Ants are the major agents of food resource removal from tropical rainforest floors

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Manuscripts

1   # Ants are the major agents of food resource removal from

2   # tropical rainforest floors

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16   CP, PE, TE, HG and LA conceived and designed the experiment; AW, FH, HG and LA

17   collected the data; HG and LA analyzed the data; HG and LA led the writing of the

18   manuscript. All authors contributed critically to the drafts and gave final approval for

19   publication.

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21   Running headline: Ants redistribute nutrients in rainforests
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Summary

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25 1. Ants are diverse and abundant, especially in tropical ecosystems. They are often

26 cited as the agents of key ecological processes, but their precise contributions

27 compared with other organisms have rarely been quantified. Through the removal of

28 food resources from the forest floor and subsequent transport to nests, ants play an

29 important role in the redistribution of nutrients in rainforests. This is an essential

30 ecosystem process and a key energetic link between higher trophic levels,

31 decomposers and primary producers.

32 2. We used the removal of carbohydrate, protein and seed baits as a proxy to quantify

33 the contribution that ants, other invertebrates and vertebrates make to the

34 redistribution of nutrients around the forest floor, and determined to what extent there

35 is functional redundancy across ants, other invertebrate and vertebrate groups.

36 3. Using a large-scale, field-based manipulation experiment, we suppressed ants from

37 approximately 1 ha hectare plots in a lowland tropical rainforest in Sabah, Malaysia.

38 Using a combination of treatment and control plots, and cages to exclude

39 vertebrates, we made food resources available to: (1) the whole foraging community,

40 (2) only invertebrates, and (3) only non-ant invertebrates. This allowed us to partition

41 bait removal into that taken by vertebrates, non-ant invertebrates and ants.

42 Additionally, we examined how the non-ant invertebrate community responded to ant

43 exclusion.

44 4. When the whole foraging community had access to food resources, we found that

45 ants were responsible for 52% of total bait removal and vertebrates and non-ant

46 invertebrates removed the remaining 48%. Where vertebrates were excluded, ants

47 carried out 61% of invertebrate-mediated bait removal, with all other invertebrates

48 removing the remaining 39%. Vertebrates were responsible for just 24% of bait

49 removal and invertebrates (including ants) collectively removed the remaining 76%.

50   There was no compensation in bait removal rate when ants and vertebrates were

51   excluded, indicating low functional redundancy between these groups.

52 5. This study is the first to quantify the contribution of ants to the removal of food

53   resources from rainforest floors and thus nutrient redistribution. We demonstrate that

54   ants are functionally unique in this role because no other organisms compensated to

55   maintain bait removal rate in their absence. As such, we strengthen a growing body

56   of evidence establishing ants as ecosystem engineers, and provide new insights into

57   the role of ants in maintaining key ecosystem processes. In this way, we further our

58   basic understanding of the functioning of tropical rainforest ecosystems.

59

60 **Keywords**: Ecosystem function, ecosystem process, soil, functional redundancy, scavenger,

61 forager, invertebrate, nutrient distribution.

62

63 **Introduction**

64

65   Tropical forests are globally important ecosystems. They hold more than half the

66 Earth's terrestrial species (Dirzo & Raven 2003) and store huge amounts of carbon (Cramer

67 *et al.* 2004; Berenguer *et al.* 2014). Within tropical forests, ants (Hymenoptera: Formicidae)

68 are a dominant invertebrate group (Lach, Parr & Abbott 2010), estimated to make up to 25%

69 of animal biomass (Hölldobler and Wilson, 1990), and are recognised as ecosystem

70 engineers (Folgarait & Folgarait 1998). Recent work has demonstrated that diversity in ants

71 and other invertebrate groups is positively associated with ecosystem functioning in

72 rainforest systems (Fayle *et al.* 2011; Griffiths *et al.* 2015). However, little is known about the

73 relative contribution of ants to ecosystem processes compared with other functionally similar

74 groups, or the capacity of organisms to compensate to maintain processes in the event of

75 anthropogenic driven changes in biotic communities.

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76   Because of their dominance and abundance within tropical forests, ants are widely

77   cited as major contributors to the maintenance of ecological processes (e.g. Holldobler &

78   Wilson 1990; Grimaldi & Engel 2005). One such process is the redistribution of non-plant

79   derived organic material, including dead animal bodies, across forest floors (Fayle *et al.*

80   2011). Ants display a wide variety of feeding strategies; the majority of species are

81   omnivorous scavengers, consuming plant, fungal and animal tissue, some are specialist

82   predators of other invertebrates, whereas others feed on seeds, honeydew, plant nectar and

83   fungi (Holldobler & Wilson 1990; Lach *et al.* 2010). Through the collection and transport of

84   material, rich in nitrogen (N) and phosphorus (P), we know that ants carry out important

85   roles in the redistribution and concentration of nutrients around ecosystems (Frouz &

86   Jilková 2008). However, we do not know the actual contribution ants make to the functioning

87   of these systems, compared with other animal groups, over large, ecologically meaningful

88   scales. This is because until now investigations have used small scale experimental

89   manipulations (e.g. Klimes *et al.* 2011; Wardle *et al.* 2011), or have been qualitative, based

90   on descriptive and/or observational data (but see Parr *et al.* 2016).

91   Recent work has demonstrated that compared with vertebrates, invertebrates are the

92   key agents of seed predation in old growth rainforests (Ewers *et al.* 2015). However, we

93   know of no investigation that quantifies the specific contribution that ants make to seed

94   removal or to the redistribution of food resources, and thus nutrients, within tropical forests,

95   when compared with other non-ant invertebrates or vertebrates. It is important that we

96   address this knowledge gap because understanding the extent to which organisms carry out

97   functionally similar roles in an environment, provides information on the resilience of that

98   ecosystem to species losses (e.g. Laliberté *et al.* 2010; Houadria *et al.* 2016). The

99   redundancy hypothesis (Grime 1997) proposes that loss of species will not affect ecosystem

100   processes as long as there are functionally similar species that act as ecological insurance

101   (Yachi & Loreau 1999), compensating for ecosystem functioning in their absence. Here, we

102   expand this hypothesis to encompass not just the ability of different species within the same

103   taxa to carry out similar functions, but propose there may also be redundancy between

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104   different taxonomic groups. For example, it is possible that the scavenging and subsequent

105   nutrient distribution role of ants in rainforests could be carried out by other invertebrates and

106   vertebrates, which would indicate a resilience of this function to changes in the structure of

107   animal communities. However, we currently lack the empirical evidence to address this

108   issue, meaning we do not know how anthropogenic driven shifts in biotic communities are

109   likely to influence the maintenance of ecosystem functioning in rainforests, which are

110   globally important, yet rapidly changing ecosystems (Hansen *et al.* 2008; Barlow *et al.*

111   2016).

