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Atmospheric change causes declines in woodland arthropods and impacts specific trophic groups

S. L. Facey [a]*, D. B. Fidler [b], R. C. Rowe [a], L. M. Bromfield [a], S. S. Nooten [a], J. T. Staley [c], D. S. Ellsworth [a] and S. N. Johnson [a]

[a] Hawkesbury Institute for the Environment, Western Sydney University, NSW, Australia

[b] School of Life Sciences, Cardiff University, Cardiff, United Kingdom

[c] Centre for Ecology & Hydrology, Wallingford, Oxfordshire, United Kingdom

*Corresponding author: email sarah.facey@westernsydney.edu.au; telephone +61 (0)2 4570 1014; fax +61 (0)2 4570 1103

Running head: Arthropod declines under elevated CO_2

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Abstract

1. Arthropod assemblages form a fundamental part of terrestrial ecosystems, underpinning ecosystem processes and services. Yet, little is known about how invertebrate communities, as a whole, respond to climatic and atmospheric changes, including predicted increases in carbon dioxide concentrations (CO_2).
2. To date, woodland Free Air CO_2 Enrichment (FACE) studies have focused entirely on northern hemisphere managed plantations. We manipulated atmospheric CO_2 in a mature, native *Eucalyptus* woodland (0.15ha, >32,000m^3) in Australia, using the EucFACE facility. We used three complementary sampling methods (vacuum sampling, pitfall and sticky trapping) to record invertebrate abundances under ambient and elevated levels of CO_2 (400 vs. 550 ppm).
3. Based on the collection of over 83,000 invertebrates, we found significant declines in the overall abundance of ground-dwelling (14.7%) and aerial (12.9%) arthropods under elevated CO_2, with significant decreases in herbivore, omnivore, scavenger and parasitoid functional groups. Even though several groups showed varying declines in abundance, elevated CO_2 did not measurably affect community composition.
4. Our results indicate that atmospheric CO_2 predicted within the next 35 years may cause declines in arthropod abundances in *Eucalyptus* woodland. Declines found in several functional groups suggest that elevated atmospheric CO_2 has the potential to affect ecosystem processes, possibly including nutrient cycling by herbivores and omnivores and biocontrol by parasitoids.

Introduction

With over one million described species, arthropods comprise the majority of terrestrial multicellular life on Earth (Mora *et al*. 2011; Scheffers *et al*. 2012) and are the main players in the bulk of terrestrial plant-based food-webs (Price, 2002). Aside from their impressive contribution to biodiversity, arthropod communities are important in a functional context, underpinning a variety of ecosystem processes (Wilson 1987). For instance, invertebrates perform substantial roles in nutrient cycling through the consumption and break down of plant material (Hunter, 2001).

Arthropod communities are shaped by complex combinations of abiotic and biotic factors, as well as biotic interactions including trophic associations (Polis 1998), which are themselves sensitive to environmental change (Tylianakis *et al*. 2008). Consequently, perturbations occurring in either the biotic or abiotic environment have the capacity to alter the structure of communities, and the interactions occurring between the species that form them, by virtue of the fact that not all species in a system will respond to change in the same way (Sanders *et al.*, 2003; Raffaelli, 2004; Pocock *et al.*, 2012).

While previous community-level studies have shown that the responses of different taxa to environmental change can be highly individualistic and species-specific (e.g. Altermatt 2003; Sanders, Belote & Weltzin 2004), invertebrate taxa sharing the same feeding strategy are likely to be affected by change in similar ways to each other, allowing some generalisations to be made (Altermatt, 2003; Hillstrom & Lindroth, 2008). For instance, sap-feeding invertebrates may be positively affected by changes in the quality of their food plants under elevated CO_2 (Bezemer & Jones, 1998). Conversely, folivores, by virtue of their different feeding habits, tend to have

reduced performance under elevated CO_2 (Stiling *et al.*, 2003; Stiling & Cornelissen, 2007), leading to reductions in folivory (Hamilton *et al.*, 2004; Knepp *et al.*, 2005). Organisms at higher trophic levels, including predators, may be more sensitive to environmental perturbations, perhaps as a result of higher metabolic costs and their dependency on the responses of organisms at lower trophic levels (Voigt *et al*. 2003; Hance *et al*. 2007). Moreover, specialist species such as endoparasitoid wasps may be more at risk from changes to the environment than generalists because they are dependent on a smaller group of hosts and therefore might be disproportionately affected if they cannot utilise alternative hosts (Hance *et al.*, 2007; Vanbergen *et al.*, 2010).

The concentration of carbon dioxide (CO_2) in the atmosphere now exceeds the range the Earth has seen in the last 800,000 years (IPCC 2013), and as such is considered an abiotic perturbation with the potential to alter ecological communities. Numerous studies have reported CO_2-induced changes in plant biomass and morphology (Pritchard *et al.*, 1999; Stiling & Cornelissen, 2007; Zhu *et al.*, 2016), altered botanical composition (Vasseur & Potvin, 1998), coupled with reductions in plant quality (Robinson *et al*. 2012). Elevated CO_2-related changes in plants could therefore have crucial implications for invertebrate herbivores (Hentley *et al.*, 2014) and their arthropod consumers, as well as the ecosystems these communities support (Tylianakis *et al.*, 2008). In spite of their recognised importance, relatively little is known about how invertebrate communities, as a whole, will respond to climatic and atmospheric changes (Jamieson *et al.*, 2012; Facey *et al.*, 2014). In order for us to adequately predict the consequences of climatic and atmospheric change on ecosystems, large scale experiments considering community-level responses to change will be necessary, complementing work in more controlled settings (Stiling *et al.*, 2003; Facey *et al.*, 2014). Free Air CO_2 Enrichment (FACE) experiments have been invaluable for assessing the impacts of elevated CO_2 for plants and invertebrates in temperate

80 forest systems (Hamilton *et al.*, 2004; Knepp *et al.*, 2005; Stiling & Cornelissen, 2007; Couture
81 & Lindroth, 2012; Couture *et al.*, 2015; Facey & Gherlenda, 2016).

82 Thus far, however, forest FACE invertebrate community studies have been limited to
83 experiments on relatively young, managed plantation trees in the northern hemisphere. Gaining
84 an adequate understanding of the responses of the terrestrial biosphere to elevated CO_2 will
85 require greater habitat representation among the next generation of FACE experiments
86 (Ainsworth & Long 2005; Facey & Gherlenda 2016; Norby *et al.*, 2016). The present study
87 redresses this gap two ways. Firstly, it is the first field-based experiment investigating arthropod
88 responses to atmospheric change in a southern hemisphere forest system, allowing comparisons
89 and generalisations to be made across studies in other systems. Secondly, our study is the first
90 experiment established in native mature natural woodland. The site consists of *Eucalyptus*
91 woodland, the second most dominant habitat type in Australia, after grasslands. This habitat is
92 estimated to cover over 890, 000km^2 of the continent (Department of the Environment and Water
93 Resources 2007). Further, the *Eucalyptus* genus is the most widely planted hardwood globally
94 (Frew *et al*., 2013), yet information concerning the responses of *Eucalyptus* communities to
95 climatic and atmospheric change is scant owing to a lack of field studies.

96 The aim of this study was to characterise the arthropod community occurring in this woodland
97 study system and assess the extent to which this community may be affected by rising
98 atmospheric CO_2 concentrations. We used a variety of sampling methods applied from ground-
99 level to the forest canopy, to obtain representative samples of the invertebrate community. We
100 focused on how different functional groups (i.e. feeding guilds) responded to elevated CO_2 i.e. if
101 specialists like parasitoids were more sensitive than generalist predator groups.

Given the generally negative effects of elevated CO_2 on plant quality, we predicted that *i)* folivorous herbivores would decline in abundance under elevated CO_2, whereas those in different feeding guilds including sap-suckers would be positively affected by alterations in food quality; *ii)* arthropods at higher trophic levels would show greater declines than groups from lower trophic levels; *iii)* specialised taxa (e.g. parasitoids) would be more strongly affected by CO_2 manipulation than generalists, and; *iii)* As a result of changes in the abundances of different taxa, invertebrate community composition would be altered under elevated CO_2 conditions.

