Wildfire impact: natural experiment reveals differential short-term changes in soil microbial communities

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Highlights

- Natural experiment compared burnt vs unburnt sites to determine wildfire impacts
- Contrasting effects in native woodland vs managed pasture soils
- Soil NH_4 increased post-fire in woodland soil whilst NO_3 increased in pasture soil
- Rapid change with greater diversity in woodland bacterial community composition

Abstract

A wildfire which overran a sensor network site provided an opportunity (a natural experiment) to monitor short-term post-fire impacts (immediate and up to three months post-fire) in remnant eucalypt woodland and managed pasture plots. The magnitude of fire-induced changes in soil properties and soil microbial communities was determined by comparing (1) variation in fire-adapted eucalypt woodland *vs.* pasture grassland at the burnt site; (2) variation at the burnt woodland-pasture sites with variation at two unburnt woodland-pasture sites in the same locality; and (3) temporal variation pre- and post-fire. In the eucalypt woodland, soil ammonium, pH and ROC content increased post-fire, while in the pasture soil, soil nitrate increased post-fire and became the dominant soluble N pool. However, apart from distinct changes in N pools, the magnitude of change in most soil properties was small when compared to the unburnt sites. At the burnt site, bacterial and fungal community structure showed significant temporal shifts between pre- and post-fire periods which were associated with changes in soil nutrients, especially N pools. In contrast, microbial communities at the unburnt sites showed little temporal change over the same period. Bacterial community composition at the burnt site also changed dramatically post-fire in terms of abundance and diversity, with positive impacts on abundance of phyla such as Actinobacteria, Proteobacteria and Firmicutes. Large and rapid changes in soil bacterial community composition occurred in the fire-adapted woodland plot compared to the pasture soil, which may be a reflection of differences in vegetation composition and fuel loading. Given the rapid yet differential response in contrasting land uses, identification of key soil bacterial groups may be useful in assessing recovery of fire-adapted ecosystems, especially as wildfire frequency is predicted to increase with global climate change.

Keywords:

Environmental disturbance; bacteria; fungi; eucalypt; Australia; fire-adapted ecosystem;

1. Introduction

Wildfires are notoriously unpredictable disturbances. However, fire is an important driver of ecosystem function, vegetation dynamics and nutrient cycling. The magnitude of fire impacts is determined by the interaction between the affected ecosystem, climate and the fire regime. Fire regimes are characterised by interactions between key components such as fire intensity, frequency, size, seasonality, type and severity (Flannigan et al., 2009). There has been considerable interest in understanding belowground fire impacts, especially on soil microbial communities where fire has direct and indirect impacts (Hart et al., 2005; Muñoz-Rojas et al., 2016; Neary et al., 1999). Direct effects result from heat transfer from the soil surface to lower depths, whereas indirect fire impacts are mediated by above- and below-ground interactions between plants and the soil environment. Soil heating affects soil microbial communities through cell death, causing reductions in biomass and diversity (Neary 1999; Dooley and Treseder, 2012). In contrast, greater fire-induced impacts on soil microbial communities, in terms of spatial extent and longevity, are mediated through changes to soil organic matter quality, soil moisture retention, soil pH and buffering capacity and changes in nutrient availability. Fire also impacts rhizodeposition, plant litter accumulation, and ash and charcoal content which alter nutrient cycling and soil microbial communities (Cobo-Díaz et al., 2015). The application of molecular techniques to post-fire studies is advancing our understanding of fire-induced changes on microbial communities, especially with detailed identification of the affected communities (Ferrenberg et al., 2013; Goberna et al., 2012; Mikita-Barbato et al., 2015); however, further work is required into immediate post-fire impacts (i.e. days since fire) and time to recovery.

Fire regimes in fire-adapted biomes have led to the evolution of plant fire survival traits (Bond and Keeley, 2005). These functional traits facilitate rapid (days to weeks) post-fire regeneration, and

include post-fire basal or epicormic resprouting (e.g. eucalypts) (Clarke et al., 2015; Gill, 1975); underground storage organs (e.g. acacia); and heat or smoke-stimulated flowering and seed germination (Bond and Keeley, 2005; Gill, 1975). Specific soil microbial fire adaptations have also been observed: some Australian fungi are pyrophilous and have underground storage organs which enable them to produce fruiting-bodies two days post-fire (McMullen et al., 2011). While fire-adapted systems have evolved protective mechanisms, they could still be substantially changed in the long-term, and sometimes irreparably, if predicted changes in climate and fire regime occur, i.e., increases in fire frequency combined with shorter recovery times (Flannigan et al., 2009). Furthermore, changes in management practices, such as more frequent low-intensity prescribed burning to control fuel loads, urban encroachment, and land use change place additional pressures on the ability of fire-adapted ecosystems to recover (Bardsley et al., 2015).

Because of their unpredictable nature, wildfire studies are reactive, often opportunistic, and may not have adequate control sites for comparison. The length of time since fire varies in wildfire studies, ranging from immediate and short-term (days, weeks, months) (Dannenmann et al., 2011; Ferrenberg et al., 2013; Muñoz-Rojas et al., 2016)) to longer-term (years, decades) (MacKenzie and DeLuca, 2006; Smithwick et al., 2009; Stephan et al., 2015). Investigating post-wildfire recovery and resilience also presents challenges in replication, establishing 'before-fire' baseline conditions and locating similar, but unburnt, control sites. Despite these challenges, understanding the relationships between soil properties, microbial communities and soil function at different post-fire timescales has the potential to identify early indicators of weakening ecosystem resilience and recovery in a range of land use systems.

A wildfire which overran a site that was part of a multi-year environmental monitoring study (de Menezes et al., 2015; Prendergast-Miller et al., 2015) provided an opportunity to characterise short-term changes in soil properties and soil microbial communities in managed pasture and remnant

native eucalypt woodland plots. We focused on short-term temporal variation, given the relatively rapid recovery of fire-adapted eucalypt woodland systems (Clarke et al., 2015; Gill, 1975; Shakesby et al., 2007). The objectives were to (1) monitor short-term temporal variation in soil and microbial parameters; (2) identify soil factors which related to temporal shifts in microbial communities; and (3) identify microbial groups which responded positively and negatively to fire-induced temporal change in soil properties. Finally, as it is difficult to directly ascertain the scale of fire impacts, the magnitude of fire as an environmental disturbance was determined by including a comparison of temporal (seasonal) variation at two unburnt (control) sites within the same locality as the burnt site. This study provided a rare opportunity to discuss temporal variation in the context of fire disturbance because data were also available from a sampling campaign which took place three weeks prior to the wildfire. We therefore tested the hypothesis that the temporal shift in soil properties and microbial communities would be different between burnt and unburnt sites in each land use.

2. Materials and Methods

2.1 Study sites and sampling design

The wildfire occurred at one of three pastoral farms (Glenrock, Bogo, Talmo) which have been previously described (de Menezes et al., 2015; Prendergast-Miller et al., 2015). A map of the study site location is provided in the Supplementary Information (Fig. S1). The naturally-occurring wildfire spread over >14000 ha of farmland which included the Glenrock farm (the burnt site). The farms at Bogo and Talmo were not affected and therefore provided unburnt pseudo-control sites for this study. The farms are within 15 km of each other and are located in the seasonally dry temperate region of New South Wales (Australia) on brown sodosols (Isbell, 2002). Glenrock is on volcanic and

sedimentary rocks of the Silurian Douro Group. Bogo and Talmo are on Mountain Creek Volcanics of the Devonian Black Range Group (Cramsie et al., 1975). As described in Prendergast-Miller et al., (2015), the main plant species in the three pasture sites was subterranean clover (*Trifolium subterraneum* L.) with some annual and perennial grasses [e.g. phalaris (*Phalaris aquatic* L.)]. The woodlands at Glenrock and Bogo consisted of remnant native woodland areas adjacent to pasture fields: the *Eucalyptus* woodland was relatively open with a native grassy understorey; the Bogo woodland had some exotic grass species. At Talmo, the pasture lay adjacent to the Burrinjuck Nature Reserve (NSW); in this remnant native woodland, *Eucalyptus* and *Acacia* tree species had a more dense cover compared to the other two woodland plots. The three study sites were located on mature (> 40 years) sheep-grazing enterprises typical of the farming landscape in rural south-eastern Australia. In this region, land clearing (by tree logging and fire) since the mid-nineteenth century, as well as soil degradation and increasing pressure on land resources has created an increasingly fragmented remnant native woodland-managed pasture landscape (Prober et al., 2002).

Monitoring sites on paired managed pasture-remnant native woodland plots were established at each farm in October 2012 (see Fig. S2). On each adjacent pasture and remnant native woodland, a plot (100 x 100 m) was gridded and 25 wireless sensor nodes were deployed (150 nodes in total). The layout of the sensor nodes was determined by spatial prediction variance (Cressie, 1993) based on the variability of soil and microbial parameters measured in de Menezes et al., (2015). The original objective of the study was to determine spatial and temporal variation in contrasting habitats using an environmental sensor network. The physical location of the nodes marked the soil sampling points to calibrate sensor- and soil-derived measurements, and the first soil samples were taken from all nodes in December 2012 (150 node samples; 25 samples per plot). The sensor nodes marked the sampling positions, and soil samples were taken within 0.5 m of the node. Due to temporal sampling, care was taken to avoid re-sampling the previous hole. Following the wildfire in January 2013, there were two sampling campaigns: (1) to collect soils at the Glenrock fire site (one

157 adjacent pasture-woodland plot) over a period of 4 months (up to April 2013) to determine post-fire
158 changes; and (2) to collect soils at the three farms to determine seasonal changes in April 2013
159 (three adjacent pasture-woodland plots). Twenty-five soil node samples were collected from each
160 pasture or woodland plot at each sampling time. The original sampling design provided replication at
161 the site level for seasonal change (n = 3 adjacent land uses). However, only the Glenrock site was
162 affected by the fire and therefore, the wildfire 'treatment' was not replicated.
163
164 Soil samples were taken at all node locations within sites in December 2012 and April 2013, to allow
165 for seasonal comparisons between burnt and unburnt sites. The wildfire burnt through the Glenrock
166 site in early January 2013 following extreme weather conditions [air temperature 42 °C, low relative
167 humidity and high wind speed at 80 kph; (RFS, 2013)]. Additional soil node samples were collected
168 post-fire at Glenrock (one week, 15 January 2013; one month, 5 February 2013, which included the
169 first post-fire rain event; and three months, 9 April 2013), to determine the impact on and dynamics
170 of soil properties and soil microbial communities. This sampling allowed detailed temporal
171 comparisons within the burnt site.
172
173 2.2 Soil sample processing
174
175 At each node for each plot, two soil cores (0-10 cm depth, 5 cm diameter) were taken and bulked.
176 Soils were kept cool (4 °C) during transfer to the laboratory and samples were processed within 48
177 hr of sampling. Soil samples were broken up by hand and homogenised, and a sub-sample was flash-
178 frozen in liquid nitrogen for molecular analyses (see below). The remaining soil sample was analysed
179 for a range of soil properties. Soil was extracted with cold (4 °C) 0.5 M K_2SO_4 (1:5 w/v ratio) (Rousk
180 and Jones, 2010), shaken for 60 min and analysed for extractable nitrogen (N) and carbon (C) pools.
181 Ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentrations were determined following Mulvaney
182 (1996) and Miranda et al., (2001) respectively; free amino acid (FAA) concentrations were quantified

using the fluorimetric o-phthalaldehyde-b-mercaptoethanol (OPAME) method (Jones et al., 2002); dissolved organic C (DOC) and total dissolved N (TDN) were analysed on a total organic C (TOC) analyser (Shimadzu TOC-VCSH/CSN þ TNM-1; Kyoto, Japan). Soil microbial biomass C (Cmic) and N (Nmic) were measured on the TOC analyser after fumigating additional soil samples with chloroform for 24 h and extracting these samples with 0.5 M K_2SO_4 (1:5 w/v ratio) (Vance et al., 1987). Microbial biomass C and N were corrected using correction factors of 0.45 and 0.54 for Cmic and Nmic respectively (Brookes et al., 1985; Wu et al., 1990). Dissolved organic N (DON) was calculated as the difference between TDN and NH_4^+ and NO_3^-. Available phosphorus (P) was determined by extracting soil samples with 0.5 M $NaHCO_3$ at pH 8.5 (1:100 w/v ratio) (Rayment and Lyons, 2011) and quantified using Malachite green (Irving and McLaughlin, 1990). Air-dried soil subsamples were milled and mid-infrared (MIR) spectroscopy was used to estimate soil C fractions (particulate, humic, and resistant organic C: POC, HOC, ROC respectively) using the prediction algorithms developed in Baldock et al., (2013). These prediction algorithms were developed on Australian agricultural soils (>500 samples, including those within the study region, Yass, NSW): spectra from the soils of this study fell within the calibration, and the error statistics associated with the predicted fraction were below threshold levels. Although not a direct measure of charcoal, the estimated ROC fraction is considered to be comprised of the poly-aryl C structures consistent with charred plant biomass and lignin-derived aryl C (Baldock et al., 2013). Soil pH was measured in a 1:2 soil:water suspension, and soil moisture content determined after drying at 105 °C overnight. All results are expressed on a soil dry weight basis.