112   Here, we quantified the contribution of ants, other invertebrates and vertebrates to

113   the removal of food resources and thus the redistribution of nutrients within a tropical

114   rainforest, to assess the roles that the different groups play in ecosystem function, and the

115   capacity for functional redundancy within and between these groups. We investigated this

116   with a large-scale manipulative field experiment in an old growth tropical rainforest in

117   Malaysian Borneo. We used the removal of food baits as a proxy for the redistribution of

118   nutrients within ant suppression and control plots. A combination of caged and open

119   treatments meant food resources were available to either the whole foraging community (all

120   invertebrates and vertebrates) or invertebrates only in control plots, whereas food resources

121   were available to either vertebrates plus non-ant invertebrates, or to non-ant invertebrates

122   only in the ant suppression plots. This design allowed us to address three questions: 1)

123   What is the relative contribution of ants, non-ant invertebrates and vertebrates to nutrient

124   redistribution around the forest floor? 2) Are non-ant organisms able to compensate to

125   maintain the same level of bait removal when ants are suppressed? 3) With ant suppression,

126   is there a change in non-ant invertebrate abundance and composition at baits? Our

127   experimental framework allowed us to partition the contribution of each group to the removal

128   of food resources and thus nutrient distribution. Additionally, we monitored the activity of

129   major invertebrate groups over the two-year duration of the experiment.

130   Given the dominance of ants within rainforest systems, we predicted that: 1) Ants are

131   the major agents of nutrient redistribution, carrying out more of the process than any other

132   group; 2) In accordance with Parr *et al* (2016), release from predation and interference

133   competition would result in an increase in the abundance of non-ant invertebrates with ant

134   suppression; 3) Although the abundance of non-ant invertebrates will increase, the role of

135   ants cannot be compensated for functionally, meaning that where they are suppressed,

136   there will be a significant decline in bait removal.

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Materials and methods

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140   FIELD SITE AND ANT SUPPRESSIONS

141   This study was carried out within an area of lowland, old growth dipterocarp rainforest in the

142   Maliau Basin Conservation Area, Sabah, Malaysia (4° 44' 35" to 55" N and 116° 58' 10" to

143   30" E; mean annual rainfall 2838 mm ± 93 mm). In October 2014, we established eight

144   experimental plots within a 42-ha area, each measuring 50 x 50 m, with an additional buffer

145   zone of 15 m surrounding treatment plots; sampling was confined to the central 50 x 50 m

146   on treatment plots. Four plots were allocated as control and four as ant suppression plots,

147   each separated by at least 100 m. We applied two poison bait types to the ant suppression

148   plots: Synergy Pro® (active ingredients: hydramethylnon and pyriproxyfen) and a custom

149   bait, which consisted of Whiskas® cat food soaked in a sugar solution (60g/L sugar in water)

150   containing Imidacloprid at a concentration of 110ppm. The combination of these two poison

151   bait types was used to ensure ants with different food preferences were attracted to the

152   baits. Suppression of ants began in October 2014 through an initial application of 7.1kg/ha

153   Synergy Pro® and 8.kg/ha custom bait, which were scattered equally by hand across the

154   entire 80 x 80 m area of each plot. In subsequent poison applications, Synergy Pro® was

155   applied at 2.5kg/ha to the central 50 x 50 m sampling area and buffer zone and the custom

156   bait was applied at 4.1kg/ha to the buffer zone only. To maintain the ant suppression

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157   treatment, while avoiding the application of excessive amounts of insecticides, we applied an

158   integrated pest management approach. If ant activity was greater than or equal to 20% of

159   that on the control plots, we reapplied baits. Using this novel, large-scale ecosystem

160   manipulation, we successfully supressed the abundance of ants arriving at bait cards by an

161   average of 90% (Appendix S1) and reduced ant abundance in the leaf litter by 87%

162   (assessed using Winkler bag extractions in 2014 and 2015).

163   This baiting approach was similar to that used by Parr et al. (2016) in that it was

164   specifically designed to minimise detrimental effects on non-target organisms in the following

165   ways: 1) the poison baits have low toxicity to terrestrial vertebrates and plants (Etigra 2006;

166   Sumitomo Chemical 2016); 2) The size and composition of the baits are designed to appeal

167   to ants, and, while they may appeal to some small mammals, we applied the baits during the

168   day when ants are at their most active and these organism are less active; 3) Once collected

169   and returned to the nest, these baits are unavailable to surface-foraging organisms; 4) The

170   quantities applied to suppression plots were below biologically relevant levels. For example,

171   the amount of insecticides in the foraging territories of even the smallest, most vulnerable

172   mammals, such as shrews and mice, were lower than the LD50s. Therefore, the amount of

173   insecticide applied would be insufficient to kill small vertebrates, even if they were able to

174   find and eat all of the bait spread over their foraging territories before it was removed by

175   ants. Finally, data from Winker bag extractions demonstrate that the abundance of non-ant

176   invertebrates on the ant suppression plots was either equal to or significantly higher

177   compared with control plots (P. Eggleton *In prep*).

178   Ant and non-ant invertebrate activity were assessed every two weeks using

179   monitoring baits. On two, 50 m transects in the centre of the plots, we placed 0.3g Whiskas®

180   cat food onto 20, 5 x 5 cm laminated cards, each separated by 5 m. These were left

181   undisturbed for one hour, after which they were checked and the numbers of ants and non-

182   ant invertebrates was recorded. It was not possible to accurately count the exact numbers of

183   ants in the field, so instead, following Parr *et al.* (2016) we estimated numbers using a

184   ranked 1-6 scale (0 = 0 ants; 1 = 1 ant; 2 = 2-5 ants; 3 = 6-10 ants; 4 = 11-20 ants; 5 = 21-50

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185   ants; 6 = >50 ants). Non-ant invertebrates were visually categorised to major group and

186   abundance recorded: wasp (Hymenoptera), cricket (Orthoptera), fly (Diptera), springtail

187   (Collembola), beetle (Coleoptera), cockroach (Blattodea), spider (Araneae) and harvestman

188   (Opiliones).

