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6

7 **Effects of environmental variation and livestock grazing on ant community**
8 **structure in temperate eucalypt woodlands**

9

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15

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17

18 Running head: Effects of grazing on ant assemblages

19

20 **ABSTRACT**

- 21 1. Grazing by livestock is a major ecological disturbance, with potential effects on
22 vegetation, soil, and insect fauna. Ants are a diverse and functionally important insect
23 group with many associations with the ground-layer, yet recent global syntheses question
24 the importance of grazing effects on ant communities relative to vegetation or soil.
- 25 2. We examined the effects of vegetation, soil and grazing on the whole ant community, ant
26 functional groups, and abundant species in temperate eucalypt woodlands, southeastern
27 Australia.
- 28 3. We found limited influence of grazing on our vegetation and soil measures, except for a
29 positive association between grazing and exotic perennial grass cover. We also found that

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30 exotic grass cover had a negative effect on overall ant abundance and richness, but not
31 functional groups or individual species. Soil C:N ratio had a positive effect on the
32 subdominant Camponotini, and leaf litter cover had a positive effect on the abundance of
33 cryptic species. Partial Mantel tests revealed an effect of both environmental and grazing
34 measures on ant assemblage composition, but constrained ordination showed that leaf
35 litter cover, grass biomass, and native and exotic perennial grass cover had stronger
36 correlations with ant community structure than grazing.

- 37 4. Our study shows that both environmental variation and grazing play a role in driving ant
38 community structure, but that key environmental variables such as grass biomass and leaf
39 litter cover are particularly important in temperate eucalypt woodlands. Monitoring of ant
40 communities to measure the benefits of changed grazing regimes for biodiversity should
41 consider contemporary grazing pressure as well as the underlying effects of variation in
42 plants and soils.

43
44 **Keywords:** agriculture, biodiversity conservation, environmental stewardship, Formicidae,
45 grazing, insect, invertebrate, modified landscape, monitoring, restoration

46 47 48 **INTRODUCTION**

49 Grazing by livestock can be a major disturbance in ecosystems due to its effects on soils and
50 plant communities (Hobbs, 1996; Milchunas & Lauenroth, 1993; Schuman *et al.*, 1999), as
51 well as associated insect fauna (Kruess & Tschamtker, 2002; Seymour & Dean, 1999). As a
52 consequence, the management of grazing is a priority for many land managers with
53 responsibility for the conservation of biodiversity (Lunt *et al.*, 2007; Rook *et al.*, 2004).

54 Grazing can alter soil and plant attributes over prolonged periods (Hobbs, 1996;
55 Milchunas & Lauenroth, 1993). For soils, this can include both physical and chemical aspects
56 such as compaction from the impact of animal hooves, as well as altered phosphorus and
57 nitrogen levels through plant consumption and redistribution via urine and dung (Beever *et*
58 *al.*, 2003; Yates *et al.*, 2000). For plants, this can include the introduction and spread of
59 exotic grass species (Driscoll *et al.*, 2014), the selective grazing of different plant species
60 (Dorrough *et al.*, 2007), and changes in the structure, biomass or composition of the ground-
61 layer plant community (Yates *et al.*, 2000). These changes to soils and plant communities
62 have consequences for associated biota, both above and below ground, with many

63 documented cases of changes in animal communities (Foster *et al.*, 2014; Hobbs, 1996;
64 Milchunas *et al.*, 1998).

65 Ants are a major component of animal biomass and biodiversity in terrestrial
66 ecosystems (Holldobler & Wilson, 1990), and play a critical role in soil health (de Bruyn,
67 1999) and ecosystem functioning (Evans *et al.*, 2011). The important role of ants in
68 ecosystems, and their often close association with soils (Bottinelli *et al.*, 2015) and plant
69 communities (Andersen, 1995) has led to their use as ‘indicators’ of ecosystem health
70 (Andersen & Majer, 2004; Barton & Moir, 2015; de Bruyn, 1999). A major part of this work
71 has been the use of ants as indicators of disturbance (Hoffmann & Andersen, 2003; King *et*
72 *al.*, 1998; Read & Andersen, 2000), particularly for grazing (Bestelmeyer & Wiens, 1996;
73 Bromham *et al.*, 1999; Hoffmann, 2010; Hoffmann & James, 2011).

74 There is a substantial literature on ant responses to grazing that has collectively shown
75 location-specific and species-specific responses are common (e.g. Bestelmeyer & Wiens,
76 2001; Bromham *et al.*, 1999; Hoffmann, 2010; Read & Andersen, 2000; Whitford *et al.*,
77 1999). Significant effort, therefore, has been invested in the search for general responses of
78 ants to disturbance and their use as bioindicators. This has been aided greatly by the use of
79 ant functional groups, modelled after plant disturbance responses such as disturbance-
80 opportunist species or disturbance-sensitive species (Andersen, 1995, 1997). Empirical
81 studies and reviews of this approach have supported the use of ant functional groups insofar
82 as providing a useful framework for building predictions about potential ant responses to
83 disturbances, including grazing regimes (Hoffmann, 2010; Hoffmann & Andersen, 2003).
84 However, important knowledge gaps remain, including the context dependence of ant
85 responses (Hoffmann, 2010), and the relative importance of fine-scale environmental features
86 in influencing ant responses (Yates *et al.*, 2011).