Materials and methods

Experimental site

The study was carried out at the *Eucalyptus* Free-Air CO_2 Enrichment ('EucFACE') site in western Sydney, Australia (33°36′59″S, 150°44′17″E), described in Duursma *et al*. (2015). In brief, the site consists of ~15 ha within a 167 ha tract of mature, native Cumberland Plain woodland, dominated by *E. tereticornis* (over 90% coverage). There are six 25m diameter ring arrays; since September 2012, the CO_2 levels have been manipulated in three randomly selected rings (ambient +150ppm, corresponding to the concentration predicted by the middle of this century under the emission scenario A1F1 (IPCC, 2007)), with the other three receiving ambient CO_2 levels. Diluted CO_2 or air (in ambient plots) is released into the vegetation within the ring from valves in the vertical vent pipes around the outside edge of the ring during the day time.

Invertebrate collections

We collected invertebrates using three different methods across seasons to obtain a broad, representative, sample of the arthropod community occupying different niches. Pitfall traps were used to sample ground-dwelling arthropods, with suction sampling to capture invertebrates from understorey vegetation and sticky traps to sample aerial (canopy) invertebrates. Suction sampling is a proven quantitative technique for sampling invertebrate populations (Brook *et al*. 2008). Sticky and pitfall trapping allow for relative comparisons of invertebrate abundance between CO_2 treatments, within sampling method (Buntin 1993; Woodcock 2005). Pitfall traps were first used in November 2013; pitfall and suction sampling was then carried out quarterly from January 2014 to January 2015 (six pitfall campaigns, five suction sampling campaigns). Sticky trapping was carried out six times throughout the experiment, on a monthly basis between the end of September and the start of December during 2013 and 2014, when most flying arthropods would be active. In each of the three niche-types, sampling was carried out in fixed locations across all sampling dates.

i) Ground-dwelling arthropods (pitfall sampling)

Within each ring, two locations were selected at random on the woodland floor. In each of these, a 500ml 9cm diameter plastic pot was buried flush with the soil level. Traps were left dry and open for one week prior to the initial sampling period in November 2013 in order to account for digging-in effects (Woodcock 2005). Thereafter, traps were active for two weeks at the beginning of each of the six sampling periods; for this they were filled to approximately one third full with water, with a droplet of scentless detergent to break surface tension. A piece of chicken-wire mesh was pegged over the mouth of the trap to prevent by-catch of non-target mammals and reptiles (Woodcock 2005), whilst only potentially excluding the very largest of

144 beetle species. A transparent plastic roof was suspended above each trap for protection during
145 rain events. A lid was placed over each trap in between sampling events.

146 *ii) Understorey arthropods (Suction sampling)*

147 Two 1×1m plots (selected at random) within each ring were used on the woodland floor. A
148 petrol-powered vacuum 'G-Vac' device (SH 86C, Stihl AG & Co. KG, Germany, Bell et al.
149 2000), fitted with an organza bag to capture dislodged debris and invertebrates, was passed over
150 the understorey herbaceous vegetation in a zig-zag pattern for 20 seconds during each sampling
151 event. Sampling was carried out when the vegetation was dry to the touch.

152 *iii) Aerial arthropods (Sticky trapping)*

153 In each of the six rings, 16 yellow card sticky traps (Bugs for Bugs, Mundubbera, Australia)
154 were secured to the central scaffold at four height intervals (2, 5, 10 and 20m) facing each
155 compass direction. This allowed a full range of arthropods occurring at different strata to be
156 sampled. Traps were left in place for one week prior to collection.

157 *Identification and processing*

158 Arthropods were counted and identified under a dissecting microscope (SZ51, Olympus, Japan)
159 to at least Order level (except for three groups taken to Subclass only – Acari, Collembola and
160 Chilognatha), and in some cases, Family level, to more reliably determine functional guild in as
161 many cases as possible (Hamilton *et al*. 2012; for a full list of identified groups and guild
162 assignments, see Table S1). Psyllidae (Hemiptera) were excluded from the study as they are the
163 focus of a concurrent study occurring at the site (Gherlenda *et al.*, 2016). Better estimations of
164 the energy flow occurring through different trophic levels within communities can be achieved

through the assessment of biomass (Saint-Germain *et al*. 2007). Thus, after abundances were taken, pitfall and suction samples were dried at 60°C to constant weight before weighing using a microbalance with 1µg accuracy (model XP6, Mettler-Toledo GmbH, Germany).

Statistical analyses

All statistics were performed in R, version 3.2.0 (R Core Team 2015). To avoid pseudoreplication, the subplots in each ring were pooled for all analyses, giving one sample per ring, per time point (n = 6, 36 plot-time samples in total for pitfall and sticky traps, 30 for suction). Separate analyses were carried out on data from each of the three sampling methods to enable assessment of the effect of elevated CO_2 on the arthropod communities in the different niches.

Abundance analyses

Total arthropod abundance, and the abundance of individual taxa and functional groups, was analysed firstly using generalized liner mixed models (GLMM) with Poisson error distributions using `glmer`. Models contained CO_2 treatment as a fixed effect and date sampled as a random factor. Model fit was verified by inspection of residual plots and overdispersion parameters from the `overdisp_fun` function (specified at http://glmm.wikidot.com/faq). In the majority of cases, data were overdispersed and so models were refitted using the negative binomial extension of GLMM, `glmer.nb`, in `lme4` (Bates *et al*. 2014). The significance of CO_2 treatment as a predictor was assessed using likelihood ratio tests between the full model and a reduced model without the fixed effect of CO_2 treatment (Faraway 2006).

Orders which were poorly represented – found in fewer than ten percent of samples or had fewer than 50 individuals - were removed from the individual Order analyses. Sanguivores were also

not analysed due to small sample size. In one case (aerial Thysanoptera), a negative binomial model did not adequately fit the data and so an observation-level random effect was included in the model to account for overdispersion (Harrison 2014).

Biomass analyses

Similar to the abundance analyses, arthropod biomass data (in terms of total sample biomass, not individual biomass) were analysed for separate functional guilds and Orders, with the same poorly-represented groups removed. Total arthropod biomass across all groups was also analysed for each sampling method. Data were modelled using linear mixed models (LMM) with the `lmer` function, with CO_2 treatment as a fixed effect and date sampled as a random factor. In all cases, biomass was rank transformed prior to analysis in order to meet assumptions of homoscedasticity of residuals. For groups with tied ranks (where zeros were present in the variable), the analysis was iterated 1000 times on retransformed data with randomly broken ties to attain stable average P and χ^2 values.

Community composition

To assess the effects of elevated CO_2 on overall community composition, we used permutational multivariate analysis of variance (PERMANOVA) coupled with non-metric multidimensional scaling (NMDS) to visualise the data (Hillstrom *et al*. 2014), with the package `vegan` (`adonis` and `metaMDS` functions, Oksanen *et al*. 2015). For community-level analyses, poorly-represented taxa were included. PERMANOVA was carried out on the three niche-types separately, with the fixed effect of CO_2 treatment, on both functional guild and Order-level abundance data. Analyses were carried out on Bray-Curtis dissimilarity matrices, permuted 999 times. The number of dimensions, k, used in each NMDS analysis was determined by visual

209 inspection of stress plots and stress values. Stress values were <0.2 across multiple runs for all
210 analyses.

211 Due to the low replication inherent in FACE designs, we set a critical *P* value of 0.1 to avoid
212 type II errors, as recommended by Lindroth & Raffa (2016) and consistent with previous studies
213 of this type (Sanders *et al*. 2004; Villalpando *et al*. 2009; Hamilton *et al*. 2012).