189

190   RESOURCE REMOVAL EXPERIMENTS

191   During September and October 2016, we established 30 bait removal stations (15 open and

192   15 caged) within the core 50 x 50 m sampling area of each experimental plot. At each

193   station, food resources were placed in an open petri-dish (6 cm width; 1.5 cm depth) either

194   directly onto the forest floor (open treatment) or within a 20 x 20 x 20 cm metal mesh cage

195   (caged treatment: Appendix S2 for photograph examples of caged and open treatments).

196   The mesh-size (1 x 1 cm) of the cages ensured no vertebrates could access the baits within

197   the caged treatment, but did not inhibit the access of the majority of invertebrates. Three bait

198   types were used: 3.05g (± 0.02g) of dried carbohydrate bait (biscuit); 3.04g (± 0.02g) of dried

199   seed bait (sunflower seed); and 1.08g (± 0.01g) of dried protein bait (fish; a smaller amount

200   of protein bait was used because it was less dense and thus occupied a larger volume than

201   the other bait types). See Appendix S2 for more details of baits used. The bait types were

202   selected to mimic the foraging resources available in the natural system such as sugar rich

203   fruits and nectar, seeds and dead animal bodies and therefore attract as wide a diversity of

204   foraging organisms as possible. We therefore used food resources that were carbohydrate,

205   protein, or seed, and importantly, selected resources that we could easily measure the

206   amount removed. Using bait assays in this way is a standard approach in ant ecology (e.g.

207   Fayle *et al.* 2011; Kaspari *et al.* 2012; Houadria *et al.* 2016). Baits were dried at 50°C for 2

208   days to a constant mass (assessed using a Ohaus^{TM} balance, 0.01g precision) before

209   placement in the field. Resource removal stations were separated by 5 m and each bait type

210   (carbohydrate/seed/biscuit) x treatment (caged/open) was randomly placed on three, 50 m

211   transects. Each transect was separated by 10 m. In each plot, bait type was replicated five

212   times per treatment (total baits, n = 30 per plot) and this was repeated temporally on 2

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213   different days (total n = 60 per plot, total n = 480; Appendix S2 for example of the plot

214   layout). Both caged and open treatments were put onto the forest floor between 09:00 and

215   11:00 and protected from the rain by a plastic cover. After 24 hours all baits were collected,

216   transported to the laboratory, dried again at $50^{\circ}C$ to constant mass and weighed.

217

218   STATISTICAL ANALYSES

219   All analyses were carried out using R version 3.2.3 (R Core Team 2015). We used

220   generalised mixed-effects models (glmer) in the 'lme4' package (Bates *et al.* 2015) to assess

221   if plot treatment (ant suppression/control), cage treatment (caged/open) and bait type

222   (carbohydrate/seed/protein) or the interaction between these factors influenced the amount

223   of bait removed from each of the stations. In this model, the proportion of dry mass that

224   remained after 24 hours in the field was the dependent variable and plot was included as a

225   random factor. Because we used proportion data as our response, a binomial error

226   distribution was specified with a logit-link function (e.g. model <- glmer(prop.gone ~ plot.treat

227   * Cage.treat * bait + (1|plot), family = binomial(link = "logit"), data=bait). Bearded pigs (*Sus*

228   *barbatus*) destroyed a total of 103 bait stations; these were removed from analyses (the

229   likelihood of a station being attacked by pigs was not significantly affected by plot treatment,

230   cage treatment or bait type: Appendix S3).

231   To investigate if the ant suppression treatment influenced the abundance of non-ant

232   invertebrates recorded at the bait monitoring cards, pooled abundances of each non-ant

233   invertebrate major group were tested in separate models. Treatment was included as a fixed

234   effect; sampling period and plot were included as separate random factors. This approach

235   was to account for lack of temporal independence arising from different plots being sampled

236   within the same time period, and for lack of spatial independence as a result of repeatedly

237   sampling on the same plot. Models were over-dispersed, and as such, we used a negative-

238   binomal glmer (using the function glmer.nb: e.g. m.fly <- glmer.nb(Fly ~ Treatment + (1|Plot)

239   + (1|day), data=invert). Finally, we performed a multivariate analysis of variance (adonis test)

240   within the 'vegan' package (Oksanen *et al.* 2016) to assess if treatment influenced the

241   community composition of the non-ant invertebrates.

242   A top-down approach was used to arrive at the best descriptive model (Zuur *et al.*

243   2009): all fixed effects and interactions were sequentially removed until a reduced minimum

244   model was obtained, including only significant terms with $P < 0.05$. Chi-squared likelihood

245   ratio tests (LRT) were used to assess the loss of explanatory power following the removal of

246   an interaction or single term predictor.

247

248   **Results**

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250   Plot treatment (ant suppression/control) ($\chi^2_1 = 17.5$, $P < 0.001$), cage treatment

251   (caged/open) ($\chi^2_1 = 14.8$, $P < 0.001$) and bait type (carbohydrate/protein/seed) ($\chi^2_1 = 25.8$, P

252   < 0.001), all significantly influenced the proportion of bait that was removed from the forest

253   floor. Less bait was removed from the ant suppression plots compared with the control plots;

254   less was removed from the caged stations compared with the open stations (Fig. 1); and

255   fewer seeds were removed compared with the carbohydrate and protein baits (Appendix

256   S4). There were no significant interactions. Bait mass of open treatments (food resources

257   available to all foragers) within the control plots declined by 80.0% (± se = 9.2%) compared

258   with a 59.5% (± se = 10.9%) decline in the caged treatments (resources available to

259   invertebrates only) in the control plots (Fig. 1). This difference suggests that vertebrates

260   remove an average of 25.6% of foraging resources and invertebrates remove the remaining

261   74.4%. Comparing the open treatments on the ant suppression and control plots enabled us

262   to quantify the contribution of ants to bait removal compared with other non-ant invertebrates

263   and vertebrates combined. We found a decline in bait mass of 80.0% (± se = 9.2%) within

264   the open baits in the control plots compared with a 38.1% (± se = 11.7%) decline in open

265   baits the ant suppression plots (Fig. 1); furthermore, this decline was consistently the case

266   for all bait types (Appendix S4). Therefore, ants were responsible for 52% of bait removal

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267 compared with all other organisms (invertebrates and vertebrates). Finally, in terms of the

268 contribution of ants to invertebrate-mediated bait removal (i.e. considering the caged

269 stations only): bait mass declined by 59.5% (± se = 10.9%) in the control compared with a

270 decline of 23.1% (± se = 9.5%) in the ant plots (Fig. 1). This difference suggests that at least

271 61% of invertebrate-mediated scavenging is carried out by ants and the remaining 39% by

272 all other invertebrates.