87 In this study, we examined ant assemblages from sites in a large-scale grazing
88 experiment in a temperate agricultural region of southeastern Australia. Our objective was to
89 examine the relative influence of a suite of grazing, plant, and soil variables on ants with the
90 aim of better understanding which variables drive patterns of ant diversity in this region
91 currently using grazing management to achieve biodiversity restoration. We split our
92 objective into two main questions: (1) how does livestock grazing and environmental
93 variation affect the abundance or species richness of functional groups and common species
94 of ant? And, (2) how does livestock grazing and environmental variation affect ant
95 assemblage composition? Previous syntheses of ant responses to grazing have suggested that
96 disturbance by grazing might be less important for structuring ant communities than natural

97 variation in vegetation and soil (Hoffmann, 2010; Hoffmann & James, 2011). Put another
98 way, the presence of livestock might not be as important as the presence of key habitat
99 attributes in determining the composition of ant communities. We therefore interpreted our
100 findings in light of this recent synthesis, as well as the generalised responses of ant functional
101 groups to disturbance and the environment described by Hoffman and Andersen (2003).
102 Specifically, we predicted that opportunists (e.g. *Rhytidoponera* spp.) would respond
103 positively to grazing disturbance (if any response), whereas other functional groups would
104 show positive associations with features of the environment, such as cryptic species (e.g.
105 *Solenopsis* spp.) preferring areas with leaf litter (Hoffmann & Andersen, 2003). We discuss
106 how our findings might inform biodiversity monitoring in temperate landscapes being
107 restored via changed livestock grazing regimes.

108

109 **METHODS**

110 **Study area and design**

111 Our study area was located in southeastern Australia, and covers an area approximately 100
112 km east to west and 150 km north to south (Figure S1). Within this area, 97 sites were
113 established during 2010-2011 on 29 different farms. All sites were located in Red gum
114 (*Eucalyptus blakelyi*) - Yellow box (*Eucalyptus melliodora*) grassy woodland, which is
115 characterised by a heterogeneous distribution of eucalypt trees interspersed by open grassland
116 (Figure S2). This type of grassy woodland was once widespread in southeastern Australia,
117 but is now a critically endangered ecological community as it has been greatly modified and
118 reduced in extent due to agricultural practices including grazing (McIntyre *et al.*, 2014).
119 Farms were grouped into three blocks, each representing a historical grazing practice of
120 either (i) continuous grazing, (ii) long-term holistic grazing (rotational grazing for greater
121 than 10 years), or (iii) short-term holistic grazing (rotational grazing for less than five years).
122 Farms with continuous grazing allow livestock access to sites all year round, whereas farms
123 with holistic grazing typically rotate higher numbers of livestock through sites, but for a
124 limited duration. On each farm, sites were established with one of three different treatments:
125 (i) grazing exclusion; (ii) stewardship; and (iii) 'business as usual'. Sites with grazing
126 exclusion were not grazed by any livestock. Stewardship sites must not be grazed for the six
127 months of the year during spring and summer periods as part of a contractual obligation to
128 the Environmental Stewardship Program of the Australian government (Lindenmayer *et al.*,
129 2012). The business-as-usual sites continued grazing in line with the usual practices of the
130 farm (*viz.* continuous grazing, long-term holistic or short-term holistic grazing). In the long

131 term, these grazing treatments will be used to assess the effectiveness of the Australian
132 Government Environmental Stewardship Program for a range of different biota
133 (Lindenmayer et al., 2012).

134

135 **Grazing and environmental variables**

136 The predominant domestic livestock grazing on farms was by sheep *Ovis aries* and cattle *Bos*
137 *taurus*. Two measures of livestock grazing pressure were obtained for each site in the
138 previous 12 months as reported by individual landholders. These data were used to generate
139 grazing variables that were used in our analyses: (i) Number of days grazed per year; and (ii)
140 Annual stocking rate. Annual stocking rate was calculated by multiplying the total number of
141 stock grazing on a site by the number of days they were present, and dividing by the area of
142 the site (hectares), and then 365 to give a value per day. Livestock numbers were first
143 standardised to 'dry sheep equivalent' to account for differences between sheep and cattle
144 Dry sheep equivalent is a standardised measure of feed requirements that allows for
145 comparisons of carrying capacity among different kinds of livestock (Mclaren, 1997). These
146 two variables were used to represent differences in grazing between the treatments
147 established on each farm.

148

149 Field surveys were conducted on each site during January and February 2012 to collect data
150 on ground-layer ecological variables. Sites consisted of a 40 x 200m fixed monitoring area
151 (0.8ha). Nested within each site were two smaller monitoring quadrats (20 x 50m) for
152 measuring vegetation variables. These plots were located at 0-50m and 150-200m along the
153 monitoring site. Within each of these 20 x 50m plots, all tree stems were recorded to 10cm
154 diameter classes (Lindenmayer et al., 2012). A 50m transect was located down the centre of
155 each plot with biometric measurements (Gibbons *et al.*, 2008) taken every metre to assess
156 ground layer native and exotic grass cover, and leaf litter cover. In addition, ground-layer
157 plant biomass was assessed using a rising plate pasture meter to determine average height of
158 ground cover present (Filip's Manual Folding Plate Meter, Jenquip, New Zealand (Correll *et*
159 *al.*, 2003).

160

161 Soil core samples were collected every 16.5m (n=12) along the centre of the 200m transect of
162 each site. Soil bulk density core samples (10cm diameter x 5cm height steel rings) were taken
163 at 0-5cm soil depth following careful removal of any surface plant and litter biomass present.
164 Following collection, samples 1-4, 5-8, and 9-12 were pooled together to provide three

165 bulked samples per depth per site (see Figure S1). Analysis was performed for each of the
166 three samples per site, and an average was then taken to give a per-site value used in
167 subsequent analyses. Samples were air dried at 35 °C for 48 h prior to processing and bulk
168 density was calculated on a sub-sample dried at 105 °C for 48 h. Air dried samples were
169 subsequently crushed, passed through a 2mm sieve, and retained for further chemical
170 analysis. The > 2mm fraction was separated into organic and non-organic components and
171 weighed and then discarded. Total carbon and nitrogen was determined with Dumas
172 combustion analysis (Vario Max, Elementar, Germany) (Matejovic, 1997).

