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

2   **communities**

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19   **Highlights**

20   -   Natural experiment compared burnt vs unburnt sites to determine wildfire impacts

21   -   Contrasting effects in native woodland vs managed pasture soils

22   -   Soil NH_4 increased post-fire in woodland soil whilst NO_3 increased in pasture soil

23   -   Rapid change with greater diversity in woodland bacterial community composition

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27

28 **Abstract**

29

30 A wildfire which overran a sensor network site provided an opportunity (a natural experiment) to

31 monitor short-term post-fire impacts (immediate and up to three months post-fire) in remnant

32 eucalypt woodland and managed pasture plots. The magnitude of fire-induced changes in soil

33 properties and soil microbial communities was determined by comparing (1) variation in fire-

34 adapted eucalypt woodland *vs.* pasture grassland at the burnt site; (2) variation at the burnt

35 woodland-pasture sites with variation at two unburnt woodland-pasture sites in the same locality;

36 and (3) temporal variation pre- and post-fire. In the eucalypt woodland, soil ammonium, pH and ROC

37 content increased post-fire, while in the pasture soil, soil nitrate increased post-fire and became the

38 dominant soluble N pool. However, apart from distinct changes in N pools, the magnitude of change

39 in most soil properties was small when compared to the unburnt sites. At the burnt site, bacterial

40 and fungal community structure showed significant temporal shifts between pre- and post-fire

41 periods which were associated with changes in soil nutrients, especially N pools. In contrast,

42 microbial communities at the unburnt sites showed little temporal change over the same period.

43 Bacterial community composition at the burnt site also changed dramatically post-fire in terms of

44 abundance and diversity, with positive impacts on abundance of phyla such as Actinobacteria,

45 Proteobacteria and Firmicutes. Large and rapid changes in soil bacterial community composition

46 occurred in the fire-adapted woodland plot compared to the pasture soil, which may be a reflection

47 of differences in vegetation composition and fuel loading. Given the rapid yet differential response

48 in contrasting land uses, identification of key soil bacterial groups may be useful in assessing

49 recovery of fire-adapted ecosystems, especially as wildfire frequency is predicted to increase with

50 global climate change.

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

54

55   **1. Introduction**

56

57   Wildfires are notoriously unpredictable disturbances. However, fire is an important driver of

58   ecosystem function, vegetation dynamics and nutrient cycling. The magnitude of fire impacts is

59   determined by the interaction between the affected ecosystem, climate and the fire regime. Fire

60   regimes are characterised by interactions between key components such as fire intensity, frequency,

61   size, seasonality, type and severity (Flannigan et al., 2009). There has been considerable interest in

62   understanding belowground fire impacts, especially on soil microbial communities where fire has

63   direct and indirect impacts (Hart et al., 2005; Muñoz-Rojas et al., 2016; Neary et al., 1999). Direct

64   effects result from heat transfer from the soil surface to lower depths, whereas indirect fire impacts

65   are mediated by above- and below-ground interactions between plants and the soil environment.

66   Soil heating affects soil microbial communities through cell death, causing reductions in biomass and

67   diversity (Neary 1999; Dooley and Treseder, 2012). In contrast, greater fire-induced impacts on soil

68   microbial communities, in terms of spatial extent and longevity, are mediated through changes to

69   soil organic matter quality, soil moisture retention, soil pH and buffering capacity and changes in

70   nutrient availability. Fire also impacts rhizodeposition, plant litter accumulation, and ash and

71   charcoal content which alter nutrient cycling and soil microbial communities (Cobo-Díaz et al., 2015).

72   The application of molecular techniques to post-fire studies is advancing our understanding of fire-

73   induced changes on microbial communities, especially with detailed identification of the affected

74   communities (Ferrenberg et al., 2013; Goberna et al., 2012; Mikita-Barbato et al., 2015); however,

75   further work is required into immediate post-fire impacts (i.e. days since fire) and time to recovery.

76

77   Fire regimes in fire-adapted biomes have led to the evolution of plant fire survival traits (Bond and

78   Keeley, 2005). These functional traits facilitate rapid (days to weeks) post-fire regeneration, and

79 include post-fire basal or epicormic resprouting (e.g. eucalypts) (Clarke et al., 2015; Gill, 1975);

80 underground storage organs (e.g. acacia); and heat or smoke-stimulated flowering and seed

81 germination (Bond and Keeley, 2005; Gill, 1975). Specific soil microbial fire adaptations have also

82 been observed: some Australian fungi are pyrophilous and have underground storage organs which

83 enable them to produce fruiting-bodies two days post-fire (McMullen et al., 2011). While fire-

84 adapted systems have evolved protective mechanisms, they could still be substantially changed in

85 the long-term, and sometimes irreparably, if predicted changes in climate and fire regime occur, i.e.,

86 increases in fire frequency combined with shorter recovery times (Flannigan et al., 2009).

87 Furthermore, changes in management practices, such as more frequent low-intensity prescribed

88 burning to control fuel loads, urban encroachment, and land use change place additional pressures

89 on the ability of fire-adapted ecosystems to recover (Bardsley et al., 2015).

90

91 Because of their unpredictable nature, wildfire studies are reactive, often opportunistic, and may

92 not have adequate control sites for comparison. The length of time since fire varies in wildfire

93 studies, ranging from immediate and short-term (days, weeks, months) (Dannenmann et al., 2011;

94 Ferrenberg et al., 2013; Muñoz-Rojas et al., 2016)) to longer-term (years, decades) (MacKenzie and

95 DeLuca, 2006; Smithwick et al., 2009; Stephan et al., 2015). Investigating post-wildfire recovery and

96 resilience also presents challenges in replication, establishing 'before-fire' baseline conditions and

97 locating similar, but unburnt, control sites. Despite these challenges, understanding the relationships

98 between soil properties, microbial communities and soil function at different post-fire timescales

99 has the potential to identify early indicators of weakening ecosystem resilience and recovery in a

100 range of land use systems.

101

102 A wildfire which overran a site that was part of a multi-year environmental monitoring study (de

103 Menezes et al., 2015; Prendergast-Miller et al., 2015) provided an opportunity to characterise short-

104 term changes in soil properties and soil microbial communities in managed pasture and remnant

105   native eucalypt woodland plots. We focused on short-term temporal variation, given the relatively

106   rapid recovery of fire-adapted eucalypt woodland systems (Clarke et al., 2015; Gill, 1975; Shakesby

107   et al., 2007). The objectives were to (1) monitor short-term temporal variation in soil and microbial

108   parameters; (2) identify soil factors which related to temporal shifts in microbial communities; and

109   (3) identify microbial groups which responded positively and negatively to fire-induced temporal

110   change in soil properties. Finally, as it is difficult to directly ascertain the scale of fire impacts, the

111   magnitude of fire as an environmental disturbance was determined by including a comparison of

112   temporal (seasonal) variation at two unburnt (control) sites within the same locality as the burnt

113   site. This study provided a rare opportunity to discuss temporal variation in the context of fire

114   disturbance because data were also available from a sampling campaign which took place three

115   weeks prior to the wildfire. We therefore tested the hypothesis that the temporal shift in soil

116   properties and microbial communities would be different between burnt and unburnt sites in each

117   land use.