214

215 **Results**

216 A total of 83,528 arthropods from 19 different taxa (16 Orders and three Subclasses) were
217 collected and identified during the experiment (14,459 ground-dwelling, 19,153 understorey and
218 49,916 aerial arthropods; Table S1). Total arthropod abundance was lower in elevated CO_2 in all
219 three of the sampled niches; this effect was significant for ground-dwelling and aerial
220 invertebrates (Table 1), which decreased by 14.7% and 12.9% respectively (ground-dwelling
221 total individuals ± SD: ambient 7,803 ± 280.17, elevated 6,656 ± 280.11; understorey: ambient
222 11,362 ± 792.56, elevated 7,791 ± 437.55; aerial: ambient 26,672 ± 384.29, elevated 23,244 ±
223 403.32, Table 1). Across all groups, total arthropod biomass did not significantly differ between
224 CO_2 treatments ($p > 0.1$, Table 1).

225 *Ground-dwelling arthropods*

226 The abundance of ground-dwelling chewing herbivores was significantly reduced under elevated
227 CO_2 conditions (Fig. 1b, Table 2), though their biomass remained unchanged ($p > 0.1$).
228 Detritivores and omnivores showed a decrease in biomass under elevated CO_2, but did not show
229 measurable declines in abundance (Fig. 1a, b, Table 2).

The abundances of ground-dwelling Hymenoptera, Isopoda and Orthoptera were significantly reduced under elevated CO_2 (Fig. 1c and d, Table 1), with latter two groups also showing decreases in biomass (Fig. 1c, d, Table 1). Acarina showed an increase in biomass (Fig. 1c, Table 1), with no evidence of change in abundance ($p > 0.1$).

Understorey arthropods

Declines were also seen in the abundance of certain groups in the understorey, though different groups were affected. Omnivores showed a significant decrease in average abundance and this was coupled with a marked decline in population biomass (Fig. 2, Table 2). None of the other feeding guilds showed a significant response to elevated CO_2 in this niche ($p > 0.1$).

At Order-level, Coleoptera were significantly decreased in abundance under elevated CO_2, but their biomass was not significantly different from ambient CO_2 (Fig. 2d, Table 1). While the data for understorey Isopoda could not be accurately modelled, this group appeared to show a trend towards lower abundance under elevated CO_2, as found for the same group in ground-dwelling samples (Fig. 2d vs. Fig. 1c).

Aerial arthropods

Elevated CO_2 generally resulted in decreased abundances of aerial arthropods. At feeding guild level, both scavengers and parasitoids experienced a significant decline in abundance (Fig. 3a, b, Table 2). At Order-level, significant decreases were seen for four of the recorded taxa; Hymenoptera, Neuroptera, Acari and Collembola (Fig. 3c, d, Table 1). However, in contrast to these declines, aerial Psocoptera showed a significant increase in abundance under elevated CO_2 (Fig. 3d, Table 1).

Community composition

While elevated CO_2 resulted in significant changes in the abundances of several different feeding guilds and Orders (summarised in Fig. 4), this did not significantly affect the community composition occurring in any of the three niche types, either in terms of functional guild or Order composition (Table S2, Fig. S1).

Discussion

Elevated CO_2 caused widespread changes in arthropod abundance and biomass

To our knowledge, this is the first study of its kind to find significant declines in the abundance of a wide range of woodland arthropods under elevated CO_2; out of the 21 taxonomic and functional groups which satisfied our analysis criteria, over half (11 groups) experienced significant declines in abundance. Previous work on soil micro-arthropod communities has found similar decreases (Hansen *et al*. 2001; Loranger *et al*. 2004), yet most previous studies looking at aboveground invertebrate communities have revealed no significant changes in abundance as a result of elevated CO_2 (Sanders *et al*. 2004; Hillstrom & Lindroth 2008; Hillstrom *et al*. 2014).

The declines in total arthropod abundance did not translate into overall declines in total biomass, though this is not unexpected as the two metrics are known to not necessarily correlate well (Saint-Germain *et al*. 2007). However, we did find significant changes in arthropod biomass at the individual functional group/Order level, five out of six of which were negative. This reinforces the findings from the abundance analyses and indicates the potential for changes in

ecosystem functioning. Reductions in biomass point to the loss of larger bodied organisms, especially in groups which did not see a corresponding reduction in abundance, such as ground-dwelling detritivores and omnivores. Larger organisms are likely to be of greater importance for trophic interactions occurring within the ecosystem (Saint-Germain *et al*. 2007), as energy flow through trophic levels is tied to body mass (Brown *et al.*, 2004). Conversely, in cases where declines in abundance were not reflected by biomass data (e.g. chewing herbivores and Coleoptera), there may be a greater proportion of larger-bodied individuals occurring under elevated CO_2 compared with ambient conditions, suggesting that ecological functionality may be more likely to be maintained for these groups, despite population declines.

Elevated CO_2 had variable effects on feeding guilds

We predicted that chewing herbivores would suffer a decrease in abundance under elevated CO_2 compared with other feeding guilds with different feeding methods, such as sap-suckers, which may even stand to benefit from such conditions via changes in phloem chemistry (Bezemer & Jones 1998). We found significant reductions in the abundances of ground-dwelling chewing herbivores, though this effect was not seen in either the understorey or aerial niches. We found declines in the abundance and biomass of omnivorous taxa in the understorey and at ground level; these animals will also have partially plant-based diets. These findings are consistent with those reported in other studies of this type (Stiling *et al.*, 2002, 2003; Hamilton *et al.*, 2012), and could indicate a reduction in herbivore-pressure and herbivore-mediated nutrient cycling in the system.

Given the decline in parasitoids and stable levels of other predatory taxa, the reduction in the abundance of herbivorous taxa at ground and understorey level is unlikely to be explained by

294 changes in top-down regulation. We based our prediction that herbivore abundance would be
295 reduced under elevated CO_2 conditions on the widely reported decrease in plant resource quality
296 observed elsewhere under the same conditions (Robinson *et al*. 2012). However, work carried
297 out at the EucFACE site concurrently with this study has revealed no change in various plant
298 quality metrics, including canopy C:N ratios (Gherlenda *et al*. 2015) and leaf area index
299 (Duursma *et al.*, 2015). This is not entirely unexpected; Hamilton *et al*. (2012) also observed
300 changes in arthropod populations under elevated conditions with no accompanying alteration in
301 C:N ratios of plant tissues. One as yet undetermined plant-mediated mechanism for these
302 declines could be alterations in plant secondary chemistry occurring under elevated CO_2, as
303 found in other studies and known to affect invertebrate herbivores (Robinson *et al.* 2012).
304 Further work is needed to link the observed changes in invertebrate abundance with plant quality
305 changes occurring in the woodland at EucFACE.

306 In contrast to the declines in chewing herbivores and omnivores, sap-sucking herbivores (Order
307 Hemiptera) did not decline in any of the three niche types. However, work by other researchers
308 at EucFACE has shown decreased abundance in the abundance of three species of psyllids under
309 elevated CO_2 (Gherlenda *et al.*, 2016). While controlled environment studies tend to report
310 enhanced abundance and performance of sap-feeders, linked with CO_2-induced changes in
311 phloem and sap chemistry (Bezemer & Jones, 1998), these often do not consider natural
312 enemies. Hentley *et al*. (2014) showed that aphid populations under elevated CO_2 were supressed
313 to population levels at ambient CO_2 when a predatory ladybird was also included in the
314 experiment. On the other hand, Percy *et al*. (2002) found that the severity of aphid infestations
315 on aspen was increased under long-term CO_2 exposure, as a result of asynchrony between aphid
316 and natural enemy populations. In our study, given the significant reduction found in parasitoid

317 abundance in the canopy, there is the potential for reduced top-down regulation of sap-feeding
318 insects in the future, and thus population growth. Such growth could increase herbivory levels
319 under elevated CO_2, as found by Couture *et al.* (2015). Long term monitoring would be needed
320 at the EucFACE experimental site to substantiate this. Presently, however, the decline in
321 chewing herbivores and omnivores and comparable levels of (non-psyllid) Hemiptera suggests
322 that herbivory will decline in *Eucalyptus* woodland as emissions of CO_2 rise, as found in other
323 northern hemisphere systems (Hamilton *et al.*, 2004; Knepp *et al.*, 2005).