2.3 Soil molecular analyses

2.3.1 Soil DNA extraction

208 DNA was extracted from 0.25 g of soil using the MO-BIO PowerSoil® kit following the manufacturer's
209 protocols except that the Qiagen TissueLizer (Venlo, Netherlands) was used (full speed for 2
210 minutes) after the introduction of buffer C1. DNA quality and quantity was determined by
211 Nannodrop and Quanti-iT™ Picogreen (Life Technologies™, Mulgrave, Australia).
212
213 2.3.2 T-RFLP processing
214
215 A T-RFLP approach was used to compare bacterial and fungal community structure before and after
216 the fire at Glenrock (burnt site). T-RFLP analysis was also performed to compare seasonal change in
217 bacterial community structure across the three farms studied (Glenrock, Bogo and Talmo). DNA
218 concentration was normalised across all samples and the bacterial 16S rRNA gene and fungal ITS
219 region were amplified using the 27f (Lane, 1991) and 519r (Lane et al., 1985) and ITS1f (Gardes and
220 Bruns, 1993) and ITS4 (White et al., 1990) primers respectively. The forward primers were labelled
221 with 6-carboxyfluorescein at the 5' end. The PCR amplification products were cleaned with
222 Agencourt® Ampure® beads (Beckman Coulter, Lane Cove, Australia) and quantified using
223 Picogreen® dsDNA quantification kit (Life TechnologiesTM, Mulgrave, Australia) according to the
224 manufacturer's instructions. Twenty-five ng of PCR products were then digested with 20 *Alu*I
225 restriction enzyme (New England Biolabs) overnight at 37°C, followed by precipitation with 150 µl of
226 cold 75% isopropanol (v/v) (Sigma-Aldrich, Sydney, Australia) for 30 minutes and then centrifuged at
227 4000 rpm for 45 minutes. PCR fragments were added to a mixture containing 9.7 µl Hi-Di™
228 formamide and 0.3 µl of GeneScan™ 600 LIZ size standard. The DNA was denatured at 94°C for three
229 minutes and the fragment lengths determined by electrophoresis using an AB3031xl Genetic
230 Analyser (Applied Biosystems, Mulgrave, Australia); the restriction fragment profiles were obtained
231 from GENEMAPPER ® (Applied Biosystems, Mulgrave, Australia). An R script was used to filter the
232 fragment profile using the method of Abdo et al. (2006) and remove spurious baseline peaks
233 (minimum height of 20 fluorescence units and peaks smaller than two times the standard deviation

234 calculated over all peaks were removed). The Interactive Binner program (Ramette, 2009) was used
235 to bin the resulting sizing data. For bacteria, the parameters used were: minimum and maximum
236 peak sizes of 40 and 520 bp, respectively, minimum relative fluorescence units of 0.099, window size
237 of 2.5 bp and shift size of 0.25 bp. For fungi, peaks smaller than 40 and larger than 600 were
238 discarded, minimum relative fluorescence units was 0.099 and a window size of 3 bp and shift size of
239 0.3 bp were used. Window size was selected based on inspection of the restriction fragment size
240 profiles using the GENEMAPPER® software.
241
242 2.3.3 Illumina MiSeq sequencing of soil bacteria from the wildfire site
243
244 In order to determine the effect of the wildfire on soil microbial groups at the burnt site (Glenrock),
245 we focused on bacterial community composition by sequencing the 16S rRNA amplicons. We
246 acknowledge that fungi, protozoa and archaea will also have been affected. Eleven sample points
247 were randomly chosen out of the 25 in each of the woodland and pasture plots at the Glenrock site
248 (i.e. 22 samples). In total 88 DNA samples representing all sampling times (December 2012, January,
249 February, April 2013) were sequenced using the Illumina MiSeq platform. DNA was quantified using
250 Qubit™ (Life Technologies™, Mulgrave, Australia), and amplified using the 27f and 519r bacterial
251 16S rRNA primers, which were adapted to contain barcodes and the Illumina linker sequence.
252 Equimolar amounts of DNA were added to one MiSeq flow cell. Paired-end sequencing was carried
253 out in the Illumina MiSeq sequencer using the 500 cycle V2 kit. Paired end reads were quality
254 checked using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), low quality
255 regions trimmed and merged using FLASH (Magoč andSalzberg, 2011), with a 20 bp minimum
256 overlap. Sequences < 400 bp and with homopolymers > 8 bp and ambiguities were removed in
257 mothur (Schloss et al., 2009) resulting in a total of 11,373,687 sequences, with a mean length of 461
258 bp. Sequences were clustered at 97% identity threshold and chimeras removed using
259 USEARCH/UCHIME (Edgar, 2010; Edgar et al., 2011). The resulting OTUs were classified in mothur

using the Greengenes reference files (DeSantis et al., 2006), with a confidence threshold of 60%. OTUs classified as eukaryotic, archaeal, mitochondrial or as plastid were removed as well as sequences not classified to domain level (bacteria). Rare sequences (those OTUs occurring < 100 times in the whole dataset, roughly corresponding to OTUs occurring at one sequence per sample on average) were also removed. The resulting dataset had a total of 7,737,445 sequences, 4513 OTUs, and the average, maximum and minimum numbers of sequences per sample were 87,925, 151,516 and 49,224, respectively. OTU abundance data was rarefied to 49,224 using the rarefy_even_depth command in the phyloseq statistical package (McMurdie and Holmes, 2013) before statistical analyses, except for DESeq2 OTU enrichment analysis for which non-rarefied data was used following the recommendations of McMurdie and Holmes (2014). Coverage of the subsampled dataset was >0.99 (Good's coverage estimator) for all samples.

3. Data analysis

Our original sample design of 150 environmental sensors deployed over three paired pasture-woodland plots was set up to maximise investment and long-term spatial-temporal data capture. As the wildfire occurred after our first sample time (December 2012) we decided to continue sampling as per our original plan (25 node samples per plot) to allow comparison with pre-fire data from the same soils in one burnt and two unburnt sites. However, this inevitably meant the post-fire study at Glenrock was pseudoreplicated, which is a consequence of investigating a real-life environmental disturbance in ecology. There is a lot of debate on how to deal with pseudoreplication (e.g. Davies and Gray, 2015; Hurlbert, 1984; Millar and Anderson, 2004) which we account for below in the analyses. This was an opportunistic study, and we have used the data to describe short-term post-fire temporal changes and compare these to seasonal changes at our study sites.

286

287 3.1 Data pre-treatment

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289 Glenrock wildfire data: the data comprised soil samples collected from December 2012 (pre-fire),
290 January, February and April 2013 (post-fire) from the Glenrock woodland-pasture site which was
291 burnt by the wildfire in January 2013. Soil properties FAA, NO_3^-, DOC and Nmic were square root and
292 NH_4^+ log(x+1) transformed to correct for skewness. For multivariate analyses, soil data were then
293 normalised and similarity between samples calculated using Euclidean distances. Bacterial and
294 fungal community structure (T-RFLP data) as well as bacterial community composition (genus level)
295 data were square root transformed to reduce the contribution of dominant TRFs/OTUs and
296 resemblance matrices created using Bray-Curtis (Clarke and Gorley, 2006).
297 Glenrock, Bogo and Talmo seasonal data: the data comprised soil samples collected from the three
298 woodland-pasture sites (Glenrock, Bogo and Talmo) in December 2012 and April 2013. Soil
299 properties FAA, NO_3^- and soil P were square root and NH_4^+ log(x+1) transformed to correct for
300 skewness. For multivariate analyses, soil property data were normalised and Euclidean distance was
301 used for the resemblance matrix. Bacterial community structure data (T-RFLP) were square root
302 transformed and Bray-Curtis was used for the resemblance matrix. Multivariate analyses were
303 conducted using PERMANOVA+ software (v7; (Anderson et al., 2008).

304

305 3.2 Temporal variation in soil and microbial parameters at the Glenrock wildfire site

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307 The wildfire at the Glenrock site provided an opportunity to compare changes in the soil
308 environment one month before and over four months post-fire. As only one site was affected, the
309 interpretation of the analyses is limited to the affected site.

310

311 Non-linear multidimensional scaling (nMDS) plots were created to visualise the multivariate
312 structure in soil properties, soil bacterial and fungal community structure (T-RFLP data) and soil
313 bacterial community composition (sequence data) at Glenrock before and after the fire across both
314 land uses (see Supplementary Info Fig. S3).

315 Temporal differences in soil properties, soil bacterial and fungal community structure (T-RFLP data)
316 and soil bacterial composition (sequence data) were tested using a permutation-based multiple
317 analysis of variance (PERMANOVA) (Table 1). PERMANOVA is a statistical technique that enables
318 parametric modelling for factors or treatments in experimental design without implicitly assuming
319 Euclidean distance and explicitly assuming a univariate or multivariate Gaussian distribution for the
320 errors in the model. The use of permutations means that statistical tests can be used that do not
321 rely on an assumed underlying distribution. The PERMANOVA tests used 9999 permutations of
322 residuals under a reduced model, with type III partial sums of squares.

323

324 As the analysis was in response to the wildfire event at one site and not a replicated experiment, the
325 factor 'time' was fixed (for repeated measures; (Anderson et al., 2008)), and land use was treated as
326 a random factor to account for sampling within one site (Millar and Anderson, 2004). A two-factor
327 crossed design was used, with time (fixed, 4 levels (Dec, Jan, Feb, April)) and land use (random, 2
328 levels (pasture and woodland)). PERMANOVA is sensitive to dispersions in homogeneity, therefore
329 significant results can indicate differences due to location in multivariate space and/or dispersion
330 (Anderson et al., 2008). Therefore, PERMDISP (a distance-to-centroid based test on multivariate
331 dispersions) was used to test for no differences in the within-group multivariate dispersion among
332 groups (Anderson et al., 2008), using the combined factor 'time and land use' as this interaction was
333 significant in the PERMANOVA results (Table 1). PERMDISP is also useful to explore changes in
334 variability, as changes in dispersion can also be used to indicate environmental stress in ecological

studies (Anderson et al., 2008). PERMDISP was performed on Euclidean (soil data) and Bray-Curtis (microbial data) resemblance matrices; the *P*-value was determined using 999 permutations.

In order to determine the magnitude of change in individual soil properties over time, pasture or woodland soil properties were tested separately by repeated measures one-way ANOVA, with sample number as subject and time as level. Where assumptions of variance were not met, the repeated measures test was performed on ranks (SigmaPlot v.13.0).