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119

120   **2. Materials and Methods**

121

122   2.1 Study sites and sampling design

123

124   The wildfire occurred at one of three pastoral farms (Glenrock, Bogo, Talmo) which have been

125   previously described (de Menezes et al., 2015; Prendergast-Miller et al., 2015). A map of the study

126   site location is provided in the Supplementary Information (Fig. S1). The naturally-occurring wildfire

127   spread over >14000 ha of farmland which included the Glenrock farm (the burnt site). The farms at

128   Bogo and Talmo were not affected and therefore provided unburnt pseudo-control sites for this

129   study. The farms are within 15 km of each other and are located in the seasonally dry temperate

130   region of New South Wales (Australia) on brown sodosols (Isbell, 2002). Glenrock is on volcanic and

5

131 sedimentary rocks of the Silurian Douro Group. Bogo and Talmo are on Mountain Creek Volcanics of

132 the Devonian Black Range Group (Cramsie et al., 1975). As described in Prendergast-Miller et al.,

133 (2015), the main plant species in the three pasture sites was subterranean clover (*Trifolium*

134 *subterraneum* L.) with some annual and perennial grasses [e.g. phalaris (*Phalaris aquatic* L.)]. The

135 woodlands at Glenrock and Bogo consisted of remnant native woodland areas adjacent to pasture

136 fields: the *Eucalyptus* woodland was relatively open with a native grassy understorey; the Bogo

137 woodland had some exotic grass species. At Talmo, the pasture lay adjacent to the Burrinjuck Nature

138 Reserve (NSW); in this remnant native woodland, *Eucalyptus* and *Acacia* tree species had a more

139 dense cover compared to the other two woodland plots. The three study sites were located on

140 mature (> 40 years) sheep-grazing enterprises typical of the farming landscape in rural south-eastern

141 Australia. In this region, land clearing (by tree logging and fire) since the mid-nineteenth century, as

142 well as soil degradation and increasing pressure on land resources has created an increasingly

143 fragmented remnant native woodland-managed pasture landscape (Prober et al., 2002).

144

145 Monitoring sites on paired managed pasture-remnant native woodland plots were established at

146 each farm in October 2012 (see Fig. S2). On each adjacent pasture and remnant native woodland, a

147 plot (100 x 100 m) was gridded and 25 wireless sensor nodes were deployed (150 nodes in total).

148 The layout of the sensor nodes was determined by spatial prediction variance (Cressie, 1993) based

149 on the variability of soil and microbial parameters measured in de Menezes et al., (2015). The

150 original objective of the study was to determine spatial and temporal variation in contrasting

151 habitats using an environmental sensor network. The physical location of the nodes marked the soil

152 sampling points to calibrate sensor- and soil-derived measurements, and the first soil samples were

153 taken from all nodes in December 2012 (150 node samples; 25 samples per plot). The sensor nodes

154 marked the sampling positions, and soil samples were taken within 0.5 m of the node. Due to

155 temporal sampling, care was taken to avoid re-sampling the previous hole. Following the wildfire in

156 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

183   using the fluorimetric o-phthalaldehyde-b-mercaptoethanol (OPAME) method (Jones et al., 2002);

184   dissolved organic C (DOC) and total dissolved N (TDN) were analysed on a total organic C (TOC)

185   analyser (Shimadzu TOC-VCSH/CSN þ TNM-1; Kyoto, Japan). Soil microbial biomass C (Cmic) and N

186   (Nmic) were measured on the TOC analyser after fumigating additional soil samples with chloroform

187   for 24 h and extracting these samples with 0.5 M K_2SO_4 (1:5 w/v ratio) (Vance et al., 1987). Microbial

188   biomass C and N were corrected using correction factors of 0.45 and 0.54 for Cmic and Nmic

189   respectively (Brookes et al., 1985; Wu et al., 1990). Dissolved organic N (DON) was calculated as the

190   difference between TDN and NH_4^+ and NO_3^-. Available phosphorus (P) was determined by extracting

191   soil samples with 0.5 M $NaHCO_3$ at pH 8.5 (1:100 w/v ratio) (Rayment and Lyons, 2011) and

192   quantified using Malachite green (Irving and McLaughlin, 1990). Air-dried soil subsamples were

193   milled and mid-infrared (MIR) spectroscopy was used to estimate soil C fractions (particulate, humic,

194   and resistant organic C: POC, HOC, ROC respectively) using the prediction algorithms developed in

195   Baldock et al., (2013). These prediction algorithms were developed on Australian agricultural soils

196   (>500 samples, including those within the study region, Yass, NSW): spectra from the soils of this

197   study fell within the calibration, and the error statistics associated with the predicted fraction were

198   below threshold levels. Although not a direct measure of charcoal, the estimated ROC fraction is

199   considered to be comprised of the poly-aryl C structures consistent with charred plant biomass and

200   lignin-derived aryl C (Baldock et al., 2013). Soil pH was measured in a 1:2 soil:water suspension, and

201   soil moisture content determined after drying at 105 °C overnight. All results are expressed on a soil

202   dry weight basis.

203

204   2.3 Soil molecular analyses

205

206   2.3.1 Soil DNA extraction

207

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^{TM} Picogreen (Life Technologies^{TM}, 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 Technologies^{TM}, 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^{TM}

228   formamide and 0.3 µl of GeneScan^{TM} 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^{TM} (Life Technologies^{TM}, 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

260   using the Greengenes reference files (DeSantis et al., 2006), with a confidence threshold of 60%.

261   OTUs classified as eukaryotic, archaeal, mitochondrial or as plastid were removed as well as

262   sequences not classified to domain level (bacteria). Rare sequences (those OTUs occurring < 100

263   times in the whole dataset, roughly corresponding to OTUs occurring at one sequence per sample on

264   average) were also removed. The resulting dataset had a total of 7,737,445 sequences, 4513 OTUs,

265   and the average, maximum and minimum numbers of sequences per sample were 87,925, 151,516

266   and 49,224, respectively. OTU abundance data was rarefied to 49,224 using the rarefy_even_depth

267   command in the phyloseq statistical package (McMurdie and Holmes, 2013) before statistical

268   analyses, except for DESeq2 OTU enrichment analysis for which non-rarefied data was used

269   following the recommendations of McMurdie and Holmes (2014). Coverage of the subsampled

270   dataset was >0.99 (Good's coverage estimator) for all samples.

271

272

273   **3. Data analysis**

274

275   Our original sample design of 150 environmental sensors deployed over three paired pasture-

276   woodland plots was set up to maximise investment and long-term spatial-temporal data capture. As

277   the wildfire occurred after our first sample time (December 2012) we decided to continue sampling

278   as per our original plan (25 node samples per plot) to allow comparison with pre-fire data from the

279   same soils in one burnt and two unburnt sites. However, this inevitably meant the post-fire study at

280   Glenrock was pseudoreplicated, which is a consequence of investigating a real-life environmental

281   disturbance in ecology. There is a lot of debate on how to deal with pseudoreplication (e.g. Davies

282   and Gray, 2015; Hurlbert, 1984; Millar and Anderson, 2004) which we account for below in the

283   analyses. This was an opportunistic study, and we have used the data to describe short-term post-

284   fire temporal changes and compare these to seasonal changes at our study sites.

285

286

287 3.1 Data pre-treatment

288

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

306

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

335   studies (Anderson et al., 2008). PERMDISP was performed on Euclidean (soil data) and Bray-Curtis

336   (microbial data) resemblance matrices; the *P*-value was determined using 999 permutations.

337

338   In order to determine the magnitude of change in individual soil properties over time, pasture or

339   woodland soil properties were tested separately by repeated measures one-way ANOVA, with

340   sample number as subject and time as level. Where assumptions of variance were not met, the

341   repeated measures test was performed on ranks (SigmaPlot v.13.0).