173

174 **Ant sampling**

175 We sampled ants using pitfall traps that were 250 ml plastic jars dug in flush with the ground
176 surface, and half-filled with a non-toxic polyethylene glycol solution. Eight traps were placed
177 in each site, with four traps at the corners of a 5 x 5m square at each end of the site, and
178 deployed for two weeks in December 2011 to collect ground-active arthropods. Three of the
179 eight traps were randomly selected and had their ants removed and sorted, with these data
180 pooled to give one sample per site. All sampling approaches have their limitations, and we
181 recognise that our sampling approach favoured spatial replication over sampling intensity
182 within sites, and favoured the more active species of the ant community.

183 All ant specimens were sorted to subfamily, genus, and species (or morphospecies) by a
184 specialist at the Australian Museum, Sydney. We placed each ant species into a functional
185 group using the classification scheme described by Andersen (1995, 1997) (see Table S1).
186 These were: (i) dominant Dolichoderinae, (ii) subdominant Camponotini, (iii) generalist
187 Myrmecinae, (iv) opportunists, (v) hot climate specialists, (vi) cryptic species, and (vii) cold
188 climate specialists.

189

190 **Statistical analysis**

191 A subset of 78 of the 97 possible sites had the full complement of soil, plant, grazing and ant
192 data collected, and these sites formed the basis of all subsequent data analysis. It is well
193 established that livestock grazing can affect soils and plants (Abbott *et al.*, 1979; Yates *et al.*,
194 2000), and we recognised that this might lead to confounding of grazing and environmental
195 effects on ants. We therefore used multivariate analysis of variance (MANOVA) to test for
196 the effect of past grazing history on the eight-environmental variables, but found no
197 significant overall effect (Rao F = 1.34, d.f. = 2, P=0.182). This is not to say that past grazing
198 has not had *any* effect on these variables, but that variation among sites within these broad

199 groupings was not substantially different from variation among sites across all grazing
200 histories. We performed a principle components analysis (PCA) of the eight plant and soil
201 variables and two short-term grazing variables to identify potential co-linearity among
202 variables and broad gradients in environmental variation (Gotelli & Ellison, 2004). We used a
203 correlation matrix of the data as the plant, soil, and grazing variables were quantified using
204 different methods and units.

205 **Question 1: How does grazing and environmental variation affect the abundance**
206 **and species richness of functional groups and individual species of ant?** We addressed
207 this first question by using all-subsets generalised linear regression to explore which set of
208 environmental and grazing variables best explained the abundance and species richness of the
209 whole ant assemblage and separate functional groups, as well as the abundance of some
210 individual species. For each model, we used a quasi-Poisson error distribution and a
211 logarithmic link function for ant response variables. Our explanatory variables included the
212 three grazing variables (included grazing history, days grazed per year, stocking rate), three
213 soil variables (soil bulk density, C:N ratio, fraction organic material >2mm) and five
214 vegetation variables (number of tree stems, grass biomass, native grass cover, exotic grass
215 cover, leaf litter cover). We weighted our models using the Schwarz Information Criterion
216 (SIC) (Schwarz, 1978), and present the best two candidate models. All models were run using
217 GenStat 16 software (VSN International, 2013).

218 **Question 2: How does grazing and environmental variation affect ant assemblage**
219 **composition?** To address this second question, we used three different multivariate analyses.
220 First, we used a multi-response permutation procedure (MRPP) to test for a difference in ant
221 assemblage composition among the three grazing history blocks. This test compares the
222 average among-site similarity within each group with the overall similarity among all sites,
223 with greater within-group similarity indicating samples are distinct from the overall set of
224 samples (McCune & Mefford, 2011; Zimmerman *et al.*, 1985). Second, we used partial
225 Mantel tests (McCune & Mefford, 2011; Sokal & Rohlf, 1995) to test the null hypothesis of
226 no correlation between among-site ant assemblage similarity and among-site variation in a
227 second matrix (either environment or grazing) while controlling for a third matrix (either
228 environment or grazing). This test enabled us to examine the independent effects of either the
229 environment or grazing, while controlling for variation in the other. We also tested for a
230 correlation with geographic proximity to see if there was any evidence of spatial
231 autocorrelation in our ant data among sites. For these tests, we used Bray-Curtis distances
232 (Bray & Curtis, 1957) for ant data, and Euclidean distances for our environmental, grazing,

233 and geographic (easting/northing) variables, and determined significance using 9999
234 permutations of the data. Third, we used Canonical Analysis of Principal Coordinates (CAP)
235 (Anderson & Willis, 2003) to examine how variation in ant assemblage composition was
236 explained by variation in our grazing and environmental variables. This ordination technique
237 uses correlation with continuous variables (our grazing and environmental variables) to
238 constrain axes of variation in ant assemblage composition derived from Principal Coordinates
239 Analysis (metric multi-dimensional scaling) (Anderson & Willis, 2003). We then used a
240 biplot to identify which ant species were strongly correlated with variation among our sites.
241 For all our multivariate analyses, we removed singletons and square-root transformed ant
242 abundance data to reduce the influence of highly abundant species.

243

244 **RESULTS**

245 **Variation in grazing and the environment**

246 Principle components analysis reduced our 10 vegetation, soil, and grazing measures to three
247 new axes that accounted for approximately 63% of total variation in these variables (Table 1).
248 The first axis (PC1) had a high negative loading for grass biomass, and positive loadings for
249 leaf litter, number of tree stems, and C:N ratio, and indicates that most variation among sites
250 can be attributed to co-variation among these variables. The sign of these loadings also
251 indicate that grass biomass decreased when litter, tree stems, and soil C:N ratio increased.
252 The second axis (PC2) had a high negative loading for large soil organic fragments, and
253 positive loadings for soil bulk density and native grass cover. The sign of the loadings
254 indicated that the soil organic fragments decreased when soil density and native grass cover
255 increased. Notably, the third axis (PC3) had high positive loadings for exotic grass cover and
256 both short-term grazing measures, indicating these variables co-varied with each other (but
257 not with the other plant and soil measures).