PERMANOVA indicated significant differences in microbial community structure between time and land use (Table 1), however, these patterns were masked when observed using unconstrained nMDS plots (see Supplementary Information, Fig. S3). Therefore, canonical analysis of principal coordinates (CAP) was used to quantify and visualise these differences in the Glenrock pasture and woodland soils (Anderson and Robinson, 2003; Anderson and Willis, 2003). The CAP procedure enables characterisation of sample groups, by visualising differences and assessing the distinction between groups in multivariate space (Anderson et al., 2008). Whereas nMDS is an unconstrained ordination, CAP is a constrained ordination technique which enables discrimination among groups along an axis through the multivariate data cloud (Anderson et al., 2008). In the CAP routine, we tested the *a priori* hypothesis of there being no difference in multivariate location among groups i.e. of the microbial community structure (bacterial and fungal T-RFLP) amongst the sampling time classes for each land use by constraining the ordination to those classes. The strength of the CAP result (Table S1) was determined by the trace statistic and the percentage cross-validation allocation success, as well as by obtaining a *P*-value using permutation tests (999 tests). Microbial community structure in pasture and woodland soils were then correlated to the respective soil properties using an overlay vector function (Pearson correlation, r). This is an exploratory tool to identify soil properties which increase or decrease with the CAP axes (Anderson et al., 2008).

Finally, land use and temporal changes in bacterial community composition (using sequenced data) at the burnt site were determined. Identification of OTU enrichment after the fire was based on the DESeq2 (Love et al., 2014) extension from the phyloseq package following the approach outlined in McMurdie and Holmes (2014). DESeq2 was run using the Wald test, with automatic filtering of low abundance OTUs, and an alpha of 0.01. Adjusted *P*-values were calculated automatically by DESeq2. The results of the DESeq2 analysis were visualised using the ggplot2 package in R (Wickham, 2009).

3.3 Comparison of seasonal shifts at wildfire and unburnt sites

Although the impact of the fire at Glenrock cannot be directly tested, the magnitude of temporal shifts in soil properties and bacterial community structure were compared between the three farms and discussed in the context of the fire disturbance.

Patterns in the multivariate data between site, month and land use for soil properties and soil bacterial community structure (T-RFLP data) were first explored using nMDS plots (Fig. S3). PERMANOVA was used to test for significant differences between these factors using a three-way crossed design (9999 permutations of residuals under a reduced model, with type III partial sums of squares): site (fixed, 3 levels: Glenrock, Bogo, Talmo); month (fixed, 2 levels: December 2012, April 2013); and land use (fixed, 2 levels: pasture, woodland) (Table 2). As PERMANOVA is sensitive to dispersions, the PERMDISP routine was performed using 'site-month-land use' as the group factor; the *P*-value was determined using 999 permutations (Table 2). PERMANOVA indicated significant differences in bacterial community structure between site, land use and sampling times, however, these patterns were masked when observed using unconstrained nMDS plots (see Supplementary Information, Fig. S3). Therefore, the CAP approach was used to test the hypothesis that the temporal i.e. seasonal shift (December 2012 *vs.* April 2013) in bacterial community structure was different between burnt (Glenrock) and unburnt (Bogo and Talmo) sites in each land use. Diagnostic results

are given in Table S1. Soil properties associated with these temporal shifts were identified using Pearson correlations. Seasonal differences to determine the magnitude of change in individual soil properties (December *vs.* April, i.e. the pre- and post-fire period at Glenrock) at the three farms but within the same land use were tested using a 2-way ANOVA (SigmaPlot v.13.0).

4. Results

4.1 Changes in soil and microbial community structure at the Glenrock wildfire site

The wildfire destroyed the monitoring site at Glenrock, and temporal samples were taken to assess the short-term variation in soil properties and microbial communities within the burnt site. Non-linear MDS plots of soil properties and bacterial and fungal community structure (T-RFLP) indicated differences in land use; in addition, temporal changes as well as an indication of increased variability were also observed in the bacterial and fungal nMDS plots (Fig. S3). PERMANOVA showed significant time x land use interactions in soil properties and microbial groups (Table 1). However, time was not significant in the soil data, and this was also inferred from the soil nMDS plot. As PERMANOVA is sensitive to dispersion, and significant effects could be due to multivariate location and/or dispersion, further tests for dispersion were conducted (PERMDISP; Table 1). For the soil data, there was no significant difference in multivariate dispersion ($P > 0.05$); therefore PERMANOVA indicated a significant land use difference in soil properties which was not due to multivariate dispersion. However, differences in dispersion were evident in the bacterial ($P = 0.02$) and fungal ($P = 0.03$) data sets (Table 1). Further PERMDISP analyses using land use or time as factors indicated that in the microbial data sets, time did show significant dispersion (bacteria $P < 0.001$; fungi $P < 0.05$), but land use did not. As dispersion can be used to infer environmental stress (Anderson et al., 2008), it is

412 possible that the significant temporal changes in dispersion in bacterial and fungal communities
413 were a consequence of the wildfire.

414

415 Fire-driven changes in soil properties are often associated with alterations in pH, inorganic N, labile C
416 and charcoal (Wan et al., 2001) which are determined by fire intensity, fuel load and land use
417 characteristics. In terms of soil properties, the temporal dynamics of the C and N pools (Fig. 1) over
418 the pre- and post-fire period at the Glenrock fire site indicated immediate differences post-fire. One
419 week after the fire (January 2013), pasture soil NO_3^- had increased from 8.6 to an average of 25 mg N
420 kg^{-1}; while pH, DOC, HOC and Nmic declined ($P < 0.05$) in the post-fire months (Fig 1; Supplementary
421 information Fig. S4). In the woodland soil ROC fraction increased from 7.1 mg g^{-1} before the fire to
422 8.4 mg g^{-1} immediately after the fire in January ($P < 0.01$; Fig. 1) and remained constant thereafter.
423 Ammonium, FAA, DON, pH, POC, DOC all increased ($P < 0.05$) in the woodland soil, while nitrate
424 which was very low pre-fire (~1 mg N kg^{-1}) declined to negligible levels in the post-fire months (Fig. 1;
425 Supplementary information Fig. S4). Declines were also measured in microbial biomass C and N.

426

427 Temporal differences in bacterial and fungal community structure (T-RFLP data) were visualised
428 using the CAP approach (Fig. 2; see also CAP diagnostics Table S1). The largest shifts in microbial
429 community structure were observed between December and January, which coincided with the
430 immediate post-fire period, in woodland soil bacteria and for both fungi and bacteria in the pasture
431 soil. The woodland soil fungal community showed a large shift between January and April. Close
432 similarity in community structure was shown between December and February in pasture soil
433 bacteria and between February and April for woodland soil bacteria. The clustering of bacterial
434 community structure was correlated to moisture in both soil types; however, in the pasture soil, the
435 shift in December-January was associated with pH, while in the woodland soil, this shift was
436 associated with FAA and DOC. Temporal shifts in fungal communities were also associated with

437 moisture in both soil types, but the December-January shift correlated with nitrate in the pasture
438 soil, and the January and April shift correlated with changes in DOC, FAA, Nmic and pH in the
439 woodland soil.
440
441 4.2 Temporal changes in bacterial community composition at the Glenrock wildfire site
442
443 Sequencing of the bacterial 16S rRNA gene indicated that at the genus-level, bacterial community
444 composition varied between months in both land uses ($P < 0.05$; Table 1). PERMDISP analysis
445 showed that there was no dispersion effect (Table 1). Differential abundances in bacterial
446 community composition after the fire were identified (Fig. 3). Immediately post-fire (December *vs.*
447 January), changes to bacterial composition were mostly negative. In this period, although the OTUs
448 declined for a similar number of phyla (four and five in woodland and pasture soil respectively), the
449 decline in OTUs in the woodland soil was greater (an 8-fold change). Throughout the monitoring
450 period, the change in the woodland soil bacterial composition was positive and greater in
451 magnitude, whereas in pasture soil, the change tended to be negative with a smaller magnitude and
452 with more phyla affected. For example, April *vs.* December had up to a 12-fold enrichment in OTUs
453 belonging to eight different phyla in the woodland soil (e.g. the Actinobacteria, Proteobacteria,
454 Bacteriodetes, Chloroflexi, Firmicutes) while the same period in the pasture soil showed a 6-fold
455 enrichment in OTUs from four different phyla, but a 3-fold decline in OTUs from eleven phyla.
456 Enrichment in bacterial composition seemed to occur earlier in the woodland soil (in February)
457 compared to pasture soil where enrichment was observed in April (Fig. 3). Bacterial composition also
458 showed contrasting patterns for the same groups: for example, immediately post-fire, the
459 Oxalobactereaceae (Proteobacteria) increased in the woodland soil but declined in the pasture soil.
460 The post-fire positive change in bacterial composition in the woodland soil was mainly seen in the
461 Firmicutes (e.g. Bacillus) and Actinobacteria. In the woodland soil, OTU enrichment rapidly increased
462 over time, with the number of OTUs increasing from two phyla immediately post-fire to eight phyla

463 3-months post-fire. In the pasture soil, OTU enrichment increased from one to five phyla, but a
464 greater number of OTUs were negatively affected.
465
466 Temporal differences in gram-negative bacteria within the order Nitrosomonadales (phylum
467 Proteobacteria) and the gram-positive spore-forming Bacillales (phylum Firmicutes) were quantified,
468 as they were identified from the soils studied and these orders also include N-cycling bacterial
469 groups (Fig. 4). The Nitrosomonodales increased one week post-fire in the pasture soil; in the
470 woodland soil, abundance was extremely low and did not change over the post-fire period. The
471 Bacillales were more abundant in pasture soil, but showed little change over time; in contrast, in the
472 woodland soil this group had a low abundance which increased one month post-fire after the first
473 rain event (February 2013) but declined thereafter.
474
475
476 4.1 Seasonal differences between burnt and unburnt sites
477
478 The analyses from the Glenrock wildfire site indicated land use as well as temporal variation in soil
479 microbial communities and identified soil properties which correlated with changes in microbial
480 community structure. However, the magnitude of these changes should be taken into consideration
481 to allow an assessment of any potential 'fire' effect. Therefore, temporal differences in soil
482 properties and bacterial community structure (T-RFLP data) were determined by comparing
483 December 2012 and April 2013 data sets collected from Glenrock (the burnt site) and the two
484 control unburnt sites (Bogo and Talmo).
485
486 Non-linear MDS plots indicated potential differences between site, land use and sampling time (Fig.
487 S3). Differences between these factors were tested using PERMANOVA (Table 2). There was a
488 significant interaction between site x land use x time for both soil properties and bacterial

community structure. PERMDISP analysis also indicated significant dispersion in both data sets. Therefore, the CAP approach was used to visualise the site and temporal differences in each land use. Seasonal differences in bacterial community structure from December 2012 to April 2013 for the three farms are shown in Fig. 5 (see Table S1 for CAP diagnostics). At the unburnt sites (Bogo and Talmo), bacterial communities showed little distinction in structure between December 2012 and April 2013 in both pasture and woodland soils. At the unburnt sites bacterial community structure was correlated with soil moisture and microbial biomass C and N contents. However, at the Glenrock burnt site bacterial community structure in both land uses between December (pre-fire) and April (three months post-fire) was more distinct in comparison to the unburnt sites, especially at Glenrock woodland. The temporal shifts in bacterial community structure at the burnt site were correlated with nitrate in the pasture soil, and with NH_4^+ and DON in the woodland soil. Therefore, change in soil bacterial communities between December and April was apparently greater at the wildfire site compared to the unburnt sites and in each land use the shift was associated with different N pools.

The temporal changes in soil properties identified at the Glenrock fire site were put into context by comparing seasonal December to April differences at all three farms. Comparison of differences in individual soil properties between the three sites (Supplementary information Fig. S5) indicated significant increases between December 2012 and April 2013 at Glenrock in pasture soil NO_3^- (average April 2013 pasture soil content 23 mg N kg^{-1}). Changes in these properties were greater than at Bogo and Talmo (average April 2013 pasture soil content 4 and 9 mg N kg^{-1} respectively). Significant increases were also observed in Glenrock woodland soil NH_4^+ (average April 2013 woodland soil content 8.7 mg N kg^{-1}) compared to Bogo and Talmo (average April 2013 woodland soil content 2.2 and 0.5 mg N kg^{-1} respectively). However, changes in other soil properties were not so dramatic when compared to the unburnt sites. For example, DON increased post-fire at Glenrock pasture and woodland: in the pasture soil, the temporal increase was greater at Bogo; in the

woodland soil, the post-fire concentration reached was similar to that measured at the Bogo unburnt site. Declines in DOC and microbial biomass N at Glenrock were also measured at the unburnt sites. The post-fire increase in Glenrock woodland soil pH (average pH 5.5 in April 2013) did not raise the pH level above that of the unburnt sites (average woodland soil pH at Bogo and Talmo 5.8 and 5.6 respectively). Soil properties such as FAA and soil C fractions (POC, ROC) showed no significant temporal change. Therefore, apart from NH_4^+ and NO_3^-, post-fire changes in most soil properties at the burnt site were similar when compared to seasonal changes in the December to April period occurring at the unburnt sites (Fig. S5).