342

343   PERMANOVA indicated significant differences in microbial community structure between time and

344   land use (Table 1), however, these patterns were masked when observed using unconstrained nMDS

345   plots (see Supplementary Information, Fig. S3). Therefore, canonical analysis of principal coordinates

346   (CAP) was used to quantify and visualise these differences in the Glenrock pasture and woodland

347   soils (Anderson and Robinson, 2003; Anderson and Willis, 2003). The CAP procedure enables

348   characterisation of sample groups, by visualising differences and assessing the distinction between

349   groups in multivariate space (Anderson et al., 2008). Whereas nMDS is an unconstrained ordination,

350   CAP is a constrained ordination technique which enables discrimination among groups along an axis

351   through the multivariate data cloud (Anderson et al., 2008). In the CAP routine, we tested the *a*

352   *priori* hypothesis of there being no difference in multivariate location among groups i.e. of the

353   microbial community structure (bacterial and fungal T-RFLP) amongst the sampling time classes for

354   each land use by constraining the ordination to those classes. The strength of the CAP result (Table

355   S1) was determined by the trace statistic and the percentage cross-validation allocation success, as

356   well as by obtaining a *P*-value using permutation tests (999 tests). Microbial community structure in

357   pasture and woodland soils were then correlated to the respective soil properties using an overlay

358   vector function (Pearson correlation, r). This is an exploratory tool to identify soil properties which

359   increase or decrease with the CAP axes (Anderson et al., 2008).

360

361 Finally, land use and temporal changes in bacterial community composition (using sequenced data)

362 at the burnt site were determined. Identification of OTU enrichment after the fire was based on the

363 DESeq2 (Love et al., 2014) extension from the phyloseq package following the approach outlined in

364 McMurdie and Holmes (2014). DESeq2 was run using the Wald test, with automatic filtering of low

365 abundance OTUs, and an alpha of 0.01. Adjusted P-values were calculated automatically by DESeq2.

366 The results of the DESeq2 analysis were visualised using the ggplot2 package in R (Wickham, 2009).

367

368 3.3 Comparison of seasonal shifts at wildfire and unburnt sites

369

370 Although the impact of the fire at Glenrock cannot be directly tested, the magnitude of temporal

371 shifts in soil properties and bacterial community structure were compared between the three farms

372 and discussed in the context of the fire disturbance.

373

374 Patterns in the multivariate data between site, month and land use for soil properties and soil

375 bacterial community structure (T-RFLP data) were first explored using nMDS plots (Fig. S3).

376 PERMANOVA was used to test for significant differences between these factors using a three-way

377 crossed design (9999 permutations of residuals under a reduced model, with type III partial sums of

378 squares): site (fixed, 3 levels: Glenrock, Bogo, Talmo); month (fixed, 2 levels: December 2012, April

379 2013); and land use (fixed, 2 levels: pasture, woodland) (Table 2). As PERMANOVA is sensitive to

380 dispersions, the PERMDISP routine was performed using 'site-month-land use' as the group factor;

381 the P-value was determined using 999 permutations (Table 2). PERMANOVA indicated significant

382 differences in bacterial community structure between site, land use and sampling times, however,

383 these patterns were masked when observed using unconstrained nMDS plots (see Supplementary

384 Information, Fig. S3). Therefore, the CAP approach was used to test the hypothesis that the temporal

385 i.e. seasonal shift (December 2012 *vs.* April 2013) in bacterial community structure was different

386 between burnt (Glenrock) and unburnt (Bogo and Talmo) sites in each land use. Diagnostic results

387 are given in Table S1. Soil properties associated with these temporal shifts were identified using

388 Pearson correlations. Seasonal differences to determine the magnitude of change in individual soil

389 properties (December *vs.* April, i.e. the pre- and post-fire period at Glenrock) at the three farms but

390 within the same land use were tested using a 2-way ANOVA (SigmaPlot v.13.0).

391

392

393 **4. Results**

394

395 4.1 Changes in soil and microbial community structure at the Glenrock wildfire site

396

397 The wildfire destroyed the monitoring site at Glenrock, and temporal samples were taken to assess

398 the short-term variation in soil properties and microbial communities within the burnt site. Non-

399 linear MDS plots of soil properties and bacterial and fungal community structure (T-RFLP) indicated

400 differences in land use; in addition, temporal changes as well as an indication of increased variability

401 were also observed in the bacterial and fungal nMDS plots (Fig. S3). PERMANOVA showed significant

402 time x land use interactions in soil properties and microbial groups (Table 1). However, time was not

403 significant in the soil data, and this was also inferred from the soil nMDS plot. As PERMANOVA is

404 sensitive to dispersion, and significant effects could be due to multivariate location and/or

405 dispersion, further tests for dispersion were conducted (PERMDISP; Table 1). For the soil data, there

406 was no significant difference in multivariate dispersion ($P > 0.05$); therefore PERMANOVA indicated a

407 significant land use difference in soil properties which was not due to multivariate dispersion.

408 However, differences in dispersion were evident in the bacterial ($P = 0.02$) and fungal ($P = 0.03$) data

409 sets (Table 1). Further PERMDISP analyses using land use or time as factors indicated that in the

410 microbial data sets, time did show significant dispersion (bacteria $P < 0.001$; fungi $P < 0.05$), but land

411 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

489 community structure. PERMDISP analysis also indicated significant dispersion in both data sets.

490 Therefore, the CAP approach was used to visualise the site and temporal differences in each land

491 use. Seasonal differences in bacterial community structure from December 2012 to April 2013 for

492 the three farms are shown in Fig. 5 (see Table S1 for CAP diagnostics). At the unburnt sites (Bogo

493 and Talmo), bacterial communities showed little distinction in structure between December 2012

494 and April 2013 in both pasture and woodland soils. At the unburnt sites bacterial community

495 structure was correlated with soil moisture and microbial biomass C and N contents. However, at the

496 Glenrock burnt site bacterial community structure in both land uses between December (pre-fire)

497 and April (three months post-fire) was more distinct in comparison to the unburnt sites, especially at

498 Glenrock woodland. The temporal shifts in bacterial community structure at the burnt site were

499 correlated with nitrate in the pasture soil, and with NH_4^+ and DON in the woodland soil. Therefore,

500 change in soil bacterial communities between December and April was apparently greater at the

501 wildfire site compared to the unburnt sites and in each land use the shift was associated with

502 different N pools.

503

504 The temporal changes in soil properties identified at the Glenrock fire site were put into context by

505 comparing seasonal December to April differences at all three farms. Comparison of differences in

506 individual soil properties between the three sites (Supplementary information Fig. S5) indicated

507 significant increases between December 2012 and April 2013 at Glenrock in pasture soil NO_3^-

508 (average April 2013 pasture soil content 23 mg N kg^{-1}). Changes in these properties were greater

509 than at Bogo and Talmo (average April 2013 pasture soil content 4 and 9 mg N kg^{-1} respectively).

510 Significant increases were also observed in Glenrock woodland soil NH_4^+ (average April 2013

511 woodland soil content 8.7 mg N kg^{-1}) compared to Bogo and Talmo (average April 2013 woodland

512 soil content 2.2 and 0.5 mg N kg^{-1} respectively). However, changes in other soil properties were not

513 so dramatic when compared to the unburnt sites. For example, DON increased post-fire at Glenrock

514 pasture and woodland: in the pasture soil, the temporal increase was greater at Bogo; in the

515   woodland soil, the post-fire concentration reached was similar to that measured at the Bogo

516   unburnt site. Declines in DOC and microbial biomass N at Glenrock were also measured at the

517   unburnt sites. The post-fire increase in Glenrock woodland soil pH (average pH 5.5 in April 2013) did

518   not raise the pH level above that of the unburnt sites (average woodland soil pH at Bogo and Talmo

519   5.8 and 5.6 respectively). Soil properties such as FAA and soil C fractions (POC, ROC) showed no

520   significant temporal change. Therefore, apart from NH_4^+ and NO_3^-, post-fire changes in most soil

521   properties at the burnt site were similar when compared to seasonal changes in the December to

522   April period occurring at the unburnt sites (Fig. S5).

523

524

525   **5. Discussion**

526

527   Post-fire wildfire studies are reactive natural experiments and may lack adequate control or unburnt

528   sites and replication for assessing fire-induced changes. In this study, a wildfire event occurred three

529   weeks after soil sampling at three paired native woodland-managed pasture plots, thus providing

530   approximate pre-fire baseline conditions in soil properties and microbial community structure. As

531   only one farm was affected (Glenrock), comparison of variation at the wildfire site with variation at

532   two unburnt sites in the same locality provided a means to assess the magnitude of potential fire-

533   induced changes in soil properties and microbial communities on two contrasting land uses.