324 We also found a significant decline in the abundance of scavengers in the canopy, likely driven
325 by the significant decrease in the abundance of mites (Acari) under elevated CO_2. The significant
326 reduction in mites is consistent with findings from other studies (Hansen *et al*. 2001; Loranger *et*
327 *al*. 2004). However, the same decline was not seen in the ground-dwelling and understorey
328 samples which contained far greater abundances of this group; indeed, the total biomass of
329 ground dwelling mites actually increased under elevated CO_2, potentially as a result of larger
330 individuals of greater body size.

331 *Specialist vs. generalist natural enemies*

332 We predicted that arthropods at higher trophic levels would show greater declines in abundance
333 than groups from lower trophic levels, given the generally greater sensitivity of higher trophic
334 levels to environmental change (Voigt *et al*. 2003). We expected this to be particularly true for
335 more specialised feeding groups such as parasitoids, because they are more restricted by tightly-
336 coupled relationships with a limited number of host species compared with generalist predators
337 which can exploit a greater range of prey species. We found significant reductions in the
338 abundance of aerial parasitoid wasps, as expected, and as such this study adds to the body of

339 evidence that specialised taxa may be more susceptible to environmental change (e.g. Hance *et*
340 *al*. 2007). Conversely, previous studies from similar sites have found increases in the numbers of
341 parasitoids or parasitism rates under elevated CO_2 (Percy *et al.*, 2002; Stiling *et al.*, 2002, 2003;
342 Hillstrom & Lindroth, 2008). Stiling *et al*. (2003) attributed their findings to the host plant
343 quality-mediated increases development time of host species, leaving them vulnerable to
344 parasitoid attack for longer periods. In our study, host species may well be experiencing reduced
345 development rates – this would require further work to determine – but the reductions seen in
346 absolute host abundance may be more important for parasitoids. The declines found across a
347 range of groups from lower trophic levels, both in terms of abundance and biomass, could be
348 responsible for the declines seen in parasitoid abundance, as their larval food sources become
349 limited.

350 Contrary to our expectations, the abundance and biomass of generalist predators such as spiders
351 did not decline in any of the niche types, despite declines in the number of many of the groups
352 likely to constitute their prey. We did find a significant decline in the abundance of aerial
353 Neuroptera, though this was the only predatory Order to show a response. Previous findings
354 concerning the responses of predatory taxa to elevated CO_2 are mixed, with some studies finding
355 increases in the abundance of carnivorous groups (Sanders *et al*. 2004; Hamilton *et al*. 2012) and
356 one reporting no change (Hillstrom & Lindroth, 2008). In our study, it is possible that highly
357 mobile predators, such as ground-walking spiders and carabid beetles could access prey external
358 to the rings from which they were caught, enabling the maintenance of ambient population levels
359 within elevated rings; however this is also true of winged parasitoids for which we still detected
360 an effect. Alternatively, the effects of elevated CO_2 at the plot level may have deterred certain
361 insects from entering the rings, resulting in the population declines seen for many of the groups

362 studied; this is an inherent issue in plot-level experiments of this type (Moise & Henry 2010) that
363 needs consideration when interpreting our results. Either way, reduced densities of these
364 invertebrate groups in elevated CO_2 suggest that conditions were less favourable for them than
365 those under ambient CO_2 levels. In addition, it could be possible that the predator population is
366 yet to respond to declines in prey availability under elevated CO_2, given the relatively short
367 fumigation time (since late 2012).

368 Our level of taxonomic identification (Order/Family) did not allow for estimations of the
369 abundance of arthropods from the fourth trophic level (intra-guild predators). The inclusion of
370 intra-guild predators within the predator group could potentially mask any CO_2 effects on the
371 abundance of third-level predatory taxa, though we might expect that fourth-level predatory taxa
372 would be negatively impacted by elevated CO_2 over the long term. The design of our study also
373 did not allow for the level of host specificity of herbivorous arthropods to be determined – this
374 could be an interesting consideration for further study, particular in the plant species-rich
375 understorey, as monophagous specialist herbivores have been shown to be more strongly
376 negatively affected by increases in CO_2 than polyphagous species (Stiling & Cornelissen, 2007).

377 *Community composition did not change under elevated CO_2*

378 Despite widespread overall declines within individual trophic groups and Orders, we found no
379 evidence for an effect of elevated CO_2 on community composition, contrary to our predictions.
380 Similarly, other studies of this type have shown weak to non-existent effects of elevated CO_2 on
381 community composition (Sanders *et al*. 2004; Hillstrom *et al*. 2014). Given that the majority of
382 the responses of the different groups to elevated CO_2 in our study, both in terms of abundance
383 and biomass, were negative in nature, this could have resulted in a compositionally-similar

384 communities comprised of fewer total individuals compared with those under ambient
385 conditions.

386 The range of sampling methods used in this study mean that we gained a broad, representative
387 sample of the community occurring in *Eucalyptus* woodland. We found many differences in the
388 responses of the individual trophic and taxonomic groups to elevated CO_2 between sampling
389 methods, indicating the potential for studies using only one sampling technique to overlook
390 effects of elevated CO_2. We therefore stress the importance of using multiple sampling methods
391 in future work in such studies, to ensure that the results more accurately reflect the responses
392 occurring in the system.

393 *Conclusions*

394 There is a growing body of evidence from community-level studies that the responses of
395 invertebrates to climatic and atmospheric change will likely be taxon-specific and idiosyncratic
396 (Sanders *et al*. 2004; Hamilton *et al*. 2012; Hillstrom *et al*. 2014). In support of this, we found
397 differences in the directions and/or strength of change for certain groups between niche types, as
398 well as differences in the responsiveness of the taxa comprising the individual feeding guilds,
399 highlighting the importance of studies across multiple trophic levels (Pocock *et al.*, 2012).
400 However, overall we found evidence for a consistent decline across a broad range of groups
401 under elevated CO_2. Particularly for those groups showing corresponding declines in biomass
402 such as detritivorous Isopoda and omnivores, these declines could indicate reductions in the
403 energy flow attributed to these organisms in the system. Significant reductions in the abundance
404 and biomass of several groups with roles in nutrient cycling and biocontrol suggest that

405 woodland ecosystem processes could potentially be affected as global concentrations of
406 atmospheric CO_2 continue to rise.

407 **References**

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Table 1: Results from likelihood ratio tests performed on GLMMs (abundance) and LMMs (rank-transformed biomass) with and without the fixed effect of CO_2 treatment, with the abundance or biomass of each of the groups collected over the course of the experiment as the dependent variable. † denotes that for this taxa, strong variation in the data made analysis unreliable. Those groups with sample sizes too small for analysis in all three niches are not shown. Significant *P* values are highlighted in bold ($\alpha = 0.1$).

Table 2: Results from likelihood ratio tests performed on GLMMs (abundance) and LMMs (rank-transformed biomass) with and without the fixed effect of CO_2 treatment, with the abundance of the arthropods in each of the recognised guilds as the dependent variable. Significant *P* values are highlighted in bold.