5. Discussion

Post-fire wildfire studies are reactive natural experiments and may lack adequate control or unburnt sites and replication for assessing fire-induced changes. In this study, a wildfire event occurred three weeks after soil sampling at three paired native woodland-managed pasture plots, thus providing approximate pre-fire baseline conditions in soil properties and microbial community structure. As only one farm was affected (Glenrock), comparison of variation at the wildfire site with variation at two unburnt sites in the same locality provided a means to assess the magnitude of potential fire-induced changes in soil properties and microbial communities on two contrasting land uses. Importantly, shifts in bacterial community structure and changes in soil properties (especially NO_3^- and NH_4^+) from December 2012 (pre-fire) to April 2013 (post-fire) were greater at the wildfire pasture and woodland plots compared to the unburnt plots. The shifts in bacterial community structure (T-RFLP data) at the unburnt sites were associated with soil moisture content, while both bacterial and fungal shifts (T-RFLP data) at the burnt site were associated with changes in pH and N pools i.e. higher contents of NO_3^- in pasture soil and NH_4^+ in woodland soil only observed at the burnt site. Additional post-fire monitoring of the Glenrock pasture and woodland plots (one week, one

541 month, three months post-fire) revealed temporal shifts in bacterial and fungal community structure
542 and bacterial community composition, as well as significant changes in soil N pools, pH, microbial
543 biomass and ROC content which correlated with the shifts in microbial community structure.
544 Therefore, the results suggest that the wildfire had an impact on soil properties and bacterial and
545 fungal communities that was greater than variation driven by seasonal changes in soil moisture
546 observed at the unburnt sites. The results also show that the magnitude of change in microbial
547 community structure was greater than the change in soil properties. Therefore, in order to
548 accurately capture fire-induced changes, monitoring post-fire changes belowground in fire-adapted
549 systems should also include an assessment of impacts on soil microbial communities as soon after a
550 fire as possible (Goberna et al., 2012; Muñoz-Rojas et al., 2016).
551
552 5.1 Temporal variation in bacterial and fungal community structure at the wildfire site
553
554 The impact of environmental disturbance such as fire on the survival and recolonisation of soil
555 microbial communities is mediated through direct effects of soil heating and indirectly through fire-
556 induced changes to pH, soil moisture retention and nutrient availability. Post-fire soil nutrients are
557 affected by changes in SOM, litter inputs and root exudation. Soil moisture-microbial relations in
558 post-fire soil may be affected by increased water repellency due to alterations of SOM. However,
559 eucalypt woodland soils can be naturally water repellent, and fire can increase or decrease this
560 phenomenon (Doerr et al., 2004; Granged et al., 2011; Shakesby et al., 2007). Therefore, post-fire
561 microbial-plant-soil interactions are complex.
562
563 Non-spore forming fungi, protozoa and some bacteria are sensitive to soil temperatures >70 °C
564 (Raison, 1979). Temperatures >200 °C may be required to kill some bacterial species (Neary et al.,
565 1999). Reductions in microbial biomass C and N are typical of fire-impacted soils (Certini, 2005;
566 D'Ascoli et al., 2005; Neary et al., 1999) and similar declines were observed at Glenrock woodland.

567 Microbial biomass C did not change in the pasture plot, and Docherty et al. (2012) also reported no
568 change in microbial biomass after fire in a grassland system. D'Ascoli et al. (2005) found microbial
569 functional diversity recovery three months after fire in a fire-adapted Mediterranean shrub land was
570 linked to increases in autumn moisture; in drier seasons, post-fire recovery was slower. The relative
571 similarity in pasture soil bacterial and fungal community structure after the first rain event post-fire
572 (February 2013) to the pre-fire community structure in December 2012 also suggests that soil
573 moisture may have been important in the recovery of microbial communities.
574
575 In general, fire has a negative impact on fungal abundance, and the magnitude of change varies with
576 fire regime and ecosystem type (Docherty et al., 2012; Dooley and Treseder, 2012). However, fungal
577 studies tend to focus more on forest habitats than on grassland ecotypes (Dooley and Treseder,
578 2012). In Australian ecosystems, determining fungal responses to fire has also focused on eucalypt
579 habitats rather than grasslands (McMullen et al., 2011). In eucalypt woodlands, fungal responses to
580 fire are variable and often site-specific, with fungal declines generally observed under repeated
581 prescribed burning (Cairney and Bastias, 2007). Indeed, some Australian woodland fungi may be
582 pyrophilous (McMullen et al., 2011), with fruit body production stimulated by fire. Consequently,
583 we speculate that changes in fungal community structure observed in this study in both pasture and
584 woodland soil could be related to an increase in the post-fire flush of ascomycetes, which is a typical
585 fire response, due to post-fire spore germination, heat stimulation of spore germination, and
586 tolerance of post-fire conditions e.g. higher pH (McMullen et al., 2011).
587
588 5.2 Temporal variation in bacterial community composition at the wildfire site
589
590 As well as post-fire rain events, changes in nutrient pools were associated with variation in microbial
591 communities. Contrasting patterns in temporal N pools were observed at the Glenrock fire site:
592 pasture soil was marked by a dramatic increase in soil NO_3^-, which became the dominant N pool;

593 whereas NH_4^+ increased in the woodland soil. Analysis of bacterial community composition indicated
594 very low abundance of the Nitrosomonadales at Glenrock woodland compared to pasture. This
595 order contains bacteria associated with N cycling, especially nitrification. This community remained
596 low in woodland soil post-fire, which suggests that this order was inherently small and was not
597 affected by fire impacts. In contrast, the Nitrosomonadales was more abundant in the Glenrock
598 pasture soil. Although we cannot directly attribute the abundance of the Nitrosomonadales to
599 increased nitrification, post-fire pasture soil was also characterised by high NO_3^- content and faster
600 nitrification rates compared to negligible rates in the woodland soil (unpublished data, Prendergast-
601 Miller).
602
603 In the woodland soil, the greatest change in bacterial community composition was the increase in
604 the OTUs classified to the Bacillales order from the Firmicutes phylum in the post-fire months. The
605 Bacillales contains many spore-formers (Vos et al., 2009), which may have allowed these bacteria to
606 recover faster. This is in agreement with previous studies that have shown both short-term (four
607 weeks) and long-term (three years) increases in abundance of the phylum Firmicutes following fire
608 (Cobo-Díaz et al., 2015; Ferrenberg et al., 2013).
609
610 Small post-fire declines were seen in the Bacteriodetes while Proteobacteria remained unchanged in
611 the woodland soil. Cobo-Diaz et al. (2015) reported greater abundance of Bacteriodetes and
612 Proteobacteria in unburned oak woodland (in the fire-adapted Mediterranean Basin) than at burnt
613 sites. Increases in the Rhizobiaceae, Chlorobiaceae and Flavobacteriaceae were seen in the
614 woodland soil, which could be linked to regeneration of N_2-fixing plants such as *Acacia* tree species.
615 Post-fire increases were also observed in the Gemmatimonadetes and Actinobacteria, and Khodadad
616 et al. (2011) showed that these bacterial groups increased in soil after six months incubation with
617 oak and grass derived biochars (synthesised charcoal), which suggests their potential role in
618 degradation of pyrogenic C.

619

620

621 5.3 Temporal variation in soil properties at the wildfire site

622

623 The extent of alteration in soil properties following fire disturbance is related to intrinsic site
624 characteristics such as biogeochemistry and aboveground vegetation (land use and fuel load), which
625 are strongly governed by season. Comparisons of the same wildfire event over different land uses
626 are rare, however, fire intensity is known to vary with density and composition of the above-ground
627 vegetation (i.e. fuel load)(Neary et al., 1999). The differences observed between pasture and
628 woodland are characteristic of each land use (e.g. negligible NO_3^- in woodland soils; higher C
629 contents in pasture soils (de Menezes et al., 2015)), and also reflect how land uses differentially
630 respond to fire. It is likely that the fire severity varied between the two land uses studied here
631 because of the different above ground vegetation composition and fuel load. Pasture (grass) fires
632 tend to spread rapidly due to the homogenous vegetation, resulting in limited heat transfer to soil
633 (Neary et al., 1999; Raison, 1979). Grass fire soil temperatures can reach 80 °C at 2.5 cm depth
634 (Raison, 1979). Therefore, this may have moderated soil responses in the pasture compared to the
635 woodland system. In contrast, eucalypt wildfires can expose soils to intense heat for longer periods
636 of time as the fire moves relatively slowly through more dense and heterogeneous woodland
637 vegetation, resulting in soil temperatures of >300 °C at 2.5 cm depth (Raison, 1979). As soil heating is
638 an important mechanism for altering the belowground soil environment following fire activity (Neary
639 et al., 1999), it is likely that the woodland soil was affected more than the pasture soil due to the
640 probable greater severity of the fire that would have occurred in the woodland vegetation. The
641 increase in woodland soil pH and ROC content and the fact that a wider variety of soil properties
642 were associated with post-fire shifts in woodland soil microbial communities also suggest that fire
643 severity was greater in the woodland compared to pasture soil. In comparison, the strongest shifts

644 (i.e. highest correlation) in pasture soil communities were associated mainly with moisture and pH
645 changes.
646
647 At the Glenrock fire site, soil pH increased by 0.3 units in woodland soil but decreased in the pasture
648 soil by 0.2 units. While pH changes were also observed at the unburnt sites, the only increase was at
649 Glenrock woodland. Wildfires tend to increase soil pH, and this change is related to ash and charcoal
650 production and their longevity in soil, which are attenuated by post-fire rain and wind (Certini, 2005;
651 Neary et al., 1999). Soil pH is a critical soil factor as it determines the availability of plant nutrients
652 and is a key driver of soil microbial communities, therefore, pH changes will have subsequent
653 impacts on soil biogeochemistry. The initial increase in woodland soil pH could be related to
654 leaching of alkaline salts from ash and charcoal (Tomkins et al., 1991) as well as organic acid
655 denaturation (Certini, 2005). In the woodland plot, the increase in soil ROC fraction reflects the
656 woody vegetation composition and the increase in ROC content could also have raised soil pH. It is
657 possible that the decline in pH at Glenrock pasture was due to seasonal change rather than fire
658 impact, as similar declines in pH were also observed at the unburnt pasture sites.
659

660 Alteration of soil N cycling is often reported following fire disturbance in a range of ecosystems (Ball
661 et al., 2010; Dannenmann et al., 2011; DeLuca and Sala, 2006; Stephan et al., 2015), and is related to
662 fire-induced changes in soil organic matter. Release of NH_4^+ as a direct consequence of SOM
663 combustion, and NO_3^- from subsequent SOM mineralisation, are typical post-fire responses.
664 Contrasting patterns in temporal N pools were observed at the Glenrock site: pasture soil was
665 marked by a dramatic increase in soil NO_3^-, which became the dominant N pool; whereas FAA and
666 then NH_4^+ increased in the woodland soil. Soil NO_3^- did not increase in the eucalypt woodland soil,
667 although studies in other forest systems (e.g. pine, oak) often report increases in soil NO_3^- and
668 nitrification rates following forest wildfire (Ball et al., 2010; DeLuca and Sala, 2006; Smithwick et al.,
669 2005). The presence of charcoal may stimulate nitrification (DeLuca et al., 2006), however, there was