534   Importantly, shifts in bacterial community structure and changes in soil properties (especially NO_3^-

535   and NH_4^+) from December 2012 (pre-fire) to April 2013 (post-fire) were greater at the wildfire

536   pasture and woodland plots compared to the unburnt plots. The shifts in bacterial community

537   structure (T-RFLP data) at the unburnt sites were associated with soil moisture content, while both

538   bacterial and fungal shifts (T-RFLP data) at the burnt site were associated with changes in pH and N

539   pools i.e. higher contents of NO_3^- in pasture soil and NH_4^+ in woodland soil only observed at the burnt

540   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

28

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

746 soil communities. Given that Australian ecosystems are fire-adapted, fire frequency will be

747 important in determining longer-term outcomes of wildfire events, such as loss of native species,

748 invasion of exotic species and decline in soil function (Prober et al., 2002; Tomkins et al., 1991).

749

750

751 **6. Conclusion**

752

753 A natural wildfire event provided an opportunity to monitor the immediate and short-term temporal

754 variation in soil and microbial parameters in contrasting managed and semi-natural land uses. Clear

755 differences were observed between managed pasture and remnant native woodland plots, which

756 could be related to fire, soil and vegetation interactions. Importantly, the magnitude of disturbance

757 was determined by comparing post-fire variation with temporal variation at two unburnt sites that

758 had similar vegetation, climate and soils as the burnt site. Australian native ecosystems are fire-

759 adapted systems and plants have evolved various traits which promote rapid recovery. Soil microbial

760 communities showed greater temporal shifts at the burnt site compared to the unburnt sites, and

761 these shifts were related to key changes in soil N pools which were not observed at the unburnt

762 sites. Importantly, although bacterial community composition was negatively affected in both land

763 uses, recovery and increases in abundance and diversity were much faster in the remnant woodland

764 soil. This suggests that fire-adapted mechanisms may have been altered following land use

765 conversion to pasture. However, differences in fuel loading due to contrasting vegetation

766 composition will also have played a role in determining fire impacts belowground. As the soil

767 microbial community showed a greater magnitude of change than the measured soil properties, it is

768 important to include detailed measures of soil microbial community structure and composition in

769 post-fire studies.

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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

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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* <

1018   0.001). Soil properties correlating with the first and second CAP axes >0.3 (Pearson correlation) are

1019   shown in bold.

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1021   Fig 3. Differentially abundant OTUs after the wildfire at Glenrock pasture (A, C, E) and woodland (B,

1022   D, F) plots. The OTUs are arranged by genus on the x axis and each dot represents an OTU, colours

1023   represent phyla. Differential abundance was analysed by comparing OTU abundance in January,

1024   February and April (2013) with the pre-fire community in December 2012 using DESeq2 extension in

1025   the phyloseq package (alpha = 0.01). Comparisons in the pasture plot are January *vs.* December (A),

1026   February *vs.* December (C), April *vs.* December (E); in the woodland plot January *vs.* December (B),

1027   February *vs.* December (D), April *vs.* December (F). The y axis indicates fold change in log base 2

1028   units. OTUs above 0 (indicated by dashed line) are considered enriched after fire, those below 0

1029   decreased in abundance compared to December 2012. The sequence data was not rarefied as per

1030   McMurdie et al., 2014. Plots were generated using ggplot2 (Wickham et al., 2009).

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1033   Fig. 4. Boxplots representing the percentage abundance of members of the orders

1034   Nitrosomonadales (A) and Bacillales (B) in Glenrock pasture and woodland before (December 2012)

1035   and after the wildfire (January to April 2013). Upper and lower box limits represent the first and

1036   third quartiles, the upper and lower lines represent the maximum and minimum abundances and

1037   dots represent outliers. Plots were generated using ggplot2 package in R (Wickham et al., 2009).

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1039   Fig. 5. Biplots showing seasonal differences from December 2012 (summer) to April 2013 (autumn)

1040   in soil bacterial community structure (T-RFLP data) in pasture (A) and woodland (B) plots at three

1041   farms. The wildfire was at Glenrock in January 2013; Bogo and Talmo farms were unburnt. Soil

1042   properties correlating (r > 0.5) with the first two axes are shown in bold. All CAP axes are significant

1043   *P* < 0.001.

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1046　　Table 1

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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