597 Table 1

Group	Niche Type	Abundance		Biomass	
		χ^2_1	*P*	χ^2_1	*P*
Overall	Ground-dwelling	3.442	**0.064**	0.190	0.66
	Understorey	2.412	0.12	0.087	0.77
	Aerial	3.878	**0.049**	-	-
Coleoptera	Ground-dwelling	1.122	0.29	0.219	0.64
	Understorey	3.129	**0.077**	2.540	0.11
	Aerial	0.493	0.48	-	-
Diptera	Ground-dwelling	0.443	0.51	0.623	0.43
	Understorey	2.002	0.16	2.005	0.16
	Aerial	0.194	0.66	-	-
Araneae	Ground-dwelling	1.546	0.21	0.000	1.00
	Understorey	0.023	0.88	0.338	0.56
	Aerial	0.658	0.42	-	-
Acarina	Ground-dwelling	0.085	0.77	2.783	**0.095**
	Understorey	1.854	0.17	2.624	0.11
	Aerial	16.486	**<0.001**	-	-
Hemiptera	Ground-dwelling	0.047	0.83	0.046	0.83
	Understorey	1.188	0.28	0.163	0.69
	Aerial	1.989	0.16	-	-
Hymenoptera	Ground-dwelling	4.646	**0.031**	0.785	0.38
	Understorey	2.580	0.11	0.941	0.33
	Aerial	3.441	**0.064**	-	-
Thysanoptera	Understorey	0.367	0.55	0.013	0.91
	Aerial	2.418	0.12	-	-
Orthoptera	Ground-dwelling	11.347	**<0.001**	5.356	**0.021**
Isopoda	Ground-dwelling	9.010	**0.0027**	14.469	**<0.001**
	Understorey	†	†	†	†
Blattodea	Understorey	0.205	0.65	0.141	0.71
Collembola	Ground-dwelling	0.824	0.36	0.081	0.78
	Understorey	0.148	0.70	0.014	0.91
	Aerial	2.708	**0.10**	-	-
Lepidoptera	Aerial	1.190	0.28	-	-
Psocoptera	Aerial	26.389	**<0.001**	-	-
Neuroptera	Aerial	5.982	**0.014**	-	-

598

599

600

601 Table 2

Group	Niche type	Abundance		Biomass	
		χ^2_1	*P*	χ^2_1	*P*
Scavengers	Ground-dwelling	2.661	0.10	0.003	0.96
	Understorey	2.619	0.11	1.709	0.19
	Aerial	9.961	**0.0016**	-	-
Detritivores	Ground-dwelling	1.712	0.19	3.379	**0.066**
	Understorey	0.116	0.73	1.005	0.32
	Aerial	<0.001	0.98	-	-
Omnivores	Ground-dwelling	1.643	0.11	3.481	**0.062**
	Understorey	3.448	**0.063**	8.471	**0.0036**
	Aerial	1.303	0.25	-	-
Chewing herbivores	Ground-dwelling	2.845	**0.092**	1.419	0.24
	Understorey	0.091	0.76	0.323	0.57
	Aerial	0.252	0.62	-	-
Sucking herbivores	Ground-dwelling	0.751	0.39	0.800	0.38
	Understorey	1.095	0.30	0.078	0.78
	Aerial	1.989	0.16	-	-
Predators	Ground-dwelling	0.597	0.44	0.069	0.79
	Understorey	0.890	0.35	0.149	0.70
	Aerial	0.535	0.47	-	--
Parasitoids	Ground-dwelling	0.305	0.58	0.296	0.59
	Understorey	0.026	0.87	0.012	0.91
	Aerial	3.422	**0.064**	-	-

602

603

Fig. 1 Mean abundance of different functional guilds (a, b) and taxonomic groups (c, d) of ground-dwelling arthropods, split by CO_2 treatment (across all dates). Ambient samples are shown with white bars; those from elevated conditions are in grey. To the right of each bar total average biomass ± SE is shown for the corresponding group. Significant differences (from GLMMs (abundance) and LMMs (biomass), Table 1 and Table 2) are denoted by asterisks (* $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$). Error bars show ± SE of the mean.

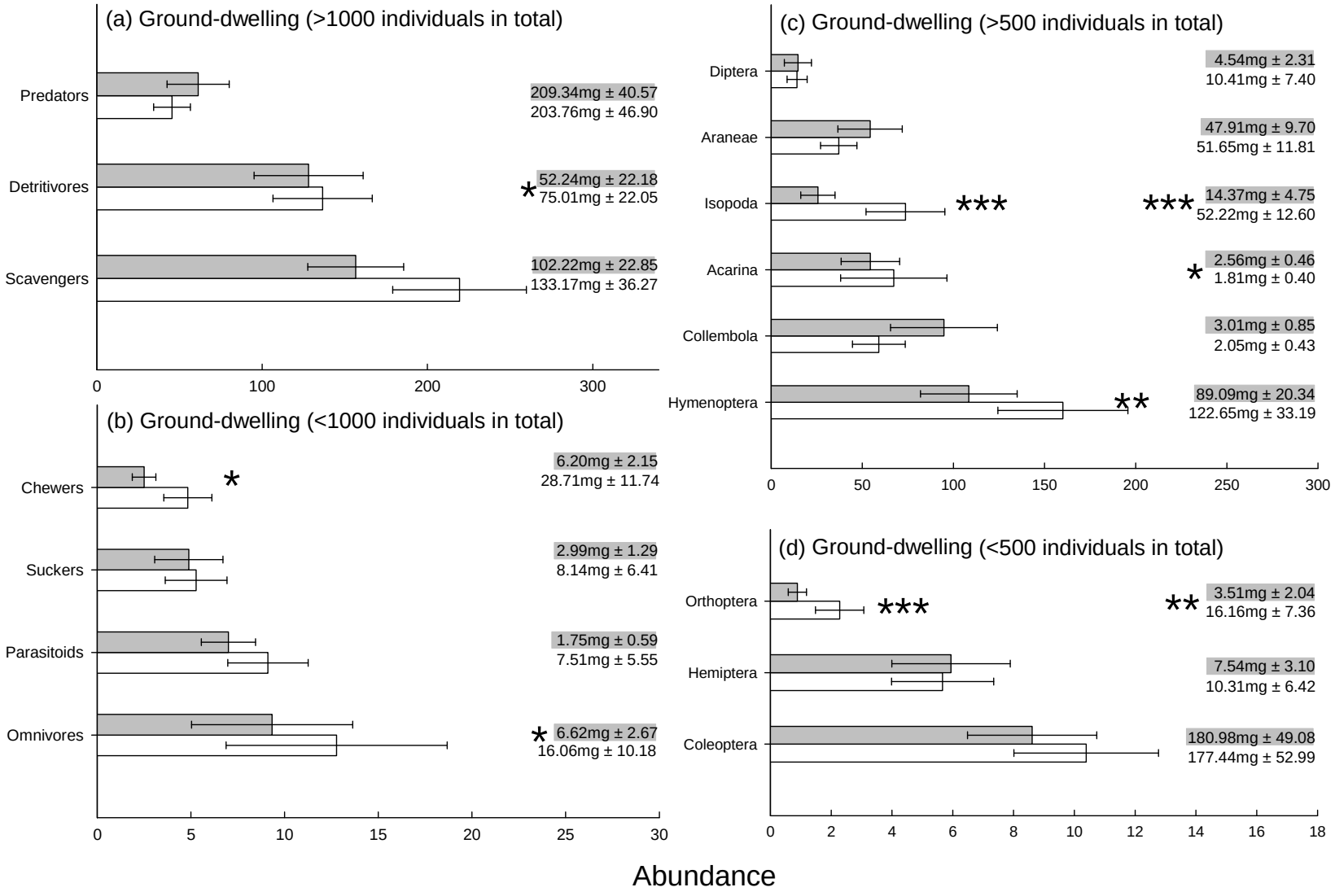
Fig. 2 Mean abundance of different functional guilds (a, b) and taxonomic groups (c, d) of understorey arthropods, split by CO_2 treatment (across all dates). Ambient samples are shown with white bars; those from elevated conditions are in grey. To the right of each bar total average biomass ± SE is shown for the corresponding group. Significant differences (from GLMMs (abundance) and LMMs (biomass), Table 1 and Table 2) are denoted by asterisks (* $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$). Error bars show ± SE of the mean.

Fig. 3 Mean abundance of different, the functional guilds (a, b) and taxonomic groups (c, d) of aerial arthropods, split by CO_2 treatment (across all dates, with no biomass data due to the sampling method). Ambient samples are shown with white bars; those from elevated conditions are in grey. Significant differences (from GLMMs, Table 1 and Table 2) are denoted by asterisks (* $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$). Error bars show ± SE of the mean.