670 no change in woodland soil NO_3^- despite the increase in woodland soil ROC content. Woodland soil
671 NO_3^- is inherently low at these sites (de Menezes et al., 2015; Prendergast-Miller et al., 2015). Low
672 soil NO_3^- is typical of eucalypt grassy woodlands in Australia (Adams and Attiwill, 1986) but may
673 increase with invasion of exotic annual species (Lindsay et al., 2010; Livesley et al., 2009; Prober et
674 al., 2002). Analysis of bacterial community composition indicated very low abundance of the
675 Nitrosomonadales at Glenrock woodland compared to pasture. However, we have no direct
676 evidence to link abundance of this order with soil NO_3^- pools in woodland or pasture soil at Glenrock.
677 Increases in post-fire soil nitrification rates have been linked to changes in soil conditions, such as
678 pH, as well as changes in microbial community composition. For example, ammonia oxidiser bacteria
679 (AOB) respond positively to post-fire nutrient dynamics (Ball et al., 2010). Although DON is the
680 dominant N form at these sites (de Menezes et al., 2015; Prendergast-Miller et al., 2015), and
681 organic N cycling occurs at similar rates in both land uses (Prendergast-Miller et al., 2015), it is clear
682 that in the short-term, the post-fire pasture soil N pool was dominated by NO_3^-. In the initial weeks
683 post-fire, pasture soil nitrifying bacteria would be able to compete for soil NH_4^+ because of the
684 absence of plant uptake, resulting in increased NO_3^- concentrations. However, the rapid increase in
685 pasture soil NO_3^- after fire requires further investigation to confirm its biotic or abiotic origin
686 (although grass-derived char and ash have a low N content (Raison, 1979)). Post-fire nitrification
687 studies are largely focused on forest systems, where NH_4^+ becomes the dominant inorganic N pool
688 due to organic matter decomposition, and the release of NO_3^- is lower and tends to have an initial
689 lag period (Prieto-Fernandez et al., 1993; Wan et al., 2001). Furthermore, differences in charcoal
690 properties between woody and grass-based ecosystems (Krull et al., 2006) could affect grassland soil
691 post-fire NO_3^- concentrations and nitrification rates. Excess NO_3^- would have implications for pasture
692 vegetation regrowth, potentially favouring the return of exotic grass species (Lindsay et al., 2010;
693 Prober et al., 2002) and affecting the balance between grass and clover (N_2 fixing) species. Higher
694 NO_3^- would also have implications for increased denitrification as well as leaching to water systems

695 especially in later months with the onset of winter rains (as is typical of temperate New South
696 Wales).
697
698 5.4 The magnitude of temporal change following wildfire disturbance
699
700 Immediate and short-term changes, from one week to three months post-fire, observed in this study
701 were put into context by comparing temporal variation at Glenrock with that of the unburnt sites. At
702 the unburnt sites, temporal shifts in bacterial community structure were different compared to the
703 burnt site, suggesting that fire disturbance may have had an additional role in driving temporal
704 variation at the Glenrock site. Furthermore, bacterial communities as revealed by both T-RFLP and
705 sequencing showed immediate changes soon after the fire (relative to the pre-fire community) and
706 soil microbial communities displayed a greater degree of change than soil properties. Temporal
707 differences in soil properties (with the exception of NO_3^- and NH_4^+) tended to be of a similar
708 magnitude and/or direction as the seasonal changes observed at the unburnt sites. This suggests
709 that soil microbial indicators of post-fire recovery and resilience need to be identified in fire-adapted
710 systems to guide assessment of monitoring schemes (Mikita-Barbato et al., 2015; Muñoz-Rojas et al.,
711 2016). Differential abundance analysis of the soil bacterial community composition revealed
712 differences in fire-induced change between woodland and pasture soil communities, in terms of
713 diversity and speed of change. Bacteria in both land uses were negatively affected one week post-
714 fire, and immediate responses, even one day post-fire, have been shown before (Goberna et al.,
715 2012; Muñoz-Rojas et al., 2016). However, the woodland soil communities showed greater and more
716 rapid stimulation post-fire than the pasture soil. Rapid recovery in woodland soil bacterial
717 communities compared to pasture soil could also reflect the impact of land use change. Microbial
718 communities in the fire-adapted native remnant woodland responded positively post-fire compared
719 to the managed pasture site where, in broad terms, bacterial community composition tended to be

720 more negatively affected by the fire. The conversion of fire-adapted native woodland to managed
721 pasture has potentially altered soil biodiversity and function, including its response to fire.
722
723 As well as short-term responses to environmental disturbance e.g. after fire events, soil microbial
724 communities and nutrient availability also vary with diurnal and seasonal variation in moisture and
725 temperature (Bardgett et al., 2005). Therefore, the seasonal (December to April) trends described
726 across the three pasture-woodland sites are part of these continual temporal fluctuations and reflect
727 plant growth dynamics and climate. At the time of this study, plant communities were transitioning
728 from (southern hemisphere) late summer growth to autumn, a period associated with cooler
729 temperatures, increasing moisture and slower plant growth. Therefore, it appears that the fire
730 resulted in only a minor disturbance to seasonal patterns which are strongly controlled by
731 temperature and moisture.
732
733 The fire-induced changes observed from this study are short-term, but post-fire ecosystem
734 responses can have a long memory effect. In some ecosystems, the impact of fire can still be
735 quantified several years or decades post-fire (MacKenzie and DeLuca, 2006; Smithwick et al., 2009;
736 Stephan et al., 2015). However, an important aspect to take into account with post-fire recovery and
737 longevity of fire impacts is the type of ecosystem involved. Australian ecosystems are fire-adapted
738 habitats, with a range of plant and microbial mechanisms that facilitate rapid recovery (e.g. days to
739 weeks) after wildfire events (Clarke et al., 2015; McMullen et al., 2011; Muñoz-Rojas et al., 2016),
740 compared to the one year recovery described following a boreal forest fire (Xiang et al., 2014).
741 Therefore, the short-term response and recovery in soil bacterial community composition within
742 three months post-fire at the Glenrock woodland site may be due to fire adaptation mechanisms.
743 There is a need for further research into the legacy of microbial adaptation in derived habitats such
744 as the pasture soil (which was converted from grassy woodland) which may be negatively affected
745 following loss of important plant traits (e.g. resprouting), resulting in the slower recovery of pasture

soil communities. Given that Australian ecosystems are fire-adapted, fire frequency will be important in determining longer-term outcomes of wildfire events, such as loss of native species, invasion of exotic species and decline in soil function (Prober et al., 2002; Tomkins et al., 1991).

6. Conclusion

A natural wildfire event provided an opportunity to monitor the immediate and short-term temporal variation in soil and microbial parameters in contrasting managed and semi-natural land uses. Clear differences were observed between managed pasture and remnant native woodland plots, which could be related to fire, soil and vegetation interactions. Importantly, the magnitude of disturbance was determined by comparing post-fire variation with temporal variation at two unburnt sites that had similar vegetation, climate and soils as the burnt site. Australian native ecosystems are fire-adapted systems and plants have evolved various traits which promote rapid recovery. Soil microbial communities showed greater temporal shifts at the burnt site compared to the unburnt sites, and these shifts were related to key changes in soil N pools which were not observed at the unburnt sites. Importantly, although bacterial community composition was negatively affected in both land uses, recovery and increases in abundance and diversity were much faster in the remnant woodland soil. This suggests that fire-adapted mechanisms may have been altered following land use conversion to pasture. However, differences in fuel loading due to contrasting vegetation composition will also have played a role in determining fire impacts belowground. As the soil microbial community showed a greater magnitude of change than the measured soil properties, it is important to include detailed measures of soil microbial community structure and composition in post-fire studies.

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996 **Table and Figure captions**

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998 TABLES

999 Table 1: PERMANOVA and PERMDISP results for differences between month and land use for soil
1000 properties, bacterial and fungal community structure (T-RFLP), and bacterial community composition
1001 (sequenced) at the Glenrock adjacent woodland-pasture plot which was destroyed by wildfire. P
1002 value (P (perm)) is derived from 9999 permutations.

1003

1004 Table 2. PERMANOVA and PERMDISP results for differences between site, month and land use for
1005 soil properties and bacterial community structure (T-RFLP) at three farms (Glenrock, Bogo and
1006 Talmo) with paired adjacent woodland-pasture plots. *P* value (*P (perm*)) is derived from 9999
1007 permutations.

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1009 FIGURES

1010

1011 Fig. 1. Temporal changes in predicted soil C fractions (A, B) and extractable soil N pools (C, D) at the
1012 Glenrock pasture (closed symbols) and woodland (open symbols) plots. Data are means (n = 25) with
1013 bars indicating ± 1 standard error. The wildfire event was in January 2013.

1014

1015 Fig. 2. Biplots showing temporal differences in community structure (T-RFLP data) for bacteria in
1016 pasture (A) and woodland (B), and fungi in pasture (C) and woodland (D) soils at Glenrock, from
1017 December 2012 to April 2013. The wildfire was in January 2013. All CAP axes are significant (*P* <

0.001). Soil properties correlating with the first and second CAP axes >0.3 (Pearson correlation) are shown in bold.

Fig 3. Differentially abundant OTUs after the wildfire at Glenrock pasture (A, C, E) and woodland (B, D, F) plots. The OTUs are arranged by genus on the x axis and each dot represents an OTU, colours represent phyla. Differential abundance was analysed by comparing OTU abundance in January, February and April (2013) with the pre-fire community in December 2012 using DESeq2 extension in the phyloseq package (alpha = 0.01). Comparisons in the pasture plot are January *vs.* December (A), February *vs.* December (C), April *vs.* December (E); in the woodland plot January *vs.* December (B), February *vs.* December (D), April *vs.* December (F). The y axis indicates fold change in log base 2 units. OTUs above 0 (indicated by dashed line) are considered enriched after fire, those below 0 decreased in abundance compared to December 2012. The sequence data was not rarefied as per McMurdie et al., 2014. Plots were generated using ggplot2 (Wickham et al., 2009).

Fig. 4. Boxplots representing the percentage abundance of members of the orders Nitrosomonadales (A) and Bacillales (B) in Glenrock pasture and woodland before (December 2012) and after the wildfire (January to April 2013). Upper and lower box limits represent the first and third quartiles, the upper and lower lines represent the maximum and minimum abundances and dots represent outliers. Plots were generated using ggplot2 package in R (Wickham et al., 2009).

Fig. 5. Biplots showing seasonal differences from December 2012 (summer) to April 2013 (autumn) in soil bacterial community structure (T-RFLP data) in pasture (A) and woodland (B) plots at three farms. The wildfire was at Glenrock in January 2013; Bogo and Talmo farms were unburnt. Soil

properties correlating (r > 0.5) with the first two axes are shown in bold. All CAP axes are significant $P < 0.001$.