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1059   Table 2

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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
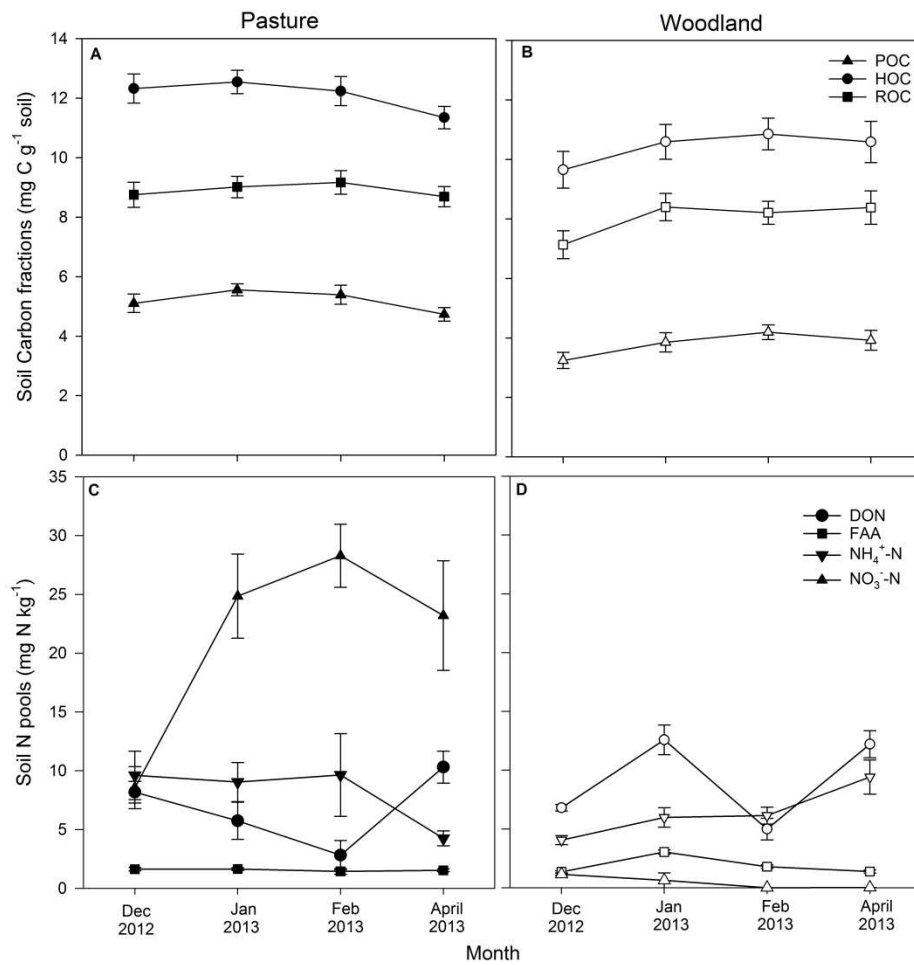
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1083   Fig 2
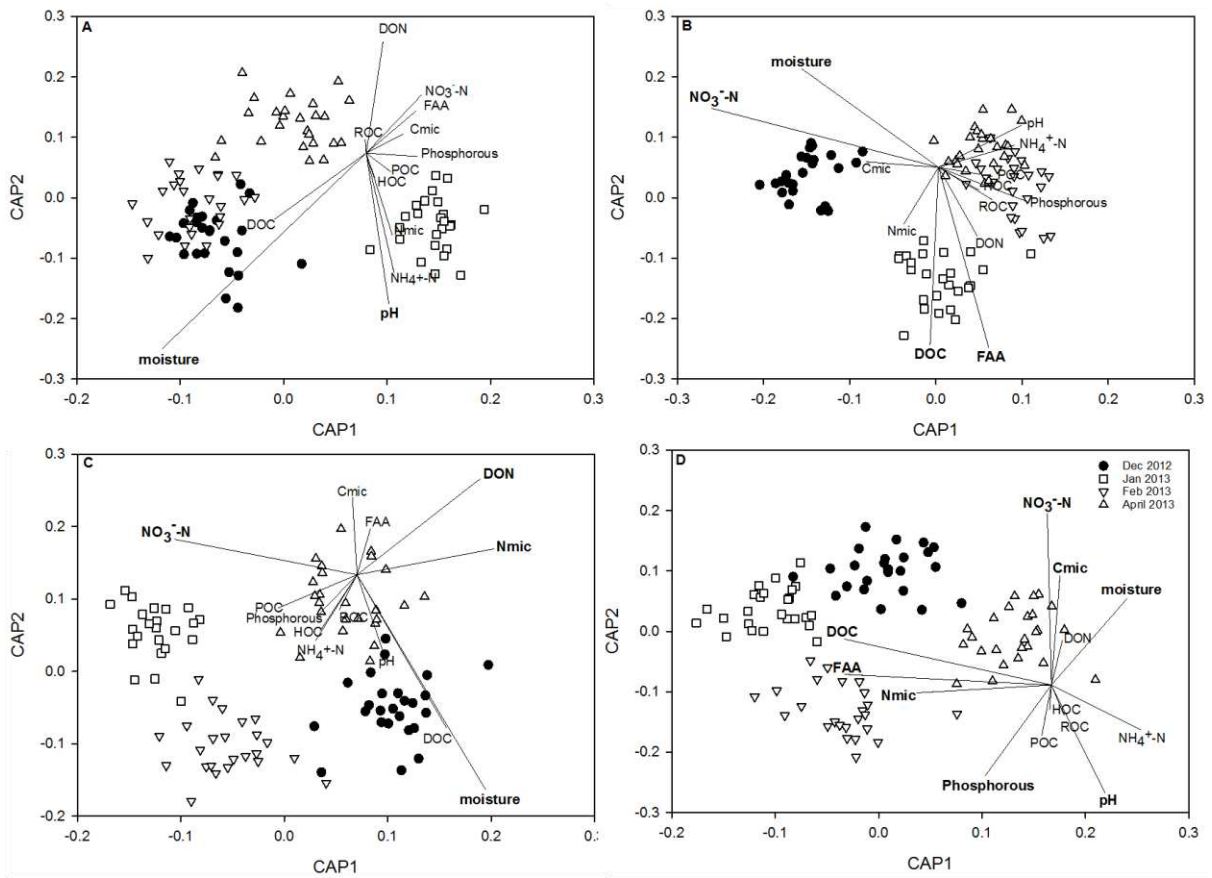
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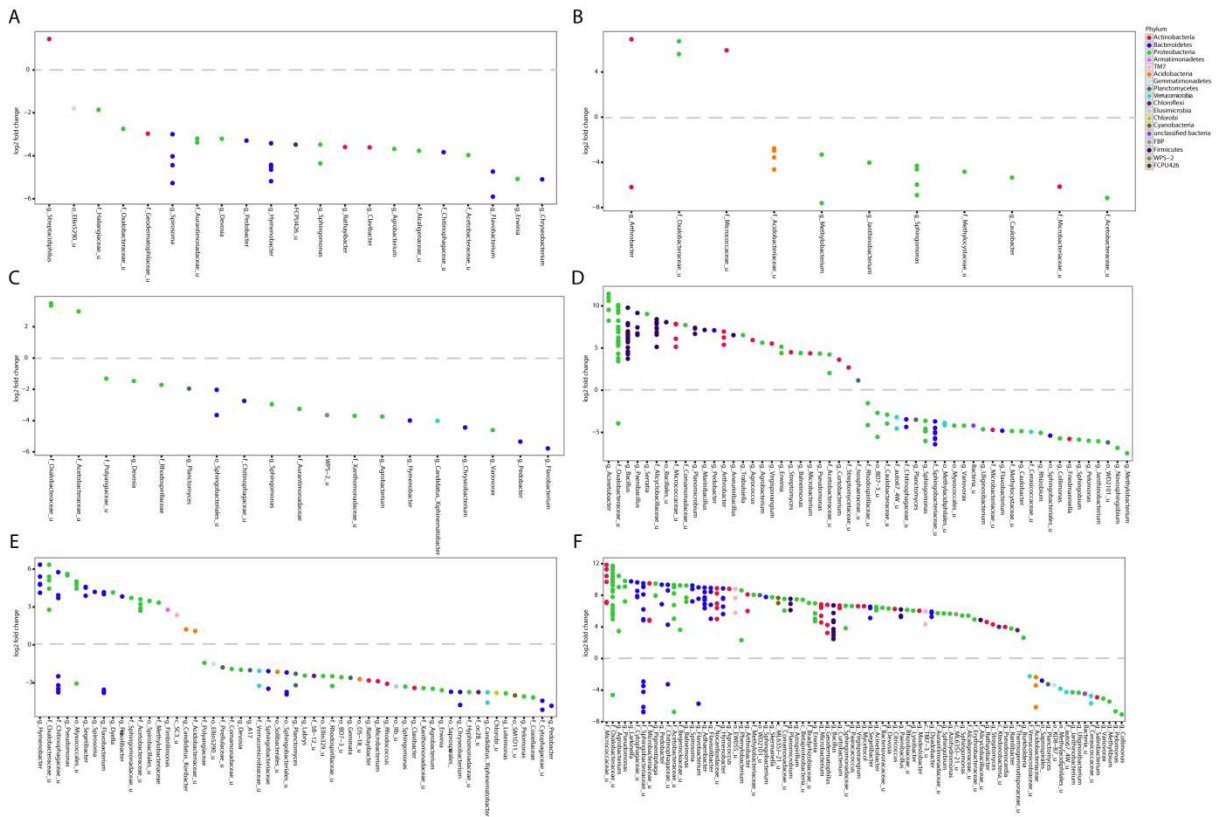
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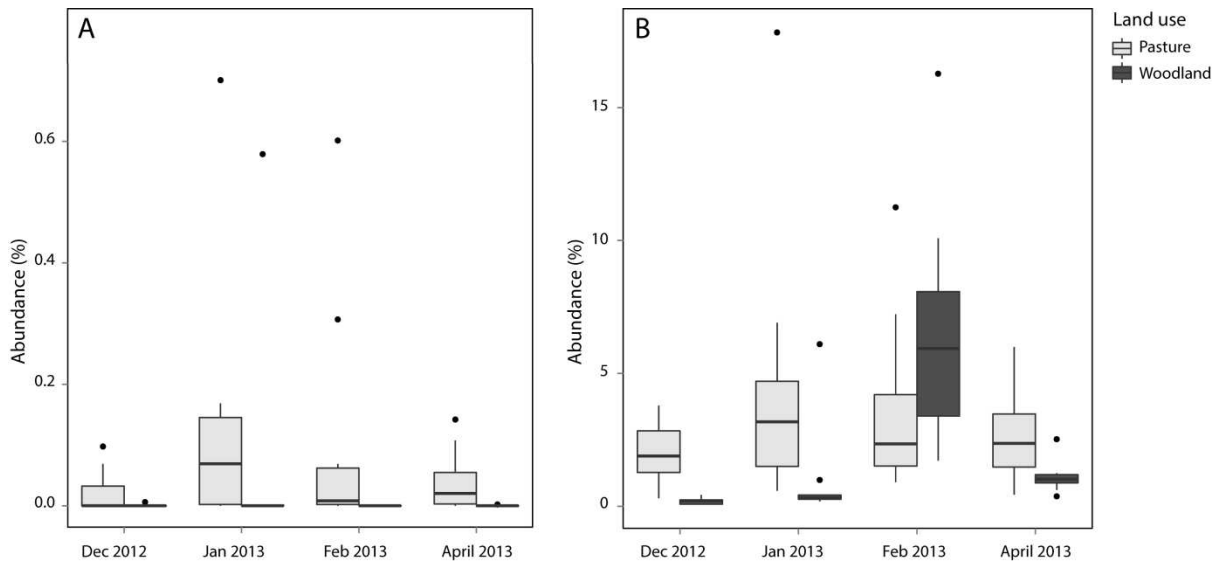
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1122   Fig 5
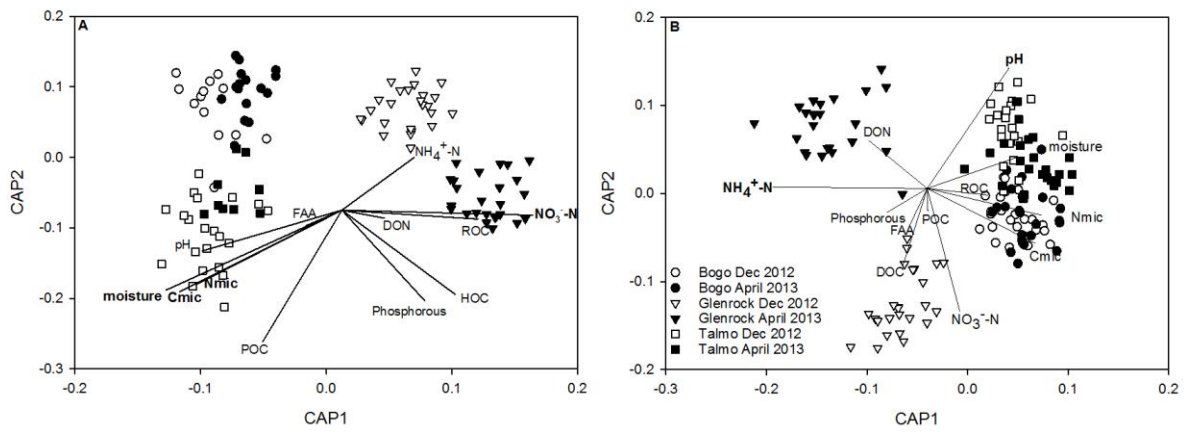
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1134   SUPPLEMENTARY INFORMATION