273 Treatment significantly affected the abundance of all non-ant invertebrate groups,

274 except beetles and spiders, observed at the monitoring baits (Table 1). In all cases, there

275 were more individuals observed in the ant suppression plots compared with the control plots

276 (Fig. 2): the abundance of flies, crickets, wasps springtails and harvestmen recorded at the

277 monitoring baits in the ant suppression plots was 80% higher than observed in the control

278 plots, while the abundance of cockroaches increased by around 50%. Consequently, there

279 was a significant shift in the composition of the invertebrate community recorded in the

280 control, compared with the treatment plots ($F_{1,6}$ = 12, P = 0.03; Fig. 3).

281

Discussion

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284 In this study we employed a novel field manipulation experiment to quantify the relative

285 contribution of ants to a key tropical forest ecosystem process. In doing so, we have

286 demonstrated for the first time, what has been long predicted, that ants are the major agents

287 of resource removal in these systems. Many papers reference ants as the most functionally

288 important invertebrate group in tropical systems – one of the "the little things that run the

289 world" (Wilson 1987). However, until now these assertions had not been verified by empirical

290 evidence in tropical forests. Previous work on ant-mediated ecosystem functioning has

291 tended to focus on seed dispersal (Gove, Majer & Dunn 2007), bioturbation of soil (Folgarait

292 & Folgarait 1998) or symbiotic food-web interactions (Currie 2001; Parr *et al.* 2016). Here,

293 we have quantified the role of ants in scavenging and thus nutrient re-distribution, which is

294　an essential and often overlooked aspect of decomposition, linking higher trophic level

295　organisms, decomposers and plants (Frouz & Jilková 2008). As such, we provide new

296　insights into the role of ants in maintaining key ecosystem processes and further our

297　understanding of the functioning of tropical rainforest ecosystems.

298　　　　Our estimates suggest that ants are responsible for a minimum of 52% of bait

299　removal when compared with all other groups (vertebrates and non-ant invertebrates), and

300　for 61% of invertebrate-mediated scavenging. Although ants display a large range of feeding

301　strategies, most forage for small, widely dispersed food, including dead vertebrates,

302　invertebrates, seeds and animal waste, which are then taken to nests (Carroll & Janzen

303　1973). This collection and transport of material, rich in nitrogen (N) and phosphorus (P),

304　results in the redistribution and concentration of nutrients around ecosystems, influencing

305　soil biota and vegetation (Frouz & Jilková 2008). For example, Wagner, Brown & Gordon

306　(1997) demonstrated that concentrations of key plant-limiting nutrients and densities of

307　micro-arthropods and protozoa were significantly higher in ant nest soils, while ant mounds

308　have been associated with increased seed production (Wagner 1997). These studies were

309　carried out in arid grasslands, not rainforests, so caution must be taken in making inferences

310　between the systems. However, these studies demonstrate the multi-trophic impact that ant-

311　mediated nutrient redistribution can have on soils and vegetation. Small scale variation in

312　soil nutrients and heterogeneity has been demonstrated to affect tropical forest diversity and

313　plant community structure (John *et al.* 2007; Xu *et al.* 2016). Therefore, ant-mediated

314　nutrient redistribution is likely to be a key process in these systems with implications for

315　forest composition and function. However, we are aware of no study to-date that has

316　focussed on the soil properties associated with ant nests in rainforest systems and as such

317　we highlight this as an area in need of further investigation.

318　　　　When only invertebrates had access to foraging resources, ants were responsible for

319　61% of bait removal, meaning that all other invertebrates combined removed the remaining

320　39% of baits. However, these figures are likely to be conservative estimates for two reasons.

321　First, our suppression treatment was effective at reducing ant activity by an average of 90%.

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322   This means that around 10% of ants may have been actively contributing to bait removal on

323   the treatment plots. Second, we observed a significant increase in non-ant invertebrates

324   arriving at monitoring cards in the ant suppression plots (explored further below). Therefore,

325   other invertebrates were contributing more to the removal of baits on the ant suppression

326   plots than would be observed under normal circumstances (i.e. with no ant suppression). It

327   is likely then, that this study underestimates the true contribution ants make to the

328   movement of food resources within tropical forests. Nevertheless, our study highlights the

329   fundamental contribution that ants make to the removal of foraging resources from tropical

330   forest floors, thus illustrating their key role in soil nutrient cycling and tropical forest function.

331   We showed that invertebrates are responsible for about three-quarters of the

332   removal of food resources from the forest floor, while vertebrates only accounted for around

333   a quarter. Although it is possible that this result is partly driven by monitoring activities on

334   experimental plots disturbing vertebrate communities, these figures are in line with work by

335   Ewers et al. (2015), who reported that invertebrates removed 72% of seeds from old growth

336   forest floors. We have built on these findings by demonstrating that this pattern holds true

337   not only for seeds but also for other food resources; providing evidence of the importance of

338   invertebrates for the cycling of both animal and plant derived products in rainforests. Ewers

339   et al. (2015) asserted that the functional importance of invertebrates was reduced in

340   secondary forest, because mammals compensate and carry out many of the functional roles

341   that are dominated by invertebrates in primary rainforests. However, it is unlikely that the

342   removal of food resources by mammals can truly replace the ecological processes carried

343   out by invertebrates, in particular ants. This is because ants concentrate nutrients in nests

344   (Bestelmeyer & Wiens 2003; Frouz & Jilková 2008), leading to greater ecosystem

345   heterogeneity and to hotspots of diversity (Wagner *et al.* 1997; Laakso & Setälä 1998;

346   Sternberg *et al.* 2007). We cannot assume therefore, that because two groups appear to

347   carry out similar processes, they have identical effects on ecosystem function. The inter-

348   phylum redundancy reported by Ewers et al (2015) may not actually mitigate the negative

349   consequences of anthropogenic habitat disturbance. Instead, in very disturbed habitats

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350   where ant diversity has declined (e.g. Luke *et al.* 2014), we may see a homogenization of

351   diversity (c.f. de Castro Solar *et al.* 2015).