258

259 **Question 1: Ant functional groups and individual species**

260 We collected 87 species of ant (22 061 individuals) from 78 sites (Table S2). The most
261 species-rich genera were the *Iridomyrmex* (13 species), *Monomorium* (11 species),
262 *Melophorus* (10 species), *Camponotus* (9 species), and *Pheidole* (9 species), and the most
263 abundant species were *Iridomyrmex rufoniger*, *Pheidole* sp B, *Monomorium sordidum*, and
264 *Rhytidoponera metallica*. Functionally, the generalist myrmecines were the most diverse
265 functional group (22 species), followed by the dominant dolichoderines (14 species).

266 All-subsets generalised linear regression revealed exotic perennial grass cover to be the
267 best predictor of the abundance and species richness of the whole ant assemblage (Table 2,
268 Fig 1a, 1b). Soil C:N ratio was important for the abundance of the subdominant Camponotini
269 (Fig 2a), the species richness of opportunists, and the abundance of the subdominant
270 Camponotini, dominant Dolichoderinae and cryptic species. Leaf litter cover was an
271 important predictor of the abundance of cryptic species (Fig 2b). Annual stocking rate was
272 found to be the single most important predictor of the abundance of cold climate specialists
273 (Fig 2c). For individual species, the best models were more complex than for functional
274 groups. Although all best models of the individual species had at least one environmental
275 predictor, seven of the eight models also had a grazing predictor, with grazing history the
276 most commonly selected variable. For example, the number of days grazed had a negative
277 effect on *Iridomyrmex rufoniger* (Fig 3a). Native grass cover, in addition to grazing history,
278 had a positive effect on the generalist *Rhytidoponera metallica* (Fig 3b). The opportunist
279 *Nylanderia* spA was the only individual species that had no grazing variable in the best
280 model and was negatively associated with the number of tree stems (Fig 3c). Additional
281 details of models are given in Tables S3 and S4.

282

283 **Question 2: Ant composition**

284 We found that ant assemblage composition was weakly significantly different among sites
285 grouped by grazing history (MRPP: $T = -1.97$, $A = 0.007$, $P = 0.037$), but that pairwise
286 comparisons between grazing blocks were not significant ($P > 0.05$).

287 Partial Mantel tests indicated a significant correlation between ant assemblage
288 composition and environmental variation when controlling for grazing ($r = 0.161$, $P = 0.002$),
289 and geographic proximity ($r = 0.156$, $P = 0.002$). We also found a significant correlation
290 between ant composition and grazing when controlling for the environment ($r = 0.176$, $P =$
291 0.012). Notably, no significant correlation was observed for geographic proximity when
292 controlling for the environmental ($r = -0.045$, $P = 0.206$). This shows there was structuring of
293 the ant community by both the environment and grazing, but not by geographic proximity.

294 The first two axes of the principal coordinate analysis explained 14.3% and 11.2% of
295 the variation in ant species composition respectively. Constraining these axes by the eight
296 environmental and two grazing variables (Figure 4) revealed that most of the environmental
297 variables were more strongly correlated with variation in the ant community than the grazing
298 variables. In particular, grass biomass and leaf litter cover were strongly correlated with ant
299 assemblage structure along the axis 1, but in opposing directions. Similarly, native and exotic

300 perennial grass cover were strongly correlated, but in opposing directions, with variation in
301 ant composition along axis 2. Several ant species were strongly correlated with the ordination
302 axes, and thus representative of distinct assemblages among our sites (Figure 4). The
303 generalist *Monomorium sordidum* and the Dominant Dolichoderine *Iridomyrmex purpureus*
304 were positively correlated with axis one, and thus characteristic of assemblages found at sites
305 with higher leaf litter. The opportunist *Rhytidoponera metallica*, generalist myrmecine
306 *Monomorium rothsteini*, and dominant dolichoderine *Iridomyrmex rufoniger* had strong
307 positive correlations with axis two, and thus characteristic of assemblages associated with
308 sites with higher native grass cover. Notably, the cold climate specialist *Heteroponera*
309 *imbellis* was the only species strongly negatively correlated with both axes 1 and 2, and was
310 characteristic of assemblages associated with higher exotic perennial grass cover.

311

312 **DISCUSSION**

313 In this study, we examined the influence of livestock grazing and environmental variation on
314 ant assemblages from sites in a temperate agricultural region in southeastern Australia. Our
315 results provide equivocal support for the global prediction that soil and vegetation have a
316 greater effect on ant community composition than grazing (Hoffmann, 2010; Hoffmann &
317 James, 2011). This is because we found that grazing history, stocking rate, and days grazed
318 per year were also important for ant abundance, species richness and compositional variation
319 among sites, and suggests a more nuanced role for these different aspects of grazing pressure.
320 Below we discuss our findings in light of the combined effects of the environment and
321 grazing, and the implications of our findings for using reduced grazing to restore ant
322 communities.

323

324 **Environmental variation is a key driver of ant community structure**

325 We found that at least one environmental variable occurred in all of the best models we
326 constructed, except for abundance of cold climate specialists. This includes the whole ant
327 assemblage, different functional groups and individual species, and demonstrates the overall
328 importance of plant and soil attributes on ant community structure. It is notable that exotic
329 grass cover was an important driver of the abundance and richness of the whole ant
330 assemblage (potentially influenced by outliers, Fig 1a), but was not important for functional
331 groups of individual species. This higher level response of the ant community, but not
332 functional groups of individual species, suggests that exotic grass cover could be a general
333 predictor of simpler ant communities with lower diversity. Our constrained ordination

334 showed that grass biomass and leaf litter cover were strongly correlated (but in opposite
335 directions) with the strongest gradient in ant compositional variation (axis 1, Fig. 4). This
336 variation is typical of the structure of grassy eucalypt woodlands, where eucalypt trees are
337 interspersed with patches of grassland (Yates & Hobbs, 1997). The heterogeneous structure
338 of grassy woodlands therefore appears to be an important driver of overall assemblage
339 composition as well as key functional groups. For example, the abundance of cryptic species
340 was best explained by leaf litter (Fig. 2b), which is their preferred habitat (Bestelmeyer &
341 Wiens, 1996; Hoffmann & Andersen, 2003). Leaf litter cover also was positively correlated
342 with the number of tree stems, and soil C:N ratio. The abundance of the subdominant
343 Camponotini was positively associated with C:N ratio, indicating they preferred more
344 wooded areas. In contrast, *Nylanderia* spA (an opportunist) was negatively associated with
345 the number of tree stems, indicating they prefer more open areas, and this was supported by
346 our ordination showing a correlation between sites characterised by *Nylanderia* and higher
347 grassy biomass and native grass cover.