1135

1136   **Wildfire impact: natural experiment reveals differential short-term changes in soil microbial**

1137   **communities**

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1140   Andrew Bissett[6], Geoff Baker[3], Mark Farrell[1], Tim Wark[7], Alan E. Richardson[3] and Peter H. Thrall[3]

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1146   [5]Data61, Private Bag 10, Clayton South, VIC 3169, Australia

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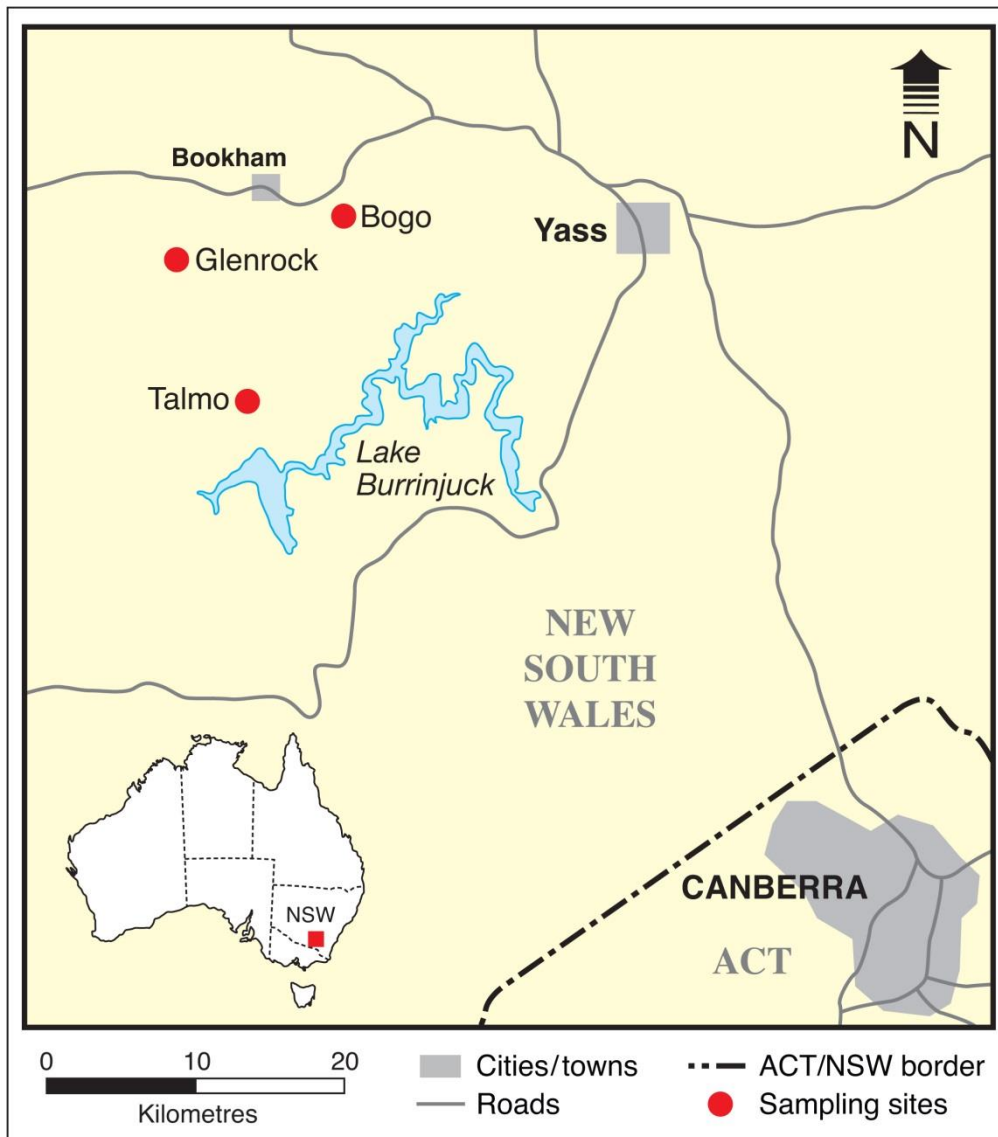1148   [7]Data61, QCAT, Pullenvale, QLD 4069, Australia

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1150   *corresponding author:* M.T. Prendergast-Miller

1151   Environment Department, University of York, Heslington, York, YO10 5NG, UK
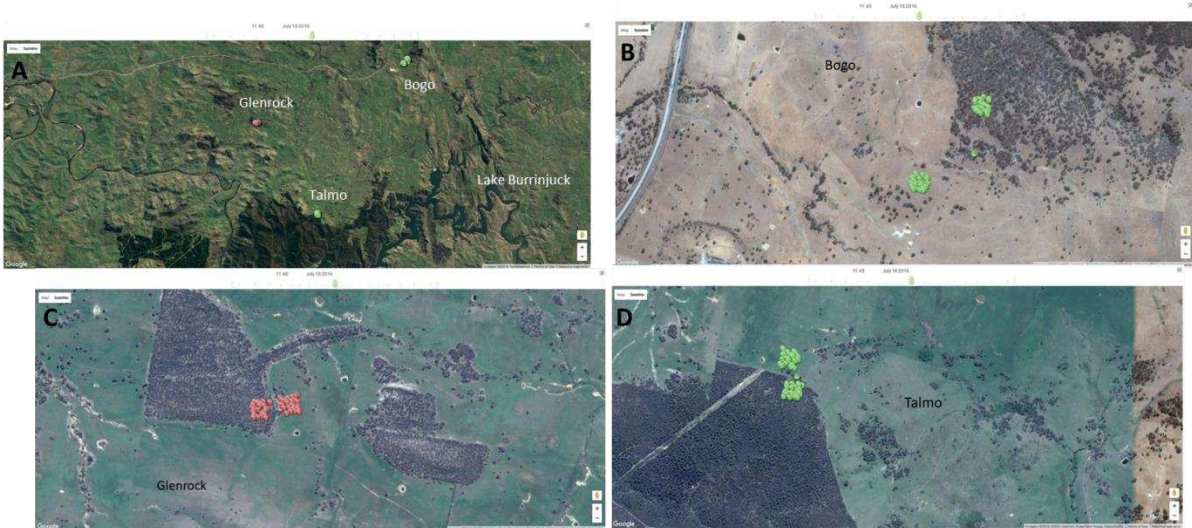
1152   Email: m.prendergastmiller@gmail.com

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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).