352   Ant suppression resulted in a shift in the abundance and composition of non-ant

353   invertebrates at monitoring baits, with a significant increase in the numbers of more than

354   50% of all groups except spiders and beetles. Thus while these groups may have removed

355   more baits than in the presence of ants, we found no evidence that this resulted in

356   compensation in scavenging rates by these other groups. Invertebrates removed an average

357   of 23% of baits from the caged stations in the ant suppression plots, while 60% of bait was

358   removed from the equivalent stations within the control plots. Ants are opportunists and have

359   been shown to find and remove food resources rapidly before other groups arrive (Fellers &

360   Fellers 1982; Wilson 1987). Our results show that when ants are removed, the rate of

361   discovery and removal of baits declines and is not compensated to any great extent by other

362   groups. If complete compensation had occurred through the activity of other invertebrates,

363   we would not expect there to be a significant difference in removal rate between the caged

364   bait stations in the ant suppression plots and control plots. Therefore, we have demonstrated

365   that it is not simply a matter of ants acting as the fastest and most efficient scavengers, but

366   that they are likely to be functionally non-replaceable in their foraging roles in rainforests.

367   This finding is important because ant diversity is sensitive to habitat disturbances such as

368   repeated logging or conversion to oil palm, (Fayle et al., 2010; Klimes et al., 2012; Luke et

369   al., 2014) and Fayle et al. (2011) found that ant species richness was directly related to the

370   rate of food resource removal across a land-use gradient. As anthropogenic habitat

371   disturbances intensify to a point where ant diversity and abundance declines, the ant-

372   mediated ecosystem processes of scavenging and nutrient redistribution are also likely

373   decline, with uncertain knock-on effects for other aspects of ecosystem functioning.

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384 **Data Accessibility**

385 Data will be made accessible through the NERC Environmental Information Data Centre.

386

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510   **Supporting Information**

511   **Appendix S1:** Ant monitoring bait results demonstrating a consistent significant reduction of

512   ant abundance in the ant suppression plots over the two-year duration of this investigation.

513   **Appendix S2:** Example photographs of the caged and open bait stations, further information

514   of the baits used, and an example of the spatial arrangement of an experimental plot.

515 **Appendix S3:** Analyses of factors affecting the numbers of baits disturbed by pigs.

516 **Appendix S4:** Results demonstrating how bait type influenced the amount of bait removed.

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544   **Table 1.** Model outputs of negative binomial generalised linear mixed effects models to

545   assess the impact of ant suppression on the abundance of non-ant invertebrates in

546   experimental plots. Groups that were significantly affected ($P \geq 0.05$) are highlighted in bold,

547   significance was determined using a likelihood ratio test.

Group	LRT	df	P
Fly	**14.03**	**1**	**<0.0001**
Cricket	**13.00**	**1**	**<0.0001**
Cockroach	**4.36**	**1**	**0.037**
Wasp	**5.93**	**1**	**0.015**
Springtail	**6.57**	**1**	**0.01**
Harvestman	**5.06**	**1**	**0.024**
Spider	*1.33*	*1*	*0.248*
Beetle	*0.94*	*1*	*0.333*

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565   **Figure 1.** The mean proportion (± SE) of food resources removed from bait stations that

566   were either caged (light grey bars: vertebrate exclusion) or open (dark grey bars: open to all

567   foragers: invertebrates and vertebrates) within ant suppression and control plots.

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569   **Figure 2.** The mean abundance (± SE) of non-ant invertebrates observed at monitoring baits

570   in ant suppression (light grey bars) and control plots (dark grey bars).

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572   **Figure 3.** A nonmetric multidimensional scaling (NMDS) ordination of the non-ant

573   invertebrate communities within ant suppression (light grey circles) and control plots (dark

574   grey triangles).

