species, and Shannon’s diversity is a measure of the relative abundance of those species). Exposure to more intense and potentially prolonged heat triggered the dominance of bacterial species (Oxalobacteraceae, Micrococcaceae, Paenibacillaceae, Bacillaceae and Planococcaceae) in all three sampling zones in comparison to the unburnt control samples. The study concludes that bacterial taxa *Massilia*, *Arthrobacter* and *Paenibacillus* are good indicators of fire severity and may be useful in evaluation of post fire recovery. Bacteria of the taxa *Massilia* and *Arthrobacter*, and the fungi *Penicillium* sp. and *Fusicladium* sp. were also reported as significant positive fire responders (present at an abundance of between 20 and 100 times that of unburnt soil) in samples collected one year post fire from fifty separate wildfire sites located primarily in Canadian boreal forests but also included some shrubland and twelve unburnt control sites [120]. The burn intensity and severity across these sites ranged from low to high and was a significant variable in bacterial and fungal diversity. Additionally, there was a significant positive relationship between burn severity gradient and weighted mean predicted 16S copy number. For both studies (Spain and Canada), the most abundant bacterial operational taxonomic unit was classified as *Arthrobacter* suggesting that it is a very strong contender as an indicator of long-term burn severity in soil. Studies suggest that gram negative *Arthrobacter* may be able to survive fires due to its ability to resist starvation, desiccation and oxidative stress and may thrive on post fire aromatic carbon sources [121]. The bacteria may also play a role in post fire nitrogen cycling and phosphorus solubilization, both factors important in encouraging plant growth [122].

Soil microbes are diverse in their macromolecular structures and metabolites [123] and therefore microbial-derived soil organic matter may reflect distinctions across communities. Identification of fire-responsive taxa may assist in elucidation of ecological strategies and help to predict the long-term ecological and biogeochemical effects of fire.

1.5 Fire effect on agricultural grassland regeneration

The capacity of a natural system to return to its initial state following a challenge event may be termed as ecological resilience [124]. In terms of agricultural grasslands, herbaceous vegetation such as perennial grasses are generally considered to be highly resilient to regrowth following fire. However this resilience relies on a host of ecosystem factors including condition of vegetal biomass, soil fertility, environmental conditions (rain, temperature, evaporation) and access to seeds from which new plants can germinate.

Grasses are some of the most resilient plants on the planet, resulting in their predominance across the biosphere and they are a vital factor in global food security as a rich source of fodder for farmed livestock [45]. Unlike many woody plants, grasses have evolved to rapidly regenerate following adverse events such as droughts, floods and fires [125]. On the other hand, their resilience may also contribute to increasing fire severity since due to their herbaceous growth form, tendency to grow in close aggregations and accumulate dense dry under-thatching, grasses make an ideal fuel for fire under the correct conditions [103].

1.5.1 Regrowth

In regions of USA, Brazil, South Africa, Ghana and Australia, grasslands are well adapted to periodic low intensity fires either through exposure to prescribed burns or annual fire seasons [103]. The beneficial aspects of these fires may be clearance of litter and old or dead plant material, encourage growth of new nutrient rich growth.

Grassland management and selection of cultivated grasses and legumes should not only consider the benefits to livestock but also the ability to maintain and support the ecosystem e.g., through carbon sequestration. This requires the monitoring of e.g., C3 and C4 grasses and an understanding of the effects of unplanned fire on the growth and density of plants capable of swift recovery and enhanced ecosystem functionality. Long term studies on grassland recovery post-fire in Texas, USA [107] showed enhanced growth of C3 grass species. In Australia the perennial herbaceous C4 species *Themeda triandra* Forssk (Kangaroo Grass) shows increased adaptation to fire since germination and proliferation are encouraged through exposure to fire [126]. Exposure to high intensity or frequent fires has been shown to encourage dominance of fire resistant native pyrophytes and other plants that may swiftly exert dominance [59, 127]. Proliferation of ecologically resilient and nutrient rich introduced pasture species, notable Buffel Grass (*Cenchrus ciliaris*), is correlated with increasing fuel load and corresponding increased burn severity [128] and the introduced perennial Gamba Grass (*Andropogon gayanus*) creates fuel beds with seven times more biomass than those created by native Australian species [129].

1.5.2 Grassland seedbank resilience to fire

Within the soil there exists a seed bank that can support passive restoration of fodder and other plants following adverse events. The seed bank is not immune to what occurs above ground and events such as fire may significantly damage the germination potential of stored seeds. High fire severities affect seed abundance in the soil, and this is especially observed when high temperatures are combined with prolonged periods of contact. Normally, there is a reduction in seed germination rate with increasing temperature, and at 300°C most of the seeds are killed. Low intensity controlled fires are an important land management tool and fulfil crucial roles such as maintaining plant densities and contributing to the nutrient profile of soils through generation of carbon-rich ash [103].

Cuello and colleagues [130] performed a study to assess the effects of heat and/or smoke on seedbank germination of soils sourced from the Eastern Hills region in Uruguay. The soils had been exposed to prescribed fire 2 hours prior to sampling and samples were taken from the top 5 cm. Additionally, unburnt samples were sourced from adjacent sites and experimentally exposed to heat (100°C) for a five-minute period to imitate a high intensity burn. Once dried, samples were encouraged to grow and were monitored for 140 days. The results suggest soil exposure to prescribed burns result in enhanced seed germination in terms of time to germination, density, and species richness. Soil exposed to 100°C for 5 minutes responded to germination similarly to unburnt control samples.

On the other hand, high intensity/severity fire may significantly affecting seedbank abundance to a depth of 10cm based on a study by Lipoma et al. [131] who report findings from a region of semi-arid shrubland in Cordoba, Argentina with soils exposed to high intensity and high severity fire and monitoring of plant diversity recovery 3 years post the fire event. They report findings of species composition relatively similar between the burned and unburned plots but the total seed number was six times lower in burnt soils in comparison to burnt soil .Reduction of seed abundance following prescribed burns in savanna grasslands was also reported from Brazil [132]. In addition to the seedbank as present in the soil at the time of burning, the generation of seeds by regrowth can be considered. Fontanele et al. [133] report the very poor quality and quantity of seeds grown from savanna grasses following a control burn event. For 17 weeks after the burn event, seeds were collected from grasses that spontaneously regrew and the fertility of the collected seeds was assessed to be low in seeds collected from burnt soils in comparison to unburnt controls.

These findings suggest that increased intensity, severity, and regularity of bushfires may exert a significant effect on soil seedbank abundance and fertility, with considerable differences between pyrophytic and/or native species and introduced species. Further studies in the wake of the recent mega-fire events would aid in clarifying the long-term effects of fire on seedbank potential.

1.6 Interventions for rapid post-fire recovery

Development and implementation of interventions and management processes to mitigate the impact of severe fire on the health and productivity of soils, seed and plants relies implicitly on a clear understanding of burn severity and intensity [88]. The research findings discussed in this review clearly indicate that mid to high severity and/or high intensity bushfires exert potentially damaging effects that may hinder rapid recovery post-fire and typically, low intensity fires do not have detrimental effects on soil or plant regrowth making a one-size-fits-all model of post-fire intervention inappropriate [88]. Currently, several intervention strategies are considered in post-fire settings and mulching, seeding or resting are most applicable for grazing systems [60].

1.6.1 Mulching

A major consequence of high intensity bushfire is erosion of burnt soils resulting in loss of macro and micronutrients. Application of agricultural straw (mulching), vegetation residues or biological geotextiles is a common post-fire management strategy employed to limit runoff and erosion at severely burnt sites [134, 135] and is intended for implementation immediately after the burn event. The practice protects soils from raindrop effects and aids in limiting erosion [136], has beneficial effects on soil water infiltration and regulating temperature fluctuations [137], enhances the activity of some earthworm species [138] and may increase water infiltration and soil quality over time, in part due to the access to organic matter and nutrients [135] .

As a post-fire intervention, mulching of soils has been assessed primarily in the context of forests and non-grazed land [134, 139-143]. Bautista et al. [144] did longitudinal examination of the regenerative capacity of straw mulching in soils exposed to unplanned fires in comparison to un-mulched burnt and unburnt control plots in the Benidorm region of Spain. Whilst this study was in a non-grazed area it assessed runoff, sediment yield, plant cover and soil dynamics over a 2-year period and revealed significant reduction of runoff and soil loss in the mulched plots. Plant growth in both the burnt mulched and burnt control plots were significantly reduced in comparison with unburnt plots with evidence of slightly stimulated vegetation growth in mulched plots.

There are key knowledge gaps in the efficacy and feasibility of mulching in the agricultural context and in particular, the economic feasibility of mulching applications in the agricultural context are not readily available and may limit functionality.

1.6.2 Seeding

Seeding is a process of rehabilitating land by the application of seeds for desired plant species and has long been a recommended practice in United States land management following exposure to wildfire [60, 144-146]. The practice is intended to reduce overgrowth of invasive species, reduce soil erosion where fire has eliminated the existing seedbank and encourage the growth of desirable species.

There is a growing body of evidence that questions the merits of the practice and whether seeding results in a desired regrowth distribution. Bruce et al. [147] report on a longitudinal study assessing the density and diversity of plants in grazed and un-grazed pastures that had been exposed to fire. The study determined no significant adaptation in plant diversity between seeded and unseeded pastures two years post-fire event.

1.6.3 Resting

Current guidelines in the United States recommends grazed land be rested for at least two seasons following a wildfire event [146] to allow optimal time for recovery of perennial plants and reestablishment of seeded species. This is a well-established recovery practice based upon the understanding that exposure to fire reduces plant regrowth, productivity, and diversity. However, recent data suggests that with growing understanding of the response of soil and vegetation to fire processes, it may be necessary to incorporate consideration of the burn intensity and burn severity but also events post-fire (e.g., rainfall, accurate measure of soil health and the density and identity of the plant regrowth). This may result in revision of required recovery time and an adaptation of management [148].

Analysis of the published research suggests a paucity in up to date and relevant recovery interventions in an environment of climate change and particularly with relevance to grasslands in the Australian agricultural setting. Further research is required to address post-fire recovery of agricultural grasslands when exposed to mega-fire events since practicality of mulching of large tracts of affected land may be unfeasible and seeding may not be beneficial in the long run. Practicality of allowing time for pastures to rest is dependent on the individual producer but a structured set of informative guidelines relevant to the Australian setting and based upon the results of experimental trials may be beneficial.

2. Objectives

- A literature review is delivered summarising the possible direct and indirect consequences of fire on the recovery of soils, pastures, and other vegetation, and options for their management from similarly affected areas in Australia, North and South America, Africa, Russia, and Europe.

- Results and summary regarding the consequences of bushfire on soil quality as measured by assessment of biogeochemistry (Section 4.4) soil microbiota (Section 4.6) is delivered.

- Insight is reported on the effect of bushfires and planned burns on nutrient values of collected pastures (Section 4.3) the growth patterns of seed banks of pasture vegetation (Section 4.5), and the effect of fire on pasture growth, normalised difference vegetation index and biomass accumulation as determined by remote sensing (Section 4.2).

- Insights to any interrelationships between nutrient content and the soil microbiome, vegetation and grazing livestock in pasture systems are discussed within individual summaries.

- An interdisciplinary toolkit for post-fire impacts and recovery to inform future post-fire pasture recovery and management was planned as an objective. Over the intervening years several excellent manuals, factsheets and web-based information portals have been generated. We have collated these resources and sought to add value through added content with regards to soil and pasture recovery post fire to complement the existing Bushfire recovery manual generated by the MLA.

3. Methodology

3.1 Granting of required research ethics and approvals

3.1.1 Ethics approvals

Ethics approval is not required to collect soil, and plant samples however, human ethics approval is required to access information pertinent to farm location. The farms selected for the project are additionally selected for sampling for the aligned MLA project B.AHE.2102: Health, welfare and biosecurity of livestock exposed to Australian bushfires: an on-farm case control study. A joint application was submitted, and the application was supported (Reference number: 2021-14224-14832-2).

3.1.2 Travel restrictions

Approval was sought and received from the University of Sydney in line with government directives that prohibited activities during the COVID-19 pandemic that involved being physically present for data collection with human participants such as face-to-face field work, experimental and cohort studies, and clinical trials. Approval to travel to conduct field sampling or to access laboratory/research space was required from several administrative levels within the University of Sydney commencing soon after Covid-19 was detected within NSW and continuing at varying levels of severity until late in 2022.

3.2 Selection of properties for sampling

3.2.1 Recruitment of planned burn site

Due to pandemic restrictions and repeated rainfall events, it was impossible to conduct a managed planned burn through the auspices of the University of Sydney. We were able to locate an alternative site for assessment of a planned fire event.

Close to Woolsthorpe in Victoria and managed by Landcare Victoria as part of the Basalt to Bay Landcare project, the property adjoins paddocks that are co-grazed with sheep and cattle. The farmland on the east of the track drains to Spring Creek at Woolsthorpe. An ecological burn under the management of local fire authorities was conducted on the south end of the Woolsthorpe site on Saturday 15 January 2022 with the aim of assisting the native grassland to out-compete exotic grass invasion, opening spaces between the tussocks to allow herbs, lilies, and other species that may be present as seed store to emerge, and to enable invasive grasses like Paspalum to be identified and treated. Researchers at The University of Sydney were able to source soil from the site the day after the planned burn (16 January 2022, collected by the land manager) and soil, seedbank and pasture 6 months later (July 2022).

3.2.2 Recruitment of bushfire exposed sampling sites in Monaro and Bega regions

The MLA project B.AHE.2102 (Health, welfare and biosecurity of livestock exposed to Australian bushfires: an on-farm case control study) had previously located and sampled a range of properties impacted by fire thus the decision was made to make use of a selection of these properties (Table 2). The reasoning behind this was as follows:

- The approximate location of fire fronts on many of these properties had been mapped. This information indicated that a substantial number of these properties contained both burnt and unburnt areas. This is important as this project investigates the role of fire on soil and pasture properties. To make this comparison, it was necessary to compare fire impacted locations with unburnt sites, whilst limiting the effects that different land management practices (on different properties) may have on any of our measurements.
- The available information suggests that these burnt/unburnt areas are in many cases on the same soil profile (Table 2). As soil profile plays a key role in all measurements that will be

undertaken in this project, it was important to sample on the same soil type (to the greatest degree possible) to compare “like-with-like” to compare the effect of burning on soils.

- The sites were selected on the basis they encompassed a wide geographical distribution (Nowra – Costal region to Bombala – Inland) and a range of agricultural practices (Table 2) and thus will give us some confidence that any results we find will be broadly applicable to bushfire impacted agricultural areas in general.
- Finally, using already established sites allowed potential for future cross comparison of collected data with the findings of the B.AHE.2102 to leverage both datasets to provide a more comprehensive understanding of the impact of fires on both pasture and livestock systems.

Table 2. Location, property identity code and soil profile of selected bushfire exposed properties.

Property ID	Location	Agricultural practice	Soil profile
RG1	Cobargo	Beef	Kurosols
RG2	Bemboka	Dairy	Kurosols/Kandasols
RG3	Bombala	Beef	Kurosols/Ferrosols/Dermasols
RG4	Moruya	Beef	Kurosols, Kandosols, Demosols
RG5	Nowra	Beef	Rudosols/Tenosols

3.3 Remote sensing

To determine the extent that the properties used in this study were impacted by fire several different sources of information were used. This data involved a combination of remotely sensed data (primarily Sentinel-2 satellite imagery which collects data across south-eastern Australia on 5-day basis) and modelled data. Combined analyses from these datasets enabled a variety of information and inferences to be drawn.

3.3.1 Fire severity

As several of the properties had been involved in a previous study that had mapped fire boundaries, this was the primary determinant of fire location on the property. In a number of cases this had not occurred, in which case we used the NSW Department of Planning and the Environment Fire Extent and Severity Mapping version 3 data (FESMv3) (https://datasets.seed.nsw.gov.au/dataset/fire-extent-and-severity-mapping-fesm-2019-20) to map the extent of fires on these properties. The FESMv3 has been developed using Sentinel 2 satellite imagery and was used to map both the extent and estimated intensity of fires across NSW during the 2019-2020 bushfire season. Based on both aerial photographs and field validation it has an overall accuracy of between 60-95% for the fire classification classes (Table 3) with an overall accuracy of 76% (DPIE FESMv3 Factsheet (December 2020)).

Table 3. Fire severity classification ruleset based on high resolution aerial photo interpretation. (DPIE FESMv3 Factsheet)

Value	Severity Class	Description	% Foliage fire affected
0	Unburnt	Unburnt surface with green canopy	0% canopy and understory burnt
2	Low	Burnt understory with unburnt canopy	>10% burnt understory >90% green canopy
3	Moderate	Partial canopy scorch	20-90% canopy scorch
4	High	Complete canopy scorch (+/- partial canopy consumption)	>90% canopy scorched, <50% canopy consumed
5	Extreme	Complete canopy consumption	>50% canopy biomass consumed

3.3.2 Fire Intensity

As fire intensity is often greater in landscapes that contain a greater proportion of trees (and was clearly the main driver of fires during the 2019-2020 bushfire season), the relationship between tree cover and fire intensity was determined using the National Forest and Sparse Woody Vegetation data (Version 5.0 – 2020 release; https://data.gov.au/data/dataset/national-forest-and-sparse-woody-vegetation-data-version-5-2020-release). This dataset categorises the Australian landscape into non-woody (<5% canopy cover = Grassland), sparse woody cover (5-19% canopy cover = Woodland) and forest (≥20% canopy cover = Forest). For the purposes of this report, grassland is classified as Non-woody, Sparse woody cover as Woodland and Forest as Forest.

3.3.3 Pasture growth

As pasture growth is primarily driven by rainfall and temperature and thus growth and recovery of pastures following the 2019-20 bushfires will be dependent on this, several metrics were used to investigate the timing and response of pasture growth subsequent to the bushfires. This data was obtained from two sources. Rainfall in the location of interest was obtained from the SILO website (https://www.longpaddock.qld.gov.au/silo), which provides interpolated (based on Bureau of Meteorology weather stations in the surrounding area) estimates of rainfall and other climate data on a 5 km^2 grid across the whole of Australia. Data was also obtained from the “Enhanced Drought Information System” (EDIS) that has been developed by the NSW DPI (https://edis.dpi.nsw.gov.au/about). The EDIS system consists of 3 indicators; a Rainfall Index (RI) and indicator of the amount of rainfall that has fallen over the previous 12 months; a Soil Water Index (SWI) which provides an estimate of the amount of water held in the soil and available for plant growth and; a Plant Growth Index (PGI), an indicator as to the extent of plant growth based on the water available for plant growth along with climatic conditions that are likely to promote plant growth. All three indexes are produced on a percentile scale of 0 - 100 which ranks current conditions against a long-term baseline (1980 – 2010) and where 0 means no rainfall/soil water/plant growth and 100 means essentially no limits to plant growth. These three indexes are then combined to produce the Combined Drought Index (CDI) which uses a six-level scale where; 5 = Intense Drought; 4 = Drought; 3= Drought affected and intensifying; 2 = Drought affected but weakening; 1 = Recovering from drought, and 0; Not in drought. See Table 4 for additional information. Each of the indexes are produced at a parish scale (which typically covers an area of 10-20 farms) and so the specific parish that encompassed each property was used. This data is only currently available to the end of 2021.

Table 4. The Combined Drought Index Phases from "Understanding CDI Phases", NSW DPI (https://edis.dpi.nsw.gov.au/cdi-drought-phases)

CDI Phase	Technical definition	Description - typical field conditions
Intense Drought	All three indicators (rainfall, soil water, plant growth) are below the 5th percentile	Ground cover is very low, soil moisture stores are exhausted, and rainfall has been minimal over the past 6-12 months
Drought	At least one indicator is below the 5th percentile	Conditions may be very dry, or agronomic production is tight (low soil moisture or plant growth). It is possible to be in Drought when there has been some modest growth, or a few falls of rain
Drought Affected (intensifying)	At least one indicator is below the 30th percentile and the rainfall trend is negative over the past 90 days	Conditions are deteriorating; production is beginning to get tighter. Ground cover may be modest, but growth is moderate to low for the time of year. When indicators are close to the Drought threshold drought conditions are severe
Drought Affected (weakening)	At least one indicator is below the 30th percentile and the rainfall trend is positive over the past 90 days	Production conditions are getting tighter, but there have been some falls of rain over the past month. It is rare to enter the Recovering phase from the Non-Drought category; Usually there is a quick (1-2 week) transition into Drought Affected or Drought. When indicators are close to the Drought threshold drought conditions are severe
Recovering	All indicators are below the 50th percentile but above the 30th percentile	Production is occurring but would be considered 'below average'. Full production recovery may not have occurred if this area has experienced drought conditions over the past six months
Non-drought	At least one indicator is above the 50th percentile	Production is not limited by climatic conditions

3.3.4 Total standing dry matter (“biomass”) and ground cover

Estimates of Total Standing Dry Matter (“biomass”) and Ground Cover were obtained from Cibolabs (cibolabs.com.au) who have developed regionally based estimates of aboveground biomass at the property scale. They are also able to provide information at the property level for a range of other environmental data including seasonal (determined for three-month season) estimates of ground cover from both living and dead plant material. Ground cover estimates are important in that groundcover (regardless of whether living or dead material) is critical in protecting soils from both wind and water erosion.

3.3.5 Normalised difference vegetation index

Normalised difference vegetation index (NDVI) is an estimate of plant “greenness” and is a well-established and historically used satellite imagery derived means by which to determine not only the extent of plant growth but also a general guide to the overall quality of forage that is growing when used in an agricultural setting. While NDVI cannot provide an estimate of pasture quality, higher values of NDVI will indicate that plants are more likely to be actively growing and taking up nutrients and thus are likely to of higher quality relative to other times of the year when they have lower NDVI values indicating that are not actively growing (senescent, water or nutrient stressed, dead). While NDVI can have a range of -1 to +1, it is typically reported as value of 0 to 1 where 0 indicates an increased likelihood of bare soil and 1 indicates an actively growing plant community which has relatively high leaf area and is likely to be of relatively high forage quality. Across much of south-eastern Australia pasture passed systems are likely to be in the order of 0.3-0.8 depending on rainfall and season with higher values found in wetter and warmer (peak-growing) times of the year. Data on NDVI for the properties used in this study were obtained using Google Earth Engine using Sentinel-2 images (earthengine.google.com). For the purposes of this part of the analysis, the NDVI for grassland only areas (on-farm Woody or Forested areas excluded) that were present on the property was determined as well as the NDVI for grasslands that were either burnt or unburnt. This was undertaken to determine how pasture systems responded to fires both in the short- and long-term.

The response of pastures to fire impacts will be confounded with the extensive drought that impacted much of south-east Australia in the two-year period prior to the fires. This will have an impact in several different ways. In the first instance, the reduction in biomass meant that on-ground fuel loads were lower and as such fire intensity was also likely lower than if the year prior to the fire had had an average (or higher) rainfall year. Secondly, due to a combination of the drought and the time of the year (peak of summer), many of the pasture species (particularly C3 that make up the bulk of improved pasture systems in south-eastern Australia) would have been in state of dormancy. This meant that depending on the intensity of patchiness of the fire in grassland area, it may be relatively unaffected or only part of it is impacted and thus with the onset of rain is able to come out of dormancy and continue to grow.

As pre-fire almost certainly impacted on the post-fire conditions, these have been included in all analyses data from 2018 until till current. The exception to this was for the NDVI measurements as the images were not available for this period.

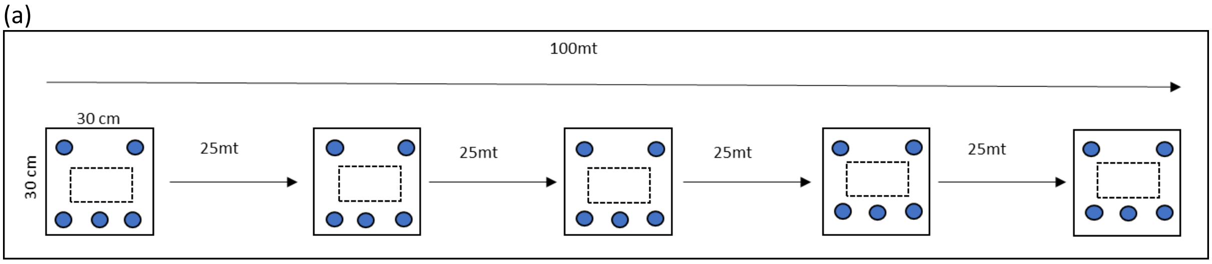
3.4 Sample collection

All five bushfire exposed properties had paddocks exposed to bushfire and those that remained unburnt or, in the case of one property (RG1, Cobargo, Table 2), were exposed to minimal burning. Repeat sampling of both burnt and unburnt areas provided additional sources for in-farm comparison (Table 5). The planned burn site had areas where no burn was carried out, samples were collected from both the burnt and unburnt areas within the site (Table 5).

The sampling template included collecting along 100 mt transects with sampling points every 25 mt; one transect (5 sampling points) for each burnt and unburnt area (Fig. 4a). Sampling squares (Fig. 4b) were employed to ensure consistency in collection volume of plant matter and soil biomass. Each sampling point was GPS (global positioning satellite) tagged to facilitate adjacent re-sampling.

Figure 4. (a) Graphical illustration of 100 m transects with sampling points set 25 m apart. Squares illustrate sampling regime blue dot indicates soil biomass core sampling and dotted rectangle indicates seed bank soil sampling. (b) Sampling square employed to ensure consistency in collection volume of plant matter and soil biomass.

(a)



(b)

Table 5. Sample collection. Full collection of soil and pasture is indicated in dark grey, partial collection is indicated in pale grey. No biological samples were collected from bushfire exposed properties (RG3 and RG5) during lockdown (1) and floods (2) as indicated in yellow.

		Bushfire sampling		Planned Burn sampling	
Property ID	Location	(1) June 2021	(2) June 2022	(1) Jan 2022	(2) July 2022
RG1	Cobargo				
RG2	Bemboka				
RG3	Bombala				
RG4	Moruya				
RG5	Nowra				
PB1	Victoria				

3.5 Plant matter nutrient quality

3.5.1 Hand plucked plant matter.

At each sampling site (burnt and unburnt paddocks) approximately 300 g of plant matter was plucked in a manner that mimicked the feeding patterns of cattle.

3.5.2 Transect plant matter sampling.

The sampling square was laid down (Fig. 4) at the determined locations (Fig. 3) in both the burnt and unburnt paddocks. All plant matter was harvested using a handheld mechanised clipper. Harvested plant matter was stored in collection bags and maintained at a cool temperature for transport.

3.5.3 Plant matter processing and analysis

Samples collected from burnt and unburnt paddocks were immediately refrigerated and sent to the University laboratory. All samples were stored at 4°C. However, due to covid restrictions (lockdown) from June 2021 and the consequent lack of personnel at the University laboratories for several months, a large proportion of the of samples collected in June 2021 were accidentally exposed to unexpected changes in storage temperature that deemed them unsuitable for subsequent analyses.

Samples were dried in a fan-forced oven at 600C for 48 h and ground to pass through 1mm screen. Chemical composition, including dry matter (DM), organic matter (OM), ash, crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), water soluble carbohydrate (WSC), dry matter digestibility (DMD), digestible OM (DOMD) and metabolisable energy (ME) were determined using the following approved analytical methods: sample preparation, dry and grind including DM (Method ID: LMOP 2-1100), ash and OM (Method ID: LMOP 2-1123), CP (DUMAS Combustion Method; AOAC 990.03; 2000), ADF (Method ID: LMOP 2-1108), NDF (Method ID: LMOP 2-1107), WSC (cold WSC Method ID: LMOP 2-1103), DMD and DOMD (pepsin cellulase digestibility; AFIA Method 1.7R Method ID: LMOP 2-1128), calculation of metabolizable energy (AFIA Method 2.2R; based on pepsin cellulase DOMD Method ID: LMOP 2-1124). All chemical analysis were conducted in duplicates at NSW DPI, Wagga Wagga Feed Quality Service.

3.5.4 Statistical analysis

Participating farms were intended to represent any similar farm in the selected areas. Thus, we analysed the data using a linear mixed model with treatment as fixed effect and farm (location) as random effect.

$Y = \mu + \text{Treatment} + (1 \mid \text{Farm}) + \text{error}$

where Y = outcome variable of interest (e.g., NDF, ADF); Treatment = affected (“Burnt”) or non-affected (“Unburnt”) areas (fixed effect; n = 2); Farm = locations (random effect; n = 6).

3.6 Biochemical analyses of soil biomass

3.6.1 Soil collection for biochemical analysis

Soil for biochemical analyses was collected through use of soil corers. Each soil core yielded samples from two depths, 0-2 cm, and 2-10 cm. The core sample was extruded from the coring device and two sections (0-2 cm and 2-10cm) of soil were separated using a ruler and knife (Fig. 5). Individual samples were transferred to labelled collection bags and stored on ice for transport.

Figure 5. Core sampling kit (excluding coring device).

3.6.2 Biochemical processing and analysis

Soil samples were first sieved (2 mm) to remove roots, gravel, and large stones. Any visible roots passing through the sieve were removed. A 10 g subsample was dried in an oven (105°C) for 48 hours to determine soil moisture, and all soil properties are expressed on a dry soil weight basis. Soil pH was measured with a pH probe (Mettler Toledo, Port Melbourne, VIC, Australia) in a 1:5 soil:water slurry. For extractable phosphorus **(**P), a 3 g moist subsample and 20 mL of 0.03 M ammonium fluoride with 0.025 M hydrochloric acid solution were placed into a centrifuge tube, shaken for 15 minutes on an end-over-end shaker, and filtered through Whatman No. 42 filter paper. Extracts were analysed for inorganic phosphorus calorimetrically using the ammonium paramolybdate and stannous chloride colouring reagent [149] on a spectrophotometer at 660 nm (UV-VIS spectrophotometer, Shimadzu, Kyoto, Japan). For extractable ammonium (NH_4^+) and nitrate (NO_3^-), a 10 g moist subsample and 40 mL of 1 M potassium chloride solution were placed into a centrifuge tube, shaken for 1 hour on an end-to-end shaker, centrifuged and filtered through

Whatman No. 42 filter paper. Extracts were analysed for NH_4^+ and NO_3^- on a flow injection analyser (Lachat Instruments, Loveland CO, USA). Available nitrogen (N) was calculated as the sum of NH_4^+ and NO_3^-.

3.7 Seed bank

Samples of soil seedbanks were collected from four fire-affected farms in southern NSW in June 2021 (field staff were required to return to Sydney due to announced lockdown so were unable to collect from the remaining planned farms) and prepared for glasshouse germination in February 2022 (delay due to pandemic lockdown conditions preventing access to laboratory/glasshouse facilities). Repeat sampling of bushfire affected properties was not performed due to long the long delay since exposure to bushfire and multiple rainfall and flood events. For the planned burn event, samples of soil seedbanks were taken from four burnt and unburnt patches in planned burn site in early July 2022 and prepared for glasshouse germination withing a week of collection.

3.7.1 Seed bank biomass collection

Sods of soil, 25 x 30 cm (Fig. 4b), were excavated to a depth of approximately 5 cm with a shovel and small hand trowel. Samples were transferred to plastic bags and stored in cool/refrigerated conditions until required. This method was a compromise of what was originally planned as it was better suited to the prevailing weather conditions and the sampling capacity available. The original intention was to bulk multiple small soil samples from a larger area (e.g., 10 x 10 m plots) as this technique has been shown to give a better representation of the soil seedbank compared to a single larger sample [150, 151].

3.7.2 Preparing seed trays and assessing germination

Each seedbank sample was evenly spread into a lined seedling tray to a depth of about 4 cm. Clods of soil were broken up by hand to ensure seed was not encased in soil aggregations. Trays were placed on benches in a temperature-controlled glasshouse (18°C) with ambient light and watered two to three times weekly as required.

Numbers of emerging seedlings were recorded at two or three time points for each trial. At the conclusion of Trial 1, seedlings representing the range of species that emerged were potted up and grown for another 4-6 weeks for potential identification.

3.8 Microbial biomass analysis

The importance of the soil microbiome to soil quality, and the growth and nutritional value of the plants that grow in it, is well-described. The microbial population of the soil, which includes bacteria, fungi, archaea, and small protists, is essential to a diverse range of biological processes, including plant germination and maturation, nutrient cycling, and the suppression of pathogenic microorganisms. As such, strategies for nurturing the soil microbiome are increasingly featured in soil management plans with the aim of improving soil quality, plant growth and nutritional value.

Climate change has been a major contributing factor in the increased incidence of bushfires and wildfires, particularly in Australia. The impact of bushfires on forests, woodlands and grasslands are well-described. The impact of bushfires on soil, particularly the soil microbiome, are less apparent, particularly the subsequent changes to nutrient cycling, and the growth and nutritional value of the plants that emerge or are sown after the fire has passed. The impact of fire on the soil microbiome is known to vary according to the intensity of the fire. Low-intensity fire can be beneficial for soil quality, by increasing organic matter and extractable cations. However, in higher-intensity fires, where soil temperatures can reach up to 600°C, there is the potential for sterilisation of the soil, which has several subsequent impacts. For example, wildfire in the USA resulted in persistent of soil damage for up to 25 months [12] and other studies report significant loss of nutrient quality of grasses post-fire in the USA [13] and Africa [14].

The aim of this study was to evaluate the impact of the 2020 bushfires on the soil microbiome of NSW farms. Soil samples were collected from five bushfire-exposed properties in NSW, from Northern NSW to Southern NSW, to represent a range of soil types and geographic locations.

3.8.1 Microbial biomass sample collection

Mock burn and other experimental research carried in Australia by on Soil Science Australia (www.soilscienceaustralia.org.au) and CSIRO [68] and researchers in Europe and the Americas [152], suggest that regardless of fire intensity, due to the speed at which fires moves through the landscape and the fact that much of the fuel is held above the ground, temperatures peak rapidly in fires on grassland and therefore soil heating at a range where biological damage is expected occurs only at the surface or to a depth of ≤10 cm. Furthermore, bushfires often result in a mosaic of fire intensities resulting in variation in burn severities.