348 A second important environmental gradient was obvious in our constrained ordination,
349 and was represented by a change from high exotic perennial grass to high native perennial
350 grass cover. Sites with high native grass cover were characterised by the occurrence of the
351 opportunist *Rhytidoponera metallica*, whereas sites with exotic grass cover were
352 characterised by the presence of the cold climate specialist *Heteroponera imbellis*. Notably,
353 we also found that exotic grass cover was the best predictor of the abundance and richness of
354 the overall ant community. However, this variable also was correlated with the two short-
355 term grazing measures, and highlights the difficulty in separating these confounded and co-
356 linear measures.

357

358 **Effects of grazing**

359 In addition to environmental effects, we also found compelling evidence of both long-term
360 (grazing history) effects and short-term (days grazed, stocking rate) effects of grazing on ant
361 assemblages. Grazing history, stocking rate, or days grazed were frequently identified as
362 important predictors of the abundance or species richness of ant functional groups we
363 examined (but not the whole community). It has been suggested that coarse response metrics
364 such as abundance or richness are often not suitable for detecting the responses of ants to
365 grazing as they can mask the responses of individual species within the assemblage
366 (Hoffmann, 2010). However, we also found a significant (albeit weak) difference in ant
367 assemblage composition between the three grazing history blocks. Past grazing practices

368 therefore appear to be an important driver of differences in ant assemblages. Variation in
369 grazing pressure was also correlated with variation in ant assemblage composition, even after
370 controlling for environmental variation, indicating it has a separate effect in addition to the
371 environment.

372 We found that eight of the nine individual ant species we analysed had at least one
373 grazing variable in the best model constructed for them. This is more than the general
374 prediction that approximately one quarter to one third of common ant species will display a
375 response to grazing (Hoffmann & James, 2011), although it was not possible to examine all
376 species. Further, the species for which grazing was important were from a mix of functional
377 groups, and not just opportunists as might be expected. For example, we found that
378 *Iridomyrmex rufoniger*, a dominant dolichoderine, had a negative association with the
379 number of days grazed. This contrasts with Lindsay et al. (2009), who found a positive effect
380 of grazing on dominant dolichoderines (i.e. *Iridomyrmex* spp.) in grassy woodland remnants,
381 although their result may have been driven by a different species of *Iridomyrmex*. These
382 apparently idiosyncratic yet common responses to grazing may be due to the different
383 measures of grazing used in our study compared with others (e.g. fixed treatments vs.
384 continuous measures), or other interacting disturbances that may not be accounted for, such
385 as fire history (Foster et al., 2014). A key conclusion from our study is that both historical
386 and contemporary grazing practices appear to be important for shaping ant communities, but
387 that these different measures of grazing may each be important in distinct ways. The builds
388 on other studies of grazing impacts on ants (e.g. Bestelmeyer & Wiens, 1996; Hoffmann &
389 James, 2011) by demonstrating that a single measure of grazing may be insufficient to
390 characterise its influence on insect biodiversity.

391

392 **Implications for biodiversity restoration**

393 We have shown that grazing, vegetation and soils were important drivers of ant community
394 structure. However, it remains unclear the degree to which historical grazing practices may
395 have altered the environment in our study area, and we found limited evidence of differences
396 in key environmental variables across the three broad grazing history blocks in our study
397 design. However, our principle components analysis did identify co-linearity between exotic
398 perennial grass cover and short-term grazing measures, suggesting some important
399 confounding among key grazing and environmental variables. It is well established that
400 grazing changes soil and plant community attributes (Milchunas & Lauenroth, 1993; Yates et
401 al., 2000), and the impact of grazing on temperate eucalypt woodlands has previously been

402 demonstrated (Bromham et al., 1999; Eldridge *et al.*, 2011; Prober & Wiehl, 2011). In
403 contrast, little is known about the historical diversity patterns of ants in temperate woodlands
404 of southeastern Australia, and it is difficult to conceptualise appropriate restoration goals for
405 ant communities. What is clear, is that restoration actions that target reductions in livestock
406 grazing should lead to improved tree regeneration (Fischer *et al.*, 2009), subsequent increases
407 in tree densities and leaf litter cover, and improved native ground cover and soil organic
408 content (Prober & Wiehl, 2011; Yates et al., 2000). This will benefit some ant functional
409 groups over others, such as cryptic species associated with litter and soil processes, and
410 generalist myrmecines and subordinate camponotini linked to many other ecological
411 processes (Bestelmeyer & Wiens, 1996; Folgarait, 1998; Hoffmann & Andersen, 2003).

412 Monitoring of ant communities to understand the benefits of changed grazing regimes
413 to restore biodiversity should consider the historical context of gazing, contemporary grazing
414 pressure, as well as the many underlying effects of plants and soils. A key challenge for ant
415 biodiversity restoration in grazing-dominated landscapes will be disentangling grazing from
416 environmental effects, and aligning this knowledge with restoration goals that focus on
417 returning both ant biodiversity and their ecological functions.

418

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422 analysis. DBL was funded by an Australian Research Council Laureate Fellowship. Ian
423 Packer from the former Lachlan Catchment Management Authority provided invaluable
424 advice in experimental design and sampling techniques.