A: Glenrock: Temporal comparison (Dec 2012-April 2013)

Soil properties

Bacterial T-RFLP

Fungal T-RFLP

Bacterial sequenced
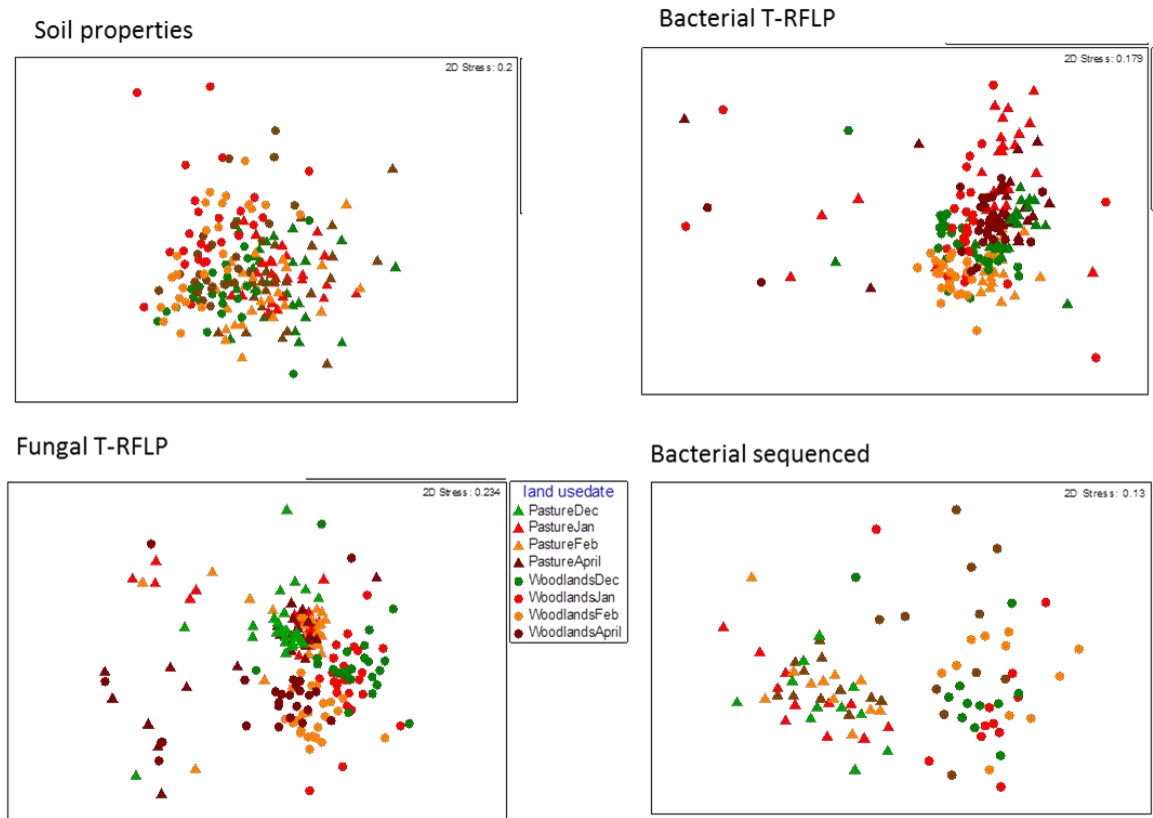
B: Glenrock, Bogo, Talmo: Seasonal comparison (Dec 2012 & April 2013)

Bacteria T-RFLP

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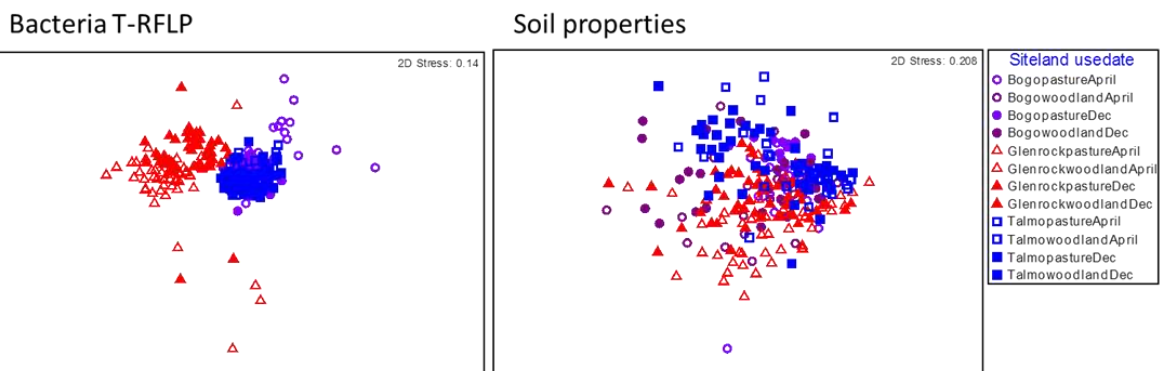
Fig. S3. nMDS plots showing unconstrained ordination of pre- and post-fire samples at (A) the Glenrock wildfire site (Dec 2012, Jan, Feb, April 2013) and (B) the seasonal comparison at Glenrock (burnt), Bogo and Talmo (unburnt sites) (Dec 2012 and April 2013).