Fig. 4 a schematic diagram summarising the main findings in this study, and showing a scaled drawing of one of the EucFACE arrays. CO_2 (or air in the case of ambient rings) is pumped in to the ring from the vertical vent pipes surrounding each array. The crane is used to access the tree canopy. Arrows show the direction of significant changes in the abundances of the taxa shown, in response to elevated CO_2. The widths of the arrows indicate their level of significance, with

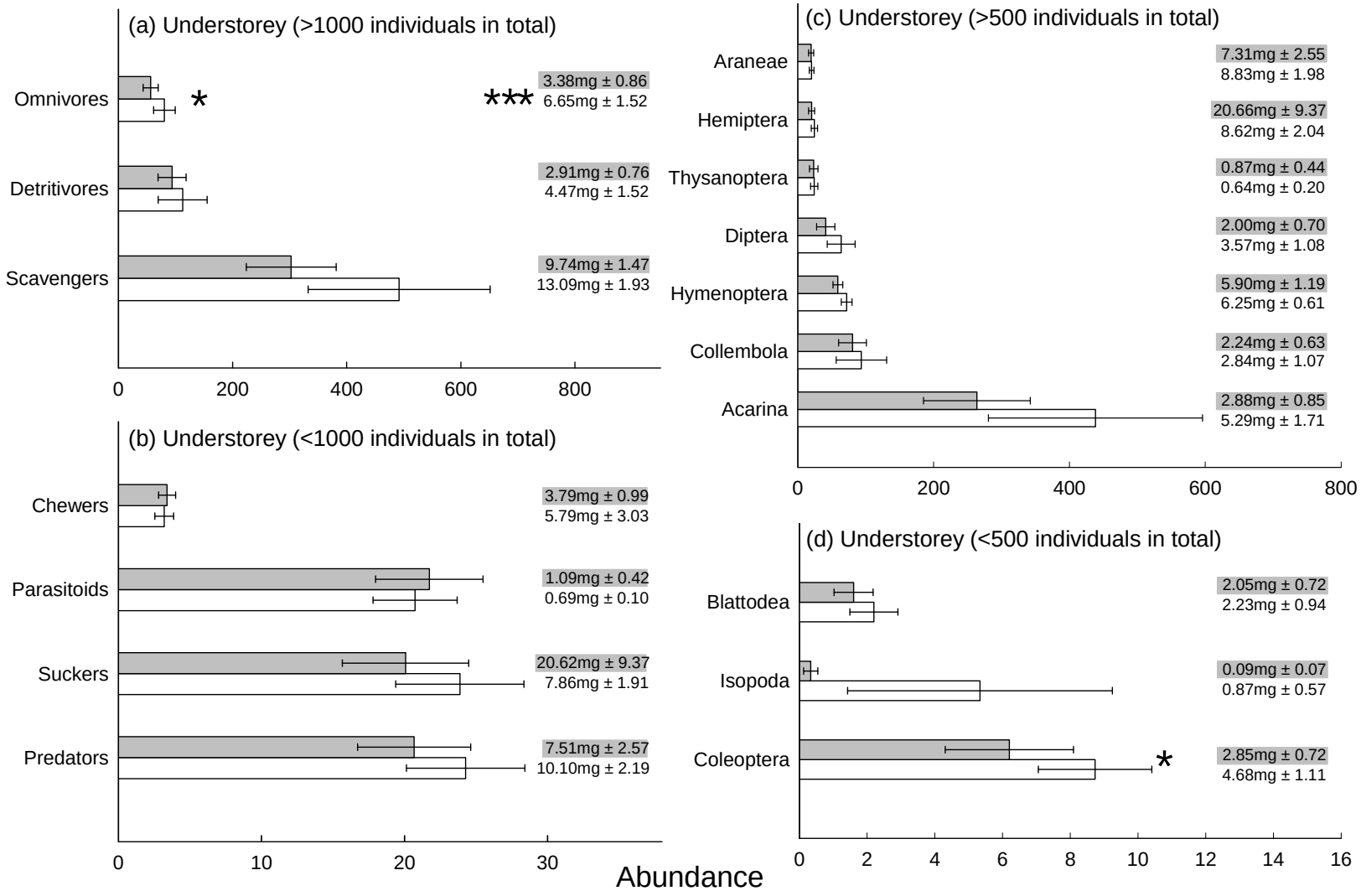
wider arrows representing smaller *P* values, and thus greater evidence against the null hypothesis.

Fig. 1


(a) Ground-dwelling (>1000 individuals in total)
Predators
Detritivores
Scavengers
209.34mg ± 40.57
203.76mg ± 46.90
52.24mg ± 22.18
75.01mg ± 22.05
102.22mg ± 22.85
133.17mg ± 36.27
0
100
200
300
(b) Ground-dwelling (<1000 individuals in total)
Chewers
Suckers
Parasitoids
Omnivores
6.20mg ± 2.15
28.71mg ± 11.74
2.99mg ± 1.29
8.14mg ± 6.41
1.75mg ± 0.59
7.51mg ± 5.55
6.62mg ± 2.67
16.06mg ± 10.18
0
5
10
15
20
25
30
(c) Ground-dwelling (>500 individuals in total)
Diptera
Araneae
Isopoda
Acarina
Collembola
Hymenoptera
4.54mg ± 2.31
10.41mg ± 7.40
47.91mg ± 9.70
51.65mg ± 11.81
14.37mg ± 4.75
52.22mg ± 12.60
2.56mg ± 0.46
1.81mg ± 0.40
3.01mg ± 0.85
2.05mg ± 0.43
89.09mg ± 20.34
122.65mg ± 33.19
0
50
100
150
200
250
300
(d) Ground-dwelling (<500 individuals in total)
Orthoptera
Hemiptera
Coleoptera
3.51mg ± 2.04
16.16mg ± 7.36
7.54mg ± 3.10
10.31mg ± 6.42
180.98mg ± 49.08
177.44mg ± 52.99
0
2
4
6
8
10
12
14
16
18
Abundance