425

426 **SUPPORTING INFORMATION**

427 **Figure S1.** Location map of our study sites, and a schematic showing arrangement of
428 sampling points for soil, vegetation and ants within each site.

429 **Figure S2.** Example of a site from our study area showing the open woodland structure that
430 is typical of the box-gum grassy woodland ecological community. Livestock grazing by
431 sheep or cattle occurred across the sites.

432 **Table S1.** Description of ant functional groups.

433 **Table S2.** List of ant species arranged by subfamily and functional group, and their count
434 from our pitfall trap samples. Ant specimens were counted and identified to species by a
435 specialist taxonomist, and retained at the Australian Museum, Sydney.

436 **Table S3.** Summary of regression models of ant functional groups.

437 **Table S4.** Summary of regression models of individual ant species.

438

439

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568

569 **Table 1.** Summary of principal component analysis of eight environmental and two grazing
 570 variables. Variables with strong axis loadings are shown in bold.

	PC1	PC2	PC3
Mean ground-layer biomass (kg/ha)	-0.454	0.087	-0.148
Leaf litter cover (%)	0.518	-0.051	-0.018
Number of tree stems (per ha)	0.451	0.102	0.063
Soil C:N ratio	0.482	0.022	-0.164
Soil organic fragments (% > 2mm)	0.048	-0.675	0.099
Soil bulk density (g/cm ³)	0.131	0.533	0.027
Native perennial grass cover (%)	-0.196	0.397	0.181
Exotic perennial grass cover (%)	-0.151	-0.262	0.407
Annual stocking rate (livestock/ha/yr)	0.054	0.093	0.613
Days grazed per year	0.080	0.066	0.601
Percentage variation explained	30.740	17.160	14.700

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574 **Table 2.** Summary of generalised linear models with top two models of environmental and grazing variables describing the abundance and
 575 species richness of the whole ant assemblage, ant functional groups, and individual ant species.

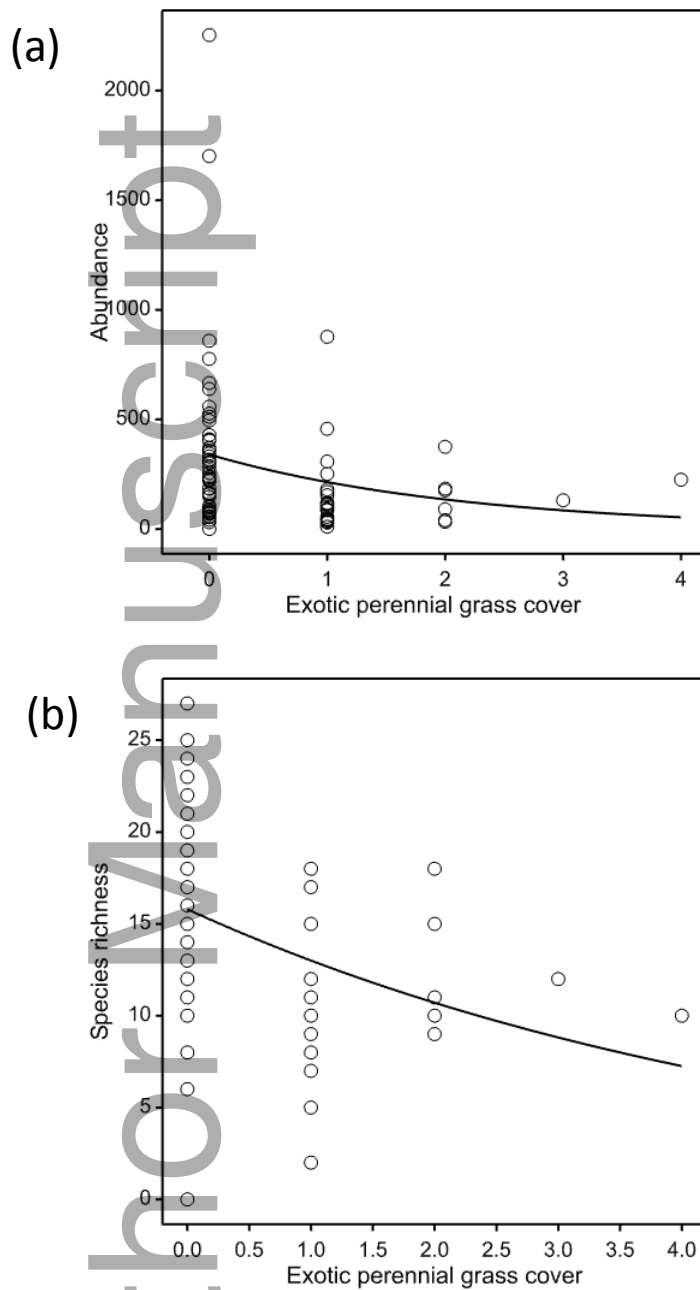
				Best Model			Second Model
		SIC	R ²	Variables [^]	SIC	R ²	Variables [^]
Whole assemblage	Abundance	81.12	8.61	EPG	82.28	12.57	EPG+ASR
	Species richness	88.31	11.55	EPG	88.88	15.70	NPG+EPG
Subdominant	Abundance	79.52	9.31	SCN	81.27	12.57	SCN+DGY
Camponotini	Species richness	87.59	17.70	SCN+ASR	86.54	23.61	SCN+ASR+DGY
Opportunists	Abundance	93.45	39.86	TS+SD+NPG	94.29	42.59	TS+SO+SD+NPG
	Species richness	84.24	31.28	SCN+EPG	87.66	32.13	SCN+EPG+ASR
Cryptic species	Abundance	81.41	11.20	LL	82.52	9.86	GB
	Species richness	76.91	4.77	SD	77.14	4.46	LL
Dominant	Abundance	91.84	21.44	GH+SCN+SD	93.24	24.68	GH+SD+EPG+DGY
Dolichoderinae	Species richness	83.25	11.91	GB+DGY	83.43	17.07	GB+NPG+DGY
Generalist	Abundance	88.60	34.64	LL+SCN+SO+ASR	90.15	37.33	LL+SCN+SO+EPG+ASR
Myrmicinae	Species richness	85.58	6.68	NPG	85.64	11.83	TS+NPG
Hot climate	Abundance	86.49	13.61	GB+DGY	87.21	17.82	GB+TS+DGY
specialists	Species richness	80.65	5.35	NPG	81.64	9.70	NPG+EPG
Cold climate	Abundance	75.10	8.34	ASR	77.15	5.51	DGY
specialists	Species richness	75.17	5.22	SO	75.52	4.72	NPG
Individual species	<i>Rhytidopoera</i>	102.79	54.67	GH+TS+SO+SD+NPG	103.30	57.04	GH+TS+SCN+SO+SD+NPG
	<i>metallica</i>						
	<i>Iridomyrmex</i>	91.37	28.88	GB+LL+SCN+DGY	89.45	35.22	GB+LL+SCN+EPG+DGY
	<i>rufoniger</i>						
	<i>Iridomyrmex</i>	93.97	41.07	GH+LL	92.19	45.74	GH+LL+NPG
	<i>purpureus</i>						
<i>Monomorium</i>	98.84	54.04	GB+LL+SD+ASR+DGY	98.88	56.73	GH+GB+LL+EPG+ASR	
<i>rothsteini</i>							