Table 1

Factor	Soil properties		Bacteria (T-RFLP)		Fungi (T-RFLP)		Bacteria (genus level)	
	Pseudo-F	*P (perm)*	Pseudo-F	*P (perm)*	Pseudo-F	*P (perm)*	Pseudo-F	*P (perm)*
Month	1.17	0.3351	2.78	0.022	2.47	0.026	2.23	0.0381
Land use	42.87	0.0001	24.0	0.001	19.49	0.001	52.12	0.0001
Month x Land use	6.81	0.0001	5.41	0.001	2.71	0.001	2.00	0.003
PERMDISP								
Group Factor	F	*P (perm)*	F	*P (perm)*	F	*P (perm)*	F	*P (perm)*
Month x land use	1.1734	0.415	4.3483	0.0027	2.7648	0.0314	1.3862	0.364

1059 Table 2

1060

PERMANOVA				
Factor	Soil properties		Bacterial data (T-RFLP)	
	Pseudo-F	*P (perm)*	Pseudo-F	*P (perm)*
Site	19.67	0.0001	60.67	0.0001
Month	24.73	0.0001	5.14	0.0001
Land use	44.66	0.0001	14.63	0.0001
Site x month	2.83	0.0015	6.70	0.0001
Site x land use	18.83	0.0001	7.35	0.0001
Month x land use	6.85	0.0001	1.44	0.1086
Site x month x land use	4.16	0.0001	4.30	0.0001
PERMDISP				
Group factor	F	*P (perm)*	F	*P (perm)*
Site x month x land use	6.75	0.001	4.80	0.001

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1068 Fig 1

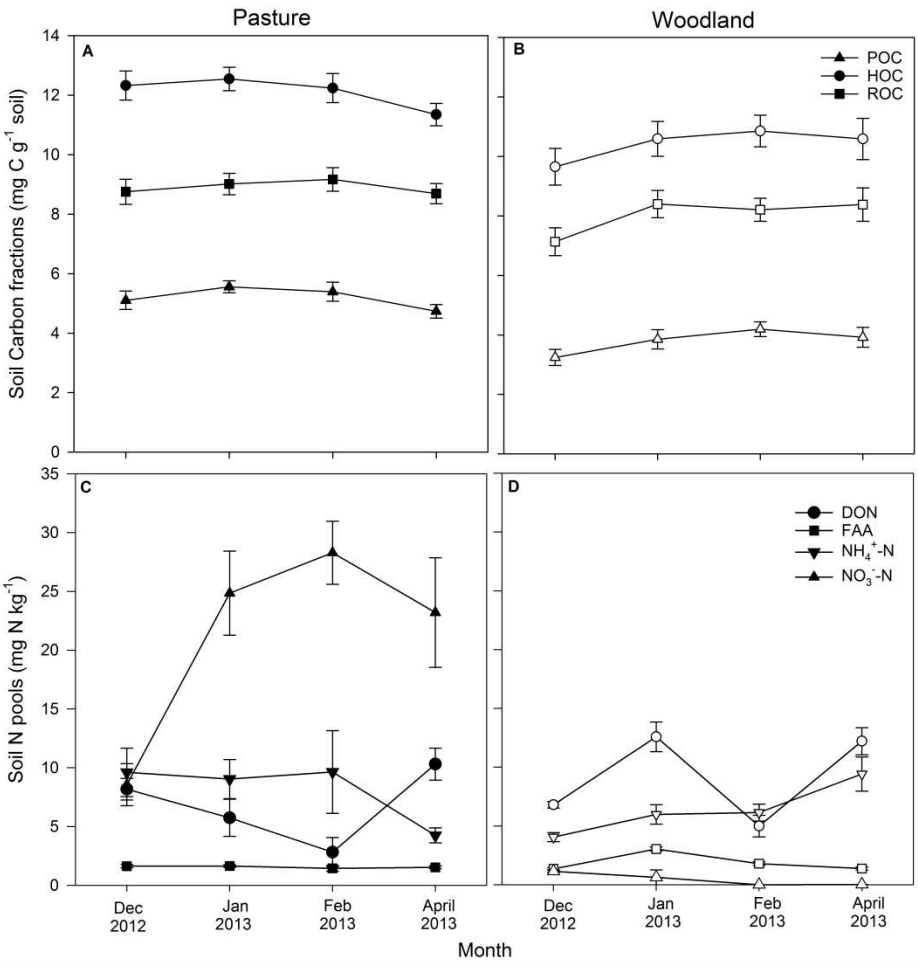

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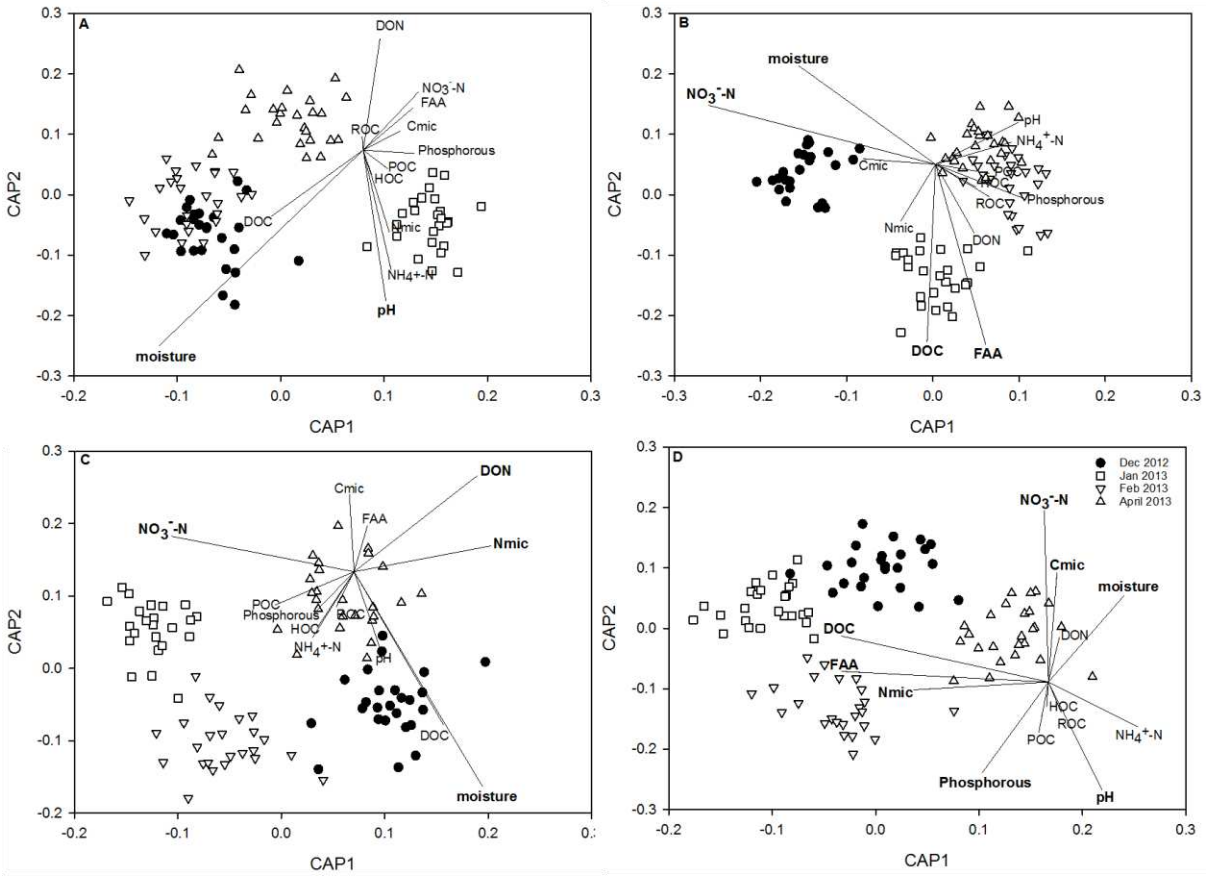
Fig 2


A
DON
NO3-N
FAA
ROC
Cmic
Phosphorous
POC
HOC
DOC
Nmic
NH4+-N
pH
moisture
CAP2
CAP1
B
moisture
NO3-N
pH
NH4+-N
Cmic
HOC
ROC
Phosphorous
Nmic
DON
DOC
FAA
C
Cmic
DON
NO3-N
FAA
Nmic
POC
Phosphorous
HOC
NH4+-N
pH
DOC
moisture
D
Dec 2012
Jan 2013
Feb 2013
April 2013
NO3-N
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moisture
DOC
DON
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ROC
POC
NH4+-N
Phosphorous
pH

Fig 3

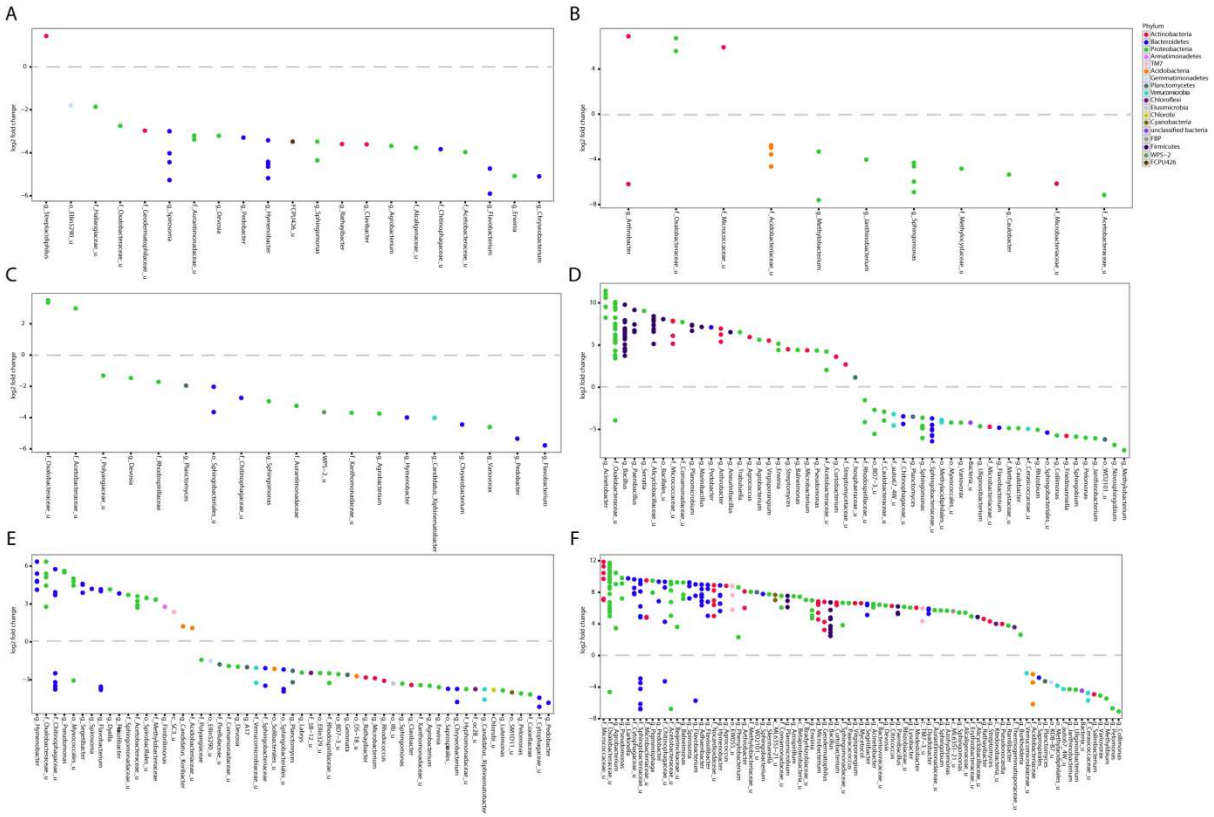

A
B
C
D
E
F
log2 fold change
Phylum
Actinobacteria
Bacteroidetes
Proteobacteria
Armatimonadetes
TM7
Acidobacteria
Gemmatimonadetes
Planctomycetes
Verrucomicrobia
Chloroflexi
Elusimicrobia
Chlorobi
Cyanobacteria
unclassified bacteria
FBP
Firmicutes
WPS−2
FCPU426