Soil samples were collected from burnt and unburnt paddocks on each of the selected properties (bushfire exposed and planned burn site). In each paddock, samples were collected along a 100 m transect at intervals of 25 m (Fig. 3) using a 30 mm soil corer at locations within the sampling square adjacent to, but independent from, soils collected for biochemical analysis. At each of the five sampling squares, soil cores were collected, and samples representing depths of 2 cm and 5 cm were separated with a sterile teaspoon (approximately 5 g per sample) and placed in separate sterile 10 mL specimen jars. Samples were stored on dry ice during transportation and transferred to a -80°C freezer upon arrival at the laboratory.

3.8.2 DNA extraction

DNA was extracted from each pooled soil sample using the DNeasy Powersoil Pro kit (Qiagen). A 220 mg (± 10 mg) sub-sample of each pooled soil sample was weighed into a Powerbead tube containing 800 µL of Solution CD1 and vortexed at high-speed for 30 s. Bead beating, which consisted of two 2-min steps at 30 Hz with a 1-min rest between steps, was performed in a TissueLyser II (Qiagen). Thereafter, DNA was extracted as per the manufacturer's protocol. DNA was eluted into 100 µL of Solution C6 and stored at -20 °C prior to analysis.

3.8.3 PCR amplification, library preparation, and next-generation sequencing

A 40 µL aliquot of each DNA extract was sent on ice to the Australian Genome Research Facility (AGRF) for PCR amplification, library preparation, and next-generation sequencing. The V3-V4 region of the bacterial 16S ribosomal RNA (rRNA) gene, and the ITS1 region of the fungal internal transcribed spacer (ITS) gene, were chosen to characterise the bacterial communities (16S microbiota) and fungal communities (mycobiota) in each soil sample, respectively. PCR amplification of the V3-V4 region was performed using the universal primers 341F (5'- CCTAYGGGRBGCASCAG-3') and 806R (5'- GGACTACNNGGGTATCTAAT-3'). PCR amplification of the ITS1 region was performed using the universal primers ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3').

Library preparation was performed using the Nextera XT DNA Library Prep Kit (Illumina). Sequencing was performed on an Illumina MiSeq system using 300 base-pair paired-end chemistry.

3.8.4 Analysis of next-generation sequencing data

The quality of raw data was evaluated with FastQC v0.11.8 [153] upon receipt from AGRF. Analysis of the sequencing data was performed using QIIME2 v2020.8 [154]. The 16S rRNA and ITS data were analysed separately.

3.8.5 Analysis of 16S rRNA (bacterial) data

The raw 16S rRNA data was imported into QIIME2 as a QIIME artifact and quality trimming, merging of paired end reads, chimera filtering, dereplication, and feature table construction were performed using the QIIME2 DADA2 plugin [155]. The forward and reverse reads were truncated based on quality scores, respectively. Twenty base-pairs were also trimmed from the 5’ end of the forward and reverse reads. The default settings were used for all other parameters. Unique sequences were grouped into amplicon sequence variants (ASVs) using a threshold of 99% similarity. Alpha rarefaction plots were generated and inspected prior to undertaking the diversity analysis to confirm that the sequencing depth was sufficient to capture the true diversity of the bacterial and fungal communities in each group of interest in each dataset. An alignment of the representative sequences for each ASV was performed with MAFFT and a phylogenetic tree was generated with FastTree using the q2-phylogeny plugin to support diversity analyses that require phylogenetic information. Alpha and beta diversity metrics were calculated and compared between groups of interest. Alpha diversity metrics included Shannon’s Diversity Index, Pielou’s Evenness Index, and Faith’s Phylogenetic Diversity. Alpha diversity metrics were used to assess the diversity and evenness within each community. The term richness refers to the number of taxa in a community. Evenness refers to the proportion of the total community that each taxon represents. When describing alpha diversity, a single value is calculated that represents the level of diversity and/or evenness within a particular community. A non-parametric Kruskal-Wallis test was then used to compare alpha diversity between groups of interest using the q2-diversity plugin.

Shannon’s Diversity Index describes both the richness and evenness of an ecological community. It is calculated by taking the total number of taxa in a community, the proportion that each taxon represents, and summing the proportion multiplied by the natural log of the proportion of each taxon. The higher the number, the greater the diversity. Pielou’s Evenness Index describes the ratio between the observed value of Shannon’s Diversity Index, and the value of Shannon’s Diversity Index if the relative abundance of all taxa was the same. Values for Pielou’s Evenness Index range from 0 (no evenness, i.e., the community is dominated by one or two taxa) through to 1 (complete evenness). Faith’s Phylogenetic Diversity differs in that it considers the phylogenetic richness of a community. It is calculated by generating a multi-sequence alignment of the representative sequences of all taxa identified in a community, generating a phylogenetic tree based on the multi-sequence alignment, and summing the lengths of the branches connecting all taxa in the community. This metric does not consider the relative abundance of each taxon.

Beta diversity metrics included Bray-Curtis distances, unweighted UniFrac distances, and weighted UniFrac distances. Beta diversity metrics measure the similarity or dissimilarity between communities. Bray-Curtis distances consider the number of species are unique to each community. When calculating UniFrac distances, branches leading to taxa that are shared between two communities are classified as “shared”, and branches leading to taxa that are present in one community only are classified as “unshared”. The distance between the two communities is then calculated as the sum of the unshared branch lengths divided by the sum of all branch lengths. Weighted UniFrac distances also consider the relative abundance of each taxa whereas unweighted UniFrac distances do not consider abundance. In both cases, a distance matrix is constructed describing the distances between communities in each group of interest, and analysed using non-parametric statistical tests, such as a permutational analysis of variance (PERMANOVA).

Taxonomy was assigned to ASVs using the q2-feature-classifier plugin with a Naïve Bayes classifier trained on the SILVA v13.8 database, with 16S sequences trimmed to retain the V3-V4 hypervariable region only using the 341F and 806R primer sequences. Chloroplast, mitochondrial, archaea and eukaryotic sequences were filtered from the feature table and the ASV representative sequences. Taxonomic data were exported as a text file and further analysed in Microsoft Excel.

3.8.6 Analysis of ITS data

The raw ITS data was imported into QIIME2 as a QIIME artifact and processed with the q2-dada2 plugin, as described above [155]. Twenty base pairs were trimmed from the 5’ end of the forward and reverse reads, however quality filtering was undertaken using the default settings only (maxEE = 2; trunc-len = 2). Sequences were grouped into ASVs, and diversity analyses were performed as described previously for the 16S rRNA data. Taxonomy was assigned to ASVs using the q2-feature-classifier plugin with a Naïve Bayes classifier trained on the UNITE v8.3 database with ITS sequences trimmed to retain the ITS1 region only using the ITS1F and ITS2 primer sequences. Chloroplast, mitochondrial, archaea and prokaryote sequences were filtered from the feature table and set of representative sequences. Taxonomic data were exported as a text file and further analysed in Microsoft Excel.

3.8.7 Identification of biomarker taxa

Linear discriminant analysis effect size (LefSe) [156], available through the Huttenhower Lab Galaxy server (http://huttenhower.sph.harvard.edu/galaxy), was used to identify biomarker taxa in groups of interest using both the 16S and ITS data. Prior to analysis on the Galaxy server, the feature table generated by QIIME2 was collapsed to the genus-level and data were converted to relative frequencies. Thereafter, the table was exported and formatted in Excel per the requirements for analysis with LefSe. Taxa with an LDA of 2 or more a deemed significant. The higher the LDA score, the greater the association between the taxa and the group of interest.

3.8.8 Phospholipid fatty acid soil analysis (PLFA)

Phospholipid fatty acid (PLFA) analysis was performed on samples sourced from the three bushfire exposed properties in July of 2021. These samples were selected as they comprised of matching samples collected from burnt and unburnt paddocks. Appropriate samples were not available from Bombora (RG1) as all grazing paddocks on the property had been impacted by fire to some degree.

This is a technique that measures microbial biomass and composition and assessment of total PLFA is an important indicator of the biomass of living microorganisms within a sample and unlike DNA-based approaches, this method of analysis is not subject to biases such as variable amounts of relic DNA or humic substances in substrates. Analysis was performed on samples sourced from the DNA described in section 3.8.2 by Microbiology Laboratories Australia (www.microbelabs.com.au).

4. Results

4.1 Selection of properties for sampling

4.1.1 Planned burn.

At the outset of the project, there was intention to carry out a planned burn under University of Sydney supervision, but restrictions set in place due to the NSW response to the global pandemic coupled with adverse weather events in the form of repeated flood and heavy rainfall prevented establishing a planned burn site. We were fortunate to be provided unique access to a limited site in Victoria held under the management of LandCare Victoria.

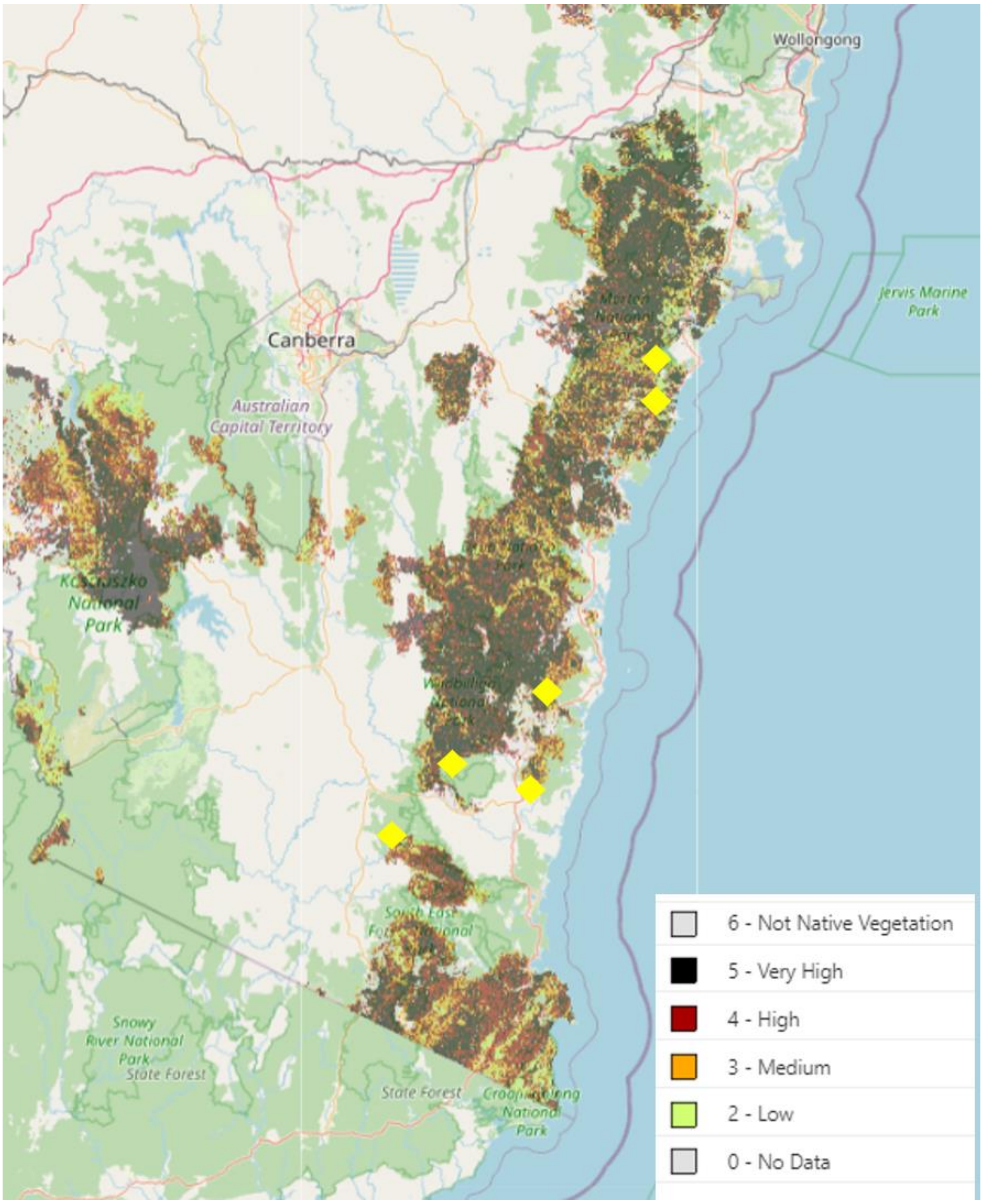
Soil samples for biochemical and microbe analysis were collected from the planned burn site 24hr post burn (Jan 2022). Due to travel restrictions, sampling was performed by the land caretaker and transported to the University of Sydney by freight transport. A second, full sampling event took place in July 2022 (Table 5) comprising of soil for biochemical, microbial, and seed bank analysis, and pasture (hand pluck and samples from transects) for pasture nutrient value estimation.

4.1.2 Bushfire exposed properties

Five properties were selected encompassing a wide geographical distribution (Nowra – Coastal region to Bombala – Inland) and a range of agricultural practices (Table 2). This broad geographic range was selected to provide confidence that results will be broadly applicable to bushfire impacted agricultural areas in general. Figure 6 indicates the locations of the selected farms (yellow diamonds). The intensity of the 2019-2020 fire exposure is overlaid on the image.

Samples were collected from each of the five properties however due to the NSW government mandated full or partial lockdown between June 2021 and October 2021, The University of Sydney pandemic response mandated limited access to laboratory workspace and complete restriction of field sampling until February 2022, and repeated flood events throughout 2021 and 2022, it was not possible to collect samples at all originally planned timepoints, sample collection is detailed in Table 5.

Figure 6. Fire exposure intensity (legend indicates intensity range) and selected farms locations (yellow diamond).



4.2 Remote sensing

4.2.1 Remote sensing analysis of bushfire exposed properties

4.2.1.1 Fire impacts on bushfire exposed properties

Grassland covered 18% to 76% of the study properties, with forest covering 19% to 71% (Tables 6-7). The fires which burnt these properties occurred between 25 November 2019 and 3 March 2020. Across all properties just over half of each property was burnt because of the six fires. Although it was not possible to include biological samples from the property in Milton due to the producer withdrawing consent for sampling access following the declaration of NSW Covid-19 lockdown in June 2021 and lack of access due to extreme flooding in June 2022, we include the remote sensing data here.

Table 6. Area and proportion of properties burnt.

Property Location	Size (ha)	Grasslands (%)	Woodland (%)*	Forest (%)*	Burnt (%)*	Date of Fire#
Bemboka	571	63	6	31	45	26/12/2019-3/3/2020
Bombala	1,481	74	3	23	19	22/1/2020-1/3/2020
Cobargo	88	76	5	19	39	26/12/2019-3/3/2020
Milton	142	43	6	51	88	25/11/2019-7/2/2020
Moruya	420	58	1	41	57	25/11/2019-15/2/2020
Nowra	111	18	12	70	61	25/11/2019-7/2/2020

* As a proportion of the property
# Start and end date of the fire that burnt the property – the property was not impacted on all dates.

Forest cover (% of total area) was correlated with % burnt, although the lowest forest cover did not have the lowest % burn and the highest forest cover did not have the highest % burn. , as tree cover increased, however, so did fire intensity with grasslands containing the greatest proportion of unburnt area (86%), woodlands having the greatest relative proportion of low intensity fires (19%) and those on-farm forest areas having the highest percentage of moderate, high, and extreme fires (Table 7).

Table 7. Impact of vegetation type on fire intensity.

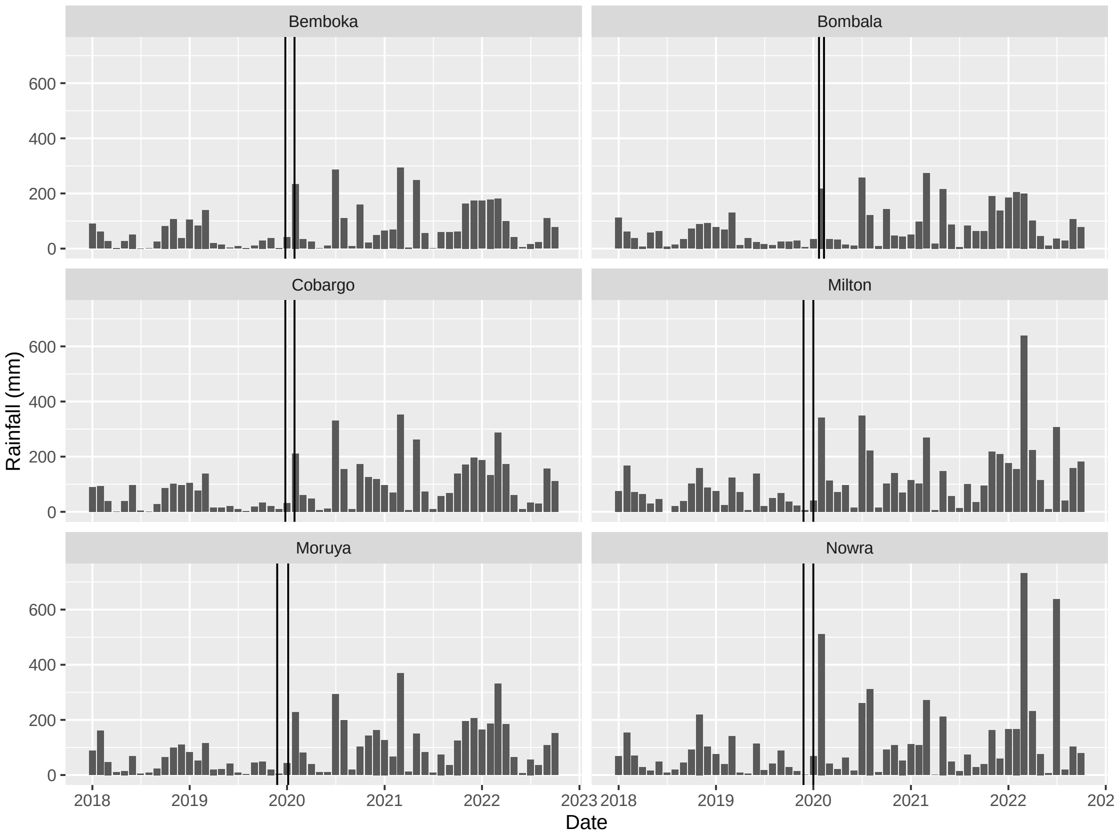
		Proportion (%) of vegetation type*				
Vegetation type (% tree cover)	Area* (%)	Unburnt	Low	Moderate	High	Extreme
Grassland (<5)	66	86	6	4	3	1
Woody (5-19)	4	52	19	15	12	1
Forest (≥20)	30	37	15	17	24	7

*As a proportion of the total area of the six properties (2,781 ha)

4.2.1.2 Drivers of pasture growth

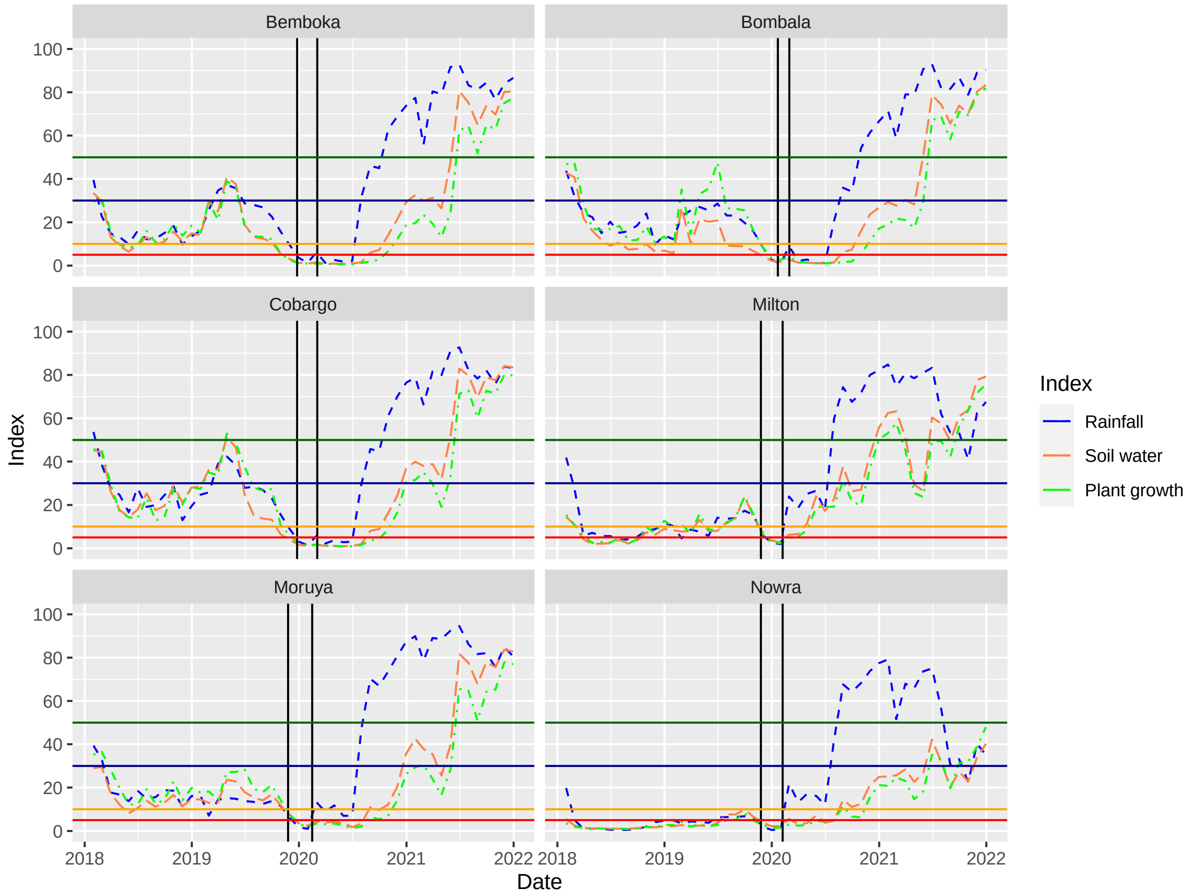
For the two years (2018-2019) prior to the 2019-2020, much of south-eastern Australia had been in the grip of a severe and long-lasting drought. This had a substantial effect on the amount of grass available to fuel the fires. Substantial rains in February 2020 contributed to bringing the fires under control in many cases (Fig. 7). However, this was, broadly speaking, a one-off event followed by several months of low to average rainfall and this resulted in little change in the Rainfall Index (Fig. 8) until mid-2020 when sustained and heavy rains continued to occur ensuring that the Rainfall Index was generally maintained well above the 30th and 50th percentile.

Figure 7. Monthly rainfall at each of the locations. The two black vertical lines indicate the period (start and end) over which the fire that impacted each property burnt.



With the cessation of fires in January and February 2020, appreciable amounts of rainfall led to an increase in soil moisture although there was a lag of five to six months before an increase in soil moisture along with plant growth was observed (Fig. 8). All locations have had well above average rainfall since mid-2020 and this sustained period of high rainfall has for most locations resulted in extremely saturated soil profiles, particularly since the second half of 2021. Consequently, under current climatic conditions, there are few limitations to growth. Any limitations to growth will most likely be the result of deficiencies in soil nutrients particularly after the sustained good growth that most pastures have enjoyed since mid-2021 and possibly to some degree water-logged soils.

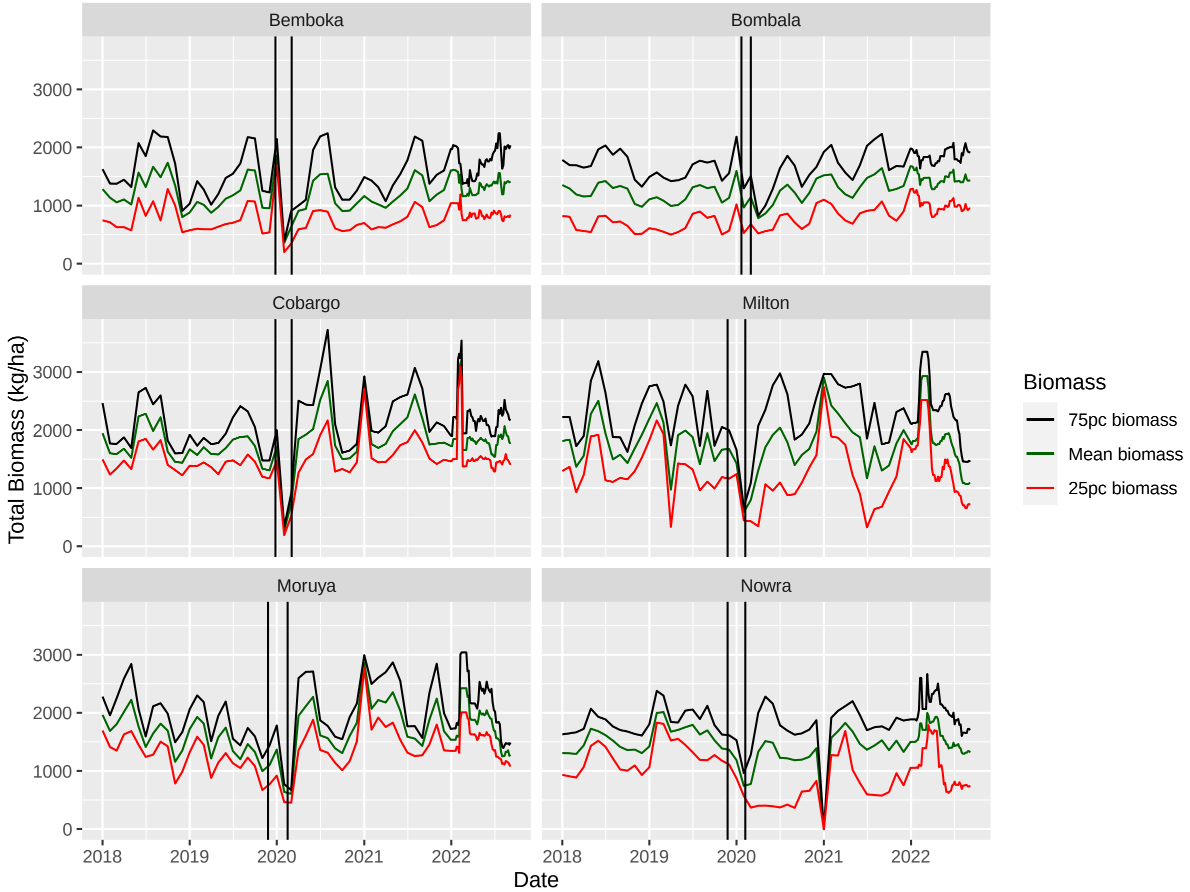
Figure 8. Index of rainfall, soil water and plant growth at each of the properties. A value of 0 indicates severe constraints while a value of 100 indicates no constraints to growth. The two black vertical lines indicate the period (start and end) over which the fire that impacted each property burnt. The dark green horizontal lines (50th percentile) indicates the Recovery Threshold, the dark blue line indicates the 30th percentile, the orange horizontal line (30th percentile) indicates the Drought Affected Threshold and the red horizontal line (5th percentile) indicated the Drought/Intense Drought Threshold.



4.2.1.3 Pasture biomass

Due to drought in the years prior to the fires, biomass was on average relatively low and oscillated around 1,000-2,000 kg/ha (Fig. 9, Table 8). For both the Moruya and Nowra properties, there had been a steady decline over 2019, resulting in a decline in pasture biomass of between 500-1,000 kg/ha from the start of 2019 to the time bushfires burnt these properties. Across all the properties, approximately 500 to 1,500 kg/ha of grass was consumed by the fires. On average (across the property), production dropped to minimal levels (500-1,000 kg) in the paddock after the fires (Fig. 9). Except for the Bombala property, the beneficial rains that occurred in February 2020 resulted in above average growth and within approximately three months of the bushfire, pasture production was the same or greater than just prior to the fires. In the case of the Moruya property, within two months forage from the end of the fires, production was almost twice that compared to a month prior the fire. Since the fire, while some properties (Bemboka, Milton, Nowra) maintained a similar level of production to the drought year prior to the fires, other properties (Bombala, Cobargo, Moruya) had an estimated increase in pasture production of around 200 kg/ha (Table 8).

Figure 9. Total aboveground biomass (average, 25th and 75th percentile estimates) both prior and following the 2019-2020 bushfire season. The two black vertical lines indicate the period (start and end) over which the fire that impacted each property burnt. The 25th and 75th percentiles provide an estimate of the greater or lower amounts of biomass present on the property.



The relatively fast recovery observed in the estimates of pasture biomass was likely impacted by several factors. Firstly, many of the properties reported a flush of growth of both clovers as well as weeds. This was not unexpected as it well known that clover growth is often inhibited due to the shading effect of grasses and thus the removal of grasses commonly sees a flush of clover growth. As there is commonly the presence of large stocks of clover seed present in the soil, with removal of grass competition they can germinate and grow relatively quickly compared to other species present in the seedbank. Weed species commonly invade areas which have been disturbed (including by fire) and that leads to a reduction in desired plant cover and associated competition from other species. The presence of RFS fire trucks along with other vehicles that were present on the properties fighting fires, and that had previously been present at multiple locations throughout the district, may well have been a source of weed seed. In addition, the strong winds that were generated by the fires as well as the normal winds that occur across a landscape also potentially brought weed seed in from other locations and thus resulted in a potentially high weed seed potential on the properties.

While in many cases there was not a lot of aboveground biomasses due to the drought, the ash produced from the fire acts as a fertiliser for new plant growth. In addition, during droughts nutrients often accumulate in soils. This accumulation is the result of the nutrient mineralization whereby organic matter is decomposed and, in the process, nutrients become available for plant uptake. While nutrients accumulate in the soil, there is a greatly reduced uptake of them by plants as the soil moisture is too low for any plant growth to occur (and plants may also be dead or in state of dormancy). Additionally, as most soil nutrients are water soluble and so uptake on nutrients from the soil to the plant roots is dependent on the mass flow of water, low soil moisture means they are unable to be taken up plants and so during droughts nutrients typically increase in a soil. With the good rains and favourable temperatures, the flush of growth that typically occurs is in part due to the presence of a relatively high amount of plant available nutrients in the soil along with the release of nutrients from lysed microbes. Finally in some cases it is likely (and observed) that landholders took the opportunity to plant a crop (particularly to provide winter feed) or put in an improved pasture system and this would also have impacted the estimated biomass production following the fires.

Table 8. Average yearly estimates of biomass production by property.

Location	**2018**	**2019**	**2020**	**2021**	**2022**
	kg/ha				
Bemboka	1,294	1,125	1,093	1,232	1,350
Bombala	1,243	1,160	1,161	1,402	1,460
Cobargo	1,799	1,649	1,688	2,012	1,900
Milton	1,786	1,810	1,517	1,902	1,845
Moruya	1,711	1,453	1,521	1,972	1,794
Nowra	1,445	1,663	1,207	1,404	1,575

4.2.1.4 Ground cover

While most properties maintained a reasonably stable degree of ground cover over 2018 (the initial year of the drought) apart from the typical decreases that might be expected due to seasonal conditions, all properties observed a substantial (~15-30%) decrease in ground cover from the 2nd and 3rd quarters onwards to the end of 2021 due to ongoing and increasing impact of the drought. While this might be expected, some locations (Milton and Nowra) saw a continued decrease in ground cover following the fires while most other properties saw ground cover increase or stay stable in the months following the fire. It should be noted however that seasonal ground cover estimates are produced every three months so the summer estimates will be based on December, January, and February and thus growth that occurred in February 2020 in response to the large rainfall events that occurred may have "biased" the ground cover estimate for the summer period.

The on-going decrease that was observed particularly at the Nowra and Milton sites was likely impacted by the very high amount of rain that occurred in those local areas in February 2020 (Fig. 7) and lead to some degree of soil erosion. The effect of this can also be observed in the estimate of pasture biomass (Fig. 10) where it appears that some pasture systems at the Nowra site took a long time to recover (i.e., the 25th percentile) and it was greater than 6 months before any increase in pasture growth and biomass production was observed. For most properties ground cover had recovered to a level comparable with pre-fire (and pre-drought) within 6 months.