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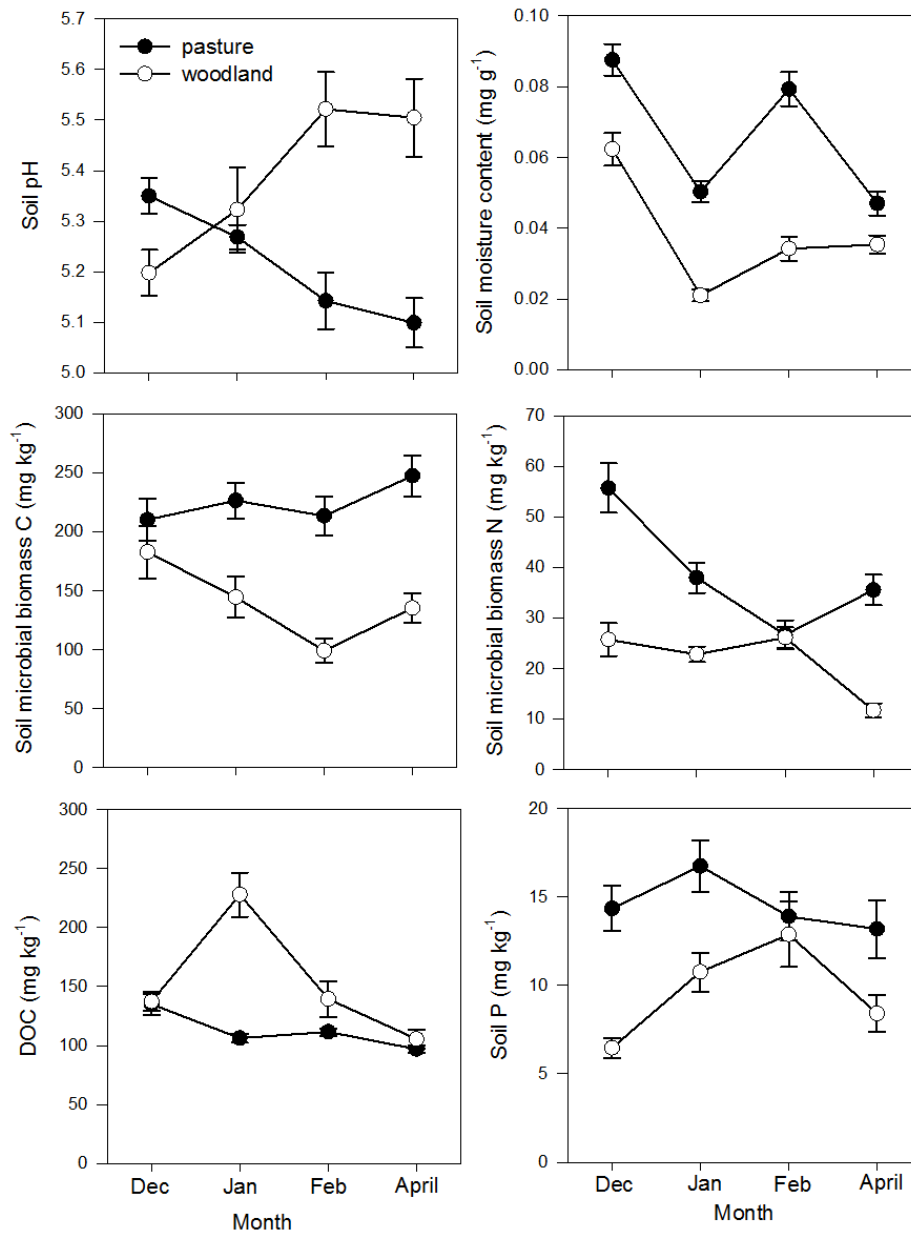

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1184　Fig. S4. Temporal changes in soil properties at the Glenrock pasture and woodland sites, December

1185　2012 (pre-fire) and post-fire (January, February and April 2013). Data are means (n = 25) with bars

1186　indicating ± 1 standard error. There was a significant (at α = 0.05) effect of month on all soil

1187　properties (except for pasture soil microbial biomass C, P > 0.05).
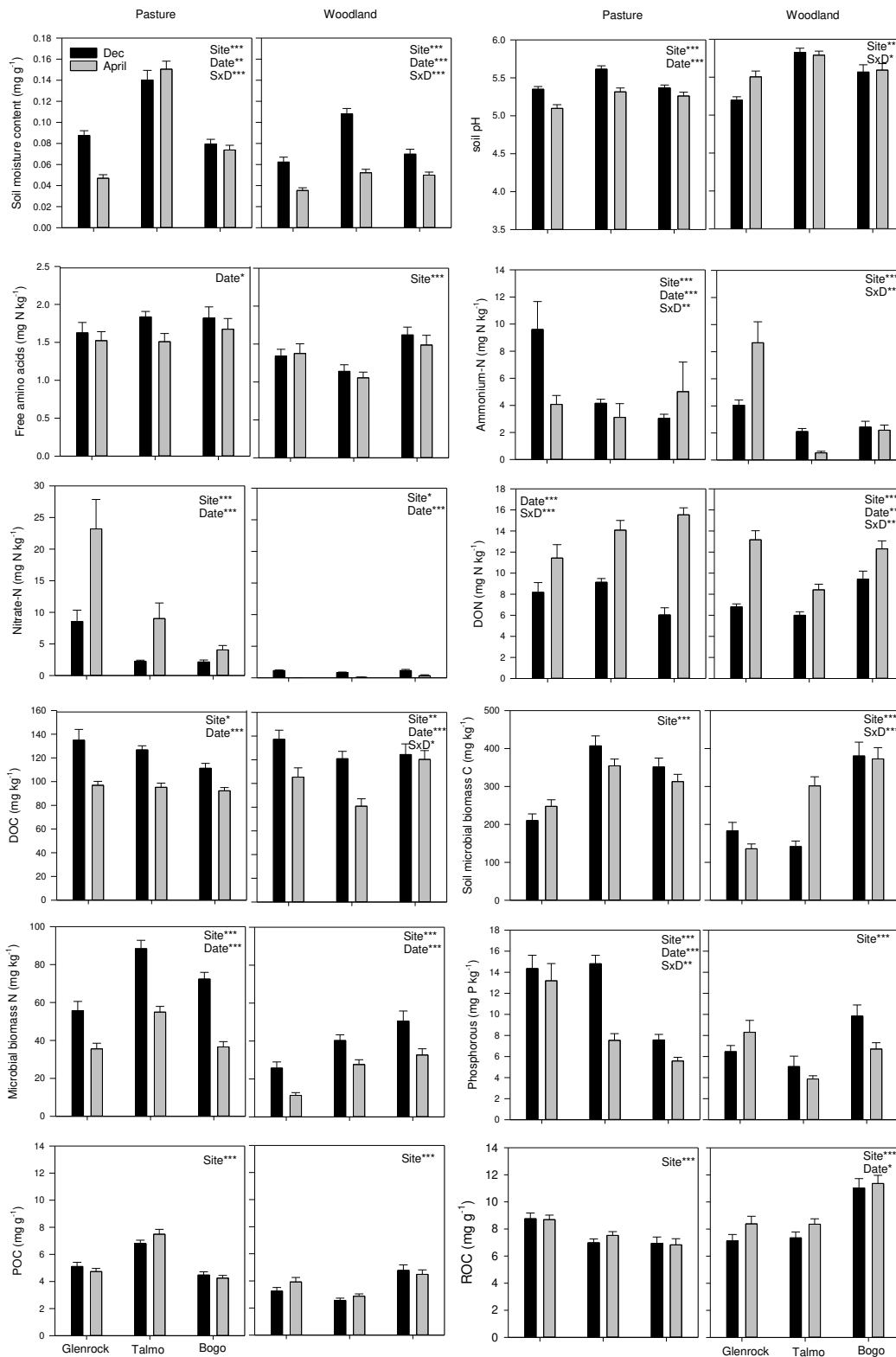
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1196   Fig. S5. Comparison of soil properties from adjacent pasture and woodland plots at Glenrock (burnt

1197   site) and Talmo and Bogo (unburnt sites) in December 2012 (pre-fire) and April 2013 (three months

1198   post-fire). Data are means (n = 25) with bars indicating ± 1 standard error. Asterisks indicate

1199   significance at *** ($P < 0.001$), ** ($P < 0.01$), * ($P < 0.05$).

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1201

1202   Table S1. CAP diagnostic statistics for analyses at the Glenrock wildfire site and for the seasonal

1203   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

1204

1205   CAP analysis finds axes through multivariate data to discriminate among *a priori* groups. CAP performs a PCO
1206   analysis on the resemblance matrix and uses these to predict group membership (using discriminant analysis).
1207   In order to avoid over-parameterisation, the CAP analysis produces diagnostics to select the appropriate
1208   subset of PCO axes used in the discriminant analysis i.e. the number of axes where the probability of
1209   misclassifying a new point to the wrong group is minimised (Anderson et al 2008).

1210   prop.G: the proportion of the variation in the data captured by the number of PCO axes selected (1.0 = 100%
1211   variation is explained)

1212   Trace statistic: the sum of the squared canonical correlations, and the associated permutation test *P* value

1213 δ_1^2: the size of the first squared canonical correlation and the associated permutation test *P* value

1214   m: the number of PCO axes selected to perform the discriminant analysis

1215   % allocation: the leave-one-out allocation success performed in the discriminant analysis

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