Fig 4

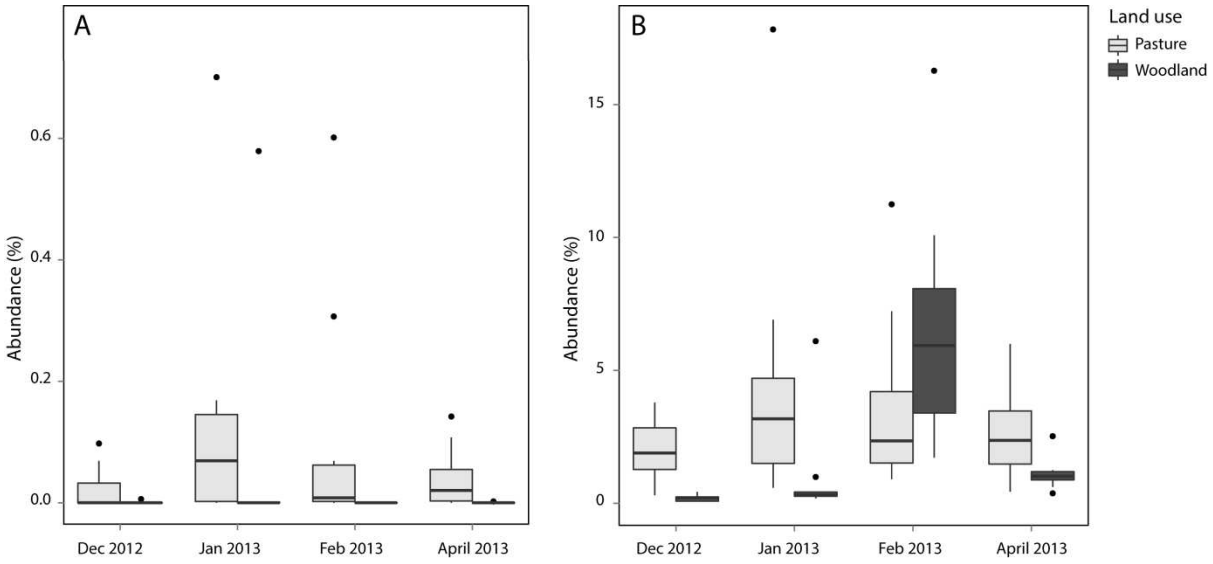
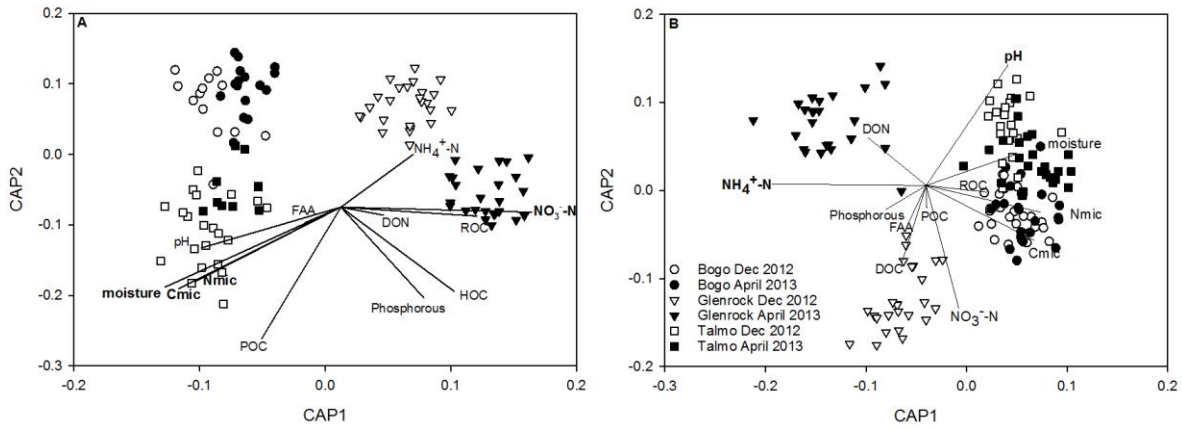

A
B
Land use
Pasture
Woodland
Abundance (%)
Dec 2012
Jan 2013
Feb 2013
April 2013

Fig 5


A
B
CAP1
CAP2
NH4+-N
NO3--N
FAA
DON
ROC
pH
Nmic
moisture
Cmic
HOC
Phosphorous
POC
DOC
Bogo Dec 2012
Bogo April 2013
Glenrock Dec 2012
Glenrock April 2013
Talmo Dec 2012
Talmo April 2013

SUPPLEMENTARY INFORMATION

Wildfire impact: natural experiment reveals differential short-term changes in soil microbial communities

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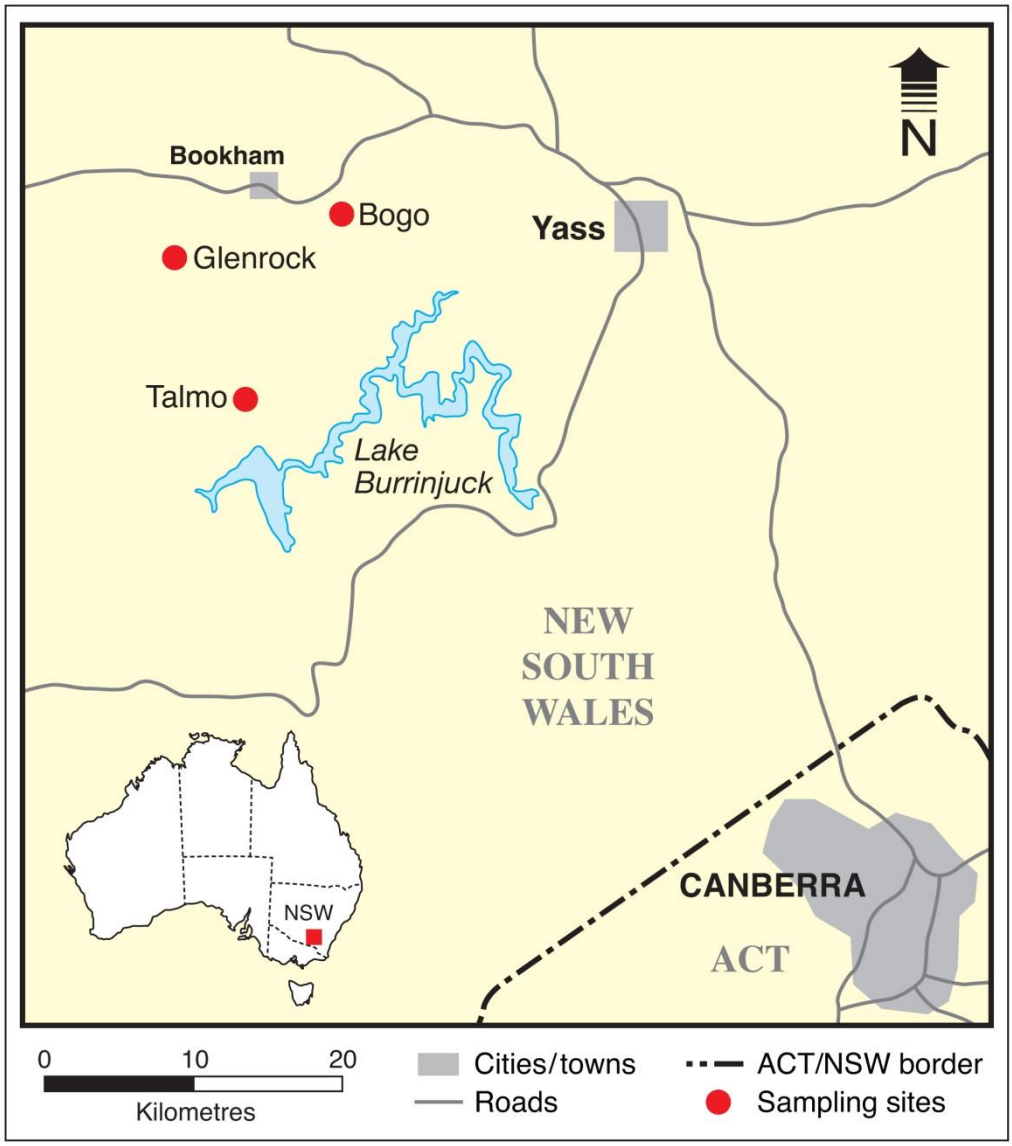

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1156 Fig. S1 Location of field sites.

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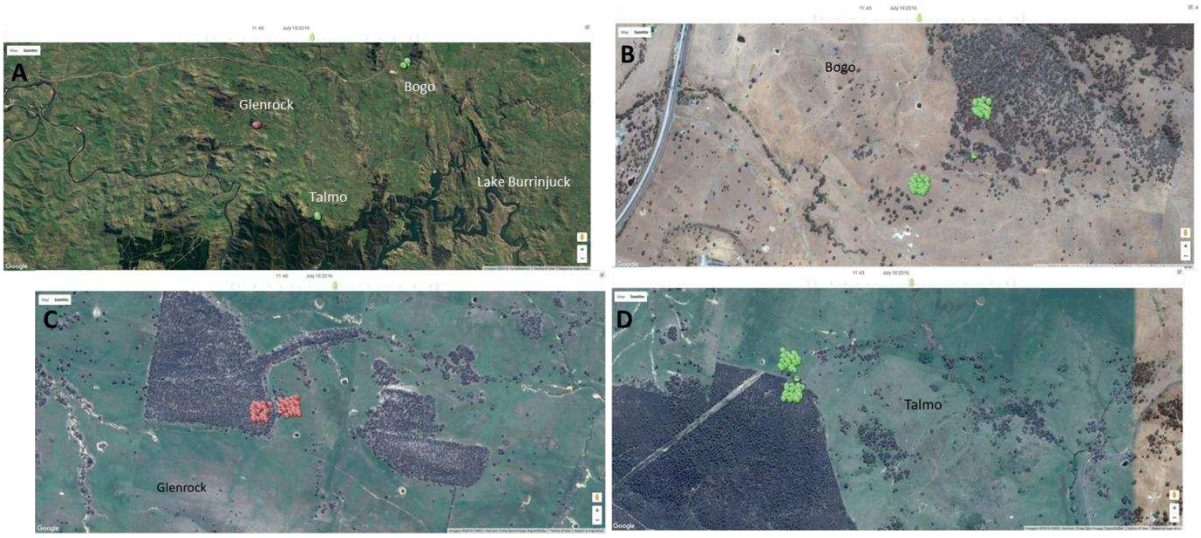

Fig. S2. Google map screen shots showing the field sites and the sensor node locations (A). Groups of 25 nodes (red and green circles) were located in 100 x 100 m plots within the remnant woodland and pasture land uses at three farms: Bogo (B), Glenrock (C) and Talmo (D). The sensor nodes were destroyed by the fire (January 2013) at the Glenrock farm (red nodes are inactive). Image taken from http://www.sensornets.csiro.au/ (accessed July 2016).