Figure 10. Ground cover estimates showing the mean and 5th and 95th percentile estimates. The two black

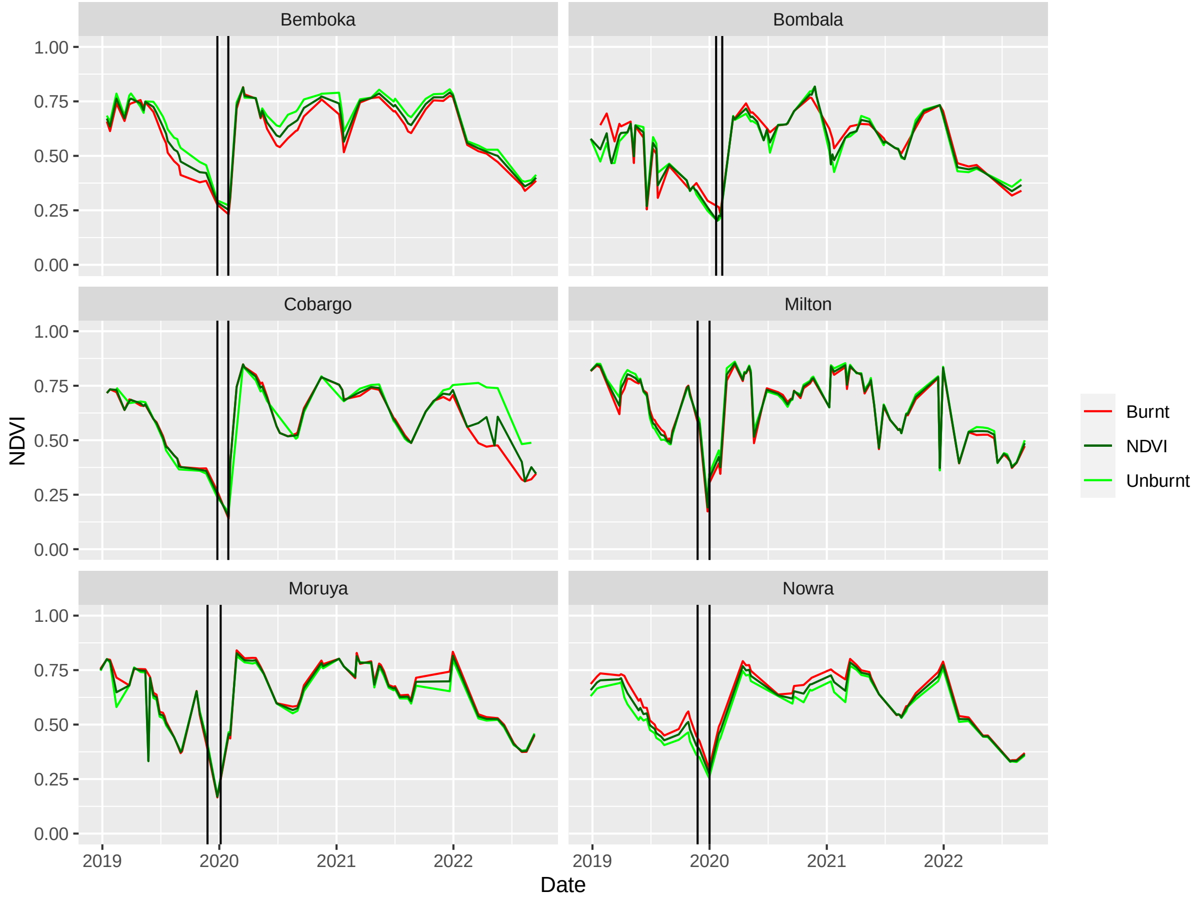
vertical lines indicate the period (start and end) over which the fire that impacted each property burnt.

4.2.1.5 Normalised difference vegetation index

The clear impact of the on-going and intensifying drought during 2019 is evident with all properties experiencing a substantial decline in NDVI (Fig. 11). While some properties observed a spike in NDVI during the spring of 2019 in response to some small spring rainfall events, those locations which experienced little spring rainfall experienced an on-going decline in NDVI in 2019. By the end of 2019 most properties were averaging an NDVI value of ~0.25 indicating a pasture consisting primarily of dead or dormant pasture. In response to the February 2020 rainfall post-fire and subsequent warm conditions, there was an immediate “greening-up” of pastures back to equal or greater NDVI in comparison to a year previously. Since February 2020, NDVI has been maintained at relatively high levels (considering the natural variation of lower growth/dormancy of plants over winter that will lead to a lower NDVI). Since 2022 all properties have observed a general decrease in NDVI although it started to pick up in September 2022 due to spring growing conditions. The sustained decrease in growth in 2022 relative to 2020 and 2021 may reflect that after two years of high rainfall, the optimal growing conditions have now exhausted the soil nutrients combined with three concurrent La Nina years resulting in many cases, in a soil profile that is extremely saturated. The waterlogging is potentially impacting on growth in many areas.

In addition to looking at property level impacts of fire on growth and a general indicator of pasture quality using NDVI, we have compared the NDVI signature of those areas of property that were burnt vs those that were not burnt to investigate if pasture systems that were burnt were impacted to a greater or less degree. As can be seen there has been no impact on pasture growth in pastures that were burnt compared to pastures that were unburnt (Fig. 11). While at the Bemboka site the burnt pasture has maintained a slightly lower NDVI after being burnt (relative to the unburnt pasture), it was also lower prior to the fire suggesting that this pasture has a lower NDVI for reasons unrelated to the fire (e.g., pasture composition, soil type, aspect).

Figure 11. Average change in NDVI ("NDVI") across all property locations from 2019 onwards as well as NDVI in burnt and unburnt pastures. The two black vertical lines indicate the period (start and end) over which the fire that impacted each property burnt.



4.2.2 Summary of remote sensing findings

Overall, pasture production while immediately impacted by the fires, resulting in an estimated loss of 500-1,500 kg/ha, bounced back to pre-fire (but drought impacted) biomass within approximately 3 months of bushfire exposure. While biomass recovered, it is likely that the composition of pastures has been modified due to the likely increased presence of clover and weeds that may reduce nutritive value of pasture biomass the overall livestock productivity of fire impacted properties.

Above average rains and the subsequent increase in soil water in the years following the 2019-2020 bushfires have maintained production at rates comparable to pre-fire conditions, and this has resulted in ground cover returning to pre-fire levels.

Based on NDVI as a proxy for pasture growth and quality and satellite estimates of pasture biomass, pastures appeared to recover relatively rapidly (immediately after rain in March 2020) although modelled data using a greater range of variables indicate that the response may be somewhat slower.

4.3 Plant nutrient analysis

Overall, the nutritive value of pasture was very low. Pastures were largely high in fibre, low in crude protein and dry matter and organic matter digestibility and therefore, low in metabolisable energy (ME) content.

"Burnt" pastures had ($p < 0.05$) greater ash and ADF content; and less WSC, OM, DMD, DOMD and ME content than "Unburnt" pastures. However, these differences were relatively small and of little significance in practice given the low quality overall (and most of them disappeared when one farm was excluded from the analysis – see below).

Table 9. Effects of treatment (Fire affected "Burnt" and non-affected "Unburnt" areas) on the nutritive value of pasture. All data (farms = 6).

	Treatment (mean|se)				
Parameter[1]	Burnt		Unburnt		P value
NDF	66.00	3.07	66.13	3.08	0.910
ADF	38.97	2.21	35.43	2.23	0.031
CP	10.25	1.39	9.54	1.39	0.315
WSC	4.37	1.44	5.56	1.44	0.038
OM	84.13	2.62	89.20	2.64	0.004
Ash	15.87	2.62	10.80	2.64	0.004
DMD	47.84	3.88	52.31	3.90	0.018
DOMD	47.24	3.34	50.91	3.36	0.025
ME	6.58	0.69	7.29	0.69	0.033

[1]NDF = neutral detergent fibreK; ADF = acid detergent fibre; CP = crude protein; WSC = water soluble carbohydrates; OM = organic matter; DMD = dry matter digestibility; DOMD = organic matter digestibility; ME = metabolizable energy. All expressed as % of the dry matter (DM), except ME (MJ/kg DM).

As expected, the variability in nutritive value among farms was large (Figs. 12-20) However, farm Bemboka, a dairy farm, presented more extreme values than any of the other farms (Figs. 12-20).

The nutritive value of "Unburnt" pastures from farm Bemboka were in line with what would be expected from high quality, fertilised grass-based pastures typically used in dairy farms. On the other hand, the extremely low nutritive value of the "Burnt" pastures for the same farm were more likely associated with soil contamination than with treatment effect. Soil contamination typically occurs when pastures are sampled in wet conditions resulting in abnormally lower content of organic matter (increased ash %).

Figure 12. Effect of treatment (Burnt vs Unburnt) on neutral detergent fibre (NDF) content (% of dry matter).

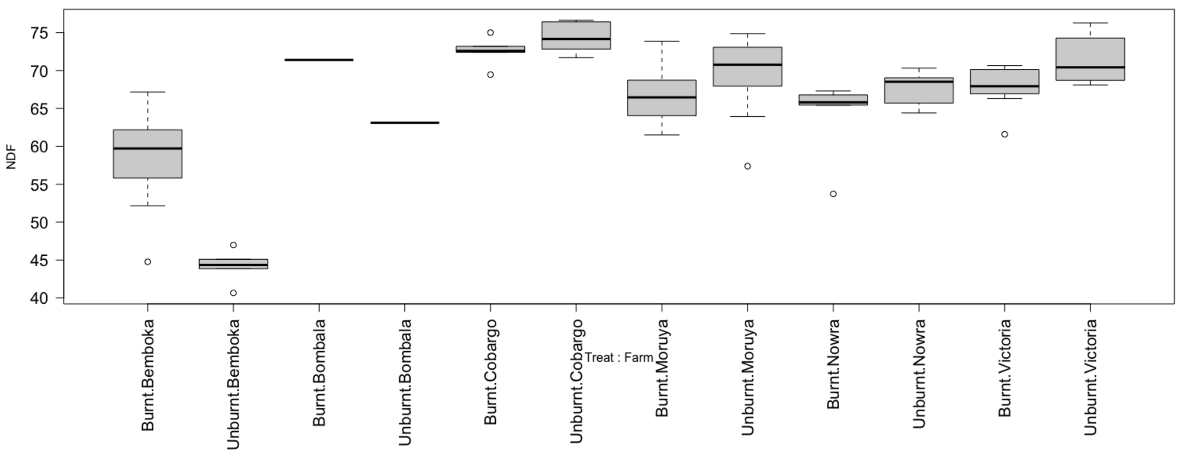


Figure 13. Effect of treatment (Burnt vs Unburnt) on acid detergent fibre (ADF) content (% of dry matter).

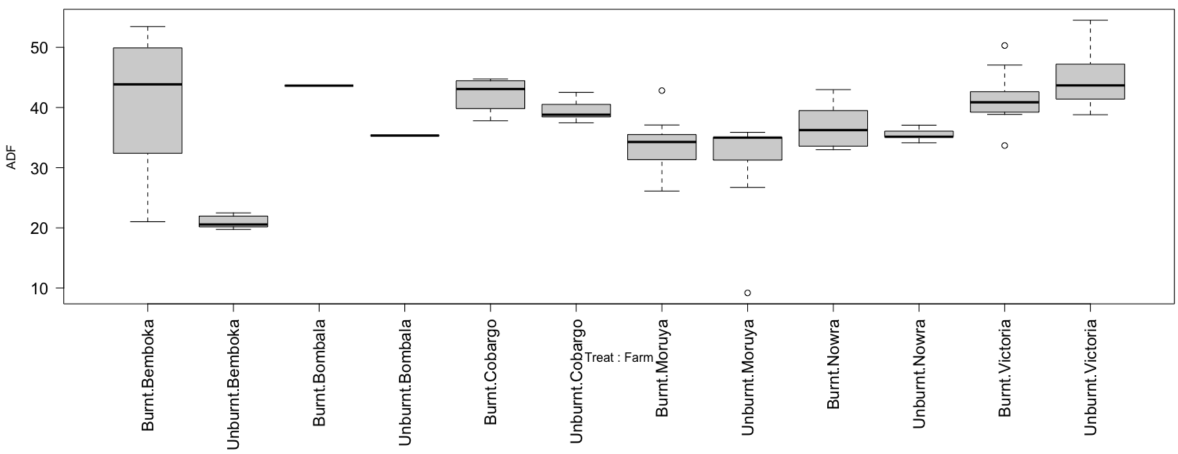


Figure 14. Effect of treatment (Burnt vs Unburnt) on crude protein (CP) content (% of dry matter).

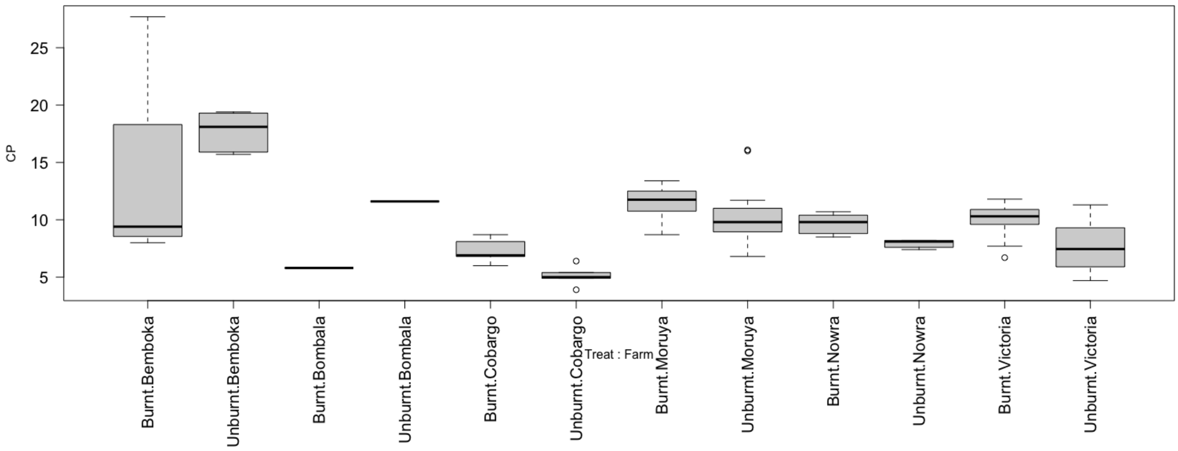


Figure 15. Effect of treatment (Burnt vs Unburnt) on water soluble carbohydrates (WSC) content (% of dry matter).

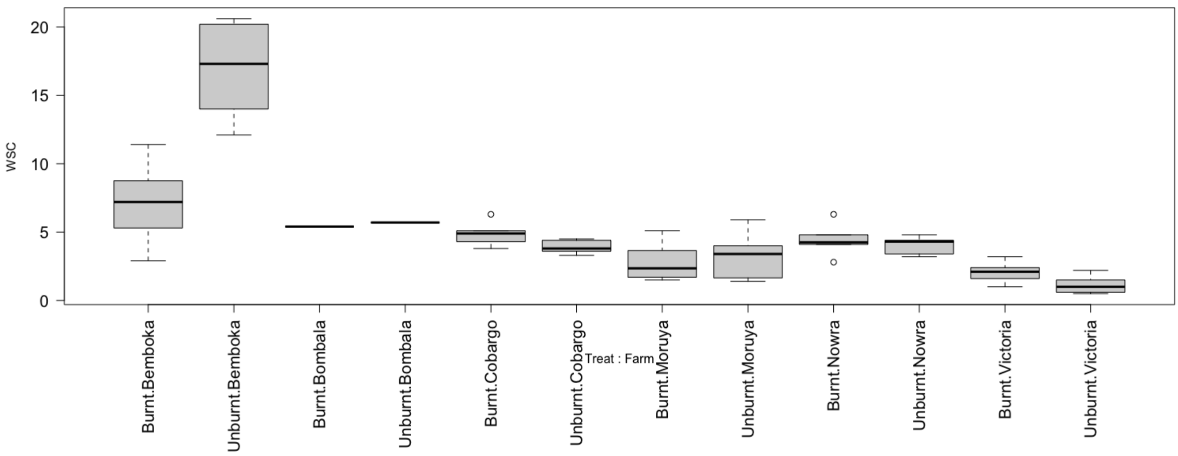


Figure 16. Effect of treatment (Burnt vs Unburnt) on organic matter (OM) content (% of dry matter).

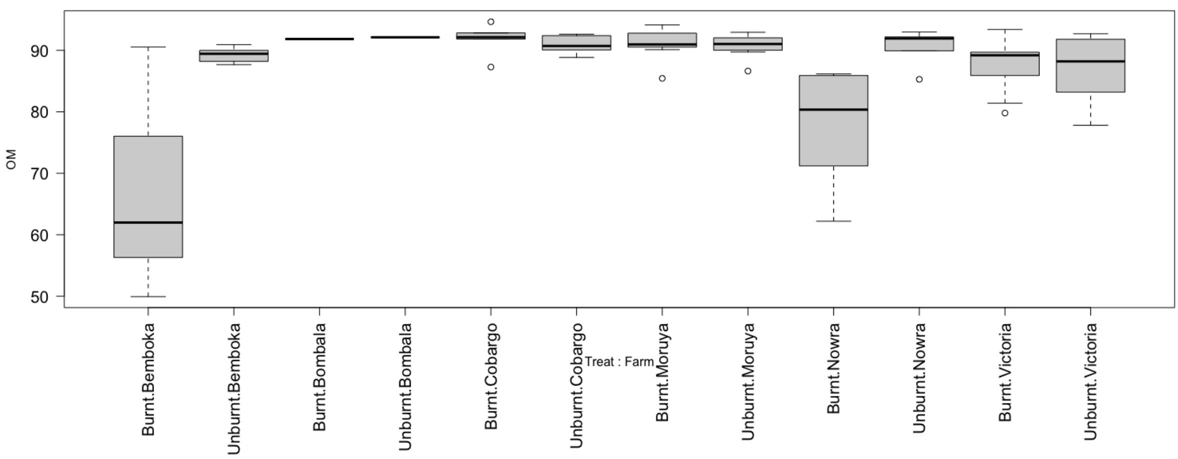


Figure 17. Effect of treatment (Burnt vs Unburnt) on ash content (% of dry matter).

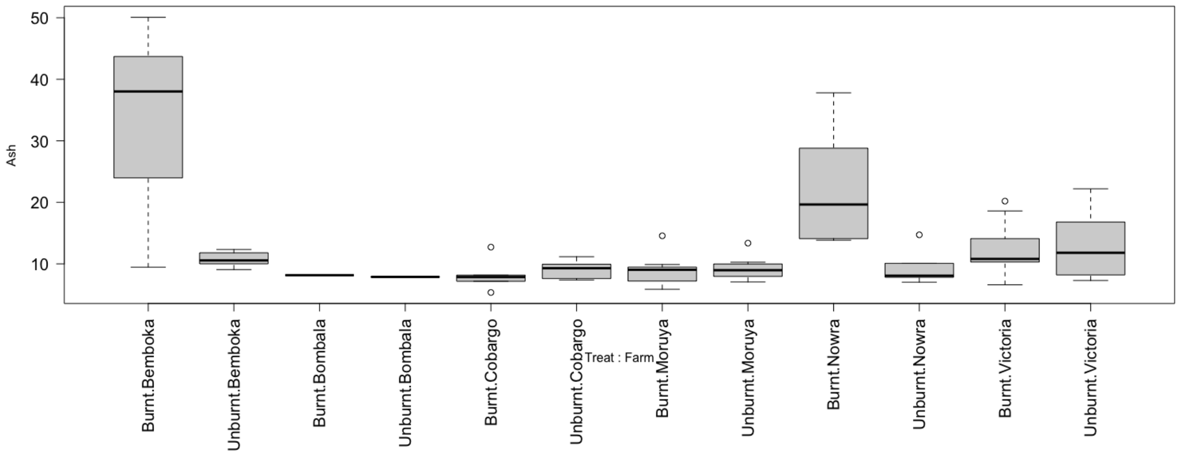


Figure 18. Effect of treatment (Burnt vs Unburnt) on digestibility of the dry matter (DMD, %).

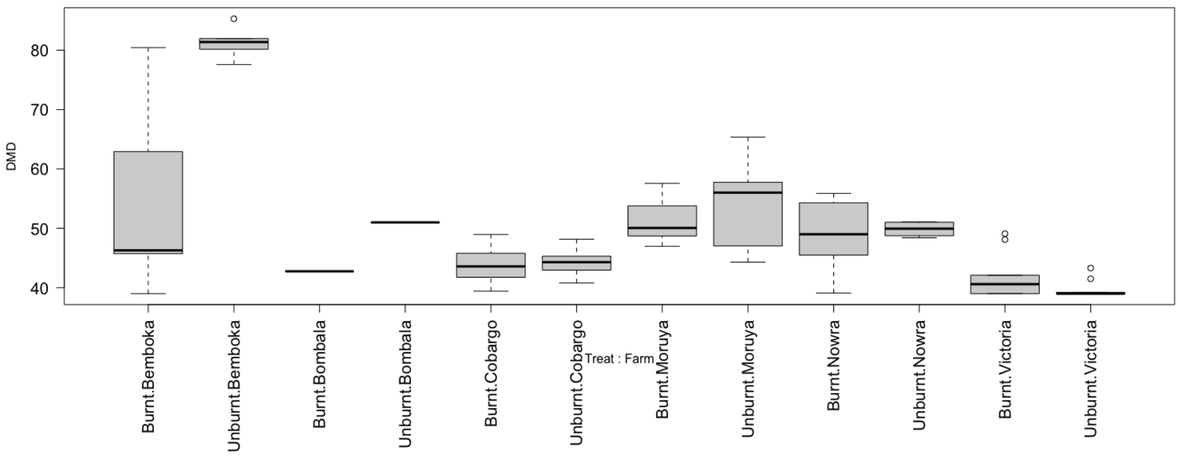


Figure 19. Effect of treatment (Burnt vs Unburnt) on digestibility of the OM (DOMD, %).

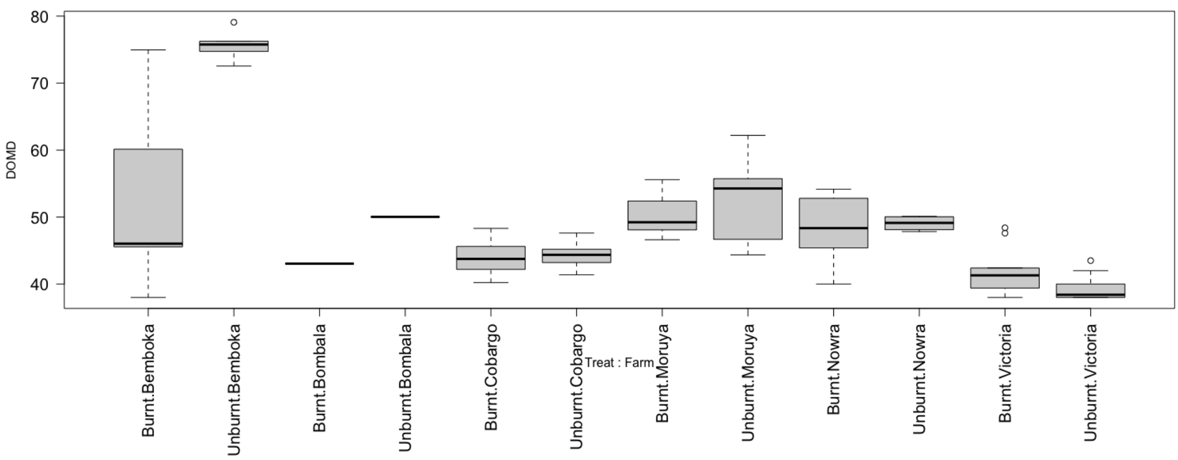
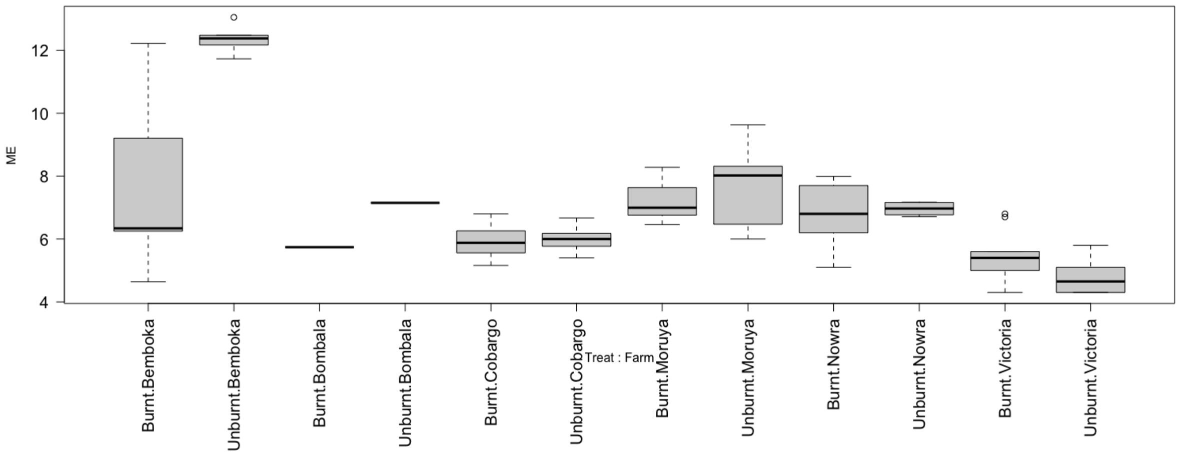


Figure 20. Effect of treatment (Burnt vs Unburnt) on metabolizable (ME) energy content (MJ/kg DM)



Due to the extreme values observed for farm Bemboka, which as outlined above, were likely influenced by soil contamination, we re-ran the statistical analysis with data from this farm excluded. Results indicate that, except for CP, for which Burnt pasture still had ~20% more than Unburnt pasture, all the other differences in nutritive value shown in Table 9 disappeared when the data from the farm that had extreme values (Bemboka) were excluded from the analysis (Table 10).

Table 10. Effects of Treatment (Fire affected "Burnt" and non-affected "Unburnt" areas) on the nutritive value of pasture. Data from Farm Bemboka excluded (unusual values).

	Treatment (mean | se)				
Parameter[1]	**Burnt**		**Unburnt**		**P value**
NDF	67.6405	1.4096	70.3071	1.41482	0.05
ADF	38.75	2.15	38.01	2.16	0.55
CP	9.44	0.85	7.98	0.85	0.03
WSC	3.81	0.67	3.49	0.67	0.22
OM	87.84	1.71	89.7	1.72	0.13
Ash	12.15	1.71	10.3	1.72	0.13
DMD	46.4	2.2	46.9	2.2	0.61
DOMD	46.1	1.9	46.3	2.0	0.76
ME	6.33	0.42	6.35	0.42	0.92

1 NDF = neutral detergent fibre; ADF = acid detergent fibre; CP = crude protein; WSC = water soluble carbohydrates; OM = organic matter; DMD = dry matter digestibility; DOMD = organic matter digestibility; ME = metabolizable energy. All expressed as % of the dry matter (DM), except ME (MJ/kg DM).

4.3.1 Summary of plant nutrient response to bushfire

Overall, all pastures, except for one farm, were of relatively low nutritive value for livestock. Despite some small, albeit adverse, effects of fire on nutritive value were apparent (Table 10), these effects largely disappeared when the farm that had the more extreme (and to some extent abnormal) values was excluded from the analysis.

The data showed large variability among farms, suggesting that sampling a larger number of farms/paddocks would be desirable to unequivocally elucidate the true effects (if any) of fire on the nutritive value of pasture.

4.4 Biochemical composition of soil in response to fire

4.4.1 Planned burn.

Immediately after the planned burn in January 2022, extractable P increased and extractable nitrate (NO_3^-) decreased in the top 2 cm of the soil compared to the unburnt control, but other parameters were not affected (Fig. 21a-e, Table 11). Six months later, in June 2022, exposure of soil to fire under planned burn conditions still caused an increase in extractable P, but this time extractable ammonium (NH_4^+) also increased, but only in the top 2 cm of soil, while other parameters were not affected (Fig. 21f-j, Table 11). Soil parameters in the subsoils (5-10 cm) were not affected by burning (Fig. 21k-o).

Figure 21. Soil properties (pH, extractable P, NH_4^+, NO_3^-, and available N) in unburnt and burnt plots sampled in January 2022 in the top 0-2cm directly after a planned burn (a-e), and in July 2022, 6 months post planned burn in surface soils (0-2 cm) (f-j) and sub soils (5-10 cm) (k-o) in VIC. Error bars represent 1 standard error (n = 12 for sampling in January 2022 and n = 15 for sampling in July 2022).

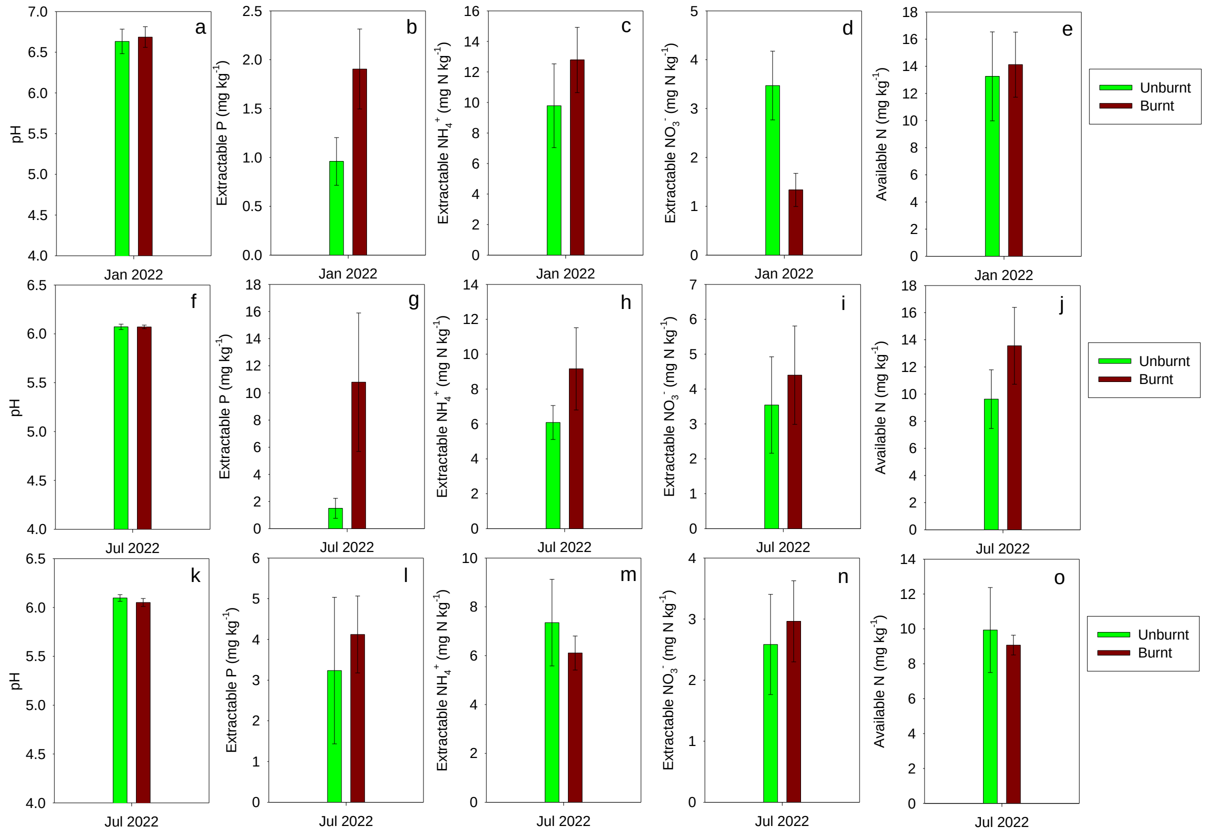


Table 11. Generalized linear mixed model results (p-values) for main and interactive effects of burning (B) and soil depth (SD) on soil pH, extractable phosphorus (extract. P), ammonium (NH_4^+), nitrate (NO_3^-), and available nitrogen (avail. N) for sites in VIC, sampled in January and July 2022. Because soil sampling in January only occurred in the top 2 cm of the soil, only main effects of burning are shown for this date. Values in italics show results that are significant at P <0.10 (appropriate for low-powered studies), in agreement with detection of a significant difference in topsoil in univariate analysis for P, NH_4^+ and NO_3^-

Date	**Treatment**	**pH**	**Extract. P**	**NH_4^+**	**NO_3^-**	**Avail. N**
Jan 2022	B	0.8	*0.06*	0.4	*0.01*	0.8
Jul 2022	B	0.6	0.3	0.7	0.8	0.7
	SD	0.7	0.3	0.5	0.2	0.2
	B×SD	0.5	*0.08*	*0.08*	0.8	0.11

4.4.2 Bushfire exposed properties

Across the Cobargo, Bemboka, and Moruya sites, for which soils were sampled both in June 2021 and June 2022, burnt plots had on average higher extractable P (Fig. 22, Table 12). Extractable P increased both in surface and sub soils and remained higher in burnt plots in 2022 (Fig. 22).

Figure 22. Soil properties (pH, extractable P, NH_4^+, NO_3^-, and available N) sampled in June 2021 and June 2022 in unburnt and burnt plots in surface (0-2 cm) (a-e) and sub soils (5-10 cm) (f-j) averaged across the field sites of Cobargo, Bemboka and Moruya, NSW (note that for light burn plots were used as “unburnt” for the Cobargo site). Error bars represent 1 standard error (n = 15).