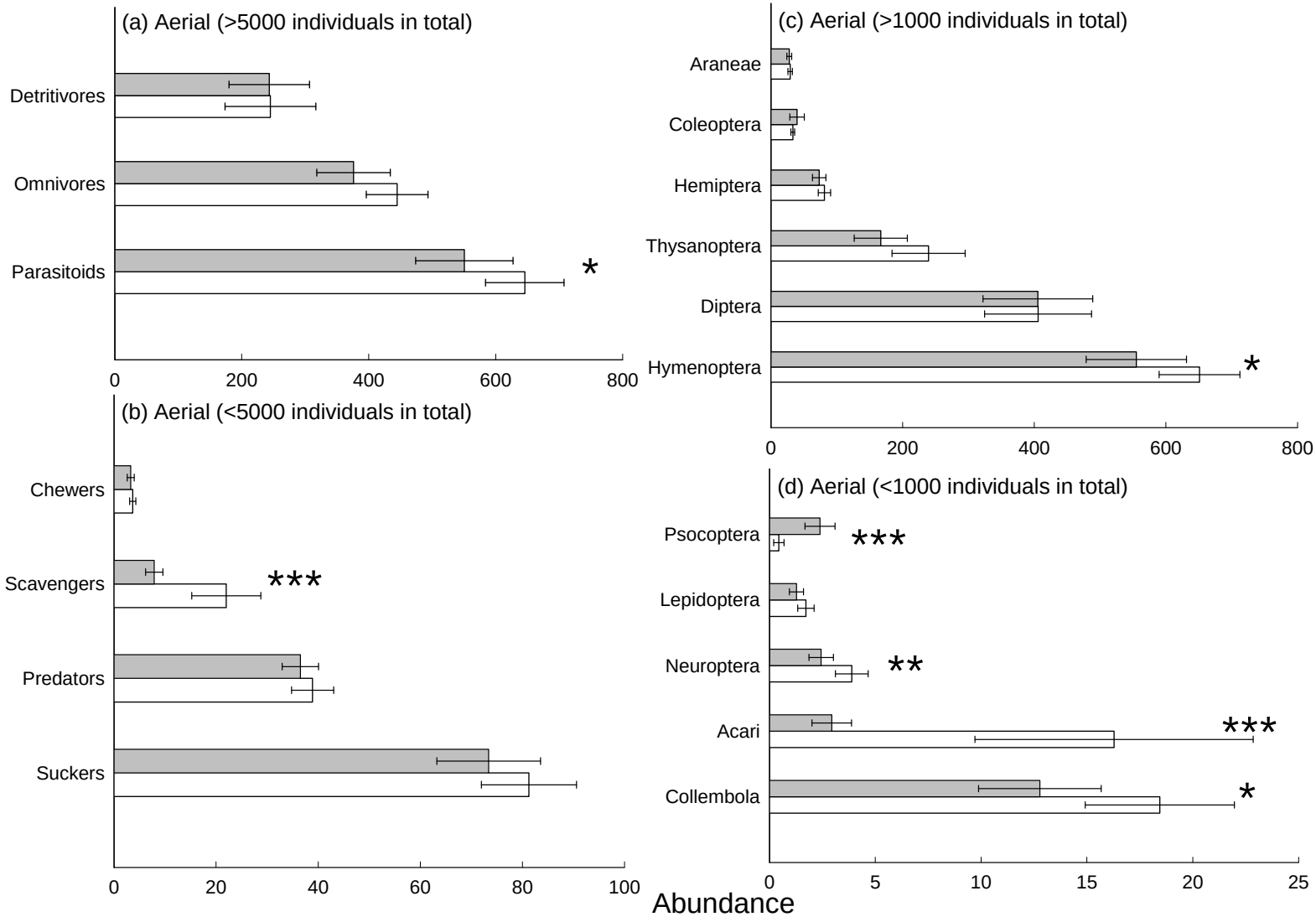
629

Fig. 2


(a) Understorey (>1000 individuals in total)
Omnivores
Detritivores
Scavengers
*
***
3.38mg ± 0.86
6.65mg ± 1.52
2.91mg ± 0.76
4.47mg ± 1.52
9.74mg ± 1.47
13.09mg ± 1.93
0
200
400
600
800
(b) Understorey (<1000 individuals in total)
Chewers
Parasitoids
Suckers
Predators
3.79mg ± 0.99
5.79mg ± 3.03
1.09mg ± 0.42
0.69mg ± 0.10
20.62mg ± 9.37
7.86mg ± 1.91
7.51mg ± 2.57
10.10mg ± 2.19
0
10
20
30
(c) Understorey (>500 individuals in total)
Araneae
Hemiptera
Thysanoptera
Diptera
Hymenoptera
Collembola
Acarina
7.31mg ± 2.55
8.83mg ± 1.98
20.66mg ± 9.37
8.62mg ± 2.04
0.87mg ± 0.44
0.64mg ± 0.20
2.00mg ± 0.70
3.57mg ± 1.08
5.90mg ± 1.19
6.25mg ± 0.61
2.24mg ± 0.63
2.84mg ± 1.07
2.88mg ± 0.85
5.29mg ± 1.71
0
200
400
600
800
(d) Understorey (<500 individuals in total)
Blattodea
Isopoda
Coleoptera
*
2.05mg ± 0.72
2.23mg ± 0.94
0.09mg ± 0.07
0.87mg ± 0.57
2.85mg ± 0.72
4.68mg ± 1.11
0
2
4
6
8
10
12
14
16
Abundance

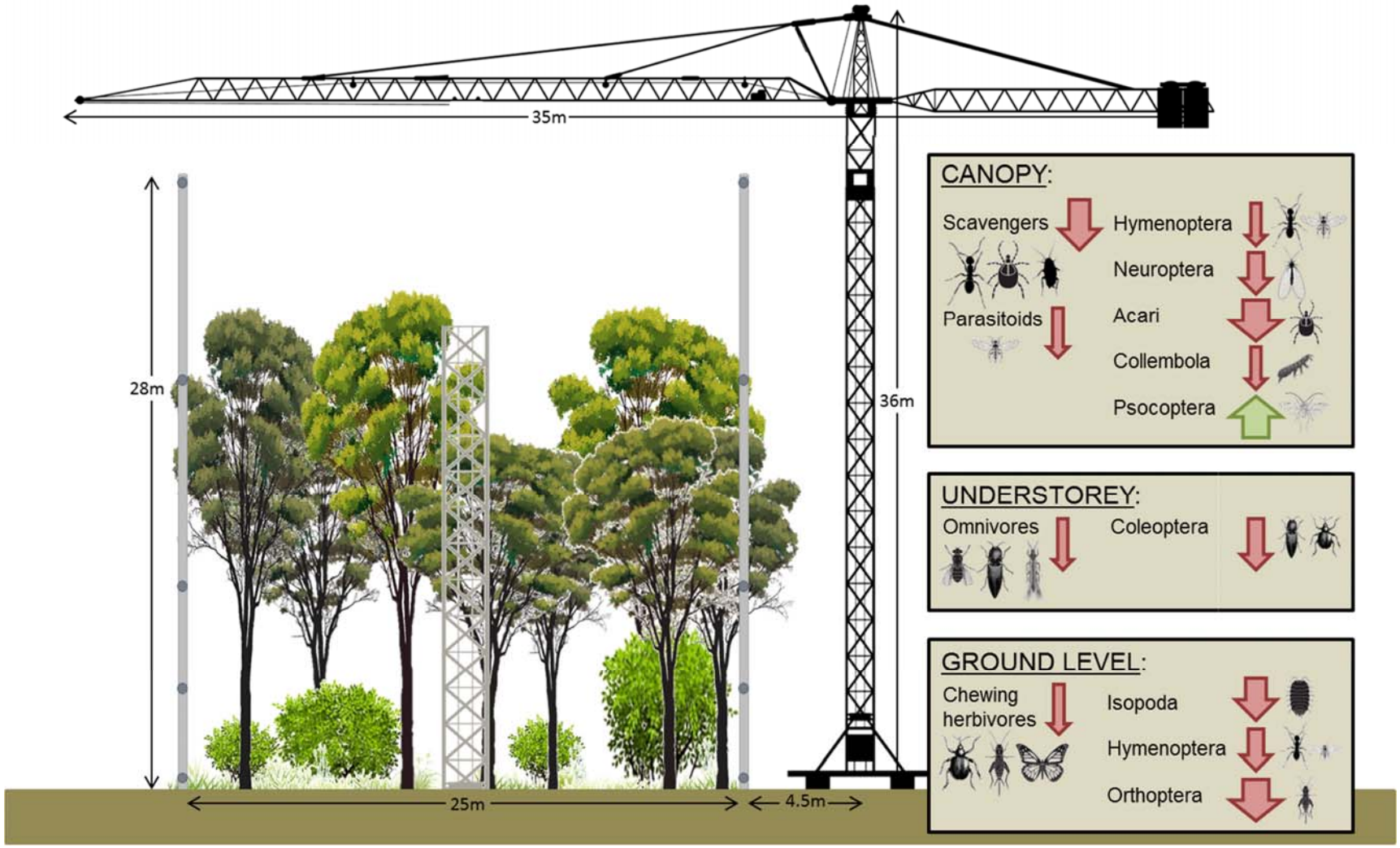
630

Fig. 3


(a) Aerial (>5000 individuals in total)
Detritivores
Omnivores
Parasitoids
*
0
200
400
600
800
(b) Aerial (<5000 individuals in total)
Chewers
Scavengers
***
Predators
Suckers
0
20
40
60
80
100
(c) Aerial (>1000 individuals in total)
Araneae
Coleoptera
Hemiptera
Thysanoptera
Diptera
Hymenoptera
*
0
200
400
600
800
(d) Aerial (<1000 individuals in total)
Psocoptera
***
Lepidoptera
Neuroptera
**
Acari
***
Collembola
*
0
5
10
15
20
25
Abundance

631

Fig. 4


35m
28m
36m
25m
4.5m
CANOPY:
Scavengers
Parasitoids
Hymenoptera
Neuroptera
Acari
Collembola
Psocoptera
UNDERSTOREY:
Omnivores
Coleoptera
GROUND LEVEL:
Chewing herbivores
Isopoda
Hymenoptera
Orthoptera

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Electronic Supplemental Material

Table S1: a list of the total abundances of the groups identified in this study and their functional guild classifications (from Barker, 2004; CSIRO, 1991; Moran & Southwood, 1982; Zimmer, 2002). Those groups marked with an asterisk were identified to Subclass level only.