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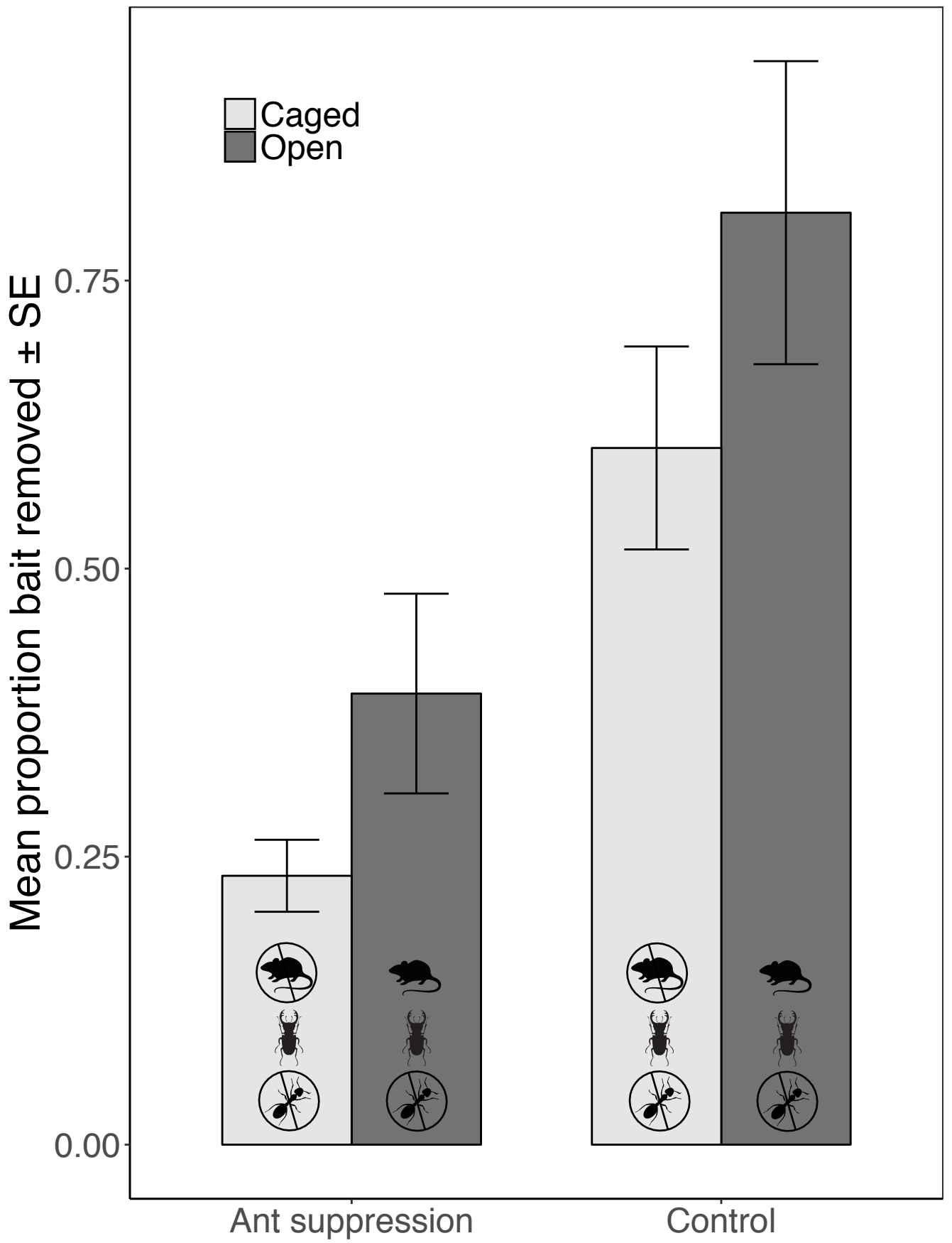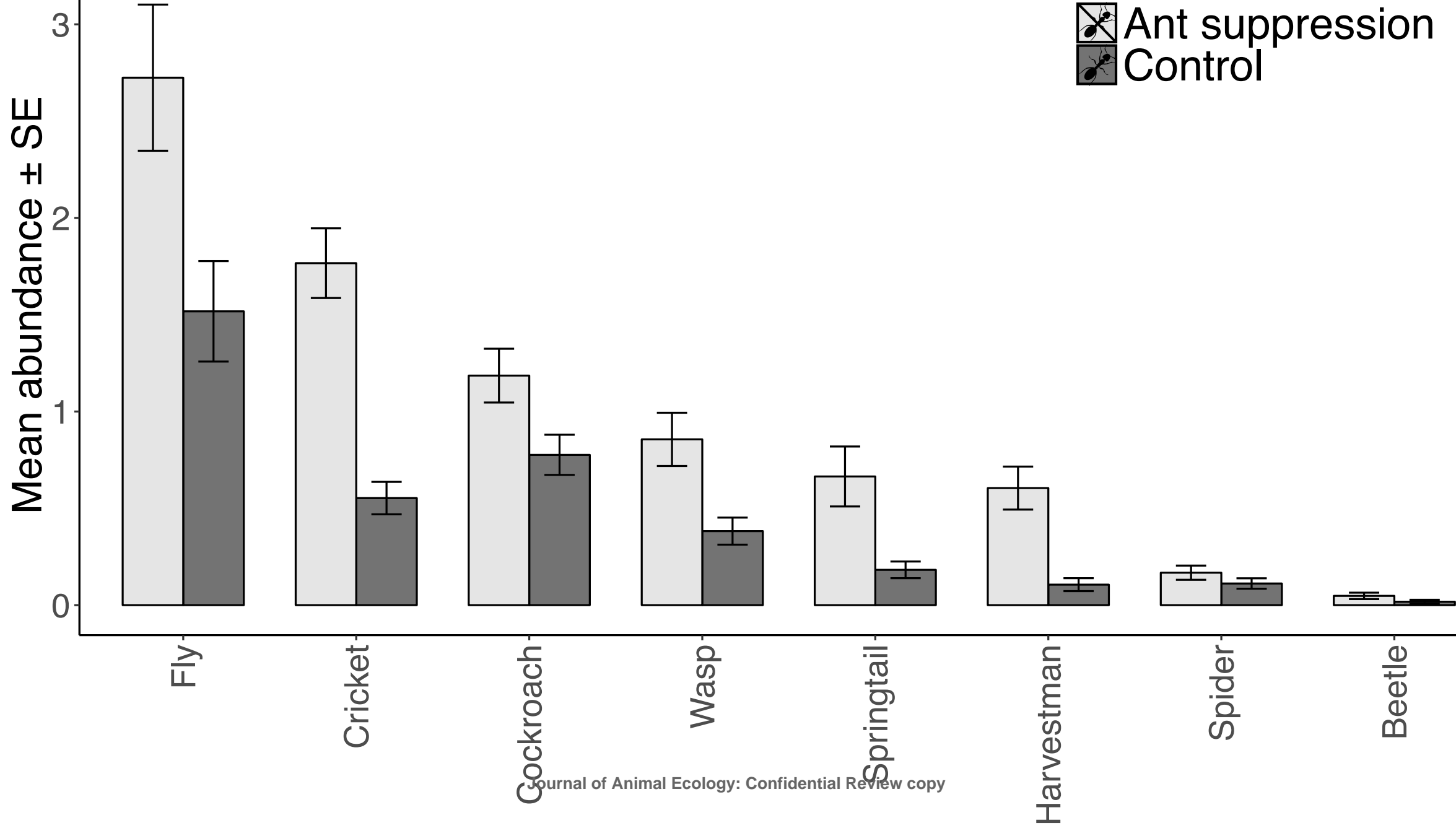
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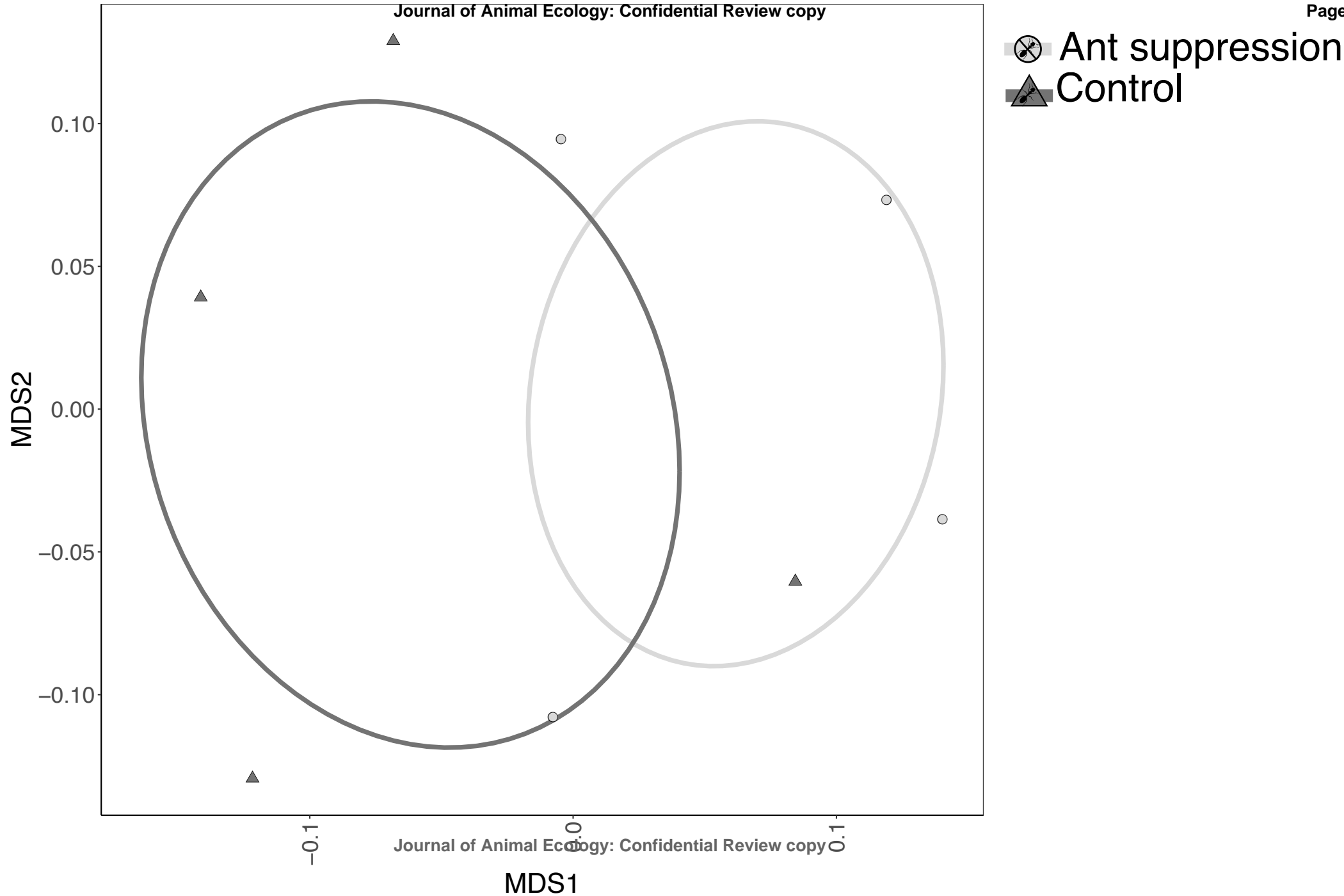
Control