<i>Monomorium sordidum</i>	100.54	65.04	GH+LL+SCN+SO+NPG+ASR	101.61	64.47	GH+LL+TS+SCN+SO+NPG
<i>Pheidole</i> spB	96.45	36.97	GH+SCN+NPG+EPG+ASR	96.59	36.84	GH+SCN+SO+EPG+ASR
<i>Nylanderia</i> spA	85.27	16.98	TS	83.38	23.70	TS+SD
<i>Notoncus ectatommoides</i>	82.46	13.35	GB+DGY	83.03	12.65	GB+ASR
<i>Camponotus consobrinus</i>	95.29	53.32	GH+GB+LL+NPG	96.98	55.08	GH+GB+LL+SO+NPG

^GH=Grazing history, GB=Grass biomass, LL=Leaf litter cover, TS=Tree stem count, SCN=Soil C:N ratio, SO=Soil organic fragments > 2mm, SD=Soil bulk density, NPG=Native perennial grass cover, EPG=Exotic perennial grass cover, ASR=Annual stocking rate, DGY=Days grazed per year

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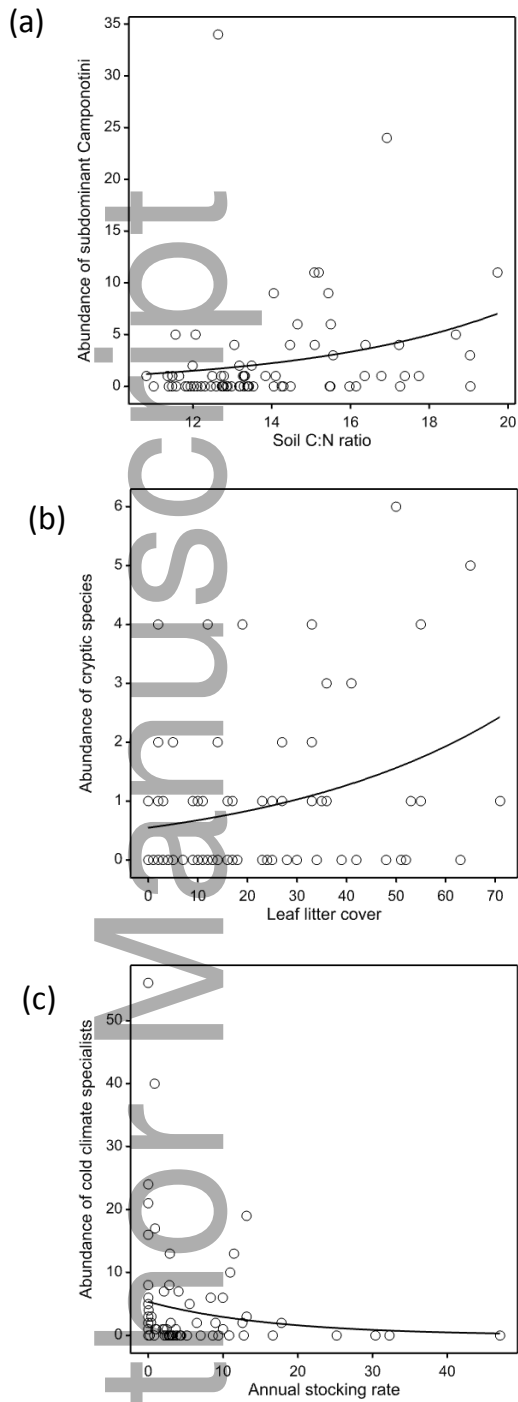
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580 **Figure 1.** Fitted models showing the relationship between percentage exotic perennial grass
 581 cover and (a) the abundance and (b) species richness of the ant assemblage. Predicted values
 582 are plotted on the original scale.