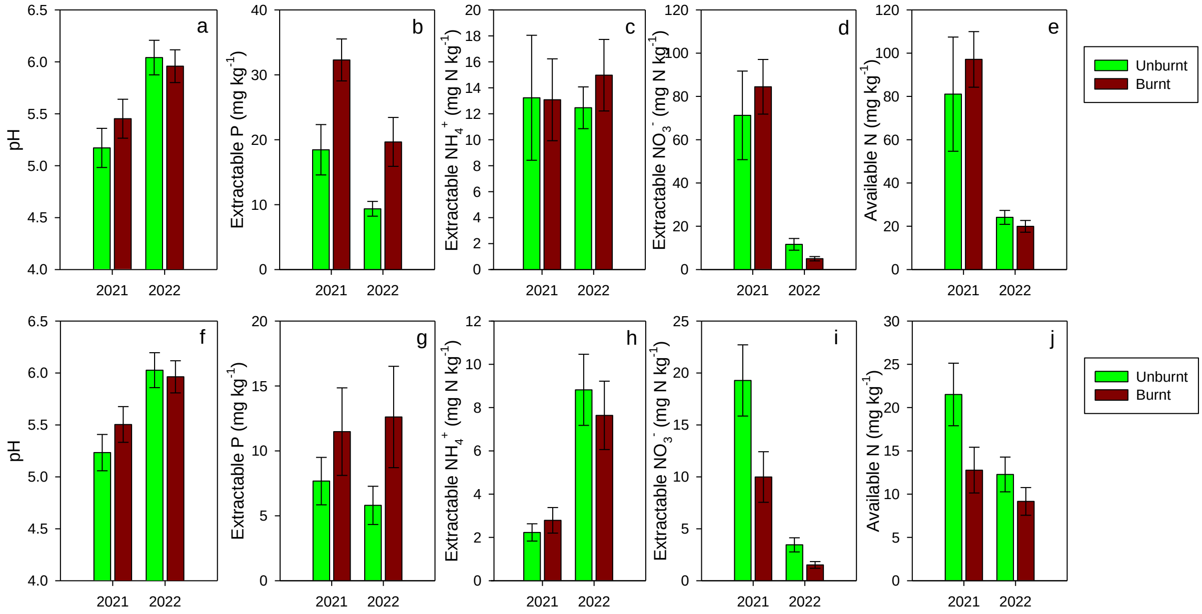


Table 12. Generalized linear mixed model results (p-values) for main and interactive effects of burning (B), soil depth (SD), farm site (FS) and time (T) on soil pH, extractable phosphorus (extract. P), ammonium (NH_4^+), nitrate (NO_3^-), and available nitrogen (avail. N) for sites in NSW. Only Cobargo, Bemboka, and Moruya sites were used for the analyses, and the light burn in Cobargo was used as the control.

	pH	Extract. P	NH_4^+	NO_3^-	Avail. N
B	0.2	0.0006	0.12	0.5	0.4
SD	0.6	0.0001	0.0004	<0.0001	<0.0001
B×SD	0.9	0.2	0.2	0.5	0.12
FS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
FS×B	0.005	0.6	0.12	0.003	<0.0001
FS×SD	0.4	0.2	0.09	<0.0001	<0.0001
FS×B×SD	0.6	0.02	0.003	0.001	<0.0001
T	<0.0001	0.003	0.002	<0.0001	<0.0001
T×B	0.03	0.8	0.6	0.6	0.5
T×SD	0.6	0.007	0.004	<0.0001	<0.0001
T×B×SD	0.8	0.5	0.3	0.2	0.2
T×FS	0.9	0.6	0.6	<0.0001	<0.0001
T×FS×B	0.07	0.05	0.04	0.0006	<0.0001
T×FS×SD	0.2	0.9	0.8	<0.0001	<0.0001
T×FS×B×SD	0.8	0.2	0.2	0.001	<0.0001

There were significant differences in all soil properties among the five sites in NSW (Figs. 15-19). Properties measured at different depths and times sometimes responded differently to burning. For instance, soil pH increased with burning at all sites in 2021, but this effect was only observed in the surface soil and disappeared in 2022 (Fig. 23). Burning did not increase extractable P at all sites (e.g., not at Bombala), and its effect varied with soil depth and time (Fig. 24). Likewise, extractable NH_4^+ increased only at specific sites, and that depended on soil depth and time (Fig. 25). Extractable NO_3^- had no clear association with burning and was in general much lower in 2022 compared to 2021 (Fig. 24). Extractable NO_3^- dominated available N, and as such, available N concentrations showed similar trends to extractable NO_3^- (Fig. 26 and 27).

Figure 23. Average soil pH in June 2021 (a, c) and June 2022 (b, d) in unburnt, lightly burnt and burnt plots in surface (0-2 cm) (a, b) and sub soils (5-10 cm) (c, d) at each farm site in NSW. Error bars represent 1 standard error (n = 5).

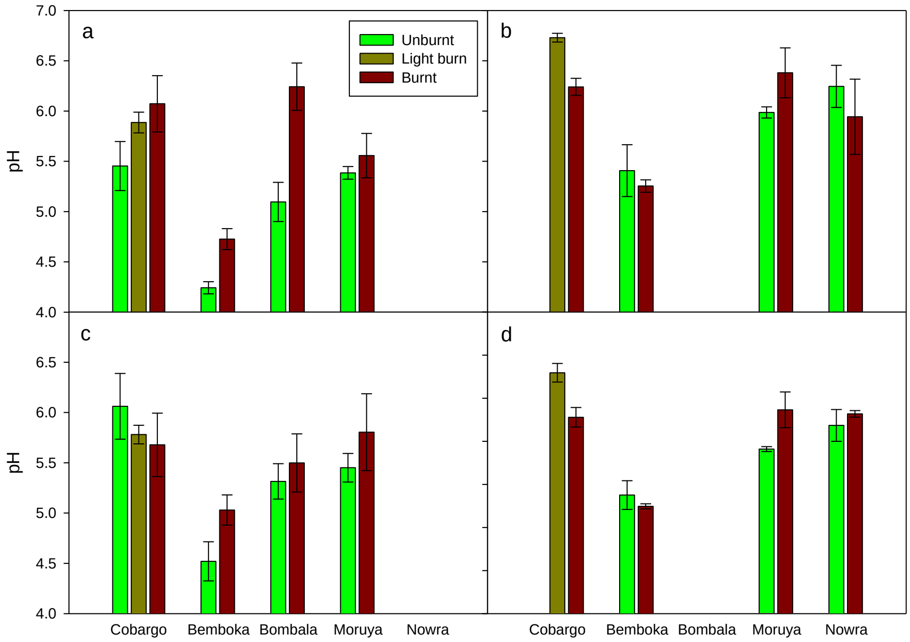


Figure 24. Average extractable phosphorus (P) in June 2021 (a, c) and June 2022 (b, d) in unburnt, lightly burnt and burnt plots in surface (0-2 cm) (a, b) and sub soils (5-10 cm) (c, d) at each farm site in NSW. Error bars represent 1 standard error (n = 5).

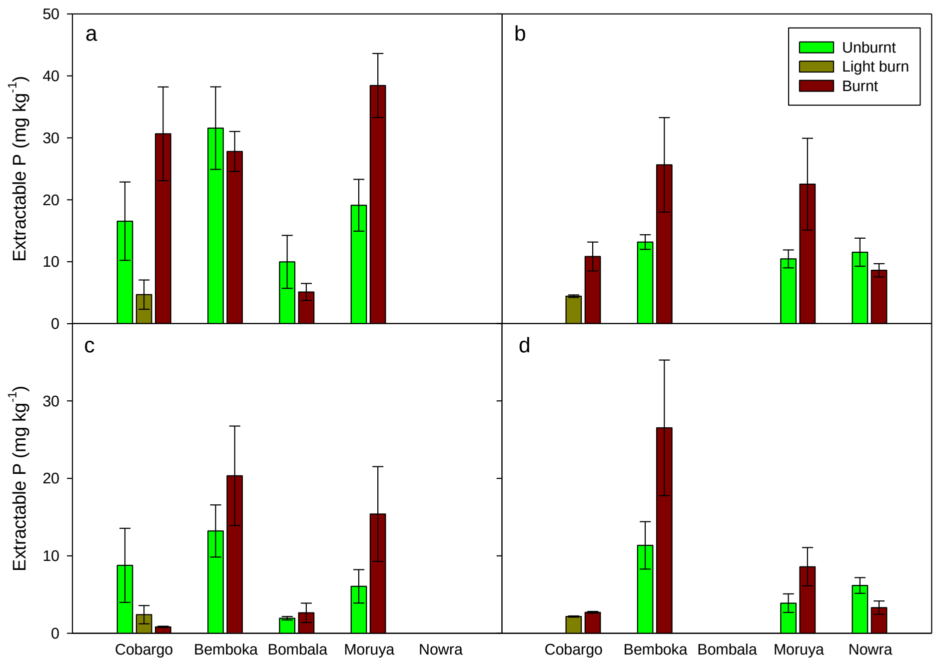


Figure 25. Average extractable ammonium (NH_4^+) in June 2021 (a, c) and June 2022 (b, d) in unburnt, lightly burnt and burnt plots in surface (0-2 cm) (a, b) and sub soils (5-10 cm) (c, d) at each farm site in NSW. Error bars represent 1 standard error (n = 5).

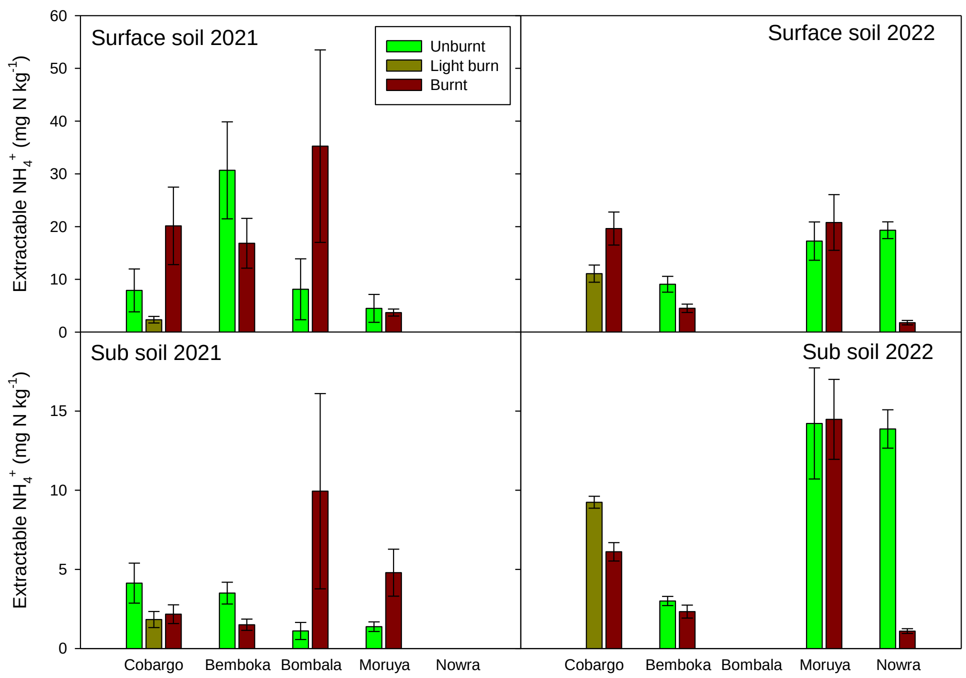


Figure 26. Average extractable nitrate (NO_3^-) in June 2021 (a, c) and June 2022 (b, d) in unburnt, lightly burnt and burnt plots in surface (0-2 cm) (a, b) and sub soils (5-10 cm) (c, d) at each farm site in NSW. Error bars represent 1 standard error (n = 5).

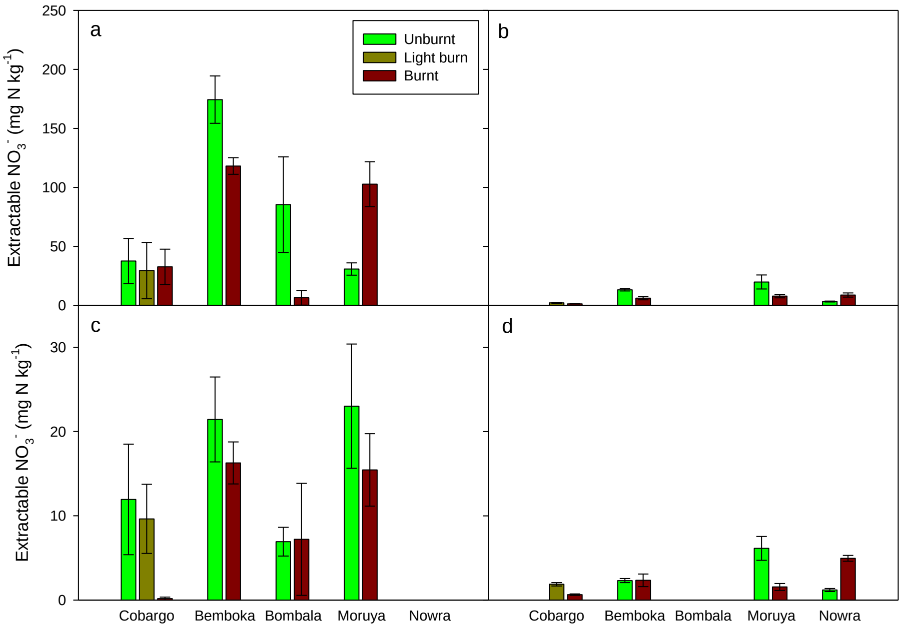
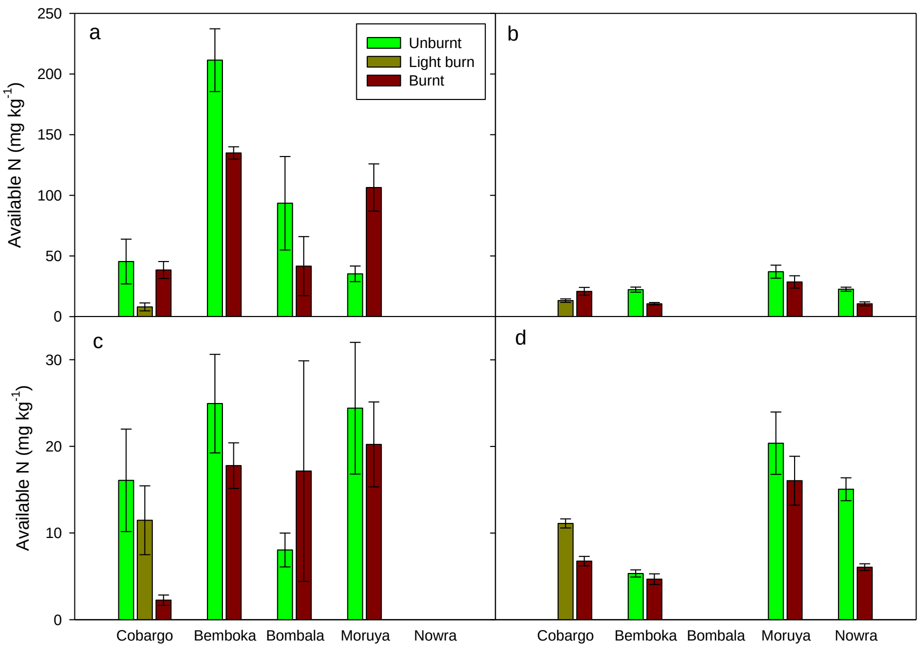


Figure 27. Average available nitrogen (N) in June 2021 (a, c) and June 2022 (b, d) in unburnt, lightly burnt and burnt plots in surface (0-2 cm) (a, b) and sub soils (5-10 cm) (c, d) at each farm site in NSW. Error bars represent 1 standard error (n = 5).



4.4.3 Summary of results of biochemical composition of soil in response to fire

The planned burning in VIC and the bushfires across all farm sites in NSW, increased extractable P up to six months after the planned burning and up to 30 months after the bushfire events in 2019-2020. Fire exposure also caused short-term increases in extractable NH_4^+ in the surface soil. Although fire temperatures of planned burning tend to be lower than for bushfires, effects on soil properties were similar. One difference was that soil pH in the surface soil increased across all farm sites after the bushfires but was not affected with planned burning. Possibly higher ash loadings after bushfires may have contributed to the increase in soil pH at farm sites in NSW. While ash deposits can cause direct and short-term increases in nutrient availability such as P, the increases in soil pH may also have long-lasting effects on soil health. For instance, an increase in soil pH may stimulate microbial activity mineralising more nutrients (i.e., P and NH_4^+). This would be beneficial for pasture growth, particularly in Australian soils that are acidic and generally poor in P availability. However, there was some variation in how bushfires affected soil properties among the different farm sites. For instance, extractable P in the surface soil only increased in two of four sites in NSW in 2021, but in three sites a year later. Factors such as pasture species composition, climate, hydrology, and soil parent material likely mediated fire effects on these soil properties, and therefore fire effects on soil health will depend on local conditions.

4.5 Seed bank viability following exposure to fire.

Soil seedbanks are important reservoirs for populations of plants recovering after disturbance. The nature of the disturbance will determine the extent of effect on soil seed banks and the conditions that surviving seed enter when they germinate. For example, removal and stockpiling of topsoil prior to mining activities will have different effect on seed stored in the soil seedbank compared to removal of leaf litter and heating of soil during the passage of a bushfire. Post-fire conditions result in changes in resource availability (e.g., light, water, nutrients) and competition from other plants.

Most of the seed stored in soil seed banks is in the layer of leaf litter and surface mineral soil [157, 158]. This is also where the greatest heating of soil occurs. Temperatures can reach up to 80°C in the top 2 cm of soil during a moderate intensity fire [159], but depends on soil type and moisture content, the amount of fuel burnt and how fast the fire is moving [160, 161].

4.5.1 Planned burn site.

Seedling germination was much lower for soils collected following the planned burn in July 2022, with half to one quarter of the number of individuals seedlings emerging compared to the results from the seedbanks collected from the bushfire exposed properties (Fig. 28). For the first sampling point 2 weeks after establishing the trial (1 August), the number of individuals counted was similar among samples from burnt and unburnt areas of the planned fire. At the second and third sampling points, there were fewer individuals in soil seedbank samples taken from burnt areas of the planned burn. There was no evidence of seedling death so it is likely that as plants grew, they could be recognised more easily as individuals (Fig. 29). As found in bushfire exposed samples, not all individuals counted represented seedlings as many may have been resprouting shoots of Onion Grass (*Romulea rosea*) from underground bulbs (Fig. 30).

Figure 28. Mean (± standard deviation) number of individual seedlings counted during the planned burn at three time points, 2, 5 and 13 weeks after the trial was set up. Soil seedbank samples (n = 7) were collected from burnt and unburnt patches after a planned burn.

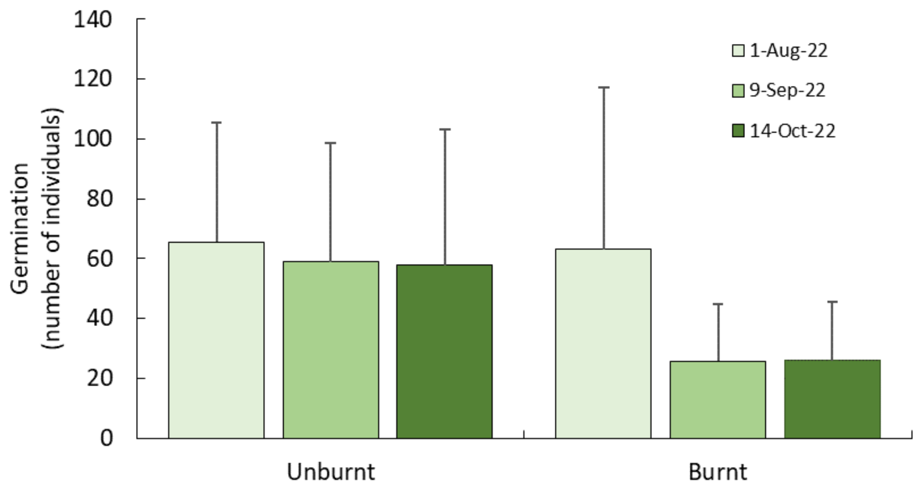


Figure 29. Seedling emergence, growth, and development over the 13-week period of the planned burn. The example provided is one of the replicates taken from an unburnt area of the planned burn area at (a) 2 weeks (1 August 2022), (b) 5 weeks (9 September), and (c) 13 weeks (14 October) after the trial was established.

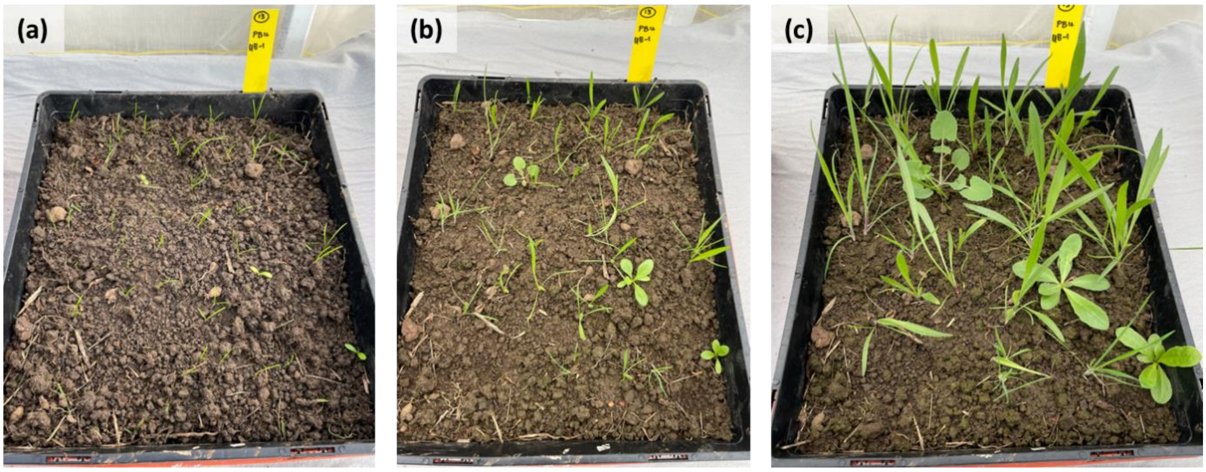
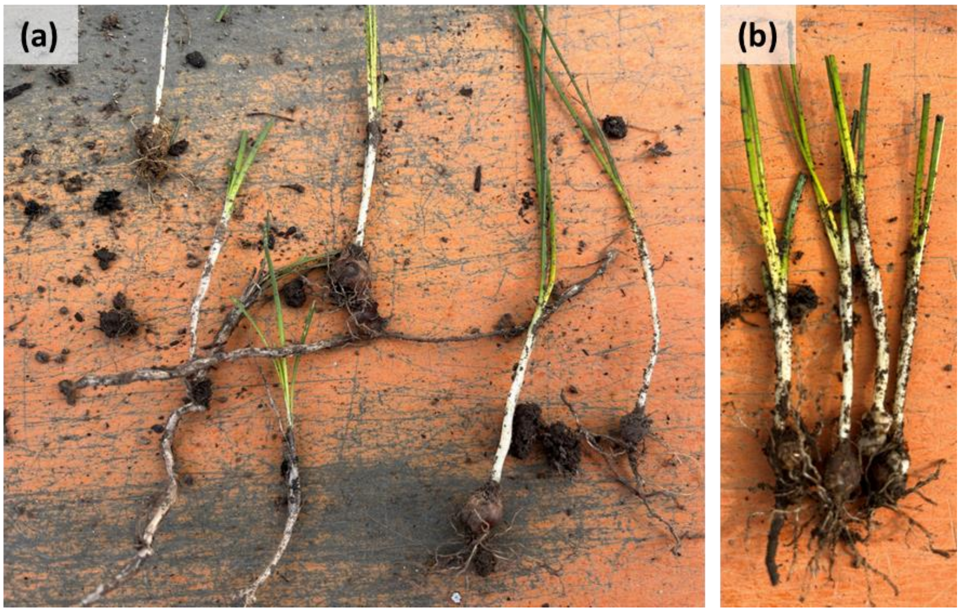


Figure 30. Examples of resprouting individuals from (a) underground horizontal stems, and (b) bulbs.



4.5.2 Bushfire exposed properties

Due to lockdown restrictions and pandemic associated limitations to laboratory access, the samples were stored for more than 8 months prior to processing. Even so, seedling germination was rapid for soil seedbanks taken from both burnt and unburnt areas of farms (with the first cotyledons appearing within a week (Fig. 31a, b). Seedling growth was similarly fast (Fig. 31c), and thick growth in seedling trays made it difficult to discern individual plants within 8 weeks from starting the trial (late March 2022; Fig. 31d).

Figure 31. Examples of seedling trays with soil seedbank samples for bushfire-affected farms. (a) Seedling trays were prepared in early February 2022, (b) cotyledons of seedlings were visible within 1 week, (c) seedling growth was rapid (late February), and (d) followed by thick growth (late March).

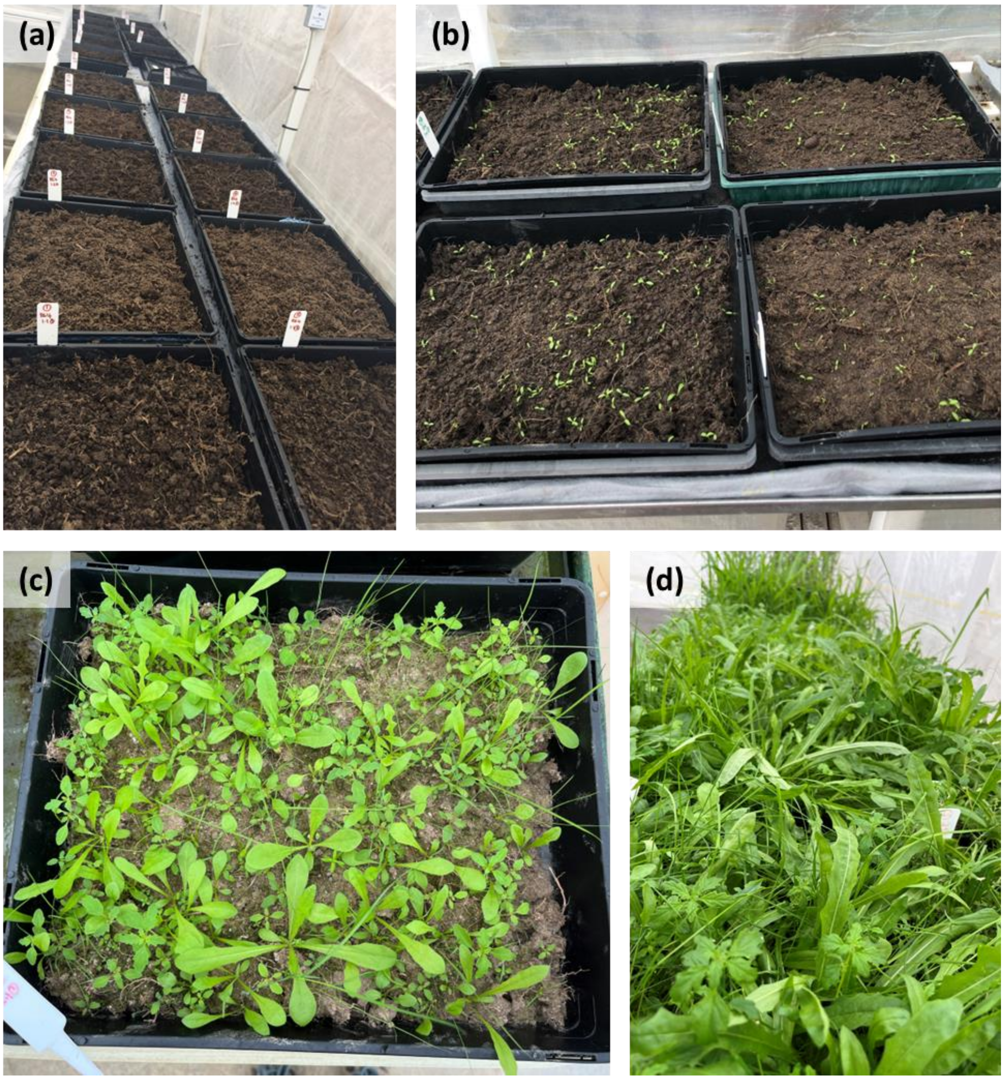
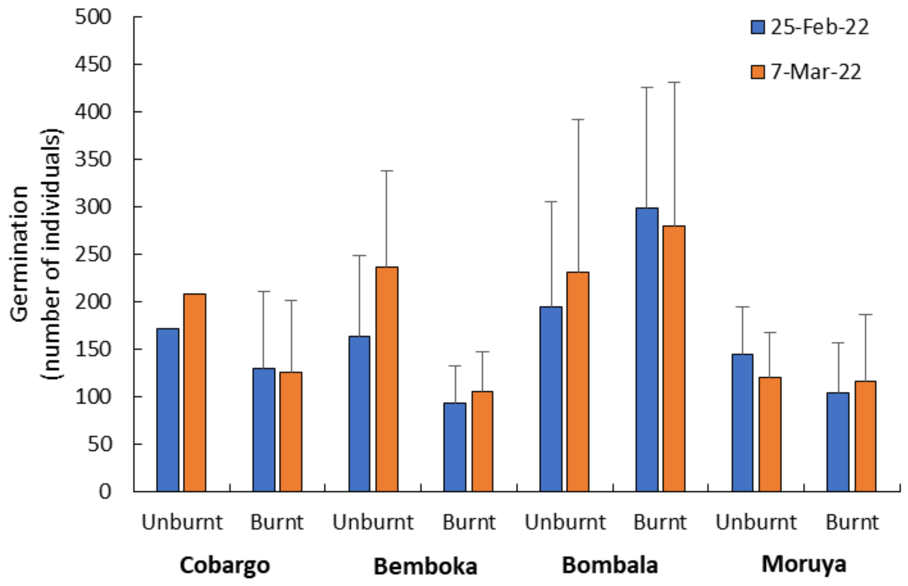


Figure 32. Mean (± standard deviation) number of individual seedlings counted during the first trial (*bushfire-affected farms*) at two time points, 2 and 3 weeks after the trial was set up. Soil seedbank samples (n = 1-10 depending on site) were collected from burnt and unburnt pasture from four farms in southern NSW burnt by bushfire in 2019-2020 bushfires.



Numbers of seedlings emerging in Sampling 1 varied widely among replicate samples with coefficient of variation (CV) ranging from 35-70%. In general, germination did not increase over time with similar numbers of individuals counted in late February (25 February) and 10 days later in early March (7 March; Fig. 32). Fewer seed germinated from the soil seedbank sampled from burnt areas on farms sampled in Cobargo and Bemboka, but it should be noted that the unburnt area of the farm in Cobargo was represented by a single soil sample due to degradation of the samples during lockdown conditions and the values for the unburnt sample fell within the range observed for burnt samples. The soil seedbank for pastures on the farm in Moruya did not appear to be affected by fire as indicated by similar numbers of emerging seedlings from seedbanks sampled from burnt and unburnt areas. In contrast, fire may have stimulated germination of some species in the seedbank of the farm in Bombala with greater numbers of seedlings emerging from soil taken from the burnt site.

Many types of seedlings could be distinguished as they emerged, but few could be identified to species (Fig. 33). Common seedling 'types' (e.g., 'grass', 'clover' and 'dandelion') were recorded in seedbank samples from all farms and from burnt and unburnt areas along with large numbers of seedlings that were classified as 'unknown'. Of the more recognisable seedling types, some were common to seedbanks representing both burnt and unburnt pastures, others were only found in soil seedbank samples from unburnt or burnt areas. Common weed species included Flaxleaf Fleabane (*Conyza bonariense*), Swamp Dock (*Rumex brownii*), Black-berry Nightshade (*Solanum nigrum*) and Yellow Wood Sorrell (*Oxalis corniculata*). Several native plant species were found including two forbs, Native Geranium (*Geranium solanderi*) and Carrot Weed (*Cotula australis*), and grasses such as Native Millet (*Panicum decompositum*). No seedlings of woody weeds were found.

It should be noted that all plant biomass that emerged was scored as a newly germinated seedling. However, there were some instances where fragments of grass rhizomes were included in the sample which may have resprouted. Sieving soil samples prior to spreading in seedling trays would have removed grass rhizomes but this processing step was not possible as soil samples were mostly saturated heavy clay.

Figure 33. Examples of recognisable seedling types from the first trial (*bushfire-affected farms*) from soil seedbank samples collected from burnt and unburnt pasture from four farms in southern NSW burnt by bushfire in 2019-2020 bushfires.

4.5.3 Summary of seed bank analysis

Bushfire and planned fire had little effect on germination of seed stored in soil seedbanks in pastures. There was evidence that fire promoted the presence of some native and weed species that had not previously been evident in aboveground biomass, but it was not clear if this was due to fire-related germination cues or changes in conditions post-fire such as reduced competition or short-term increase in nutrient supply.

Although identification of the individual species responding to fire was not the aim of this study it would be worthwhile identifying weed species to inform farmers about post-fire weed control and knowing what native species may remain in seedbanks to understand the natural capital of pastures.

4.6 Response of soil microbiota to fire

4.6.1 Planned burn.

Samples sourced from the planned burn site on January 2022 did not provide usable DNA for microbiota analysis despite repeat extractions from the collected soil samples. Results of analysis of samples sourced from July 2022, six months post the initial burn event are described below.

4.6.1.1 Soil microbiota variations in planned burn samples

4.6.1.1.1 Community diversity

The results from the planned burn provide insights into the short-term impacts of fire on the composition of the microbial community of soil. Diversity analyses were undertaken using several alpha- and beta-diversity metrics to compare the bacterial communities (microbiota) in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 cm and 5 to 7 cm below the soil surface.

At a depth of 0 to 2 cm, there was no significant difference in alpha diversity between samples collected from burnt and unburnt paddocks according to any of the alpha diversity metrics evaluated (Table 13). At a depth of 5 to 7 cm, alpha diversity was significantly different in samples collected from the burnt paddocks according to Shannon's Diversity Index ($p = 0.014$) and Faith's Phylogenetic Diversity ($p = 0.014$) (Table 13). There was no significant difference in alpha diversity between the burnt and unburnt paddocks at this depth according the Pielou's Evenness Index ($p = 0.142$) (Table 13).

There was a significant difference in beta diversity between samples collected at a depth of 0 to 2 cm from burnt and unburnt paddocks according to analysis of the Bray-Curtis distances ($p = 0.045$) and weighted UniFrac distances ($p = 0.043$), but not the unweighted UniFrac distances ($p = 0.177$) or the Bray-Curtis distances ($p = 0.069$). There was a significant difference in beta diversity between samples collected at a depth of 5 to 7 cm from burnt and unburnt paddocks according to analysis of the unweighted UniFrac distances (p = 0.036) and the weighted UniFrac distances (p = 0.038) but not the Bray-Curtis distances (p = 0.058).