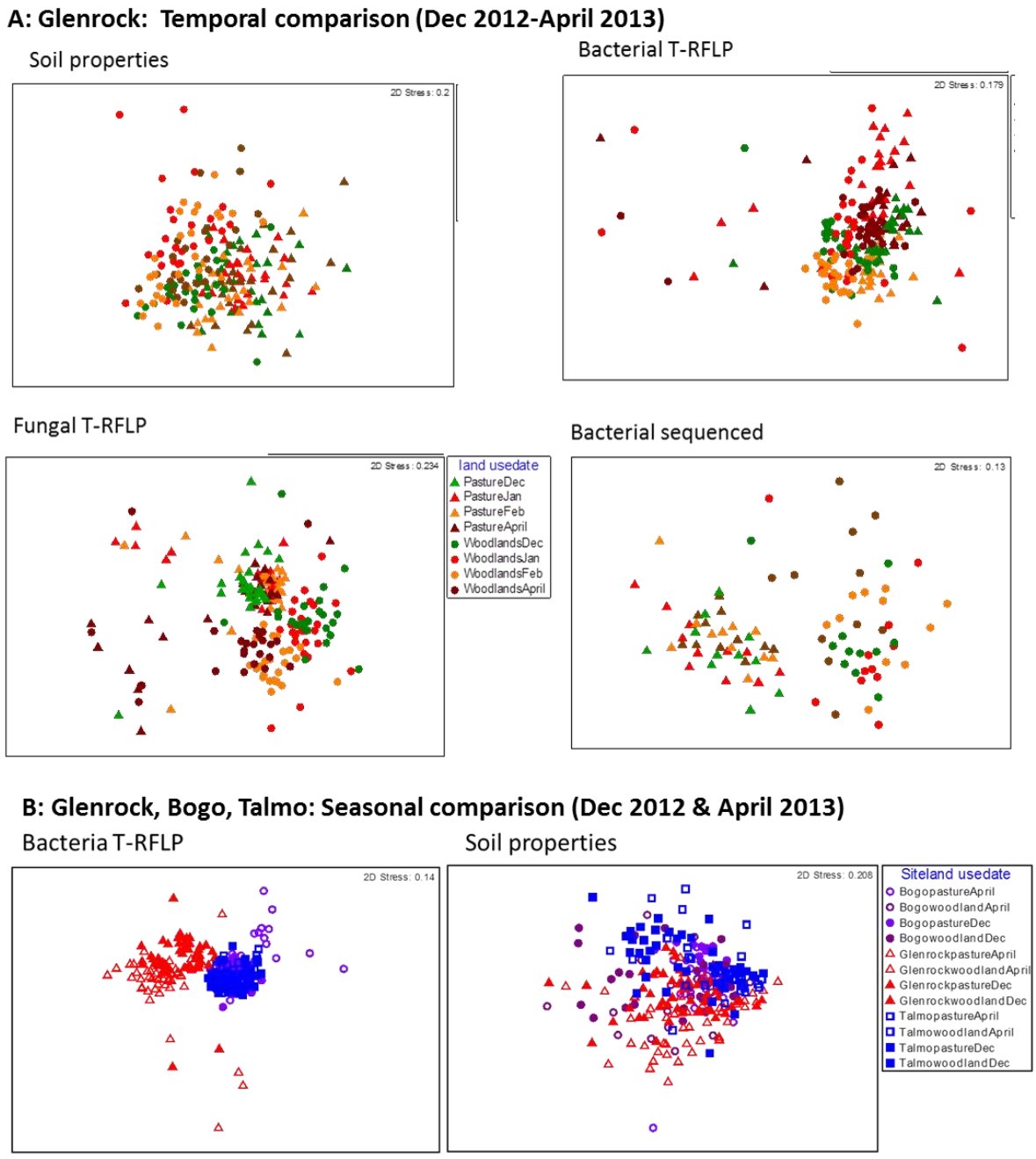

Fig. S3. nMDS plots showing unconstrained ordination of pre- and post-fire samples at (A) the Glenrock wildfire site (Dec 2012, Jan, Feb, April 2013) and (B) the seasonal comparison at Glenrock (burnt), Bogo and Talmo (unburnt sites) (Dec 2012 and April 2013).

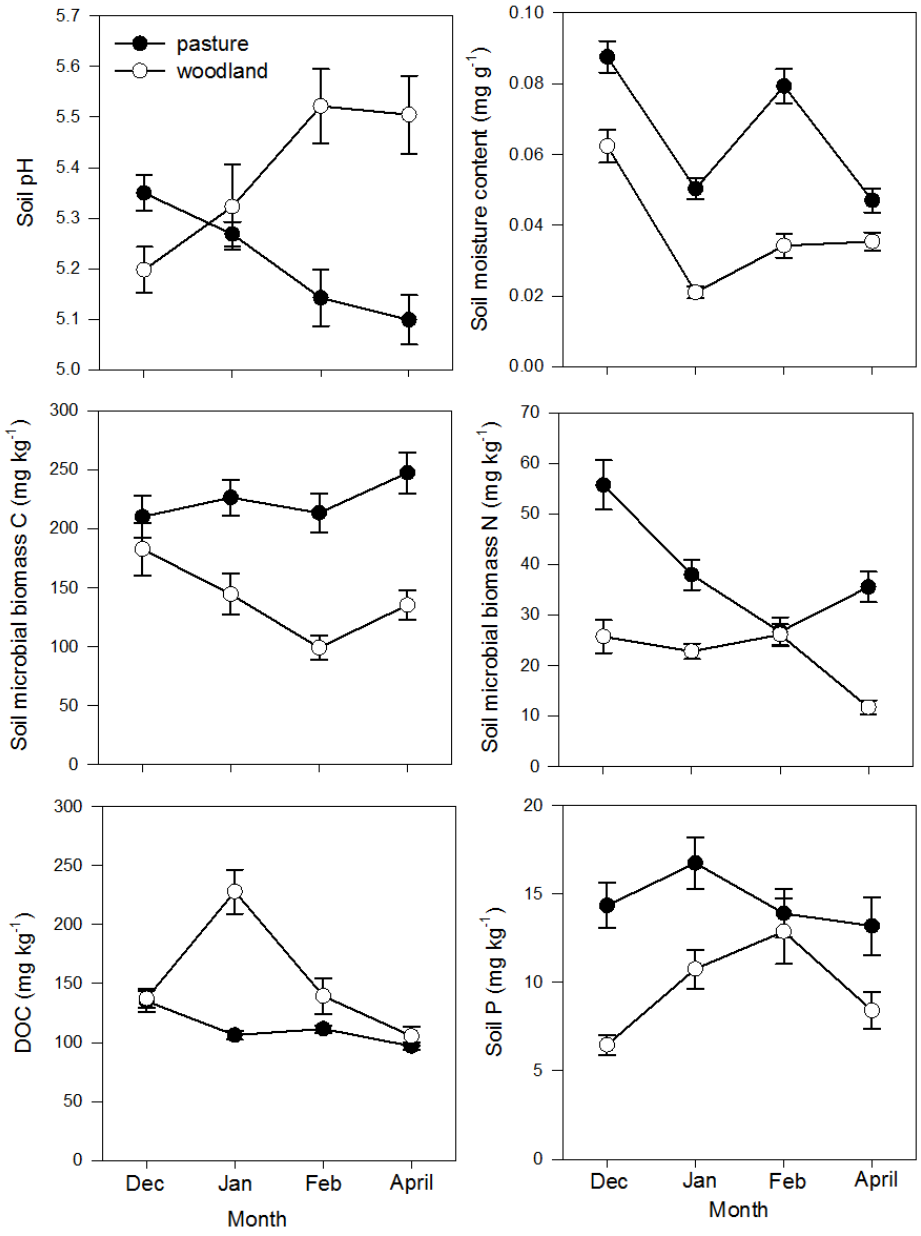

Fig. S4. Temporal changes in soil properties at the Glenrock pasture and woodland sites, December 2012 (pre-fire) and post-fire (January, February and April 2013). Data are means (n = 25) with bars indicating ± 1 standard error. There was a significant (at $\alpha = 0.05$) effect of month on all soil properties (except for pasture soil microbial biomass C, $P > 0.05$).

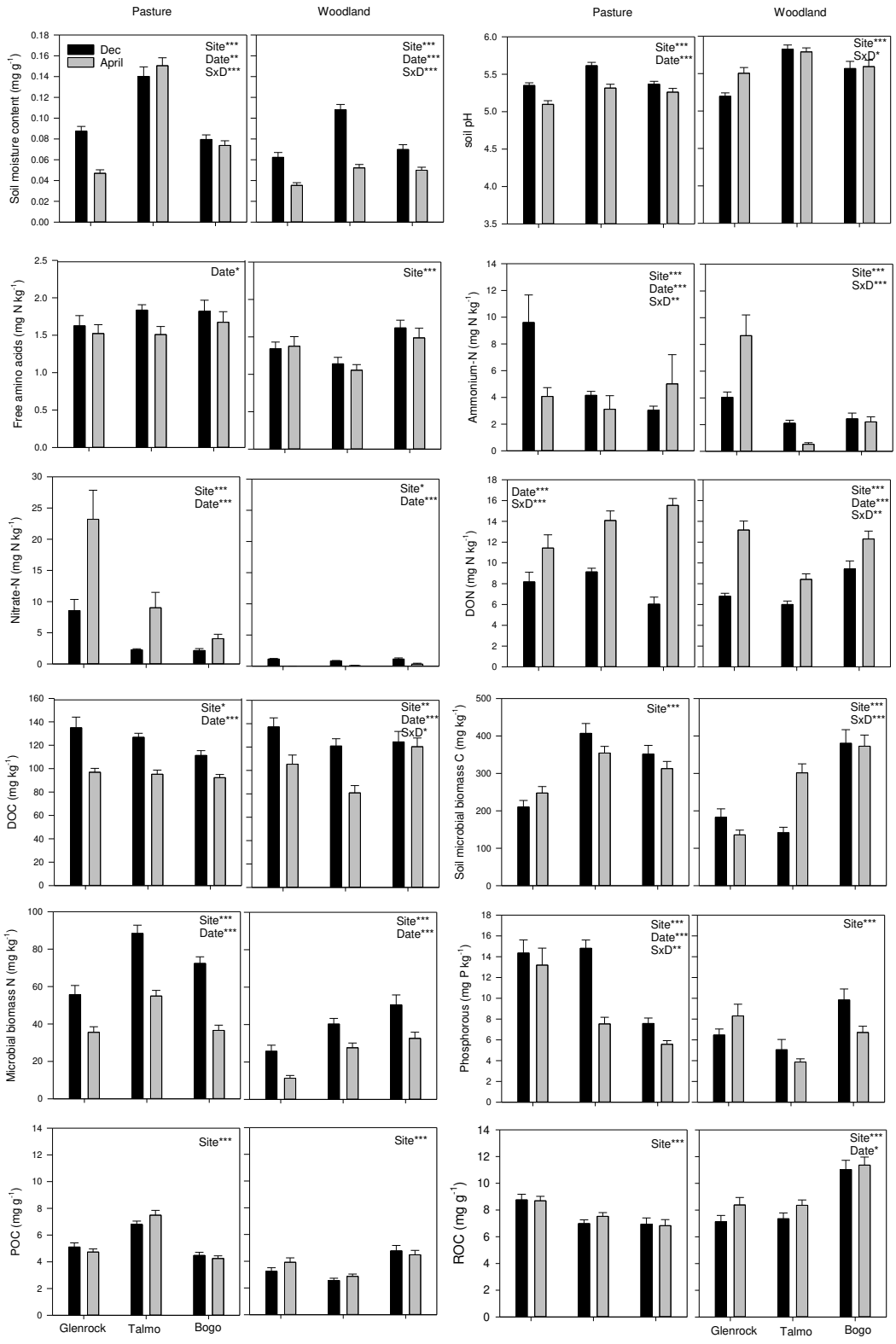

Fig. S5. Comparison of soil properties from adjacent pasture and woodland plots at Glenrock (burnt site) and Talmo and Bogo (unburnt sites) in December 2012 (pre-fire) and April 2013 (three months post-fire). Data are means (n = 25) with bars indicating ± 1 standard error. Asterisks indicate significance at *** ($P < 0.001$), ** ($P < 0.01$), * ($P < 0.05$).

Table S1. CAP diagnostic statistics for analyses at the Glenrock wildfire site and for the seasonal comparison over three sites (Glenrock, Bogo and Talmo).

Site	Data set	Prop. G	Trace statistic (*P value*)	First squared canonical correlation (δ_1^2) (*P value*)	Number of PCO axes (*m*)	Cross-validation allocation success (%)
Glenrock wildfire site	Bacteria T-RFLP pasture	0.84	2.19 (0.001)	0.91 (0.001)	16	91
(group factor = month)	Bacteria T-RFLP woodland	0.78	2.52 (0.001)	0.91 (0.001)	13	98
	Fungi T-RFLP pasture	0.94	2.20 (0.001)	0.88 (0.001)	34	83.5
	Fungi T-RFLP woodland	0.91	2.32 (0.001)	0.86 (0.001)	34	87.8
Glenrock, Bogo and Talmo sites	Bacteria T-RLFP pasture	0.99	3.17 (0.001)	0.96 (0.001)	27	81.7
(group factor = site-month)	Bacteria T-RFLP woodland	0.93	3.13 (0.001)	0.91 (0.001)	19	87.5

CAP analysis finds axes through multivariate data to discriminate among *a priori* groups. CAP performs a PCO analysis on the resemblance matrix and uses these to predict group membership (using discriminant analysis). In order to avoid over-parameterisation, the CAP analysis produces diagnostics to select the appropriate subset of PCO axes used in the discriminant analysis i.e. the number of axes where the probability of misclassifying a new point to the wrong group is minimised (Anderson et al 2008).

prop.G: the proportion of the variation in the data captured by the number of PCO axes selected (1.0 = 100% variation is explained)

Trace statistic: the sum of the squared canonical correlations, and the associated permutation test *P* value

δ_1^2: the size of the first squared canonical correlation and the associated permutation test *P* value

m: the number of PCO axes selected to perform the discriminant analysis

% allocation: the leave-one-out allocation success performed in the discriminant analysis

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