Appendix 1 – Ant monitoring bait results

Data from ant monitoring baits demonstrate that the ant suppression treatment significantly reduced the abundance of ants that arrived at monitoring cards (LRT = 8.93, df = 2, *P* = 0.01; determined by an ordered logistic regression using the clmm package in R; example syntax: m1 <- clmm(activity.score ~ Treatment + (1| Date) + (1| Plot), data = cards); Fig. S1). The mean activity score on bait cards in control plots was 2.5 (indicating a mean abundance of between 5 and 10 ants), whereas the mean score on the ant suppression plots was 0.24 (indicating a mean abundance of less than one ant). This demonstrates that the suppression treatment reduced the activity of ants on treatment plots by an average 90% compared to ant activity on control plots.
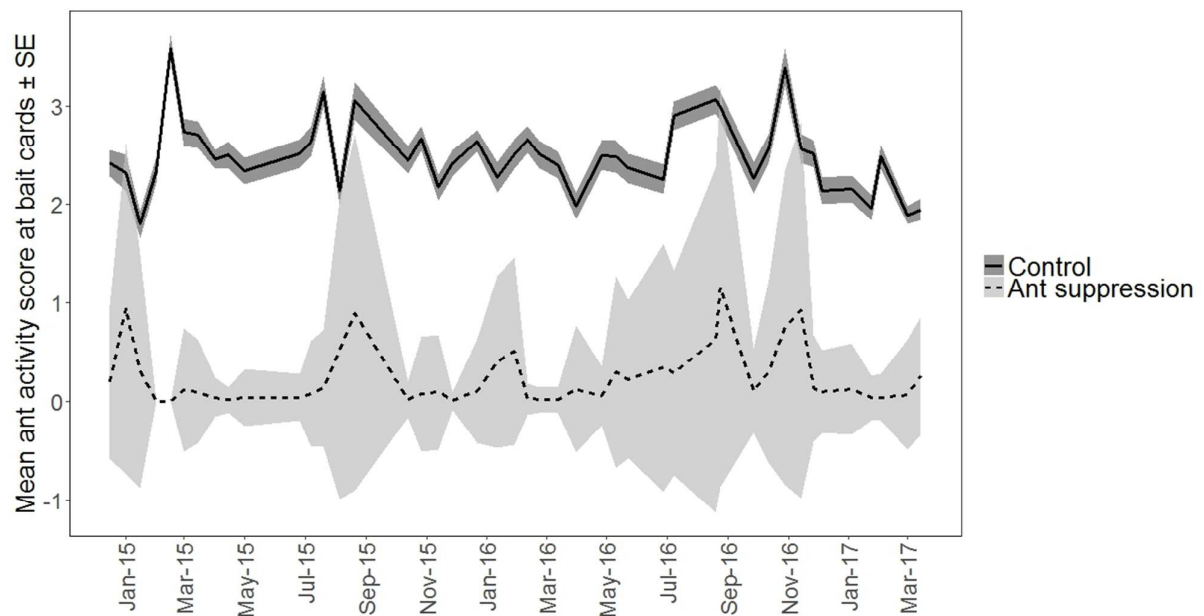

Figure S1.1 Mean ant activity scores (± SE: shaded ribbons) at bait cards, assessed every two weeks for two years on control (solid line) and ant suppression plots (dashed line).

Appendix S2 – Details of cage treatment, baits, and plot spatial design

Cage treatments

Figure S2.1. Caged bait stations, which restricted the access vertebrates to the food resources, containing seed (left photo) and carbohydrate bait (right photo)

Figure S2.2. Open bait stations, allowing access by all foragers to the food resources, containing seed (left photo) and carbohydrate bait (right photo)

Bait details

Carbohydrate bait:

We used a wheat-based biscuit readily available in supermarket stores:

Tigar Biskuat Choc

Ingredients: Wheat flour, sugar, palm oil, cocoa powder, raising agents (ammonium bicarbonate), acid sodium pyrophosphate, liquid glucose, vitamins and minerals (A, B1, B2,

B3, B5, B6, B12, D, E, Calcium carbonate, Magensium, Iron, Phosphorus, Iodine, Zinc), Milk

powder, tapioca starch, salt, flavour.

Protein bait:

We used a dehydrated fish snack readily available in supermarket stores:

Wanfa Snek Ifan

Ingredients: Fresh fish meat, Wheat starch, Wheat flour, sugar, salt, chilli, flavourings

This product was chosen over fresh or tinned fish because it facilitated the quantification of

dry mass removed by scavenger, whereas this would have been problematic with tinned or

fresh fish.