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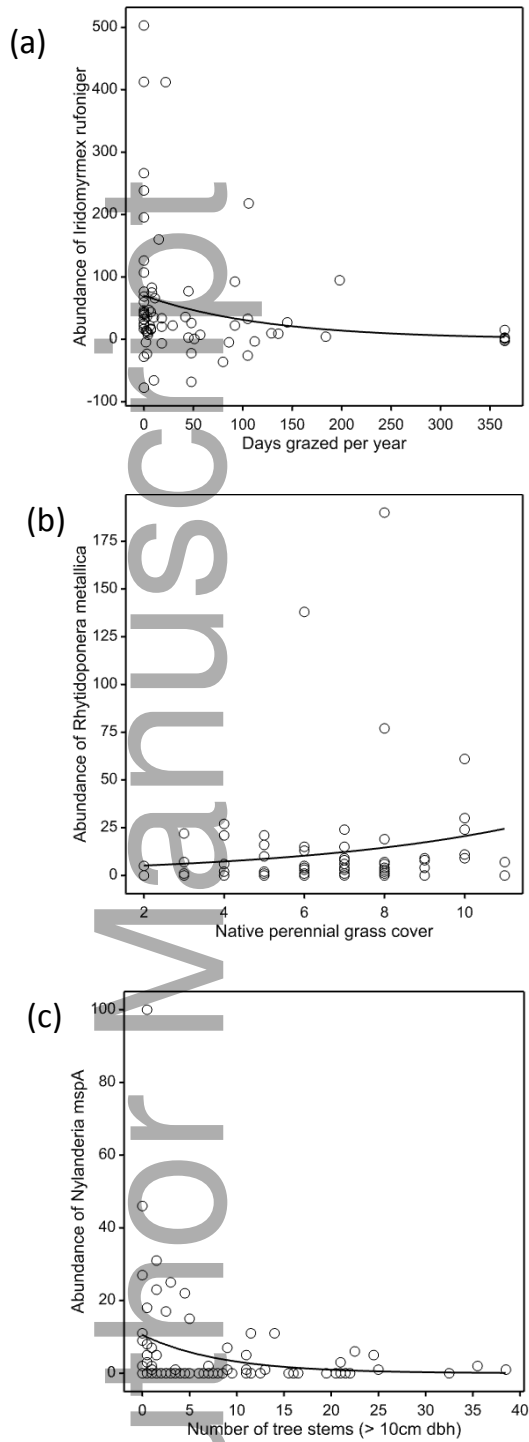
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587 **Figure 2.** Fitted models showing the relationship between soil nitrogen, leaf litter and
 588 stocking rate variables, and the abundance of (a) subordinate Camponotini, (b) cryptic
 589 species, and (c) cold climate specialists, respectively. Predicted values are plotted on the
 590 original scale.



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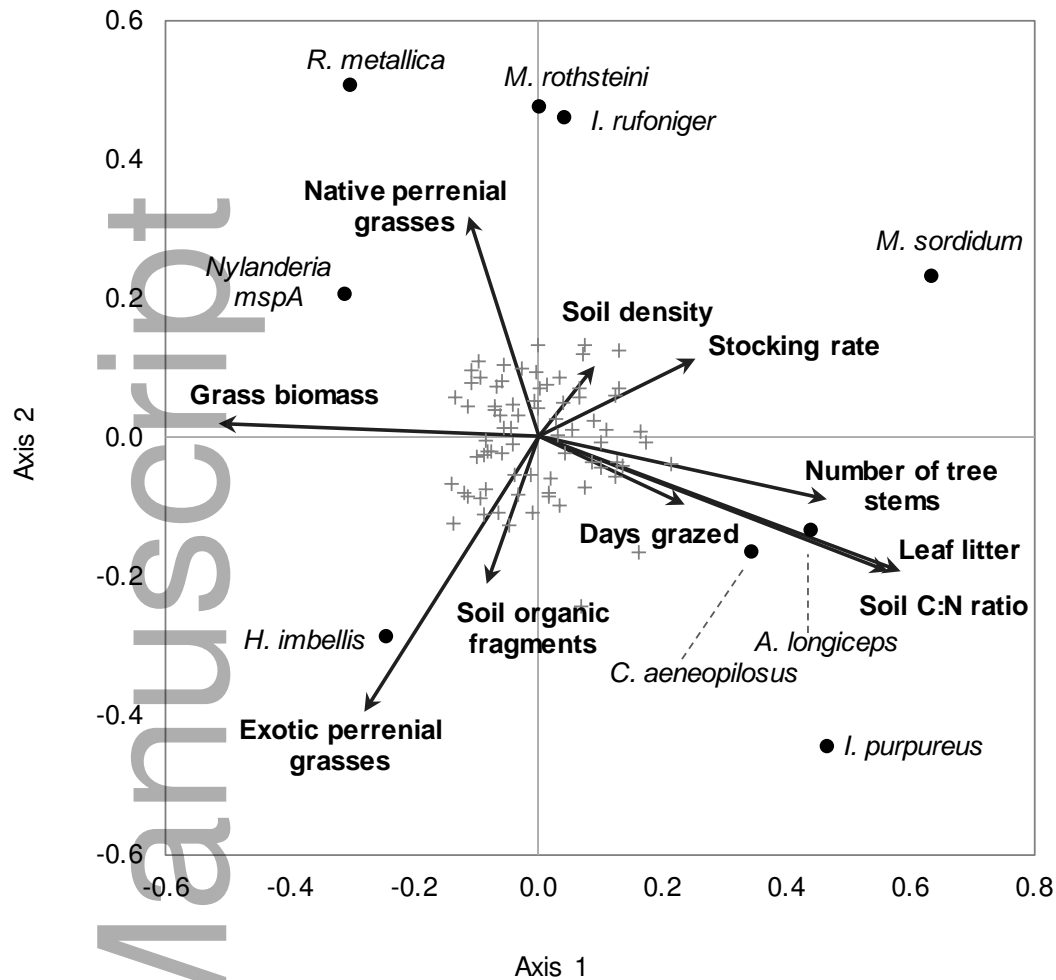
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Figure 3. Fitted models showing the relationship between grazing and environmental variables and the abundance of (a) *Iridomyrmex rufoniger*, (b) *Rhytidoponera metallica*, and (c) *Nylanderia spA* . Predicted values are plotted on the original scale.



596
 597 **Figure 4.** Canonical Analysis of Principal Coordinates ordination showing variation in ant
 598 species composition among sites (crosses) and strength and direction of correlations of
 599 constraining environmental and grazing variables (dark arrows). Individual ant species (black
 600 dots) strongly correlated with the axes in each quadrant of the ordination are also shown to
 601 indicate their relative influence on species composition among sites.