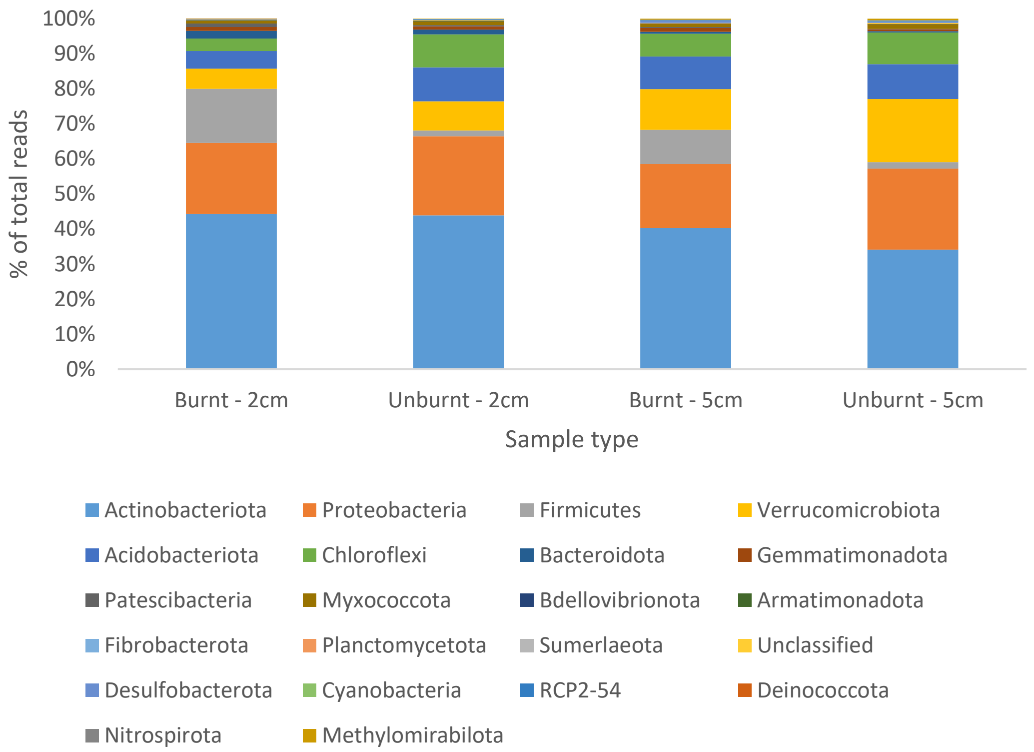
Table 13. Alpha diversity analysis for soil samples collected during the planned burn conducted in 2022. The average alpha diversity value calculated for each group is presented. For each gene target, alpha diversity metrics were compared between burnt and unburnt soil samples at each depth using a non-parametric Kruskal-Wallis test.

		Alpha Diversity Metric								
		Shannon's Diversity Index			Pielou's Evenness Index			Faith's Phylogenetic Diversity		
Target	**Depth**	**Unburnt**	**Burnt**	**P-value**	**Unburnt**	**Burnt**	**P-value**	**Unburnt**	**Burnt**	**P-value**
16S	2 cm	8.34	8.27	1.000	0.92	0.90	0.462	38.53	40.35	0.806
	5 cm	7.90	8.54	0.014	0.90	0.92	0.142	32.50	41.50	0.014
ITS	2 cm	5.38	5.27	1.000	0.69	0.68	0.806	44.29	44.15	0.462
	5 cm	3.33	5.21	0.049	0.47	0.69	0.049	29.67	37.27	0.127

4.6.1.1.2 Taxonomic analysis

The composition of the bacterial communities (microbiota) present in soil samples collected from depths 0 to 2 cm and 5 to 10 cm in the burnt and unburnt paddocks were characterised and compared at both the phylum- and genus-level. At the phylum-level, the dominant phyla were mostly similar in samples from the burnt and unburnt paddocks at both depths (Fig. 34); however, there was an increase in the relative abundance of the phylum Firmicutes in samples from burnt paddocks at both depths. In the burnt paddocks, at a depth of 0 to 2 cm the most abundant bacterial genera were *Bacillus* (9.33%), *Blastococcus* (4.28%), species in the clade Candidatus Udaeobacter (3.89%), *Acidothermus* (3.68%), and *Conexibacter* (3.21%). In soil samples collected from unburnt paddocks at this depth, the most abundant bacterial genera were an unclassified bacterium belonging to the family Geminicoccaceae (7.30%), an uncultured bacterium belonging to the class Oligoflexia (4.94%), an uncultured bacterium belonging to the order Thermomicrobiales (4.79%), *Anaeromyxobacter* (4.27%), and *Asticacaulis* (3.86%).

Figure 34. Relative abundance of bacterial phyla identified in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 and 5 to 7 cm from the planned burn conducted in 2022. The relative abundances are based on pooled data from all four farms.



In soil samples collected from burnt paddocks at a depth of 5 to 7 cm, the most abundant bacterial genera identified were Candidatus Udaeobacter (9.95%), *Bacillus* (6.37%), an uncultured bacterium belonging to the family Xanthobacteraecea (6.39%), *Acidothermus* (5.14%), and uncharacterised bacterium belonging to the order Solirubrobacterales (4.38%). In soil samples collected from unburnt paddocks at this depth, the most abundant bacterial genera identified were Candidatus Udaeobacter (15.9%), an uncultured bacterium belonging to the family Xanthobacteraecea (9.19%), Acidothermus (6.39%), an uncultured bacterium belonging to the order Gaiellales (4.99%), and an uncharacterised bacterium belonging to the phylum Chlorflexi (3.93%).

Lefse was used to identify bacterial genera that were preferentially abundant in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 cm (Fig. 35) or 5 to 7 cm (Fig. 36). Genera with an LDA score of two or more were regarded as significantly abundant in the group of interest. Genera that could be definitively identified are reported. Sixty-eight bacterial taxa were preferentially abundant in soil collected from a depth of 0 to 2 cm from burnt paddocks, 28 of which could be resolved to the genus-level: *Psychroglaciecola*, *Pseudarthrobacter*, *Microvirga*, *Fonticella*, *Roseomonas*, *Methylorosula*, *Lysinibacillus*, *Psychrobacillus*, *Caulobacter*, *Planomicrobium*, *Modestobacter*, *Ferruginibacter*, *Roseisolibacter*, *Clostridium sensu stricto*, *Tumebacillus*, *Ramlibacter*, *Gemmatimonas*, *Paraburkholderia*, *Methylorubrum*, *Chthoniobacter*, *Marmoricola*, *Paenarthrobacter*, *Devosia*, *Geodermatophilus*, *Solirubrobacter*, *Massilia*, *Blastococcus*, and *Bacillus*. One genus, *Vicinamibacter* was identified as preferentially abundant in soil samples collected from a depth of 0 to 2 cm from unburnt paddocks. Four bacterial taxa were preferentially abundant in soil collected from a depth of 0 to 2 cm from unburnt paddocks, one of which could be resolved to the genus-level: *Vicinamibacter*.

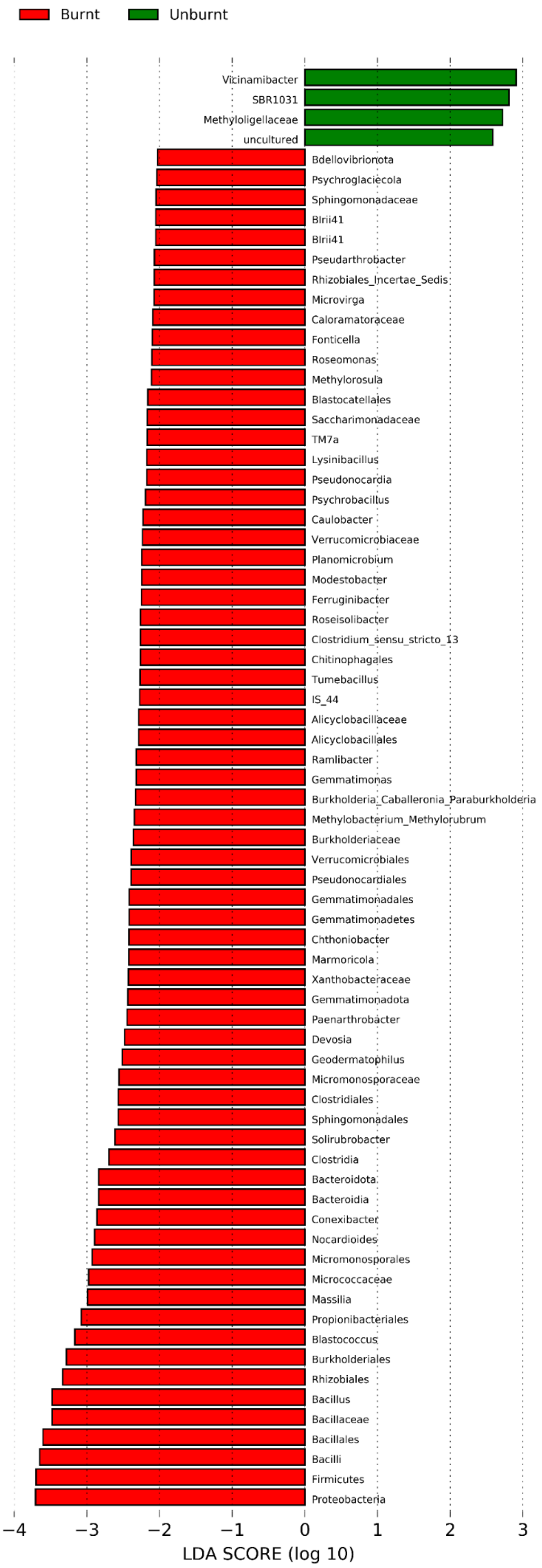
Many of the genera that were significantly more abundant in the burnt paddocks, such as *Psychroglaciecola*[162] , *Microvirga* [163]*Roseomonas* [164], and *Ramlibacter*[165], have previously been reported to respond positively to fire and to be abundant in soil post-fire, but for some of these genera their ecological significance is not well-described. A number of these genera, including *Pseudarthrobacter* [166] are reported to have plant growth-promoting properties.

The genus *Lysinibacillus* is reported to aid soil regeneration due to its' ability fix nitrogen and solubilize phosphorus [167] . The genus *Gemmatimonas* is part of the rhizosphere and supports plant growth [168].

Four bacterial taxa were preferentially abundant in soil collected from a depth of 0 to 2 cm from unburnt paddocks, one of which could be resolved to the genus-level: *Vicinamibacter* .

Members of the genus *Vicinamibacter*, which belongs to the phylum Acidobacteria, has been described in a range of environments but their ecological role is not well-understood bacteria within the phylum Acidobacteria as they are difficult to isolate. However, there is evidence that the genus may play a role in plant growth-promotion [169].

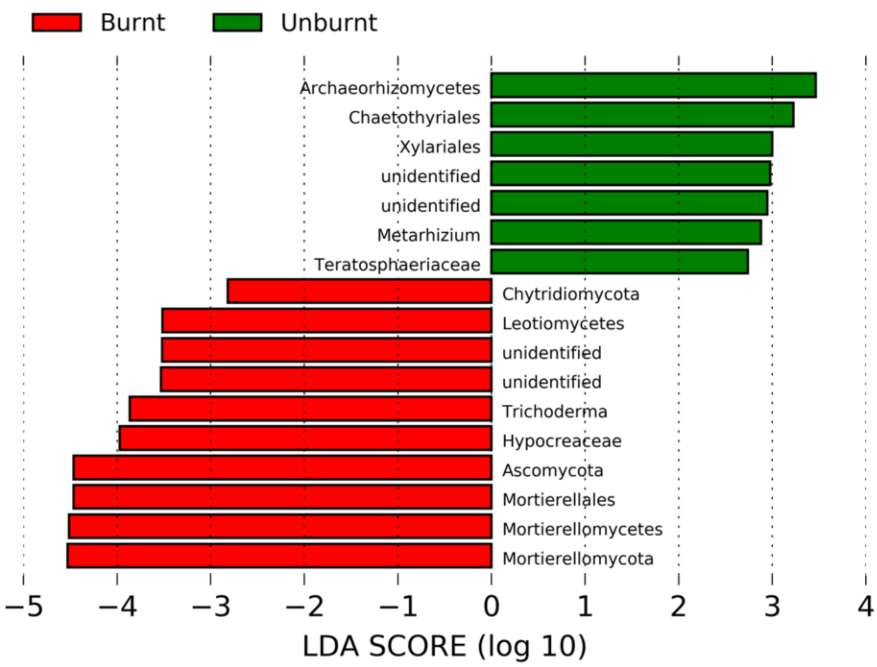
Figure 35. Lefse results for analysis of the bacterial communities in soil collected from burnt and unburnt paddocks at a depth of 0 to 2 cm following a planned burn conducted in 2022.



Ten bacterial taxa were preferentially abundant in soil collected from a depth of 5 to 7 cm from the unburnt paddock, one of which could be resolved to the genus-level: *Metarhizium* (Fig. 36). This genus was also identified as significantly more in the 2021 and 2022 datasets. Seven bacterial taxa were preferentially abundant in soil collected from a depth of 5 to 7 cm from unburnt paddocks, one of which could be resolved to the genus-level: *Trichoderma*.

Members of the genus *Trichoderma* are abundant in soil and often constitute a large proportion of the mycobiome in soil post-fire [170]. *Trichoderma* are known to be important plant growth-promoting fungi, rapidly colonising soil and changing the rhizosphere environment to enhance plant growth [171].

Figure 36. Lefse results for analysis of the bacterial communities in soil collected from burnt and unburnt paddocks at a depth of 5 to 7 cm following a planned burn conducted in 2022.



4.6.1.2 Soil mycobiota results in planned burn samples (ITS gene)

4.6.1.2.1 Community diversity

Diversity analyses were undertaken using several alpha- and beta-diversity metrics to compare the fungal communities (mycobiota) in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 cm and 5 to 7 cm below the soil surface (Table 14).

At a depth of 0 to 2 cm, there was no significant difference in alpha diversity among samples collected from burnt and unburnt paddocks according to any of the alpha diversity metrics evaluated. At a depth of 5 to 7 cm, alpha diversity was significantly higher in samples collected from the burnt paddocks according to Shannon's Diversity Index (p = 0.049) and Pielou's Evenness Index (p = 0.049). There was no significant difference in alpha diversity between the burnt and unburnt paddocks according to Faith's Phylogenetic Diversity (p = 0.127).

Table 14: Alpha diversity analysis for soil samples collected during the planned burn conducted in 2022. The average alpha diversity value calculated for each group is presented. For each gene target, alpha diversity metrics were compared between burnt and unburnt soil samples at each depth using a non-parametric Kruskal-Wallis test.

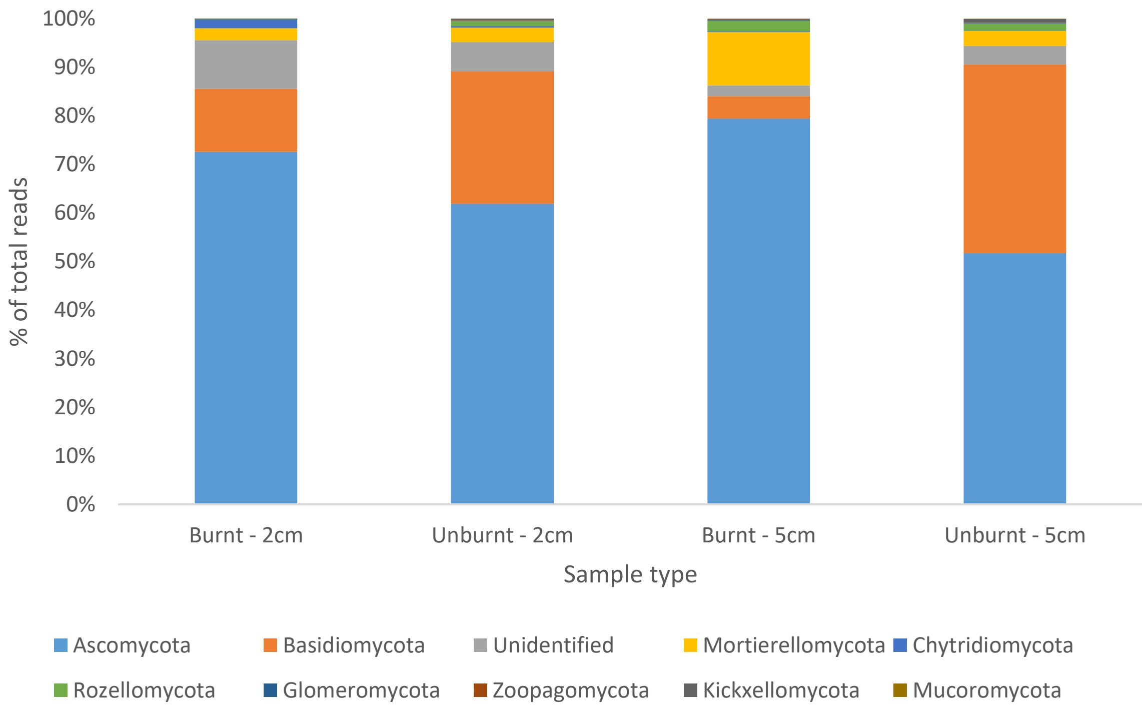
		Alpha Diversity Metric								
		Shannon's Diversity Index			Pielou's Evenness Index			Faith's Phylogenetic Diversity		
Target	**Depth**	**Unburnt**	**Burnt**	**P-value**	**Unburnt**	**Burnt**	**P-value**	**Unburnt**	**Burnt**	**P-value**
16S	2 cm	8.34	8.27	1.000	0.92	0.90	0.462	38.53	40.35	0.806
	5 cm	7.90	8.54	0.014	0.90	0.92	0.142	32.50	41.50	0.014
ITS	2 cm	5.38	5.27	1.000	0.69	0.68	0.806	44.29	44.15	0.462
	5 cm	3.33	5.21	0.049	0.47	0.69	0.049	29.67	37.27	0.127

There was a significant difference in beta diversity between samples collected from a depth of 0 to 2 cm from burnt and unburnt paddocks according to analysis of the Bray-Curtis distances (p = 0.045) and the weighted UniFrac distances (p = 0.043) but not the unweighted UniFrac distances (p = 0.177). There was no significant difference in beta diversity between samples collected from a depth of 5 to 7 cm from burnt and unburnt paddocks according to any of the beta diversity metrics evaluated.

4.6.1.2.2 Taxonomic analysis

The composition of the fungal communities (mycobiota) present in soil samples collected from depths 0 to 2 cm and 5 to 7 cm in the burnt and unburnt paddocks were characterised and compared at both the phylum- and genus-level. In soil samples collected from burnt paddocks, at a depth of 0 to 2 cm the most abundant fungal genera were *Penicillium* (24.43%), an unclassified fungus belonging to the phylum Ascomycota (11.18%), an unclassified fungus (9.80%), an unclassified fungus belonging to the family Didymellaceae (5.40%), and *Solicoccozyma* (4.85%). In soil samples collected from unburnt paddocks, the dominant fungal genera were *Penicillium* (22.98%), an unclassified fungus (5.85%), an unclassified fungus belonging to the class Tremellomycetes (4.69%), *Sebacina* (4.17%), and an unclassified fungus belonging to the phylum Ascomycota (3.94%) (Fig. 37).

Figure 37. Relative abundance of bacterial phyla identified in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 and 5 to 7 cm from the planned burn conducted in 2022. The relative abundances are based on pooled data from all four farms.



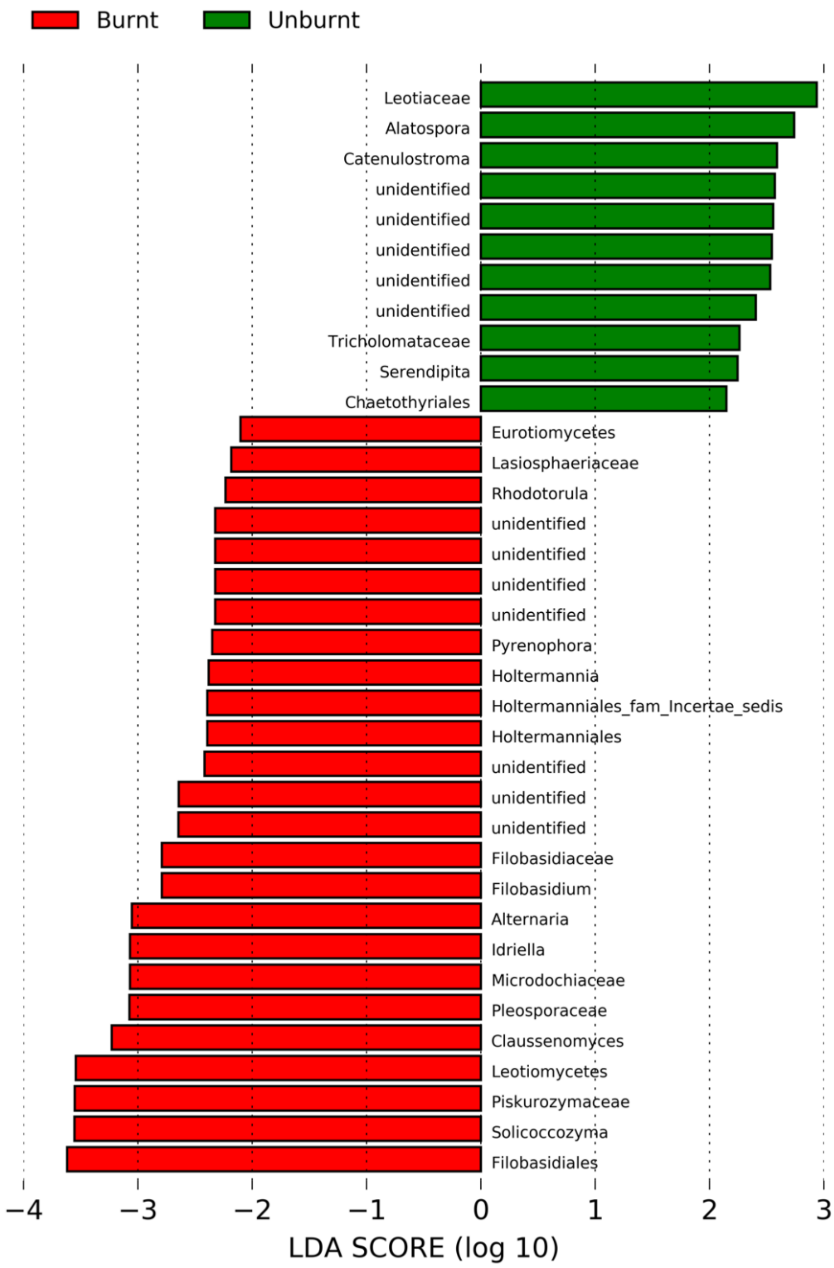
In soil samples collected from a depth of 5 to 7 cm from unburnt paddocks, the most abundant fungal genera identified were an unclassified fungus belonging to the phylum Basidiomycota (14.51%), *Penicillium* (11.85%), *Ilyonectria* (6.93%), an unclassified fungus belonging to the family Clavariaceae (6.68%), and *Archaeorhizomyces* (5.65%). In soil samples collected from this depth from the burnt paddocks, the most abundant fungal genera were an unclassified fungus belonging to the phylum Ascomycota (56.71%), *Penicillium* (11.12%), an unclassified fungus belonging to the order Mortierellales (6.49%), *Tympanis* (3.18%), and another unclassified fungus belonging to the order Mortierellales (3.13%).

Lefse was used to identify fungal genera that were preferentially abundant in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 cm or 5 to 7 cm. Genera with an LDA score of two or more were regarded as significantly abundant in the group of interest. Genera that could be definitively identified are reported.

Twenty-five fungal taxa were preferentially abundant in soil collected from a depth of 0 to 2 cm from burnt paddocks, seven of which could be resolved to the genus-level: *Rhodotorula*, *Pyrenophora*, *Holtermannia*, *Filobasidium Alternaria*, *Idriella*, and *Solicoccozyma* (Fig. 38).

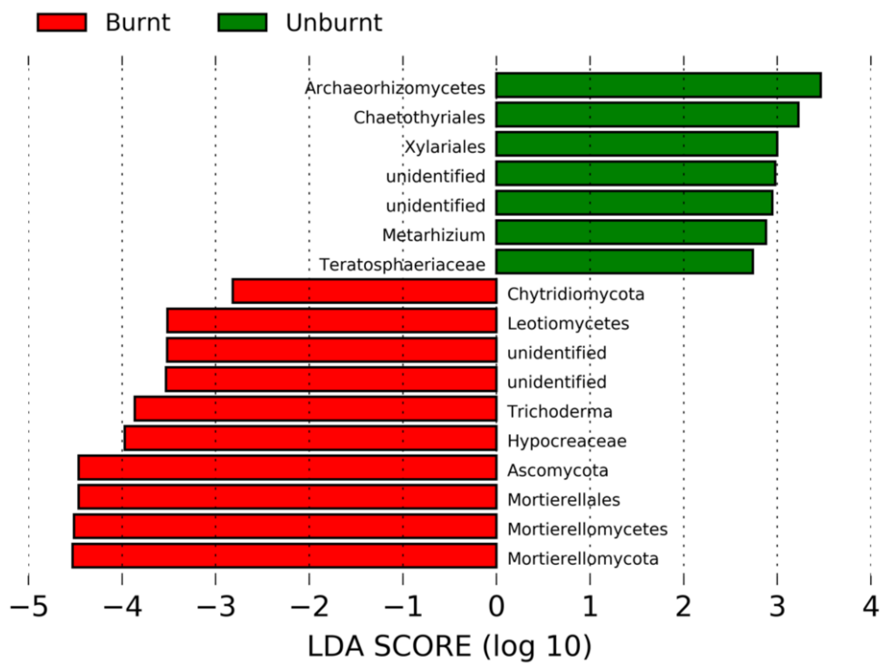
Eleven fungal taxa were preferentially abundant in soil collected from a depth of 0 to 2 cm from unburnt paddocks, three of which could be resolved to the genus-level: *Alatospora* (LDA score = 2.74), *Catenulostroma*, and *Serendipita*.

Figure 38. Lefse results for analysis of the fungal communities in soil collected from burnt and unburnt paddocks at a depth of 0 to 2 cm following a planned burn conducted in 2022.



Ten fungal taxa were preferentially abundant in soil collected from a depth of 5 to 7 cm from burnt paddocks, one of which could be resolved to the genus-level: *Metarhizium* (Fig. 39). Seven fungal taxa were preferentially abundant in soil collected from a depth of 5 to 7 cm from unburnt paddocks, one of which could be resolved to the genus-level: *Trichoderma.*

Figure 39. Lefse results for analysis of the fungal communities in soil collected from burnt and unburnt paddocks at a depth of 5 to 7 cm following a planned burn conducted in 2022.



4.6.2 Response of microbiota to bushfires Sampling 1 (June 2021)

4.6.2.1 Analysis of soil microbiota (16S rRNA gene)

4.6.2.1.1 Community diversity

Diversity analyses were done using several alpha- and beta-diversity metrics to compare the bacterial communities (microbiota) in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 cm and 5 to 7 cm below the soil surface across all four farms and at the farm-level. In general, when considering the number of taxa present in each community and their relative abundances, the bacterial communities were similar in both the burnt and unburnt paddocks.

Overall, when considering data from all four farms, the diversity of the bacterial communities present in soil from the burnt paddocks was significantly higher according to most of the diversity metrics evaluated (Table 15). At a depth of 0 to 2 cm, alpha diversity was significantly different between samples collected from the burnt paddocks according to Shannon's Diversity Index (p = 0.011) and Faith's Phylogenetic Diversity (p = 0.020). There was no significant difference according to Pielou's Evenness Index (p = 0.586). At a depth of 5 to 7 cm, alpha diversity was significantly different in samples collected from the burnt paddocks according to Shannon's Diversity Index (p = 0.021) and Faith's Phylogenetic Diversity (p = 0.037). There was no significant difference according to Pielou's Evenness Index (p = 0.530).

Table 15. Alpha diversity analysis for soil samples collected from farms in 2021. The average alpha diversity value calculated for each group is presented. For each gene target, alpha diversity metrics were compared between burnt and unburnt soil samples at each depth using a non-parametric Kruskal-Wallis test.

			Alpha Diversity Metric								
			Shannon's Diversity Index			Pielou's Evenness Index			Faith's Phylogenetic Diversity		
Target	**Farm**	**Depth**	**Unburnt**	**Burnt**	**P-value**	**Unburnt**	**Burnt**	**P-value**	**Unburnt**	**Burnt**	**P-value**
16S	All	2 cm	8.71	8.90	0.011	0.90	0.90	0.586	63.71	70.25	0.020
		5 cm	8.53	8.64	0.021	0.88	0.89	0.530	61.68	63.86	0.037
	RG2	2 cm	8.51	8.75	0.347	0.88	0.88	0.602	65.04	70.21	0.465
		5 cm	7.93	8.56	0.175	0.82	0.87	0.028	60.87	69.20	0.117
	RG3	2 cm	9.04	8.87	0.251	0.91	0.91	0.347	68.06	75.30	0.117
		5 cm	8.81	8.80	0.602	0.90	0.89	0.754	65.23	68.75	0.347
	RG4	2 cm	8.45	8.62	0.251	0.91	0.90	0.602	53.27	61.90	0.016
		5 cm	8.28	8.37	0.251	0.90	0.90	0.754	49.25	50.89	0.602
ITS	All	2 cm	4.84	5.09	0.107	0.59	0.62	0.049	54.11	59.76	0.930
		5 cm	3.74	4.26	0.791	0.49	0.56	0.665	41.46	43.02	0.878
	RG2	2 cm	4.95	4.67	0.465	0.63	0.55	0.251	45.00	50.19	0.175
		5 cm	4.85	5.07	0.601	0.63	0.66	0.601	44.75	45.66	0.917
	RG3	2 cm	5.60	5.34	0.917	0.69	0.65	0.465	53.18	59.33	0.602
		5 cm	4.07	3.68	0.602	0.54	0.50	0.754	39.86	34.76	0.347
	RG4	2 cm	5.99	5.23	0.462	0.69	0.64	0.462	73.38	60.08	0.086
		5 cm	3.18	4.16	0.465	0.42	0.54	0.465	38.81	44.55	0.465

There was a significant difference in beta diversity between samples collected at a depth of 0 to 2 cm from burnt and unburnt paddocks according to analysis of the unweighted UniFrac distances (p = 0.006), but not the weighted UniFrac distances (p = 0.123) or the Bray-Curtis distances (p = 0.069). There was no significant difference in beta diversity between samples collected at a depth of 5 to 7 cm from burnt and unburnt paddocks according to any of the three beta diversity metrics evaluated. These results indicate that there were some significant differences regarding the number of taxa identified in each sample type, but the communities were not significantly different when the relative abundance of each taxon was taken into consideration.

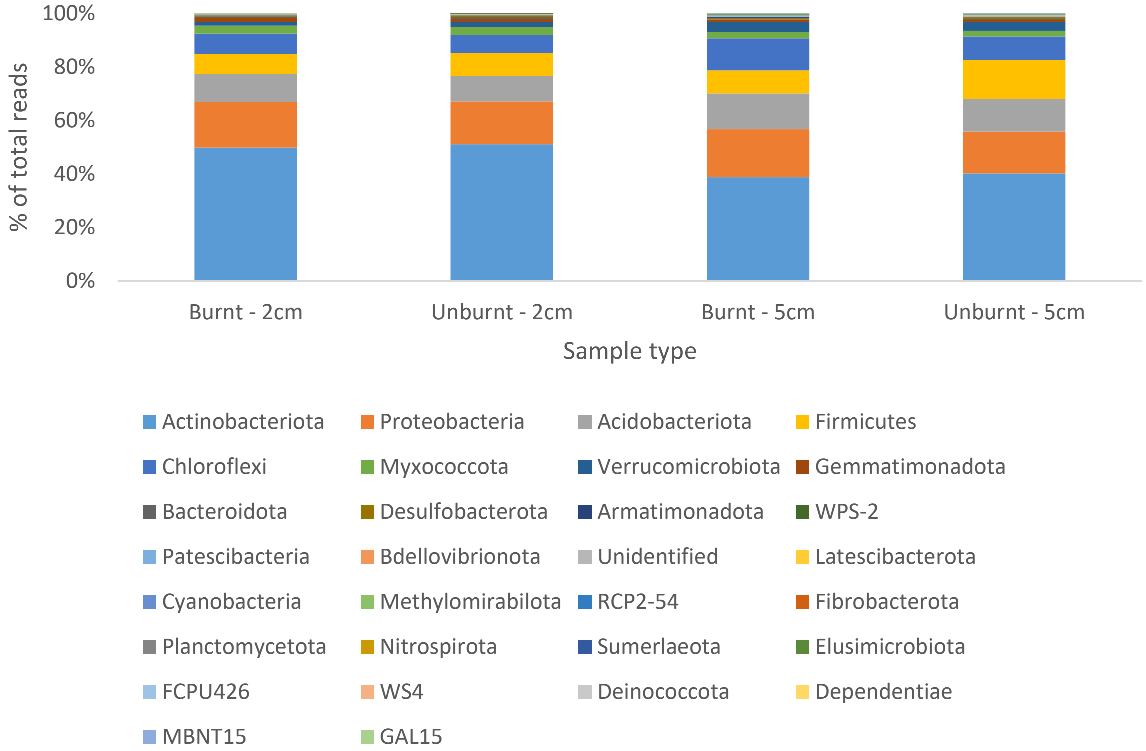
Data obtained from the 2021 samples were also analysed at the farm-level for RG2, RG3, and RG4. Soil samples from a representative unburnt paddock was not available from RG1 as fire had impacted the whole property, so comparisons between burnt and unburnt paddocks could not be made for this farm. On Farm RG2, there were no significant differences in alpha diversity between the bacterial populations present in the soil of burnt and unburnt paddocks at a depth of 0 to 2 cm (Table 15). There was a significant difference at a depth of 5 to 7 cm according to Pielou's Evenness Index (p = 0.028), which indicates that similar taxa were present in both sites but that their relative abundances differed. On Farm RG3, there were no significant differences in alpha diversity between the bacterial populations present in the soil of burnt and unburnt paddocks at either depth. On Farm RG4, there was a significant difference between the bacterial populations of burnt and unburnt paddocks at a depth of 0 to 2 cm according to Faith's Phylogenetic Diversity (p = 0.016).