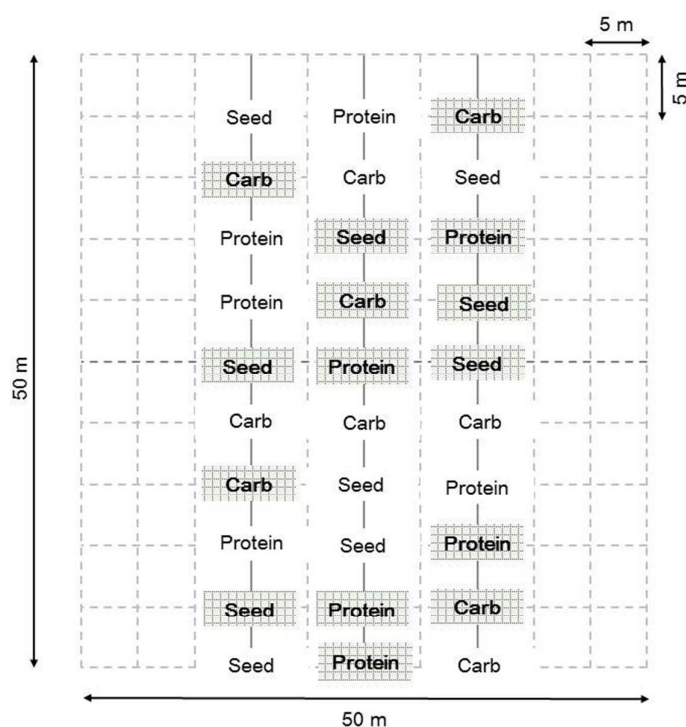## Spatial arrangement of bait stations on experimental plots

Figure S2.3. Bait x treatment spatial arrangement on an experimental plot. Each bait type

was replicated five times per caged (hashed words)/open treatment and placed randomly on

three, 50 m transects. Bait stations on the transects were 5 m apart and each transect was

separated by 10 m. This resulted in n = 30 bait stations (5 bait replicates x 3 bait types x 2

cage treatments) for each of the eight plots (4 x ant suppression and 4 x control). This was

repeated twice per plot totalling n = 480 bait stations for the experiment as a whole (60 baits

x 4 plot replicates x 2 plot treatments [ant suppression/control])

Appendix S3 – Bait stations attacked by pigs

We used a binomial glmer to assess whether plot treatment, cage treatment, or bait type influenced the likelihood that pigs disrupted bait stations. In total 103 bait stations (21.5%) were removed from the main analyses because of pig interference. No factor significantly affected the likelihood of pig attack (fig. 1).
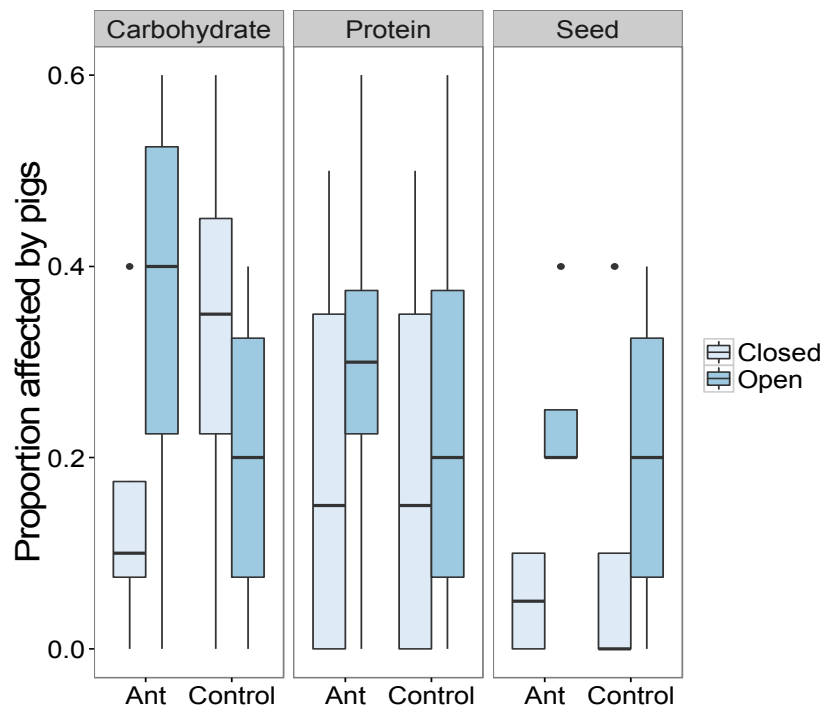

Figure S3.1. Median proportion and interquartile range of bait stations (carbohydrate, protein, or seed) excluded from analyses experimental plots (ant suppression and control) because they were affected by pigs in the field. Caged baits are light blue boxes and baits open to all foragers are dark blue boxes. Outliers are displayed by black dots.

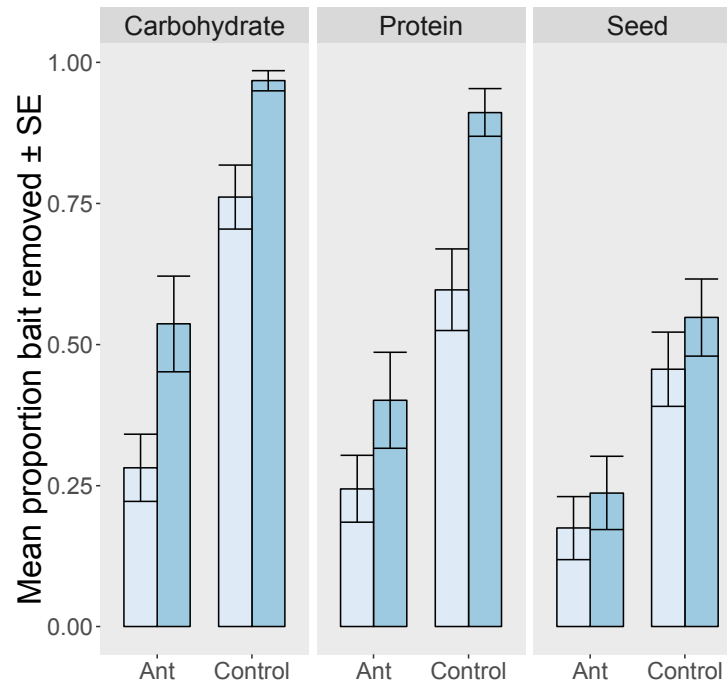## Appendix S4 – Differences in bait removal

Figure S4.1. The mean (± SE) proportion of bait removed from ant suppression (Ant) and control plots (Control) from caged (light blue bars: vertebrate exclusion) and open bait stations (dark blue bars: open to all foragers).

225x225mm (72 x 72 DPI)