Groups identified	Total abundance			Functional guild classification
	ground-dwelling	understorey	aerial	
Diptera:	(520	1576	14605)	-
Sciaridae	179	368	8144	Detritivores
Mosquitoes	0	9	0	Sanguivores
Psychodidae	1	7	38	Detritivores
Syrphidae	0	0	29	Predators
Asilidae	0	0	1	Predators
Other Diptera	340	1192	6393	Omnivores
Coleoptera:	(342	224	1314)	-
Elateridae	8	0	12	Omnivores
Carabidae	157	7	0	Predators
Tenebrionidae	2	0	0	Scavengers
Stapylinidae	45	32	109	Predators
Scarabaeidae	23	0	0	Detritivores
Curculionidae	66	32	66	Chewing herbivores
Cantharidae	0	1	12	Omnivores
Coccinellidae	0	0	72	Predators
Other Coleoptera	41	152	1043	Omnivores
Hymenoptera:	(4833	1966	21711)	-
Non-ant Hymenoptera	290	637	21540	Parasitoids
Formicidae	4543	1329	171	Scavengers
Neuroptera	3	0	114	Predators
Lepidoptera	9	22	54	Chewing herbivores
Hemiptera:	(209	673	2784)	-
Predatory Hemiptera (e.g. Reduviidae)	26	14	0	Predators
Other (non-psyllid) Hemiptera	183	659	2784	Sucking herbivores
Thysanoptera	9	715	7317	Omnivores
Orthoptera	57	45	5	Chewing herbivores
Psocoptera	4	23	51	Detritivores
Blattodea	32	57	21	Scavengers
Mantodea	0	1	4	Predators
Lithobiomorpha	25	1	0	Predators
Scolopendromorpha	15	0	0	Predators
Chilognatha*	1	0	0	Detritivores
Araneae	1644	598	1028	Predators
Pseudoscorpiones	8	21	0	Predators
Acari*	2191	10531	346	Scavengers
Isopoda	1788	85	0	Detritivores
Collembola*	2769	2615	562	Detritivores
GRAND TOTAL:		83,528		

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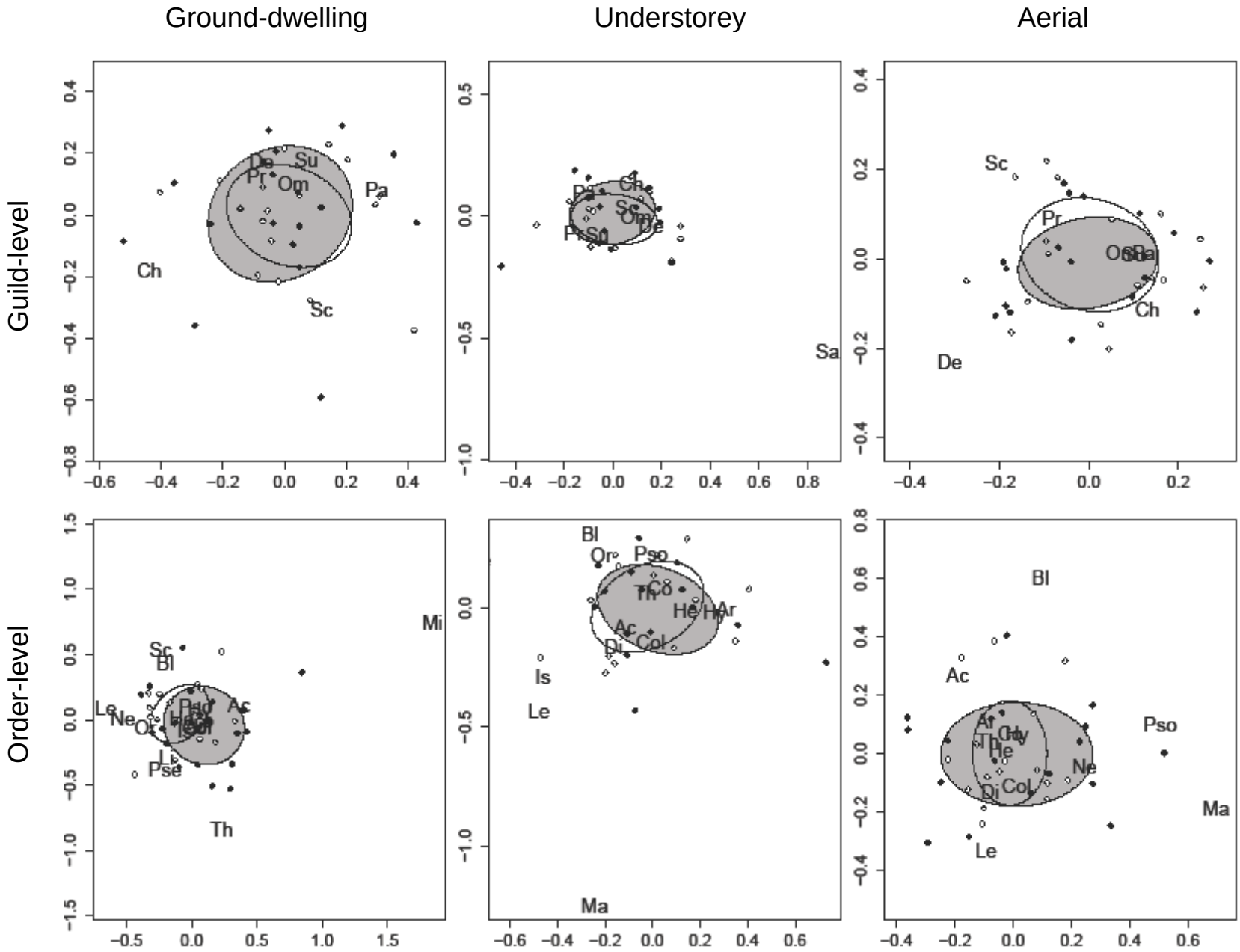
Table S2: Results from multivariate permutational analysis (PERMANOVA) of the effect of CO_2 treatment on community data from the three different niche-types, partitioned by Order identity and functional feeding guild classification (FG).

Niche type	Community tested	d.f. (treatment, residual)	SS	MS	Pseudo-F	R_2	*P* (perm)
Ground-dwelling invertebrates	Order	1, 34	0.204	0.204	1.119	0.032	0.326
	FG	1, 34	0.124	0.124	0.837	0.024	0.479
Understorey invertebrates	Order	1, 28	0.068	0.068	0.372	0.013	0.823
	FG	1, 28	0.055	0.055	0.323	0.011	0.812
Aerial invertebrates	Order	1, 34	0.064	0.064	0.882	0.025	0.421
	FG	1, 34	0.066	0.066	1.076	0.031	0.338

Fig. S1: NMDS plots of arthropod community data in each of the three niche types, partitioned by functional guild classification and Order identity. Ambient CO_2 samples are shown in white, with elevated CO_2 in dark grey/black. Ellipses show the standard deviation around each community centroid. All sampling dates were included in the analysis. Pr Predators, Pa Parasitoids, Sc Scavengers, De Detritivores, Om Omnivores, Su Suckers, Ch Chewers, Sa Sanguivores; Di Diptera, Co Coleoptera, Ar Araneae, Ac Acarina, He Hemiptera, Th Thysanoptera, Bl Blattodea, Is Isopoda, Col Collembola, Hy Hymenoptera, Pse Pseudoscorpiones, Or Orthoptera, Le Lepidoptera, Li Lithobiomorpha, Ma Mantodea, Pso Psocoptera, Ne Neuroptera, Mi Millipedes (Chilognatha), Sc Scolopendromorpha. Stress values remained below 0.2 for all analyses, with *k*=3 dimensions.


Ground-dwelling
Understorey
Aerial
Guild-level
Order-level
Su
De
Pr
Om
Pa
Ch
Sc
Sa
Ch
Sc
De
Mi
Sc
Bl
Le
Ne
Ac
Pse
Th
Bl
Or
Pso
Ac
Col
Is
Le
Ma
Bl
Ac
Pso
Ne
Le
Ma