On Farm RG2, beta diversity differed between samples from the burnt and unburnt paddocks at a depth of 0 to 2 cm according to analysis of the unweighted UniFrac distance matrix only (p = 0.022). At a depth of 5 to 7 cm, beta diversity different significantly according to analysis of the Bray-Curtis (p = 0.018), unweighted UniFrac (p = 0.035) and weighted UniFrac (p = 0.007) distance matrices. On Farm RG3, there was no significant difference in beta diversity at either depth. On Farm RG4, beta diversity differed between samples from the burnt and unburnt paddocks at a depth of 0 to 2 cm according to analysis of the Bray-Curtis (p = 0.009), unweighted UniFrac (p = 0.006) and weighted UniFrac (p = 0.011) distance matrices. At a depth of 5 to 7 cm, beta diversity differed significantly according to analysis of the Bray-Curtis (p = 0.009), unweighted UniFrac (p = 0.004) and weighted UniFrac (p = 0.005) distance matrices.

4.6.2.1.2 Taxonomical analysis

The composition of the bacterial communities (microbiota) present in soil samples collected from depths 0 to 2 cm and 5 to 7 cm in the burnt and unburnt paddocks were characterised and compared at both the phylum- and genus-level. Broadly, when considering the presence and absence of specific taxa, and their relative abundances, the composition of the microbial communities was similar in the burnt and unburnt paddocks across all five farms (i.e., the dominant taxa were similar in both sample types). The phyla Actinobacteria and Proteobacteria were the most abundant phyla, with similar relative abundances in each sample type (Fig. 40). In the burnt paddocks, at a depth of 0 to 2 cm the most abundant bacterial genera were *Acidothermus* (11.86%), *Bacillus* (4.99%), an uncultured bacterium belonging to the order Gaiellales (3.48%), *Conexibacter* (3.46%), and *Pseudonocardia* (3.00%). In soil samples collected from unburnt paddocks, the most abundant genera were *Acidothermus* (11.35%), *Bacillus* (6.31%), an uncultured bacterium belonging to the order Gaiellales (4.45%), *Conexibacter* (3.19%), and an uncultured bacterium belonging to the family Xanthobacteraceae (3.01%).

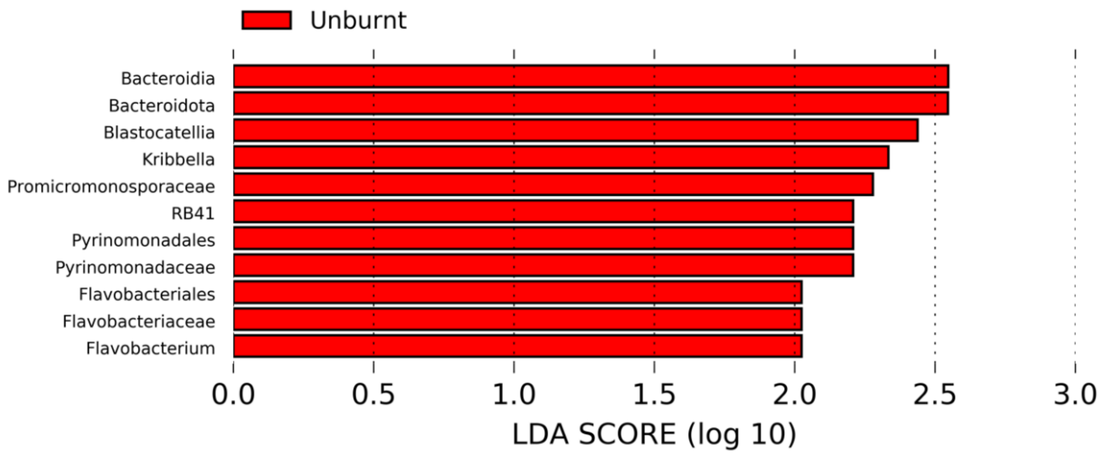
Figure 40. Relative abundance of bacterial phyla identified in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 and 5 to 7 cm from four farms in 2021. The relative abundances are based on pooled data from all four farms.



At a depth of 5 to 7 cm, in soil collected from burnt paddocks the most abundant genera identified were *Acidothermus* (17.63%), *Bacillus* (6.56%), an uncultured bacterium belonging to the family Xanthobacteraceae (5.17%), an uncultured bacterium belonging to the order Acidobacteriales (3.96%), and an uncultured bacterium belonging to the order Gaiellales (3.72%). In soil collected from the unburnt paddocks, the most abundant genera were *Acidothermus* (13.41%), *Bacillus* (11.29%), an uncultured bacterium belonging to the family Xanthobacteraceae (4.87%), an uncultured bacterium belonging to the order Gaiellales (4.84%), and *Conexibacter* (3.11%).

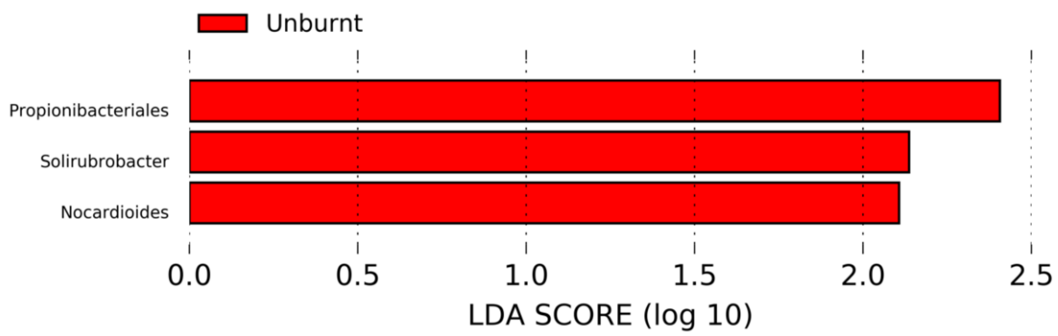
There were significant differences in the diversity and relative abundance of several less-abundant taxa between the burnt and unburnt paddocks. Lefse was used to identify bacterial genera that were preferentially abundant in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 cm or 5 to 7 cm. Genera with an LDA score of two or more were regarded as significantly abundant in the group of interest. Eleven bacterial were identified as preferentially abundant in soil collected from a depth of 0 to 2 cm from unburnt paddocks, two of which could be resolved to the genus-level*: Kribbella,* and *Flavobacterium* (Fig. 41).

Figure 41. Lefse results for analysis of the bacterial communities in soil collected from burnt and unburnt paddocks at a depth of 0 to 2 cm in 2021.



Three bacterial taxa were identified as preferentially abundant in soil collected from unburnt paddocks at a depth of 5 to 7 cm, one of which could be resolved to the genus-level: *Solirubrobacter*. No bacterial genera were preferentially abundant in soil samples collected from burnt paddocks at either depth (Fig. 42).

Figure 42. Lefse results for analysis of the bacterial communities in soil collected from burnt and unburnt paddocks at a depth of 5 to 7 cm in 2021.



The genus *Kribella* (previously named *Nocardioides*) was first described in 1999 [172]. Members of this genus have been identified in the rhizosphere microbiome and are associated with resistance to soil-borne pathogens [173]. Members of this genus are also reported to respond positively to fire, thriving in pyrogenic organic material [174]. Members of the genus *Flavobacterium* has also been shown to respond positively to fire; this is most likely due to their capacity to degrade recalcitrant organic compounds produced by fire, such as polycyclic aromatic hydrocarbons [175]. The greater abundance of these genera in unburnt paddocks in the present study is surprising given their positive association with fire, but this might be a result of the long interval between the fire event and sample collection. The genus *Solirubrobacter* is known to respond poorly to fire events [176]. This genus has a role in the nitrogen cycle and provides a source of bioactive compounds, so as decrease in the relative abundance of this genus in soil following a fire event may be an indicator poor soil quality.

4.6.2.2 Analysis of soil mycobiota (ITS gene)

4.6.2.2.1 Community diversity

Diversity analyses were undertaken using several alpha- and beta-diversity metrics to compare the fungal communities (mycobiota) in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 cm and 5 to 7 cm below the soil surface across all four farms and at the farm-level.

There were few significant differences in alpha diversity between the burnt and unburnt soil samples. At a depth of 0 to 2 cm, alpha diversity was significantly different in soil samples collected from burnt paddocks according to Pielou's Evenness Index ($p = 0.049$) (Table 15). There was no significant difference in alpha diversity between the burnt and unburnt paddocks at this depth according to Shannon's Diversity Index ($p = 0.107$) and Faith's Phylogenetic Diversity ($p = 0.878$) (Table 15). At a depth of 5 to 7 cm, there was no significant difference in alpha diversity between samples collected from burnt and unburnt paddocks according to any of the three alpha diversity metrics (Table 15).

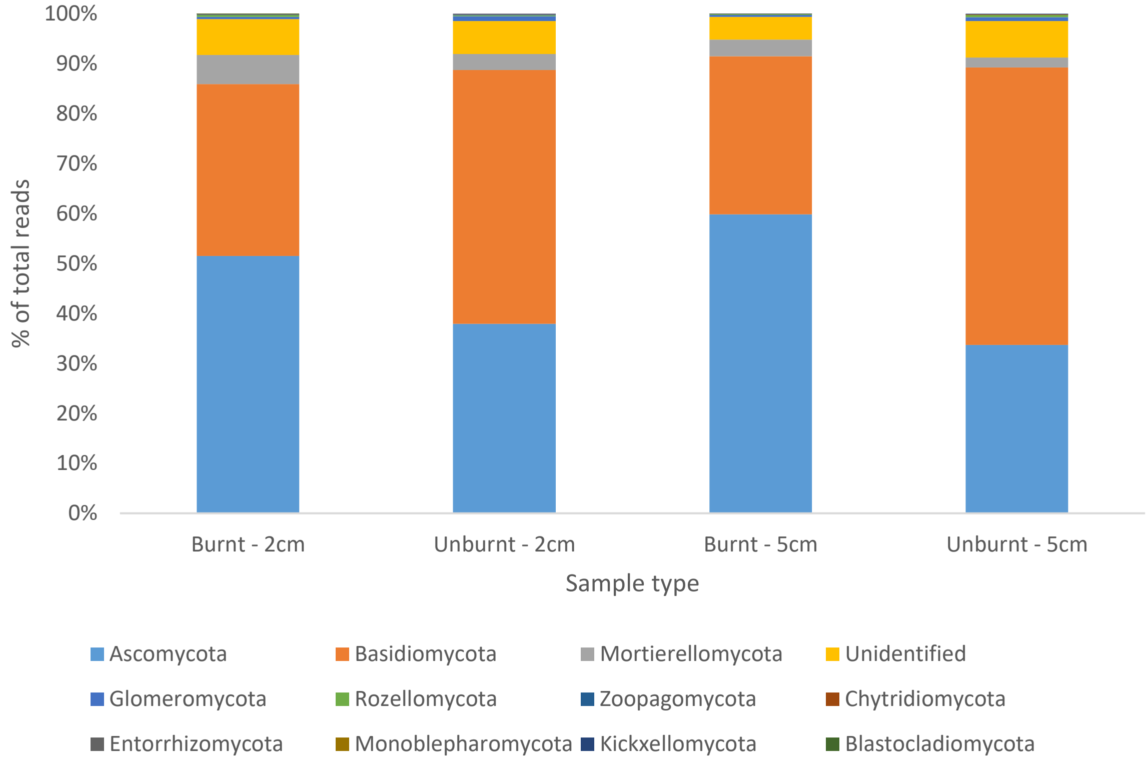
There was a significant difference in beta diversity between samples collected from a depth of 0 to 2 cm from burnt and unburnt paddocks according to analysis of the Bray-Curtis distances ($p = 0.003$) and the unweighted UniFrac distances ($p = 0.043$) but not the weighted UniFrac distances ($p = 0.335$). There was a significant difference in beta diversity between samples collected from a depth of 5 to 7 cm from burnt and unburnt paddocks according to analysis of the Bray-Curtis distances ($p = 0.027$) and the unweighted UniFrac distances ($p = 0.043$), but not the weighted UniFrac distances ($p = 0.155$). Again, these findings indicate that the composition of the microbial communities was similar in the burnt and unburnt paddocks, but that the presence or absence low-abundance taxa differed between sites. The results of this study are also consistent with those of previous studies that have indicated that the long-term impact of bushfire is greater on soil bacteria than on soil fungi.

At the farm-level, there was no significant difference in alpha diversity at either depth according to any of the alpha diversity metrics evaluated (Table 15). On Farm RG2, there was a significant difference in beta diversity between samples collected from burnt and unburnt paddocks at a depth of 0 to 2 cm (p = 0.009) and 5 to 7 cm (p = 0.01) according to analysis of the unweighted UniFrac distance matrix. There were no significant differences in beta diversity on Farm RG3 at either depth. On Farm RG4, at a depth of 0 to 2 cm, beta diversity different significantly according to analysis of the Bray-Curtis (p = 0.007), unweighted UniFrac (p = 0.011) distance matrices. At a depth of 5 to 7 cm, beta diversity different significantly according to analysis of the Bray-Curtis (p = 0.011), unweighted UniFrac (p = 0.012) and weighted UniFrac (p = 0.027) distance matrices.

4.6.2.2.2 Taxonomical results

The composition of the fungal communities (mycobiota) present in soil samples collected from depths 0 to 2 cm and 5 to 7 cm in the burnt and unburnt paddocks were characterised and compared at both the phylum- and genus-level. The dominant phyla were similar in soil from the burnt and unburnt paddocks, although there was an increase in the relative abundance of Ascomycota in the burnt paddocks, and a corresponding decrease in the relative abundance Basidiomycota (Fig. 43). The dominant genera were similar across the burnt and unburnt paddocks at both depths. In the burnt paddocks, in soil samples collected from a depth of 0 to 2 cm the most abundant fungal genera identified were *Archaeorhizomyces* (6.67%), *Fusarium* (6.49%), an unclassified fungus (5.79%), *Mortierella* (5.52%), and *Marasmius* (5.52%). In soil samples collected from unburnt paddocks, the most abundant fungal genera identified were *Clavaria* (13.27%), an unclassified fungus from the order Agaricales (6.43%), *Fusarium* (6.01%), an unclassified fungus (5.84%), and an unclassified fungus from the class Agaricomycetes (4.62%).

Figure 43. Relative abundance of fungal phyla identified in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 and 5 to 7 cm from four farms in 2021. The relative abundances are based on pooled data from all four farms.



In soil samples collected from burnt paddocks at a depth of 5 to 7 cm, the most abundant fungal genera identified were *Archaeorhizomyces* (12.86%), an unidentified fungus belonging to the class Archaeorhizomycetes (12.81%), *Hygrocybe* (6.46%), an unidentified fungus belonging to the family Hyaloscyphaceae (4.71%), and an unidentified fungus belonging to the family Thelephoraceae (4.66%). In soil samples collected from the unburnt paddocks at this depth, the most abundant fungal genera identified were *Clavaria* (17.46%), an unclassified fungus belonging to the order Agaricales (14.00%), an unclassified fungus (6.76%), *Archaeorhizomyces* (6.65%), and an unclassified fungus belonging to the family Clavariaceae (6.62%).

Lefse was used to identify fungal genera that were preferentially abundant in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 cm or 5 to 7 cm. Genera with an LDA score of two or more were regarded as significantly abundant in the group of interest. Genera that could be definitively identified are reported. Four fungal taxa were preferentially abundant in soil collected from a depth of 0 to 2 cm from unburnt paddocks, two of which could be resolved to the genus-level: *Metarhizium* and *Apiotrichum* (Fig. 44). No fungal genera were preferentially abundant in soil samples collected from burnt paddocks at this depth. Two genera were preferentially abundant in soil collected from a depth of 5 to 7 cm from unburnt paddocks (Fig. 45): *Metarhizium* and *Apiotrichum*. Five fungal taxa were preferentially abundant in soil collected from burnt paddocks at a depth of 5 to 7 cm, two of which could be resolved to the genus-level: *Leohumicola* and *Talaromyces*. Three fungal taxa were preferentially abundant in soil collected from unburnt paddocks at this depth, two of which could be resolved to the genus-level: *Metarhizium* and *Apiotrichum*.

Members of the genus *Metarhizium* are pathogenic to arthropods (entomopathogenic). As such, several biological control agents based on this genus have been developed targeting arthropods, including crop pests [177]. *Metarhizium* spp. are also reported to increase plant growth through the provision of arthropod-derived nitrogen [178]. The relative abundance of this genus was lower in soil samples from the burnt paddocks, which could have implications for plant growth and productivity post-fire. Fungi within the genus *Apiotrichum* (formerly members of the genus *Trichosporon*) are abundant in soils and are involved in nutrient cycling and the decomposition of organic material [179], but their significance in fire-affected landscapes has not been directly investigated. The genus *Leohumicola* is reported to be heat-resistant and has been isolated from a range of environments post-fire [180]. The growth of heat-resistant genera such *Leohumicola* after fire enhances the decomposition of organic matter and the release of nutrients, which could aid the recovery of pasture post-fire. Members of the genus *Talaromyces* also rapidly colonise soil post-fire, which may also enhance soil restoration and plant growth [181].

Figure 44. Lefse results for analysis of the fungal communities in soil collected from burnt and unburnt paddocks at a depth of 0 to 2 cm in 2021.

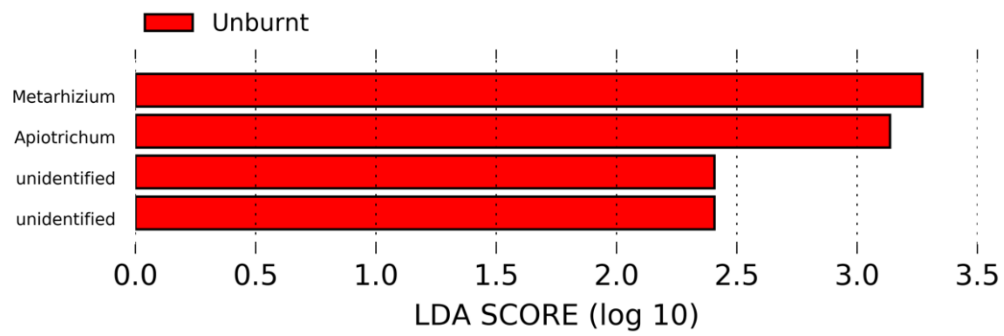
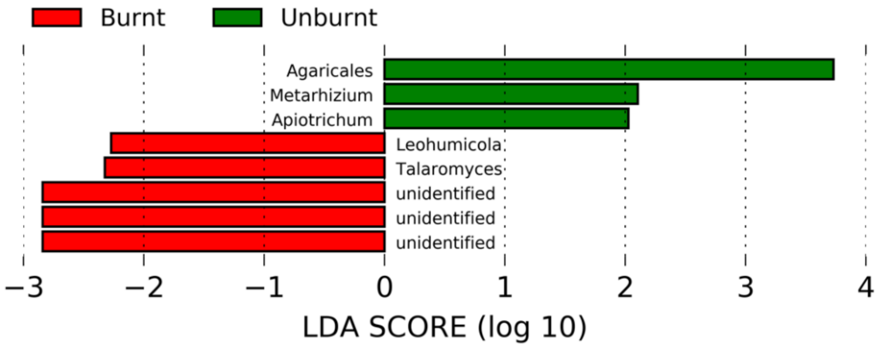


Figure 45. Lefse results for analysis of the fungal communities in soil collected from burnt and unburnt paddocks at a depth of 5 to 7 cm in 2021.



4.6.3 Response of microbiota to bushfires Sampling 2 (June 2022)

4.6.3.1 Soil microbiota (16S rRNA gene)

4.6.3.1.1 Analysis of community diversity

Diversity analyses were done using several alpha- and beta-diversity metrics to compare the bacterial communities (microbiota) in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 cm and 5 to 7 cm below the soil surface across all four farms and at the farm-level (Table 16).

Table 16. Alpha diversity analysis for soil samples collected from farms during 2022. The average alpha diversity value calculated for each group is presented. For each gene target, alpha diversity metrics were compared between burnt and unburnt soil samples at each depth using a non-parametric Kruskal-Wallis test.

			Alpha Diversity Metric								
			Shannon's Diversity Index			Pielou's Evenness Index			Faith's Phylogenetic Diversity		
Target	**Farm**	**Depth**	**Unburnt**	**Burnt**	**P-value**	**Unburnt**	**Burnt**	**P-value**	**Unburnt**	**Burnt**	**P-value**
16S	All	2 cm	8.71	8.90	0.036	0.90	0.90	0.689	63.71	70.25	0.008
		5 cm	8.53	8.64	0.209	0.88	0.89	0.841	61.68	63.86	0.688
	RG2	2 cm	8.61	8.33	0.480	0.93	0.88	0.034	52.57	62.00	0.034
		5 cm	8.56	8.23	0.034	0.92	0.88	0.034	53.75	60.06	0.289
	RG4	2 cm	7.78	8.14	0.297	0.89	0.90	0.655	48.32	52.42	0.180
		5 cm	8.06	8.41	0.248	0.91	0.91	1.000	45.07	51.89	0.248
	RG5	2 cm	7.84	8.48	0.050	0.88	0.92	0.014	39.46	48.62	0.624
		5 cm	8.23	8.28	0.117	0.89	0.90	0.028	52.59	56.19	0.251
ITS	All	2 cm	4.84	5.09	0.643	0.59	0.62	0.504	54.11	59.76	0.681
		5 cm	3.74	4.26	0.310	0.49	0.56	0.757	41.46	43.02	0.003
	RG2	2 cm	5.97	5.84	0.289	0.76	0.81	0.480	48.62	35.79	0.077
		5 cm	5.96	5.84	0.480	0.76	0.81	0.289	51.27	35.89	0.067

RG4	2 cm	5.99	5.84	0.289	0.76	0.80	0.480	48.98	35.82	0.077
	5 cm	5.96	5.85	0.280	0.76	0.81	0.471	50.15	35.81	0.157
RG5	2 cm	5.93	5.82	0.289	0.75	0.80	0.480	50.90	35.75	0.157
	5 cm	5.96	5.84	0.289	0.76	0.81	0.479	50.84	35.44	0.077

At a depth of 0 to 2 cm, there was a significant difference in alpha diversity according to Shannon's Diversity Index ($p = 0.036$) and Faith's Phylogenetic Diversity ($p = 0.008$) but not Pielou's Eveness Index ($p = 0.689$), which indicates that the dominant taxa were similar in both sample types but that some low-abundance taxa differed. There were no significant differences in alpha diversity at a depth of 5 to 7 cm.

Beta diversity differed between samples from the burnt and unburnt paddocks at a depth of 0 to 2 cm according to analysis of the Bray-Curtis ($p = 0.019$) and weighted UniFrac ($p = 0.034$) distance matrices but not the unweighted UniFrac distance matrix ($p = 0.064$). At a depth of 5 to 7 cm, beta diversity different significantly between the two sample types according to analysis of the Bray-Curtis ($p = 0.020$) and unweighted UniFrac ($p = 0.022$) distance matrices but not the weighted UniFrax distance matrix ($p = 0.089$).

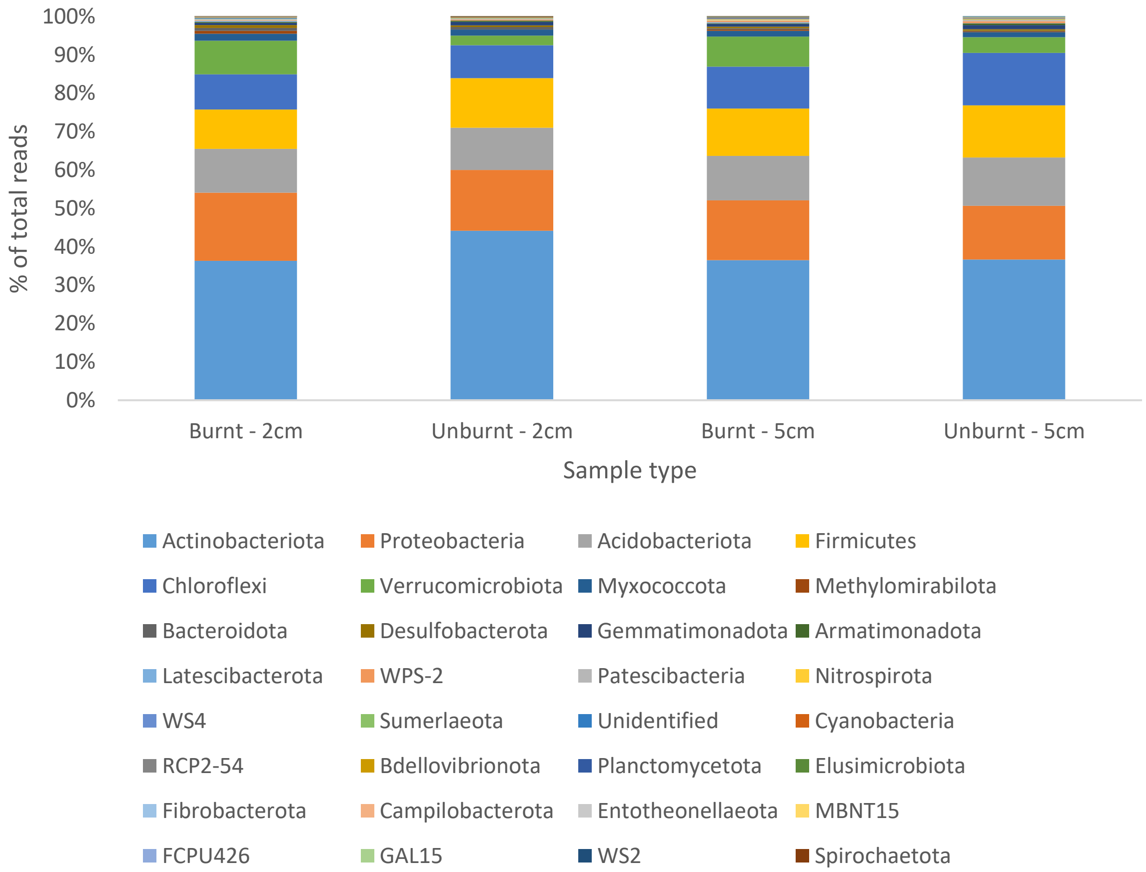
On Farm RG2, alpha diversity differed between samples collected from the burnt and unburnt paddocks at a depth of 0 to 2 cm according to Pielou's Evenness Index ($p = 0.034$) and Faith's Phylogenetic Diversity ($p = 0.034$). At a depth of 5 to 7 cm, there was a significant difference according to Shannon's Diversity Index ($p = 0.034$) and Pielou's Evenness Index ($p = 0.034$). On Farm RG4, there were no significant differences at either depth. On Farm RG5, alpha diversity differed significantly at both depths according to Pielou's Evenness Index ($p < 0.05$).

On Farm RG2, beta diversity was significantly different between samples collected from burnt and unburnt paddocks at a depth of 0 to 2 cm according to analysis of the Bray-Curtis ($p = 0.024$) distance matrix. At a depth of 5 to 7 cm, there were significant differences according to analysis of the Bray Curtis ($p = 0.032$), unweighted UniFrac ($p = 0.035$), and weighted UniFrac ($p = 0.037$) distance matrices. On Farm RG4, beta diversity was significantly different between samples collected from burnt and unburnt paddocks at a depth of 0 to 2 cm according to analysis of the Bray Curtis ($p = 0.025$), unweighted UniFrac ($p = 0.023$) distance matrices. There were no significant differences at 5 to 7 cm. On Farm RG5, at a depth of 0 to 2 cm, there were significant differences according to analysis of the Bray Curtis ($p = 0.007$), unweighted UniFrac ($p = 0.014$), and weighted UniFrac ($p = 0.008$) distance matrices. There were also significant differences at a depth of 5 to 7 cm according to analysis of the Bray Curtis ($p = 0.010$), unweighted UniFrac ($p = 0.013$), and weighted UniFrac ($p = 0.009$) distance matrices.

4.6.3.1.2 Taxonomical diversity

The composition of the bacterial communities (microbiota) present in soil samples collected from depths 0 to 2 cm and 5 to 7 cm in the burnt and unburnt paddocks were characterised and compared at both the phylum- and genus-level. At the phylum level, the dominant taxa were similar in the burnt and unburnt paddocks at both depths, with Actinobacteria the dominant phyla (Fig. 46). At the genus-level, the dominant taxa were similar across the burnt and unburnt paddocks at both depths. In the burnt paddocks, at a depth of 0 to 2 cm the most abundant bacterial genera were *Acidothermus* (7.62%), Candidatus Udaeobacter *(7.36%*), *Bacillus* (7.28%), an uncultured bacterium belonging to the family Xanthobacteraceae (5.03%), and an uncultured bacterium belonging to the order Gaiellales (4.63%). In soil samples collected from unburnt paddocks, the most abundant genera were *Acidothermus* (10.08%), *Bacillus* (8.74%), *Conexibacter* (5.03%), an uncultured bacterium belonging to the order Gaiellales (4.22%), and an uncultured bacterium belonging to the phylum Chloroflexi (3.42%).

Figure 46. Relative abundance of bacterial phyla identified in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 and 5 to 7 cms from four farms in 2022. The relative abundances are based on pooled data from all four farms.



At a depth of 5 to 7 cm, in soil collected from burnt paddocks the most abundant genera identified were *Acidothermus* (11.58%), *Bacillus* (9.10%), Candidatus Udaeobacter (6.56%), an uncultured bacterium belonging to the family Xanthobacteraceae (6.53%), and an uncultured bacterium belonging to the order Gaiellales (5.81%). In soil collected from the unburnt paddocks, the most abundant genera were *Acidothermus* (12.39%), *Bacillus* (9.72%), *Conexibacter* (5.22%) an uncultured bacterium belonging to the order Gaiellales (4.45%), and an uncultured bacterium belonging to the family *Xanthobacteraceae* (3.85%).

A total of 16 bacterial taxa were identified as preferentially as preferentially abundant in soil collected from the burnt paddocks at a depth of 0 to 2 cm, one of which, Candidatus Udaeobacter, could be resolved to the genus-level (Fig. 47). A total of 21 bacterial taxa were identified as preferentially abundant in soil collected from burnt paddocks at a depth of 5 to 7 cm (Fig. 48), two of which, Candidatus Udaeobacter and *Gaiella*, could be resolved to the genus-level. No bacterial genera were preferentially abundant in soil from unburnt paddocks.

The genus Candidatus Udaeobacter is an oligotroph that is known to proliferate in nutrient-poor conditions. As such, this genus could be used as a bioindicator species to identify soil in poor a nutrient state [182, 183]. The ecological role of species in the genus *Gaiella* is not well-described, but the genus is reported to be heat-tolerant [184], and may have a role in nutrient-cycling and resistance to soil-borne pathogens [185]. In contrast to the present study, previous studies have reported that fire results in a decrease in the relative abundance of the genus *Gaiella* [176]. The long interval between the fire event and sample collection in the present study may explain this discrepancy.

Figure 47. Relative abundance of bacterial phyla identified in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 and 5 to 7 cm from four farms in 2022. The relative abundances are based on pooled data from all four farms.

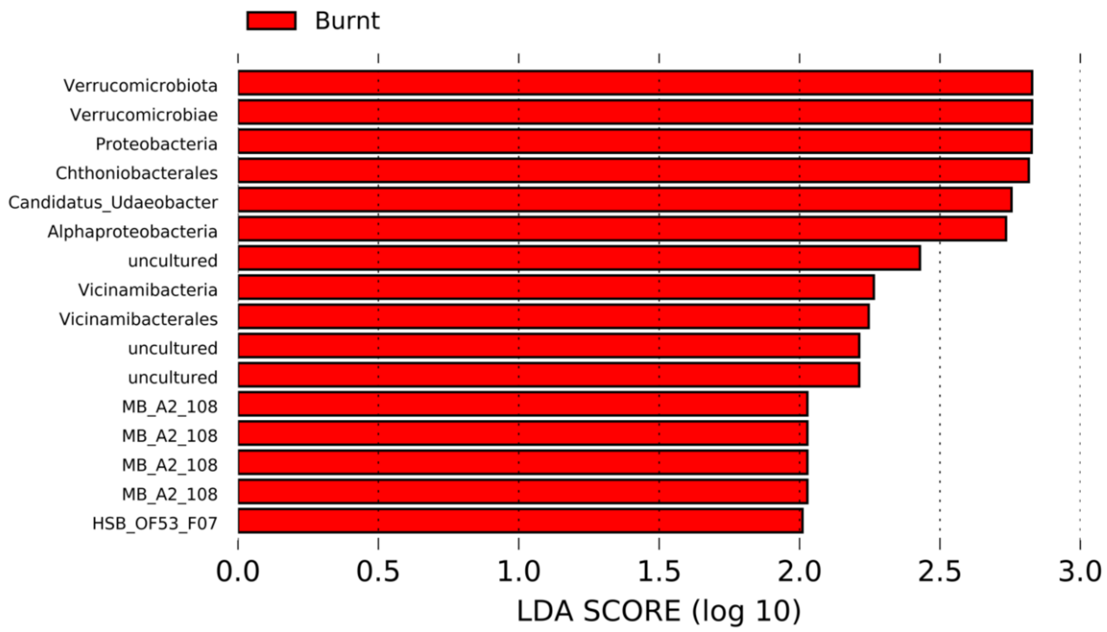
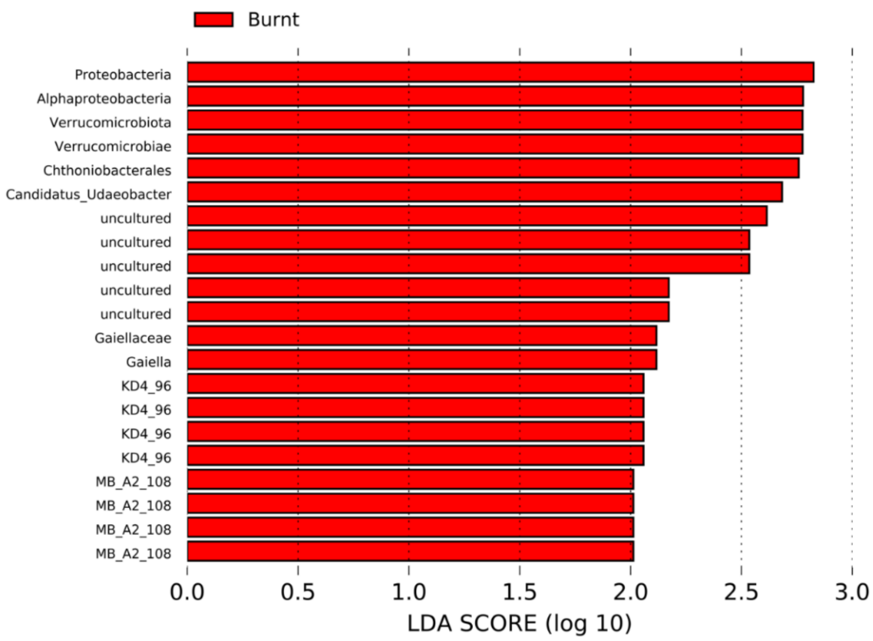


Figure 48. Lefse results for analysis of the bacterial communities in soil collected from burnt and unburnt paddocks at a depth of 5 to 7 cm in 2022.



4.6.3.2 Soil mycobiota (ITS gene)

4.6.3.2.1 Community diversity

There was a significant difference in alpha diversity overall at a depth of 5 to 7 cm according to Faith's Phylogenetic Diversity (p = 0.003) (Fig. 16). There were no other significant differences in alpha diversity at either depth overall or at the farm-level. Beta diversity was significantly different between samples collected from burnt and unburnt paddocks according to analysis of the Bray-Curtis distance matrix (p = 0.041). At a depth of 5 to 7 cm, beta diversity was significantly different according to analysis of the Bray-Curtis (p = 0.010), unweighted UniFrac (p = 0.001) and weighted UniFrac (p = 0.046) distance matrices.

On Farm RG2, there was a significant difference in beta diversity between samples collected from the burnt and unburnt paddocks at a depth of 0 to 2 cm according to analysis of the Bray-Curtis (p = 0.024), unweighted UniFrac (p = 0.041), weighted UniFrac (p = 0.022) distance matrices. There were also significant differences at a depth of 5 to 7 cm according to analysis of the Bray-Curtis (p = 0.029), unweighted UniFrac (p = 0.033), weighted UniFrac (p = 0.037) distance matrices.

On Farm RG4, there were no significant differences in beta diversity at a depth of 0 to 2 cm. There were significant differences at a depth of 5 to 7 cm according to analysis of the Bray-Curtis (p = 0.036), unweighted UniFrac (p = 0.032), weighted UniFrac (p = 0.033) distance matrices.

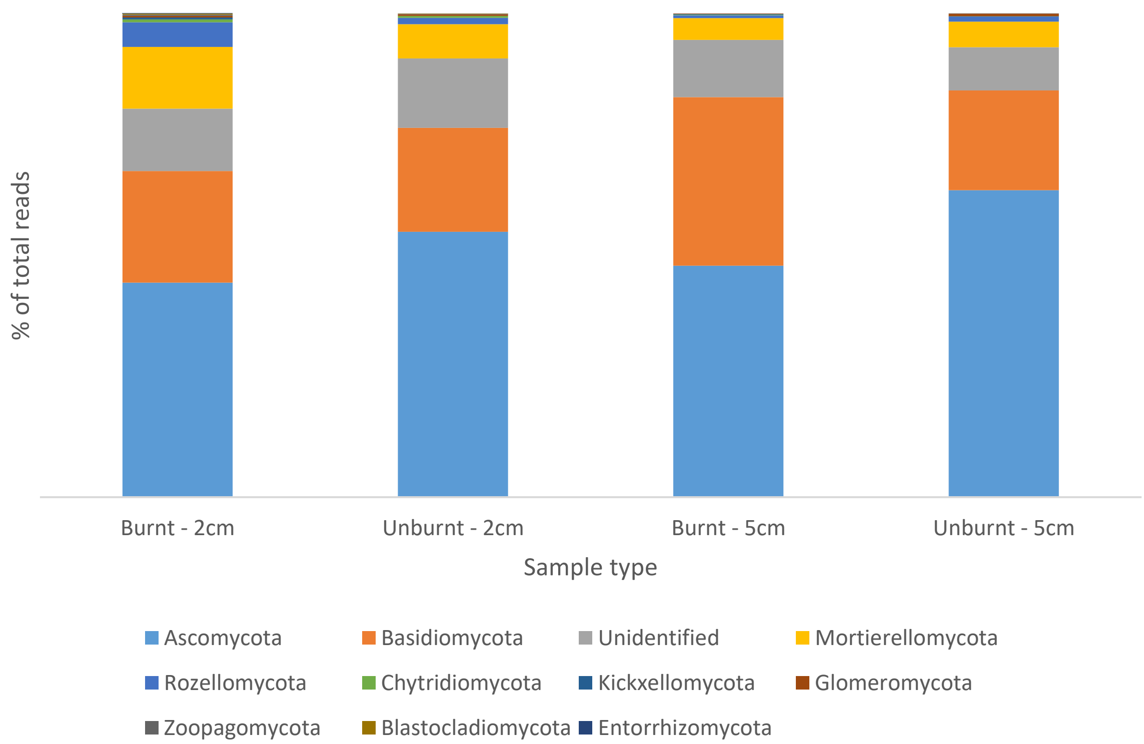
On Farm RG5, there was a significant difference in beta diversity between samples collected from the burnt and unburnt paddocks at a depth of 0 to 2 cm according to analysis of the Bray-Curtis (p = 0.031), unweighted UniFrac (p = 0.034), weighted UniFrac (p = 0.033) distance matrices. There was also a significant difference at a depth of 5 to 7 cm according to analysis of the Bray-Curtis (p = 0.036), unweighted UniFrac (p = 0.037), weighted UniFrac (p = 0.031) distance matrices.

On Farm RG2, there was a significant difference in beta diversity between samples collected from the burnt and unburnt paddocks at a depth of 0 to 2 cm according to analysis of the Bray-Curtis (p = 0.014), unweighted UniFrac (p = 0.019), weighted UniFrac (p = 0.008) distance matrices. There were also significant differences at a depth of 5 to 7 cm according to analysis of the Bray-Curtis (p = 0.029), unweighted UniFrac (p = 0.033), weighted UniFrac (p = 0.037) distance matrices.

4.6.3.2.2 Taxonomical diversity

The composition of the fungal communities (mycobiota) present in soil samples collected from depths 0 to 2 cm and 5 to 7 cm in the burnt and unburnt paddocks were characterised and compared at both the phylum- and genus-level (Fig. 49). The dominant phyla were mostly similar in samples from burnt and unburnt paddocks at both depths. However, in contrast to the 2021 samples, the relative abundance of the phyla Ascomycota was greater in samples from the burnt paddocks than from the unburnt paddocks. In soil samples collected from burnt paddocks, at a depth of 0 to 2 cm the most abundant fungal genera were an unclassified fungus (12.84%), *Mortierella* (11.74%), *Solicoccozyma* (4.97%), *Fusarium* (4.73%), and an unclassified fungus belonging to the class Rozellomycotina cls Incertae sedis (3.82%). In soil samples collected from unburnt paddocks, the dominant fungal genera were an unclassified fungus (13.49%), *Solicoccozyma* (12.56%), *Aleuria* (10.45%), *Fusarium* (7.04%), and *Mortierella* (5.18%).

Figure 49. Relative abundance of fungal phyla identified in soil samples collected from burnt and unburnt paddocks at depths of 0 to 2 and 5 to 7 cm from four farms in 2022. The relative abundances are based on pooled data from all four farms.



In soil samples collected from a depth of 5 to 7 cm from the burnt paddocks, the most abundant fungal genera were an unclassified fungus belonging to the family Clavariaceae (17.45%), *Pseudeurotium*

(13.82%), an unclassified fungus (11.15%), *Solicoccozyma* (4.94%), and *Mortierella* (4.37%). In soil samples collected from this depth from the unburnt paddocks, the most abundant fungal genera were an unclassified fungus belonging to the phylum Ascomycota (21.47%), *Archaeorhizomyces* (15.08%), an unclassified fungus (8.46%), *Solicoccozyma* (7.39%), and an unclassified fungus belonging to the class Archaeorhizomycetes (5.84%).

A total of 11 fungal taxa were identified as preferentially as preferentially abundant in soil collected from the burnt paddocks at a depth of 0 to 2 cm, two of which, *Mortierella* and *Chaetomium*, could be resolved to the genus-level (Fig. 50). One fungal taxon was identified as preferentially abundant in soil collected from burnt paddocks at a depth of 5 to 7 cm, but this taxon could not be resolved beyond the class-level (Fig. 51).

Members of the genus *Mortierella* are widespread plant growth-promoting fungi (PGPF) that have been identified in bulk soil, the rhizosphere, and plant tissues [186]. Species within the genus *Chaetomium* are regarded as a pyrophilous fungi, which are fungi that respond positively to fire. *Chaetomium* spp. proliferate early after fire events and produce large fruiting bodies and extensive mycelia that bind and stabilise the soil, reducing erosion and increasing moisture retention [187].

Figure 50. Lefse results for analysis of the fungal communities in soil collected from burnt and unburnt paddocks at a depth of 0 to 2 cm in 2022.

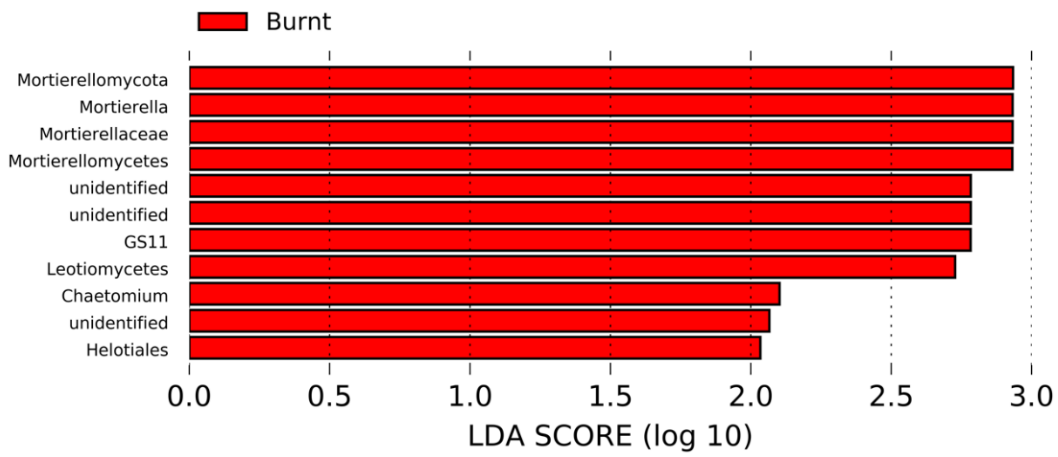
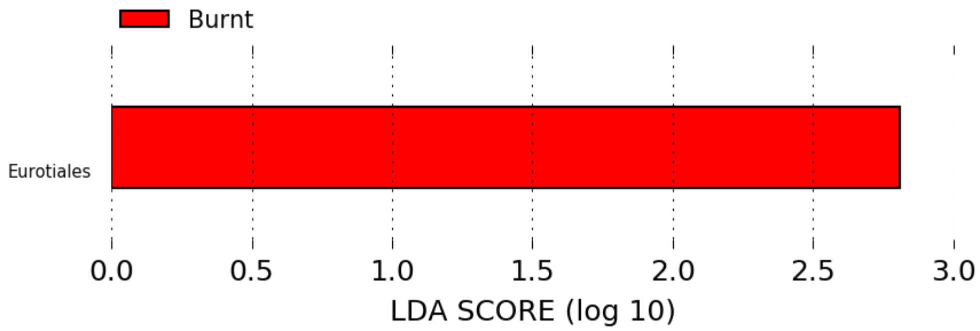


Figure 51. Lefse results for analysis of the fungal communities in soil collected from burnt and unburnt paddocks at a depth of 5 to 7 cm in 2022.



4.6.4 Summary of microbiota diversity results

The results of this study indicate that bushfire has both short- and long-term impacts on the composition of microbial communities in soil. There were significant differences in the composition of the bacterial and fungal communities present in the soil of burnt and unburnt paddocks on properties affected by bushfires in 2019/2020, and from a single property on which a controlled burn was conducted in 2022.

A range of alpha and beta diversity metrics were used to characterise and compare the diversity of the bacterial and fungal communities present in soil in the burnt and unburnt paddocks. Broadly, these metrics consider both the diversity of the communities (i.e., the number of taxonomic groups that make up the community), and the evenness of the communities (i.e., the relative abundances of each of the taxonomic groups that make up the communities). On the properties affected by the 2019/2020 bushfires, there were marked differences in the composition of the bacterial communities in the soil of burnt and unburnt paddocks, particularly at a depth of 0 to 2 cm. The diversity of the bacterial communities present in soil from the burnt paddocks was significantly higher according to most of the diversity metrics evaluated. However, the genera that were unique to the soil samples from the burnt paddocks represented a relatively small proportion of the total dataset (i.e., the dominant bacterial genera were similar in soil from the burnt and unburnt paddocks). The implications of this are unclear and warrant further investigation. Low-abundance taxa can have a disproportionately large impact on the function of a bacterial community, which could have implications for pasture growth and nutrient content.

The long-term impacts of bushfire on the fungal communities were less marked on these properties. The diversity analyses indicated that bushfire had no significant impact on the diversity of the fungal communities at either depth, but that at a depth of 0 to 2 cm the relative abundances of the dominant genera had shifted. There were no apparent differences in the composition of the fungal communities at a depth of 5 to 7 cm.

Differences in the composition of the bacterial and fungal communities between the burnt and unburnt paddocks were also apparent for soil samples collected after the planned burn. The diversity analyses revealed that exposure to fire resulted in an increase in the overall diversity of both the bacterial and fungal communities, largely low-abundance taxa, as well as some shifts in the relative abundance of the dominant genera, but that the dominant bacterial genera remained similar in soil from the burnt and unburnt paddocks.

4.6.5 Phospholipid fatty acid soil analysis (PLFA)

This report presents a condensed and detailed overview of 59 PLFAs soil tests. The data was analysed after PLFAs were extracted and quantified from different soils, which were sampled from three different sites at two soil depths (0-2 and 5-10 cm) and from two fire conditions (burnt and unburnt).

4.6.5.1 Bemboka, NSW

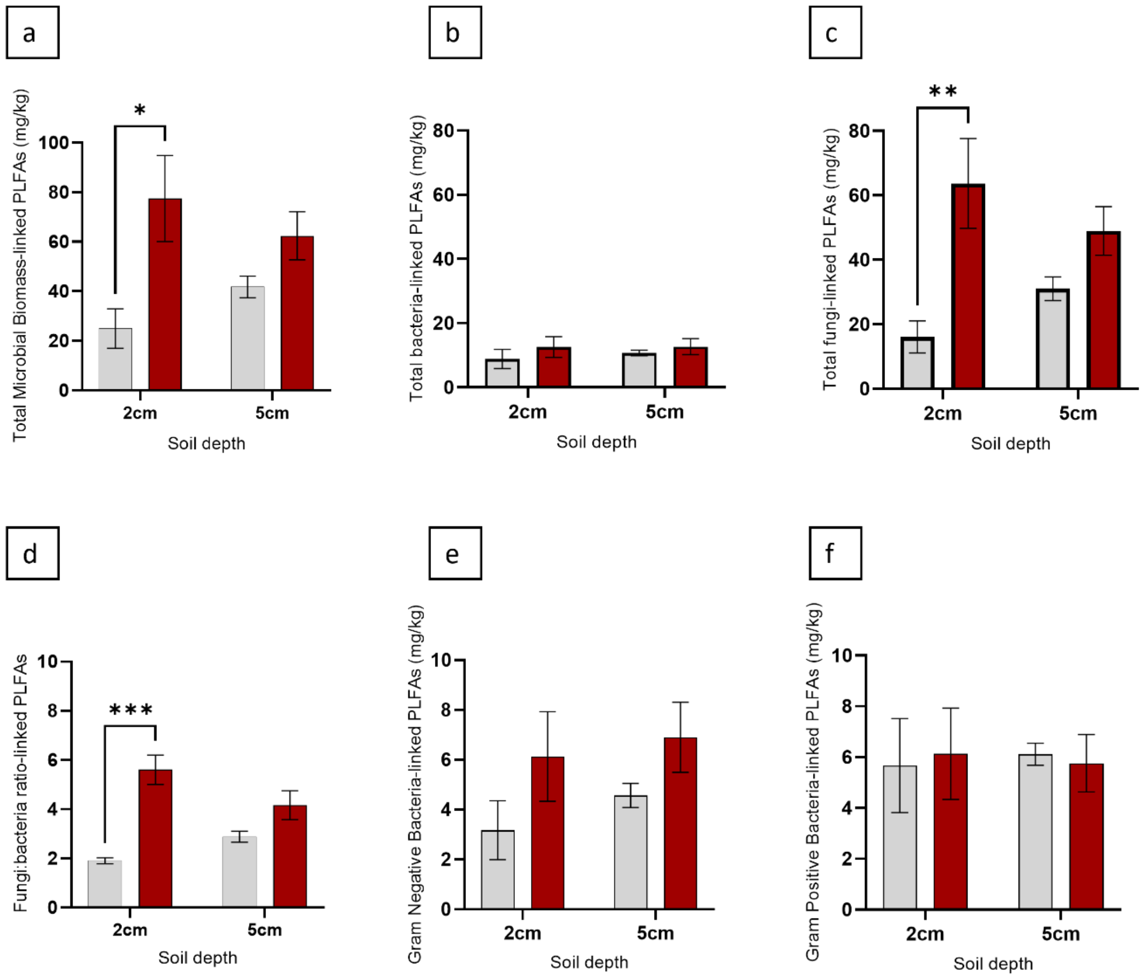
The two-way ANOVA tests showed no statistical differences for the bacteria-linked PLFAs such as Total bacteria, Gram (+) and (-), and *Pseudomonas*, either by soil depth (0-2 and 5-10 cm) or by fire condition (burnt and unburnt) (Table 17). The result indicated little or no effect of the fire on bacteria biomass in this site. However, the PLFAs-linked to total fungi, mycorrhizal fungi, and the fungal to bacteria ratio were always higher in the burnt samples at both soil depths. Due to higher levels of PLFAs-linked to fungi, the total biomass of microorganisms was greater in the soil from burnt sites at both 2 and 5 cm (Figure 51 a-i).

The PLFAs indicators for microbial diversity (Shannon diversity index) showed no differences among any of the parameters (Figure 52). However, the index was slightly higher at 5-10 cm in soil from both burnt and unburnt sites.

Table 17. Summary of the two-way ANOVA PLFAs-linked microbiology analysis for site Bemboka, NSW

Source of variation	Total microbes	Total Bacteria	Total fungi	Gram (+)	Gram (-)	Pseudo-monas	Actino-mycetes	AM fungi
Soil depth	0.9346	0.7061	0.9917	0.9804	0.0602	0.4809	0.6090	0.7497
Fire condition	0.0041	0.2887	0.0014	0.9700	0.4199	0.0316	0.7392	0.0124
Interaction	0.1641	0.7472	0.0996	0.7784	0.8139	0.7305	0.9791	0.2184
Significant?	**Yes, Burnt vs Unburnt at 2 cm**	No	**Yes Burnt vs Unburnt at 2 cm**	No	No	No	No	**Yes, Burnt vs Unburnt at 2 cm**

Figure 51. PLFA Bemboka. (a) total microbial biomass linked PLFAs, (b) total bacterial linked PLFAs, (c) total fungi linked PLFAs, (d) fungi: bacterial ratio linked PLFAs, (e) Gram negative linked PLFAs, (f) Gram positive linked PLFA's, (g) Mycorrhizal fungi-linked PLFAs (mg/kg), (h) Actinomycetes-linked PLFAs (mg/kg), (i) Pseudomonas-linked PLFAs (mg/kg). Where significant (*= P value ≤0.05, **= P value ≤0.005). Unburnt samples are grey and burnt samples are indicated in red.



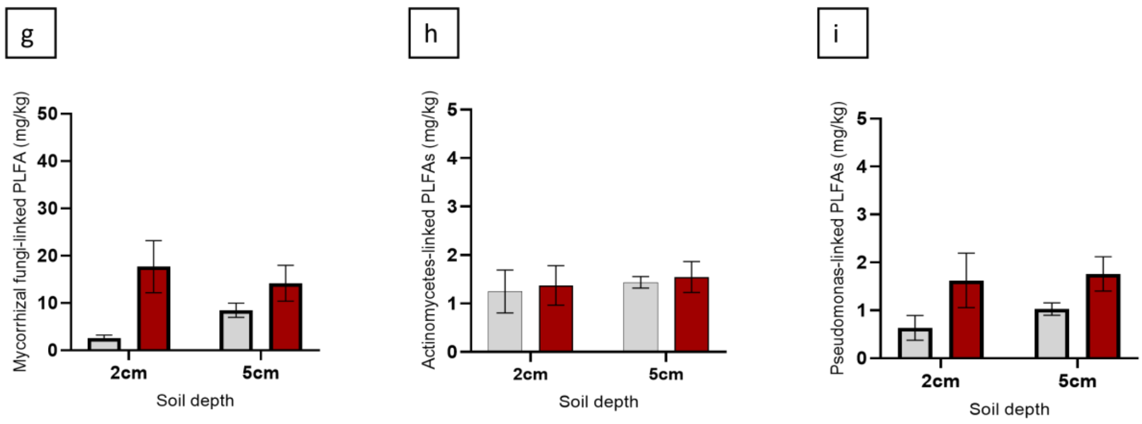
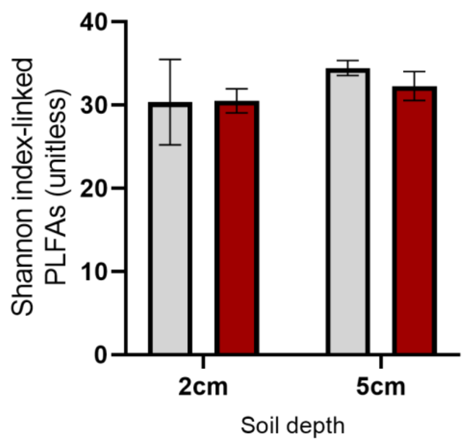


Figure 52. indicators for microbial diversity Bemboka. Where significant (*= P value ≤0.05, **= P value ≤0.005). Unburnt samples are grey and burnt samples are indicated in red.



4.6.5.2 Bombala, NSW

The two-way ANOVA analysis showed different interactions between the measured factors (Table 18). However, variation between samples was high and were not always statistically significant (Fig. 53 a-I, 54). In general, PLFAs in the soil from burnt sites were lower than those from unburnt sites, except for PLFAs-linked to total fungi and total microbial biomass, which were higher at 2 cm. However, they were not at 5 cm, indicating that fungi may not be affected by fire in the same way as bacteria because the PLFAs for total bacteria were always lower in the burnt samples (Fig. 53 a-c).

Gram positive and Gram-negative bacteria (Fig. 53 e and f), *Pseudomonas*, Actinomycetes, and mycorrhizal fungi were all affected by the fire condition with lower PLFAs found in the soils from burnt sites (Fig, 53 g-i). In addition, these microbial-linked PLFAs were slightly lower at 5-10 cm when compared to 0-2 cm depth.

The PLFAs indicator for microbial diversity (Shannon diversity index) showed no differences among any of the parameters. However, the index was slightly higher in the unburnt soil at both 2 and 5 cm (Fig. 54, Table 19).

Table 18. Summary of the two-way ANOVA PLFAs-linked microbiology analysis for site Bombala, NSW.

Source of variation	Total microbes	Total Bacteria	Total Fungi	Gram (+)	Gram (-)	Pseudo-monas	Actinos	VAM fungi
Soil depth	0.7881	0.6609	0.8946	0.5562	0.7855	0.7020	0.4525	0.8172
Fire condition	0.9898	0.0570	0.8264	0.0134	0.2349	0.2502	0.0068	0.2638
Interaction	0.3393	0.3603	0.3352	0.3179	0.4138	0.6025	0.2262	0.4809
Significant?	No	No	No	**Yes, 5cm burnt vs unburnt**	No	No	**Yes, 5cm burnt vs unburnt**	No

Figure 53. Total PLFA Bombala. (a) total microbial biomass linked PLFAs, (b) total bacterial linked PLFAs, (c) total fungi linked PLFAs, (d) fungi: bacterial ratio linked PLFAs, (e) Gram negative linked PLFAs, (f) Gram positive linked PLFA's, (g) Mycorrhizal fungi-linked PLFAs (mg/kg), (h) Actinomycetes-linked PLFAs (mg/kg), (i) Pseudomonas-linked PLFAs (mg/kg). Where significant (*= P value ≤0.05, **= P value ≤0.005). Unburnt samples are grey and burnt samples are indicated in blue.

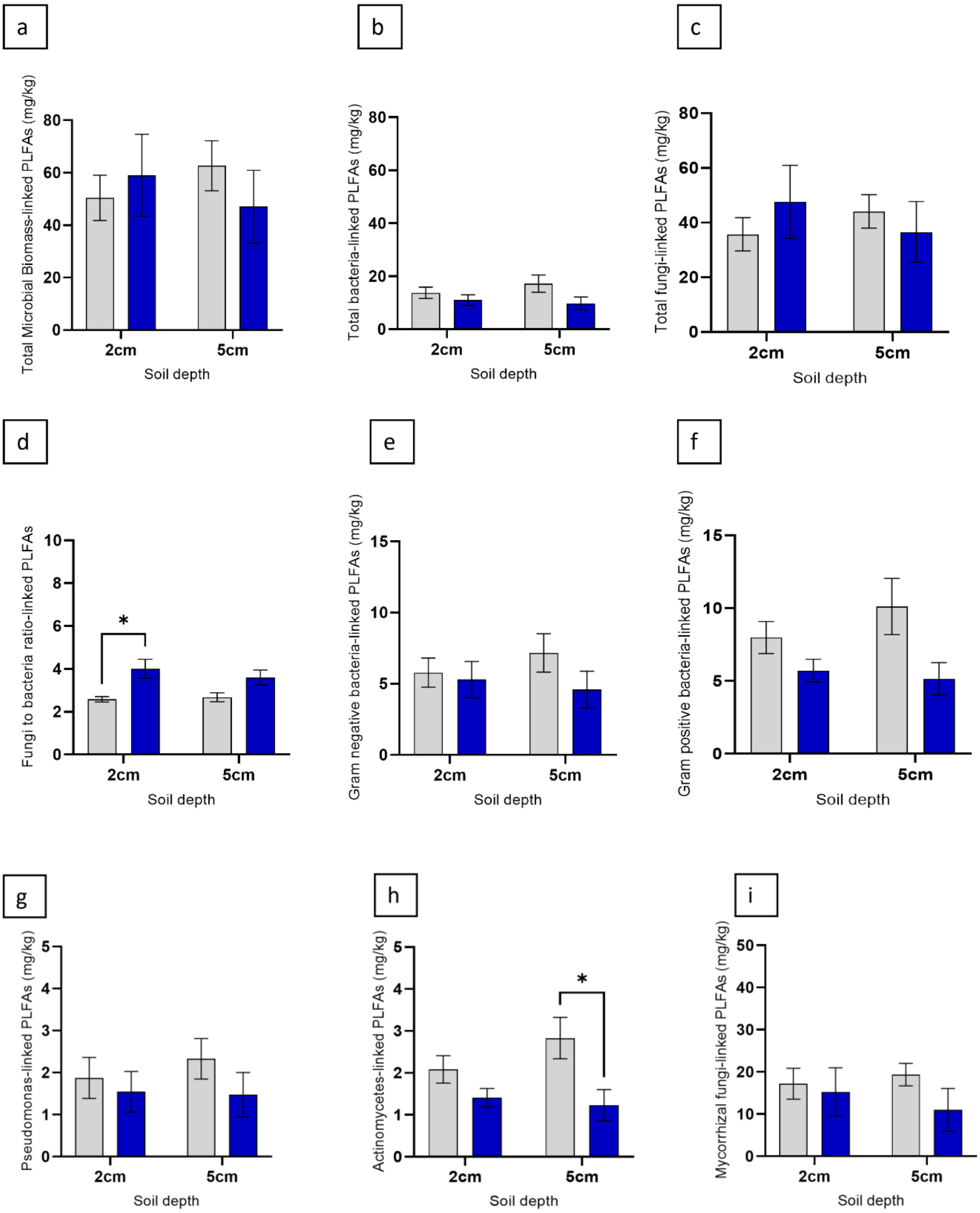
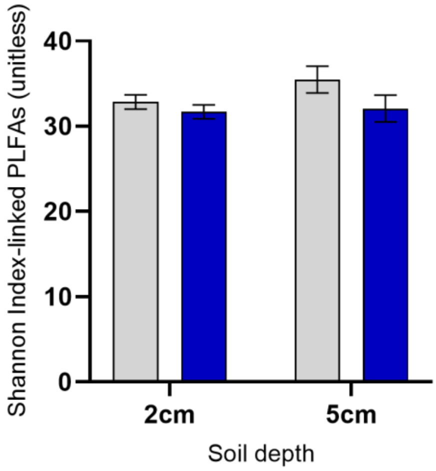


Figure 54. indicators for microbial diversity Bemboka. Where significant (*= P value ≤0.05, **= P value ≤0.005). Unburnt samples are grey and burnt samples are indicated in blue.



4.6.5.3 Moruya, NSW

The two-way ANOVA analysis showed that the microbial-linked PLFAs were always lower in the burnt soils for all the microbial groups, indicating an effect of fire (Table 19). In the specific case of mycorrhizal fungi-linked PLFAs, the fungi-linked PLFAs were only detected in the unburnt soil. No PLFAs were recovered from the burnt soil at any depth. *Pseudomonas* were also low in this site, but the lowest values were always recorded in the burnt soils with a higher effect at 5 cm. As in the other sites, variation among the samples was high and statistical differences were not always evident. In addition, in both burnt and unburnt samples, the highest levels of microbial-linked PLFAs were recorded at 5 cm compared to 2 cm (Fig. 55 a-i).

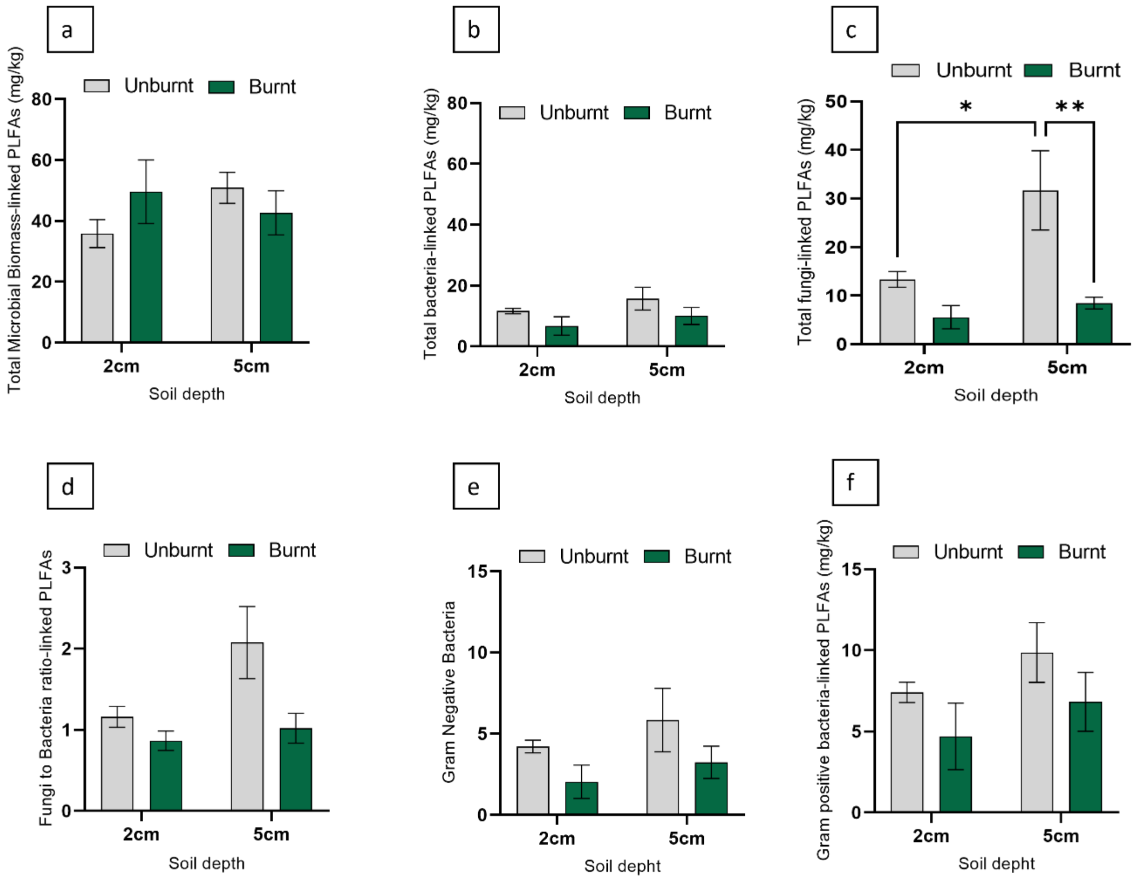
In relation to the Shannon index for microbial diversity (Fig. 56), values were higher for the unburnt soils at both 2 cm and 5 cm. However, the differences were not statistically significant even though there was a much lower diversity in the burnt soils at 2 cm (Table 29).

The fungi to bacteria ratio was also higher in the unburnt soils, with higher values at 5-10 cm, indicating more PLFAs-linked to fungi in the deeper layers, as expected. There were no obvious differences between 0-2 and 5-10 cm in the soil from the burnt site for this variable.

Table 19. A summary of the two-way ANOVA PLFAs-linked microbiology analysis for site Moruya, NSW.

Source of variation	Total microbes	Total Bacteria	Total Fungi	Gram (+)	Gram (-)	Pseudo-monas	Actinos	VAM fungi
Soil depth	0.0254	0.1939	0.0127	0.1915	0.2302	0.0115	0.1106	0.0106
Fire condition	0.0027	0.0711	0.009	0.1065	0.0517	0.0108	0.0214	0.0061
Interaction	0.1783	0.8876	0.0577	0.9248	0.8501	0.0215	0.9025	0.0106
Significant?	**Yes, 5cm burnt vs unburnt**	No	**Yes, 2cm unburnt vs 2 and 5c, burnt & unburnt**	No	No	**Yes, 5cm unburnt vs 2 & 5cm burnt & unburnt**	No	**Yes, 5cm unburnt vs 2 and 5cm burnt & unburnt**

Figure 55. Total PLFA Bombala. (a) total microbial biomass linked PLFAs, (b) total bacterial linked PLFAs, (c) total fungi linked PLFAs, (d) fungi: bacterial ratio linked PLFAs, (e) Gram negative linked PLFAs, (f) Gram positive linked PLFA's, (g) Mycorrhizal fungi-linked PLFAs (mg/kg), (h) Actinomycetes-linked PLFAs (mg/kg), (i) Pseudomonas-linked PLFAs (mg/kg). Where significant (*= P value ≤0.05, **= P value ≤0.005). Unburnt samples are grey and burnt samples are indicated in green.



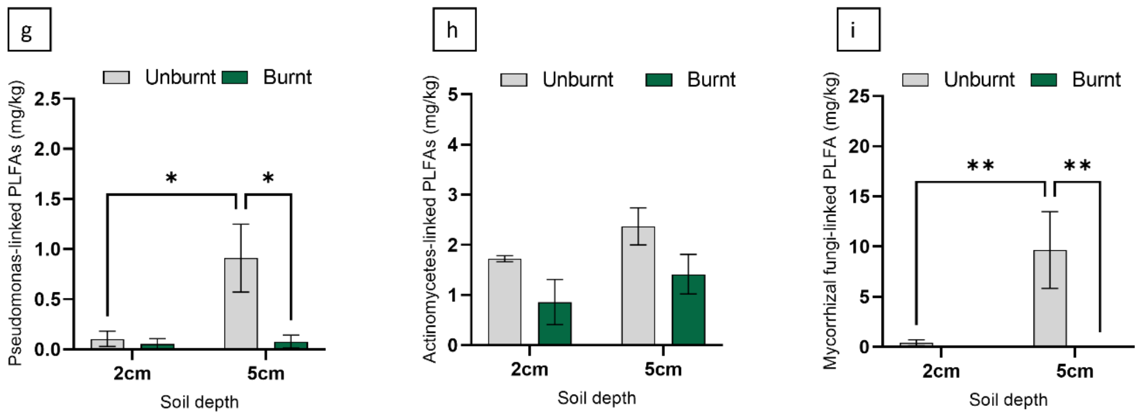
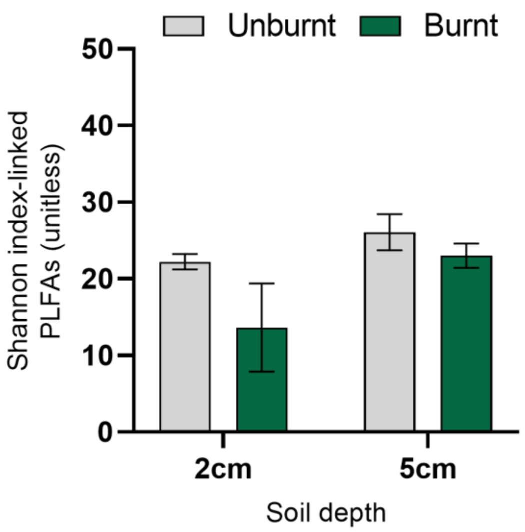


Figure 54. indicators for microbial diversity Bemboka. Where significant (*= P value ≤0.05, **= P value ≤0.005). Unburnt samples are grey and burnt samples are indicated in green.



4.6.5.4 PLFA discussion

In recent years, fire in forests and on agricultural land has been an important factor of disturbance. With more disturbance due to fire to come due to current global warming conditions, it is important to acknowledge and set up thresholds of the effect of this disturbance on soil health. The effect on soil microbiology may be a key parameter to measure due to the importance of the microbial groups on nutrient cycling. Different responses to soil microbiology have been reported depending on the intensity of the fire, the duration of burn and the soil depth at which the samples were taken. However, the responses are mostly negative in the short-term, with some parameters such as microbial community structure (PLFAs pattern) still impacted after 5 years [188].

In relation to specific microbial groups, fungi and bacteria change considerably due to changes in the carbon composition of the burned soil [189]. Fire severity can have a greater effect on fungi compared to bacteria. Our data indicated this type of response for the farm in Moruya but not for the Bemboka site, where fungi levels were significantly greater in soils from burnt sites even when collected 18 months after exposure to fire. Bacteria levels were statistically similar in the soil from burnt and unburnt sites. However, their levels were slightly higher in the unburnt soils at Bombala and Moruya. Similar results were found when analysing samples taken after a fire in Granada, Spain [190]. This study highlighted the importance of the timing of sampling and the significance of other soil measurements such as C-biomass and organic-C as additional parameters to explain the effect of fire on fungi and bacteria.

When more specific microbial groups were measured, for example, Gram positive (+) and Gram negative (-) bacteria including *Pseudomonas* and Actinomycetes, the results varied depending on the site, and were directly related to the intensity of the fire. For example, at Bombala and Moruya, the soils from unburnt sites showed higher levels of both types of bacteria, in contrast to Bombala in which soil from the burnt sites were only higher in Gram negative (-) bacteria. Gram positive (+) bacteria in soil from unburnt sites did not show any differences. This contrasts with findings in another study where PLFAs-linked to Gram positive (+) and Gram negative (-) bacteria were always higher in soils from burnt sites, which was attributed to the type of carbon present after a fire event [191].

In the case of mycorrhizal fungi, the data varied depending on the site. For example, at Moruya, the mycorrhizal fungi linked PLFAs were reduced considerably. However, the contrary was found at the Bemboka site, with no clear pattern at Bombala. In a study by Hebel et al. [192], it was found that mycorrhizal fungi were reduced in a ‘red’ soil which was severely burned, but not in a ‘black’ soil which was not as severely burned highlighting the importance of awareness not only of the soil type but also vegetation in formulating a response to fire exposure. This suggests the need for further research assessing the recovery in relation to soil characteristics, vegetation (as fuel load) as well as soil biochemistry.

5. After bushfire – key information and resources for soil and pasture recovery

In the years since the 2019/2020 bushfire event, Australian National and Local Government and external organisations have developed frameworks, pathways, and sources of information for multiple aspects of support and recovery for agriculture after bushfire including the following:

MLA bushfire recovery links: *https://www.mla.com.au/research-and-development/dealing-with-natural-disasters/bushfire-recovery/*

National Landcare Program: https://www.agriculture.gov.au/agriculture-land/farm-food-drought/natural-resources/landcare/national-landcare-program/landcare-facilitators

National soil strategy: https://www.agriculture.gov.au/agriculture-land/farm-food-drought/natural-resources/soils

Western Australia Department of Agriculture: https://www.agric.wa.gov.au/fire/farm-recovery-after-fire-%E2%80%93-western-australia

Victoria Department of Agriculture: https://agriculture.vic.gov.au/farm-management/emergency-management/bushfires

Landscape South Australia (Hills and Fleurieu): https://www.landscape.sa.gov.au/hf/our-priorities/land/fire-recovery/cudlee-creek-fire-recovery/land-livestock-pasture-care-after-fire

Effects of bushfires on fresh produce safety: https://fpsc-anz.com/wp-content/uploads/2021/12/FPSC-FactSheet-Bushfires-and-Produce-Safety.pdf

NSW DPI Pasture recovery advice:
https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0019/320626/Pasture-recovery-after-bushfires.pdf

Australian weed strategy:
https://www.agriculture.gov.au/sites/default/files/sitecollectiondocuments/pests-diseases-weeds/consultation/aws-final.pdf

A review of the potential impacts of different fire regimes on soil erosion and sedimentation:
https://www.environment.nsw.gov.au/~/media/6676FDEC72B546F5B849301424B29835.ashx

WWF report on the impacts of the 2019/2020 bushfires on food and agriculture in Australia:
https://www.wwf.org.au/ArticleDocuments/353/WWF%20Report-Fire%20on%20the%20Farm_converted.pdf.aspx

While these are extensive and informative, a consultation with primary producers revealed gaps in on-line resources currently available and several common queries and comments emerged. Based upon this feedback, the toolkit addresses these queries in a Q&A format and provides links to appropriate tools and services including a glossary of key terms, evaluation of the effects of fire, soil condition after bushfire, and plant recovery after bushfire. It is the recommendation of this group that a web-based toolkit be developed providing easy access to updated national and regional information.

Background

Fire is a strong evolutionary force that has contributed to shaping the landscape upon which we live. It has influenced human evolution and society in terms of geographic settlement, food procurement, and the development of agriculture and technology. In Australia, the indigenous peoples have long utilised the tradition of anthropogenic grassland burning to support and shape their surroundings [23-26]. Many ecosystems and native plant and animal species are well adapted to local fire regimes, for example, the germination of several plant species (pyrophytes) have evolved to be contingent upon exposure to fire [27, 28].

Shifts in fire regimes can test the resilience of plant and animal species and ecosystems. For human communities, changing fire regimes represent a destructive force responsible for loss of life and accumulating high socio-economic losses. Australia is one of the most fire prone regions in the world with annual variations in fire seasons across the continent. In 2007, the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the Australian Bureau of Meteorology projected changes to temperature, evaporation, and annual rainfall over the next 30 years foreseeing changes in frequency, intensity, and duration of extreme weather and related events including the potential for extended and more severe fire seasons [33]. In 2019-2020, this prediction was borne out by a catastrophic fire season across regions of Eastern Australia. Adverse conditions were exacerbated with multiple years of drought. The first bushfires were triggered by the arrival of hot dry windy weather conditions and ignitions in early September 2019 in northern NSW and spread south as extreme 'mega-fire' weather conditions continued throughout spring and early summer [34, 35]. Between September 2019 and January 2020, an estimated 7.38 million hectares were burned, including 0.53 million hectares of agricultural land. These bushfires are estimated to have killed 1.25 billion animals (included wildlife, domesticated animals, and livestock) and, although it is difficult to estimate the exact numbers of livestock impacted, it is estimated to have significantly affected 8.6 million sheep and 2.3 million cattle in NSW and Victoria [36].

After bushfire, initial attention centres around aspects that are essential for maintaining immediate health and welfare of human communities, livestock, and wildlife populations. This primarily involves aboveground activities such as clearing of burnt matter and rebuilding infrastructure. In terms of recovery of agricultural systems, what happens below the surface (i.e., to the soils), often determines the level of successful recovery of pastures after fire.

Pasture supporting grasslands occupy over 30% of the global landmass and forms the backbone of the agricultural food web performing a vital role in global food supply [46]. Pasture plants are a significant contributor to regulation of atmospheric carbon concentration through sequestration of soil organic carbon and the incorporation of photosynthetic pathways in grass species to incorporate carbon dioxide into three- (C3 grasses) or four-carbon (C4 grasses) compounds [47].

Bushfire events impacting agricultural grasslands and pastures often result in rapidly moving fires with low intensities in comparison to tree-dense land with high fuel loads. However, exposure to bushfire may change the botanical composition of pastures and grasslands and the structure and function of soil. Both impacts may lead to a reduced plant growth and carrying capacity of the pasture in the following season or seasons.

After fire, soils are often vulnerable to erosion since aboveground organic material is lost and exposure to heat and accumulation of ash may result in alteration of soil hydrophobicity, pH, and nutrient availability. Additionally, fire may alter the balance of microbial communities in soil that support resilience in pasture production, potentially impacting nutrient and organic matter cycling functions.

Evidence from past and recent bushfire events in grasslands have shown that low severity fires may exert beneficial impacts on soil properties resulting in improved vegetation density [29]. Examples of beneficial fires are prescribed or planned fires done during autumn and winter seasons for landscape management [30]. Beneficial consequences of controlled fires to soil and plant productivity may include production of ash rich in carbon, an increase of soil organic matter, pH, electrical conductivity, and extractable cations such as calcium, magnesium, sodium, and some forms of nitrogen such as ammonia, important for regrowth of vegetation [30, 31].

Agricultural lands have the capacity to recover from exposure to bushfires. However, recently burned areas need to be carefully managed to support rapid recovery for optimal pasture growth. Successful recovery is reliant on multiple factors including having an insight of the conditions during the fire (e.g., fire intensity), the area affected by fire (e.g., soil type and fertility, subsoil moisture availability, pasture type, farming system), and past and future seasonal conditions.

Glossary of key terms

In Australia, the term ***bushfire*** describes any vegetation fire while globally the term ***wildfire*** is commonly utilised to describe any unplanned vegetation fire and encompasses grass fires, forest fires, and scrub fires [60].

The primary drivers that determine the potential for and the intensity of a bushfire are: ***Ignition*** (source of fire, either by human action or from natural sources such as lightning), ***biomass/fuel abundance*** or ***fuel load*** (relative measure of the fuel based on the arrangement, structure, composition, proportion of dead material, and thickness of the fuel elements), ***fuel dryness*** (the moisture content of fuel and whether it can support combustion or not) and; ***appropriate weather conditions*** for fire spread (hot, dry, and windy) [61].

Fire intensity or how hot a fire burns represents the energy released over the duration of the fire measured in kilowatts per metre (kW/m) of fire front [65]. It may also be defined as the rate of heat energy released per unit time/per unit of the length of the fire line [66]. Fire intensity is generally classified according to three sub-categories (mild to cool burn, medium intensity or hot burns, and extreme intensity or very hot burn). It should be noted that the categorisation is largely reliant on data sourced from forest fires and the average temperature range for grassfires may generally be within the mild to cool burn range.

Fire severity or ***burn severity*** is used to describe the alteration of the surface and sub-soil properties of soil caused by heat from fire and, although there is some lack of clarity regarding a concise definition, the term is increasingly being utilised to quantify the response of the ecosystem to fire.

For further information, a useful glossary of fire related terms prepared by the Australasian Fire Authorities Council may be sourced here: https://www.afac.com.au/docs/default-source/doctrine/bushfire-terminology.pdf and here: https://www.forestry.org.au/fire-terminology

Q&A

Question 1. How do I assess the damage to my soil in the short- and long-term?

Soils are the foundation of every farming enterprise and underpin the capacity of managed ecosystems to support pasture growth and, ultimately, livestock production. Soil health is a dynamic relationship encompassing minerals, organic matter, air, water and living macro- and microorganisms that interact with plants to provide nutrients, control pests and other benefits, such as the capacity to increase plant nitrogen. Soil conditions impact on plant regeneration and growth, translating into variations in nutritive value of livestock feed.

The following video links are an informative first step in gaining insight to soil responses to bushfire and suggested management: https://www.youtube.com/watch?v=ONv-PFK9qwE and https://youtu.be/b3ACS_pJS5c. There are several organisations that provide support, training and information regarding Australian soils including: https://www.soilscienceaustralia.org.au/ (Soil Science Australia) who have generated a useful factsheet on the management of soils after bushfire:

(https://www.soilscienceaustralia.org.au/wp-content/uploads/2020/01/202003-FACT-SHEET-Soil-fire-impacts-and-management.pdf), Soil and Landscape Grid of Australia (https://esoil.io/TERNLandscapes/Public/Pages/SLGA/), Australian Soil Resource Information System (https://www.asris.csiro.au/), and CSIRO SoilMapp (https://www.csiro.au/en/research/technology-space/it/SoilMapp).

Bushfires can increase the capacity of soil to repel water (***soil hydrophobicity***). This may lead to reduction of water availability to plants and prevent optimal plant recovery but, depending on the burn intensity and severity, it may lead to risk of erosion. The extent of fire-induced change is influenced by ***burn intensity*** (Table 1) and the subsequent soil temperatures. At low- to mid-fire intensity, the situation most common for burnt pasture or grassland, changes in soil water repellency are not significant. In other types of farm vegetation, increasing fire intensity may result in increases in soil water repellency (e.g., nearby bushland or remnant patches of trees and shrubs). ***Burn severity*** (Table 2) has a primary role in ***soil erosion*** and deep burning (complete burn of all vegetation) in combination with increased soil water repellence results in increased risk of soil erosion.

Use the Tables 1 and 2 to assess the paddocks damage on your property post burn to accurately determine burn intensity and burn severity.

To support plant growth, soils should contain a steady supply of ***nutrients*** such as ***organic carbon***, ***macronutrients***, and ***micronutrients***. Soil organic matter forms through decomposition of plant and animal material and leads to the formation of soil organic carbon via mineralisation. The quantity of belowground organic matter in soil varies widely dependant on factors such as vegetation type, temperature, and moisture. Macronutrients supplied by the soil (nitrogen, phosphorus, potassium, sulphur, magnesium, and calcium) are essential for the formation of crucial cellular components in the growth of plants such as proteins and nucleic acids. Micronutrients contribute as cofactors of plant enzyme activity and are also known as trace elements. Within soil, the essential micronutrients and are molybdenum, copper, zinc, manganese, iron, nickel, boron and chlorine [98]. Several factors contribute to the capacity of plants to take up nutrients from soil and are therefore important for propagation of soil fertility. These include soil properties (pH, texture, groups), soil microbial community (influence organic carbon and organic decomposition), soil organic matter (regenerative source of nutrients), and soil hydraulic properties. Exposure to fire leads to a change in the abundance, form and distribution of available and total nutrients present in soil [60, 67]. Nutrients may be lost in gaseous form (volatilisation, gasification) or dispersed in particulate form in smoke during fire [99].

Table 1. Characterisation of burn/fire intensity.

Burn intensity	Maximum soil surface temperature (C°)	Description in pasture/grassland pasture setting
Mild or *cool-moderate burn*	250	Cool- moderate burns occur at sites of minimal dry grass cover and result in patchy growth and survival of some seed, perennial grasses, and clovers.
Medium intensity or *hot burn*	400	Hot burns occur at sites of with accumulation of heavy plant cover such as lightly grazed pasture and results in bare/charred soil and minimal remaining plant matter.
Extreme intensity or *very hot burn*	900	Very hot burns occur at sites with heavy fuel loads (soils with thick roots, adjacent tree cover, hay bales). The soil is sterilised as all plant matter, seeds and top organic matter within the soil is burnt.

Table 2. Characterisation of burn severity.

Burn severity	Description
Unburnt	Plant parts are green and undamaged, no direct effect from heat.
Scorched	Unburnt but plants exhibit leaf loss from radiated heat.
Light	Trees scorched but leaves remain; surface litter, mosses and grasses charred; soil organic layer mostly intact and charring limited to shallow depth (mm).
Moderate or severe surface burn	Canopy cover consumed, some charring but few leaves remain in trees; all understorey shrubs and larger plants charred or consumed; soil organic layer largely consumed.
Deep burning	Canopy trees killed; surface litter and soil organic layer largely consumed; white ash deposition and charred organic matter to depth of several centimetres.

Before considering any costly management strategies it is advisable to undertake soil testing to fully assess the scope of damage in relation to soil nutrient content and related factors including pH, organic carbon, macronutrients, and micronutrients, and if possible, soil microbial communities. This should be done as soon as practical using replicate samples from paddocks burnt during bushfire and repeated over time when necessary. There are numerous soil testing services available that can provide this service in your local area and it is advisable to seek recommendations from local agricultural agencies or access the following link to identify a certified professional soil scientists https://www.soilscienceaustralia.org.au/cpss/.

Question 2. What do I need to do in the short and the long term to improve the quality of my soil?

In the short term it is advisable to gain an accurate understanding of the status of your soil using the methods outlined in Answer 1. Once this has been done it is possible to determine the next steps in the short and long term.

Previous research indicates that mid to high severity and/or high intensity bushfires result detrimental effects on soil health in terms of nutrient availability while a cool – moderate burn may result in plant regrowth as normal within the next growth season (depending upon seasonal variability, e.g., rainfall). There is not a one-size-fits-all model of post-fire intervention since the variability that exists across Australia makes it impossible to formulate such a plan.

Currently, several intervention strategies are considered in post-fire settings and mulching, seeding or resting are most applicable for grazing systems.

Mulching: A major consequence of high intensity bushfire is erosion of burnt soils resulting in loss of macro- and micronutrients. Erosion will be more extreme on sloping areas of land. Application of agricultural straw (mulching), other vegetation residues or biological geotextiles is a common post-fire management strategy used to limit runoff and erosion at severely burnt sites [134, 135] and is intended for implementation immediately after the burn event. Studies have explored the short- and long-term effects of mulching on agricultural lands; the practice protects soils from raindrop effects and aids in limiting erosion [136], has beneficial effects on soil water infiltration and regulating temperature fluctuations [137], enhances the activity of some earthworm species [138], and may increase water infiltration and soil quality over time, in part due to the access to organic matter and nutrients [135]. It should be noted that most of the studies assessing the efficacy of mulching have been done on non-grazed land and there are key knowledge gaps in the efficacy and feasibility of mulching in the agricultural context and the economic feasibility of mulching applications in the agricultural context are not readily available and may limit functionality. Material bought onsite may also pose the risk of introduction of weed seeds, insect pests, and pathogens so sourcing mulch from reputable sources is highly recommended.

Seeding: Seeding is a process of rehabilitating land with the application of seeds for desired plant species. It has long been a recommended practice in United States land management following exposure to wildfire [60, 144-146]. The practice is intended to reduce vigorous growth of invasive species, reduce soil erosion where fire has eliminated the existing seedbank, and encourage the growth of desirable species.

Resting: Current guidelines in the United States recommends grazed land be rested for at least two seasons following a wildfire event [146] to allow optimal time for recovery of perennial plants and reestablishment of seeded species. This is a well-established recovery practice based upon the understanding that exposure to fire reduces plant regrowth, productivity, and diversity. However, recent data suggests that with growing understanding of the response of soil and vegetation to fire processes, further information may be required, and it may be necessary to incorporate consideration of the burn intensity and burn severity alongside post-fire conditions (e.g., rainfall, soil health and the density and identity of the plant regrowth). This may result in revision of required recovery time and an adaptation of management [148], particularly in an Australian context and may be site specific. For example, if the site is on a slope or particularly exposed and therefore prone to erosion it should be seeded with pasture species or some other vegetative biomass to prevent post-fire erosion should there be a rain or wind event. Mulching is common in the United States but is not recommended due to the potential introduction of seed of unwanted weed species.

Question 3. I have noticed a lot of weeds in my burnt paddocks. Many were weeds previously unseen in my area and I could not easily identify them.

Unplanned fires create an opportunity for competitive weed species to dominate in burnt sites and the movement of equipment, machinery, stock, and people associated with fire response and post-fire recovery efforts can facilitate the inadvertent spread of weeds. Weed management, regulated under the *Biosecurity Act 2015* (NSW), is an important part of the process of restoring fire affected communities.

For weeds that have not been seen previously on properties it is important to remember that they may already be on-site in the soil seed bank, be transported by animal, insect, and human activities, or be transported passively via wind or water. Seeds of weed species are not generally long-lived as they fit a functional group of fast-growing plants that invest in lots of small short-lived seed for maximum dispersal but there are always exceptions. Grass seeds tend to be less persistent than seeds of broadleaf weeds. Seeds of many native species can be long-lived and, although they may not have been seen in pastures for many years previously, may germinate after fire and become locally abundant. For example, seeds of some species of *Acacia* can live (remain viable) for up to 50 years. As pasture species recover or are reintroduced through seeding, many native and weed species alike cannot compete for space, light, and nutrients and will eventually disappear.

It should also be remembered that not all plants will be killed by bushfire, many have underground tubers, rootstocks, or runners that can be protected from heat by a layer of soil. Although the aboveground matter might be burnt during fire, many grasses and perennial pasture species can recover from underground parts. This plant recovery response is a common sight in burnt bushland with new green shoots appearing on eucalypt trees, grass trees and understorey species within weeks of the bushfire, particularly if rain follows soon after an area is burnt.

For identification of weeds, useful links include: https://weeds.org.au/, Conservation Management Networks plant database: https://begavalley.nsw.gov.au/environment/managing-weeds-after-bushfire, factsheets: https://www.landcarevic.org.au/assets/Uploads/Landcare-after-the-fires-Weed-Control.pdf, https://cdn.environment.sa.gov.au/landscape/docs/hf/weed-management-techniques.pdf and the mobile phone enabled applications, iNaturalist: (https://inaturalist.ala.org.au) and PictureThis: (https://www.picturethisai.com). The following link describes practical methods for assessing the density of weed regrowth https://www.agric.wa.gov.au/grains-research-development/assessing-weed-population-density .

Question 4. What impact does bushfire have on plant quality and quantity?

Herbaceous vegetation such as perennial grasses are generally considered to be highly resilient to regrowth following fire. However, this resilience relies on a host of ecosystem factors including condition of biomass of vegetation, soil fertility, environmental conditions (e.g., rain, temperature, evaporation), and access to seeds from which new plants can germinate.

Grasses are some of the most resilient plants on the planet, resulting in their predominance across the biosphere and they are a vital factor in global food security as a rich source of fodder for farmed livestock [45]. Unlike many woody plants, grasses have evolved to rapidly regenerate following adverse events such as droughts, floods and fires [125].

Useful links reviewing pasture crops and their respective responses to bushfire can be found here: https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0019/320626/Pasture-recovery-after-bushfires.pdf , and here https://www.agric.wa.gov.au/fire/pasture-recovery-after-fire

As describe earlier, there is a seedbank within the soil that can support passive restoration of pasture and other plants following adverse events. However, the seed bank is influenced by what happens aboveground and bushfire may damage the germination potential of stored seed. High fire severities affect seed abundance in the soil, particularly when high temperatures are combined with prolonged periods of contact with heat. Normally, there is a reduction in seed germination rate with increasing soil temperature, and at 300°C, most of the seeds are killed. Low intensity controlled fires are an important land management tool and fulfil crucial roles such as maintaining plant densities and contributing to the nutrient profile of soils through generation of carbon-rich ash [103]. Increasing prevalence of high intensity and high severity fires exert potentially harmful short- and long-term consequences to soil and this in turn may impact the capacity of soil to nurture seeds and support growth of nutrient-rich pasture for livestock consumption [17, 45, 59].

6. Key findings

- There is limited capacity to report on the immediate and interrelated effects of bushfires on soil, pasture, and the microbiome due to significant issues:
  - Collection of biological samples (soil, plant) was not commenced until 18 months after bushfires due to delays in finance and contract approvals.
  - Post-fire but pre-sampling there was repeat exposure of all properties to abnormally high rainfall events.
  - Lack of accessibility limited planned repeat sampling due to persistent flood events and a global pandemic. This resulted in only two major sampling events at bushfire exposed properties (June 2021 and June 2022).

- Use of satellite imagery and comparison with long range data shows:
  - The effects of the bushfire are relatively minor in relation to biomass following multi-year drought however there is evidence of a rebound in biomass across all properties.
  - The results must be considered in the context of three sets of (ab)normal natural conditions, i.e., multi-year drought, megafire and flood events. Despite this, results are indicative of relatively low level of productivity from pasture – a concern for livestock industries.

- Use of satellite imagery is an important and useful method for gaining a broader overview of property status and puts into context a range of factors including biomass estimations however satellite imagery needs to be matched with biological sampling as it is susceptible to geographic conditions (cloud cover, tree cover).

- Biochemical analysis of soil samples collected in bushfire exposed and unexposed paddocks as well as soils exposed to a planned burn has revealed persistent variation in extractable phosphorus (P), nitrate (NO_3^+), and available nitrogen (N) indicative of a soil with improved nutrient content for pasture growth in severely burnt pastures in comparison to unburnt sites. This finding suggests a positive aspect to bushfire exposure in the context of grassland fires however it must be stressed that this finding may be confounded by properties closely adjoined with forests since the predicted temperatures of these soils may be higher and this present study was not able to compare the effects of shade cover/tree proximity to fire affected soil.

- Nutritional analyses of plant tissue indicate that for most sites, pastures were of relatively low nutritive value for livestock. The effects of fire were small and not unequivocal, with very large variability observed within and among sampling sites (farms).

- Exposure to fire has no apparent detrimental effect on seed germination in the short term (six months post burn) as measured in the planned burn site and the long term (eighteen and thirty-months post bushfire event).

- Verbal reports from all the producers in this study and biological evidence in the plant matter growth in the seed bank analysis from soils collected 18 months post bushfire suggest that an unexpected issue faced by producers post bushfire was rapid and vigorous overgrowth of previously unseen weed species. Producer feedback and consultation with experts suggests the reasons may be multifactorial but include biosafety issues (transferal of seeds) brought about by e.g. high traffic of emergency and support vehicles post fire event, high winds because of the fire event transferring native and other seeds with greater resilience and lower nutrient requirements in comparison to the desired livestock fodder plant species. Tools/links for monitoring and addressing this issue are suggested in Section 5.

- Analysis of the microbiota (bacteria) and mycobiota (fungi) in soil collected from planned burn at six months post fire event, and bushfire exposed/unexposed soils at 18- and 30-months post fire exposure, found no consistent differences between burnt and unburnt soils at the community-level; however, there was evidence of a difference between proportions of lesser-known taxa that were more abundant in unburnt paddocks, which may have implications for soil quality and plant growth. For example, several taxa known to support plant growth were less abundant in the burnt paddocks than in unburnt paddocks. However, there was also evidence of a greater abundance of fire-resilient (pyrophilous) taxa within burn paddocks. These taxa thrive in a post-fire environment and increase nutrient availability, enhancing plant regeneration.

- Phospholipid fatty acid soil analysis of samples sourced from bushfire exposed properties eighteen months post fire the fire event show variation between properties for the total volume of fungi and bacteria measured in the soils. Analysis of the volumes of more specific microbial groups (Gram-positive and Gram-negative bacteria), show variation in response correlated to fire intensity with a greater volume of bacteria groups present in the unburnt soils. The data suggests that soil type may play a role in bacterial and fungal responses to fire exposure.

- In the years since the 2019/2020 bushfire event, Australian National and Local Government agencies and external organisations have developed frameworks, pathways, and sources of information for multiple aspects of support and recovery for agriculture after bushfire however consultation with primary producers revealed gaps in on-line resources currently available and several common queries and comments emerged. Based upon this feedback, the toolkit/recommendations outlined in section 5 addresses these queries in a Q&A format and provides links to appropriate tools and services including a glossary of key terms, evaluation of the effects of fire, soil condition after bushfire, and plant recovery after bushfire. It is the recommendation of this group that a web-based toolkit be developed providing easy access to updated national and regional information.

7. Future research and recommendations

- Ensure funds and procedures are in place in case of future bushfire events to allow for accurate and rapid assessment of biological variables soon after the bushfire event. This might be achieved by putting in place a repository of collection kits that could be sent to producers soon after the bushfire event with easy-to-follow instructions. Collection of soil and plant matter soon after the event is essential to accurately gain an understanding of the consequence of bushfire to the agricultural process.

- While previous research has shown that satellite imagery is an important and useful method for gaining a broader overview of property status, further extensive research is required to assess value and specificity of the method in relation to agricultural land and in as a complement of biological sampling. This future research should include.
    - Expansion of sampling conditions
    - Establishing a baseline/database of pasture quality across the varied geographic areas/conditions within Australia
    - Collate database of all past trials e.g., estimates of biomass in disparate regions. There is much unpublished evidence that is held within government and other reports that would be vital to build a comprehensive database for future referral and will allow for the development of modelling algorithms/programmes.

- There is a paucity in published data relevant to soil biochemistry post bushfire in Australian agriculture settings. Development of a repository of such data that included variables such as soil type, and microbial and nutrient content variations across the Australian sub-continent and across varying times of the year would allow for improved insight.
    - Section 5 provides information and links to soil assessment tools, it is recommended that an easy to access website is commissioned by the MLA providing information on soil collection methods, sites to access collection tools/kits and access to baseline data for comparison. The proposed website could serve as a repository for primary producer submitted soil sampling data and over time, such a resource would be invaluable.

- Post bushfire, primary producers should put in place weed management systems and maintain vigilance to ensure that new invasive and possibly damaging (poisonous) species do not gain a foothold in the fire affected paddocks. Section 5 provided links to methods for assessment of weed density and plant identification tools.

- Although there are several excellent online and mobile phone application-based weed/plant application tools (see Section 5), a comprehensive and easily accessible compendium of weeds showing images of weeds at multiple stages of growth and highlighting at-risk plants (poisonous to livestock) and suggesting management methods to enable producers to rapidly identify and eradicate weed matter would be useful.

- While the results of this study have provided insights to the long term (18 to 30 months) consequence of bushfire to microbiota within the soil of cattle supporting paddocks, there remains a paucity in data related with the immediate and long-term effects of bushfire on soil microbiota and an understanding of the potential benefits of adaptation of soil microbiota to improve nutrient availability for the optimised support of pasture growth.
    - Conduct meta-analysis of past data and carry out collections of soils across the Australian agricultural range with varying productivity/soil types and other important variables to establish baselines.
    - Introduce microbiota testing as an annual test alongside soil nutrient value testing and create a comprehensive accessible database to allow for future modelling and management decisions.

- The literature review identified key knowledge gaps in the efficacy and feasibility of mulching in the agricultural context and in particular, the economic feasibility of mulching applications in the

Australian agricultural context are not readily available and may limit functionality. A comprehensive study of the efficacy of mulching in bushfire recovery through use of planned burn simulations may provide producers with increased confidence.

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