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 Boom and bust: rapid feedback responses between insect outbreak dynamics and canopy leaf area impacted by rainfall and CO₂

Andrew N. Gherlenda, Jessica L. Esveld, Aidan A. G. Hall, Remko A. Duursma, Markus Riegler

 Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia

Corresponding author: Markus Riegler, Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith NSW 2751, Australia.

Email: m.riegler@westernsydney.edu.au; Phone: +61-2-4570 1229

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Abstract

Frequency and severity of insect outbreaks in forest ecosystems are predicted to increase with climate change. How this will impact canopy leaf area in future climates is rarely tested. Here, we document function of insect outbreaks that fortuitously and rapidly occurred in an ecosystem under free-air CO$_2$ enrichment. Over the first two years of CO$_2$ fumigation of a naturally established mature Eucalyptus woodland we continuously assessed population responses of three sap-feeding insect species of the psyllid genera Cardiaspina, Glycaspis and Spondyliaspis for up to ten consecutive generations. Concurrently, we quantified changes in the canopy leaf area index (LAI). Large and rapid shifts in psyllid community composition were recorded between species with either flush (Glycaspis) or senescence-inducing (Cardiaspina, Spondyliaspis) feeding strategies. Within the second year, two psyllid species experienced significant and rapid population build-up resulting in two consecutive outbreaks: first, rainfall stimulated Eucalyptus leaf production increasing LAI, which supported population growth of flush-feeding Glycaspis without impacting LAI. Glycaspis numbers then crashed and were followed by the outbreak of senescence-feeding Cardiaspina fiscella that led to significant defoliation and reduction of LAI. For all three psyllid species, the abundance of lerps, protective coverings excreted by the sessile nymphs, decreased at e[CO$_2$]. Higher lerp weight at e[CO$_2$] for Glycaspis but not the other psyllid species provided evidence for compensatory feeding by the flush-feeder but not the two senescence-feeders. Our study demonstrates that rainfall drives leaf phenology, facilitating the rapid boom-and-bust succession of psyllid species, eventually leading to significant defoliation due to the second but not the first outbreaking psyllid species. In contrast, e[CO$_2$] may impact psyllid abundance and feeding behaviour, with psyllid species-specific outcomes for defoliation severity, nutrient transfer and trophic cascades. Psyllid populations feeding on Eucalyptus experience rapid boom-and-bust cycles depending on availability of suitable foliage driven by rainfall patterns and leaf phenology.
Introduction

At high population densities, defoliating forest insects can severely impact plant productivity and ecosystem function (Barbosa et al., 2012). Conversely, plant diversity, nutrient content, phenology and productivity drive forest insect herbivore population dynamics and community composition (Berryman, 1988). Such trophic interactions between plants, herbivores and their antagonists can form the basis for cyclical population peaks of defoliating forest insects (Berryman, 1996). Environmental factors, in particular temperature (Battisti et al., 2005) and water stress (White, 1969), also contribute to the abrupt proliferation of defoliating insect populations. Therefore, climate change is expected to increase the severity and frequency of forest insect outbreaks (Haynes et al., 2014; Trumbore et al., 2015), or it may contribute to the collapse of cyclical insect population dynamics (Esper et al., 2007). While the impact of temperature on forest insects and their outbreak potential has been investigated (Visser & Holleman, 2001; Johnson et al., 2010), the influence of elevated atmospheric CO₂ concentrations (e[CO₂]) and rainfall on insect communities at endemic levels is less certain (Hamilton et al., 2012; Hillstrom et al., 2014; Gherlenda et al., 2016) and the effect of e[CO₂] and rainfall on insect communities at epidemic levels is unknown.

Herbivores have been extensively studied with regard to the plant-stress hypothesis. This hypothesis was established based on observations of psyllid outbreaks on Eucalyptus following soil water deficits which are thought to result in physiological stress and nitrogenous fluxes in trees (White, 1969). Because of some conflicting results between controlled studies and field observations this hypothesis was refined to the pulsed-stress hypothesis, based on the incorporation of feeding-guild specific responses to water stress-induced nutrient fluxes in plants (Huberty & Denno, 2004). Further changes were made through the sub-categorisation of plant-sap feeding and chewing herbivores into species preferentially feeding on either flush- or senescent leaves (White, 2015).
Within the guild of plant sap-feeders, aphids have so far received considerable attention in climate change research, with an overall prediction that they will profit from plant-mediated effects of e\(\text{CO}_2\), which is likely contingent on species-specific nutrient requirements, such as plant nitrogen content (Newman et al., 2003; Robinson et al., 2012). Other plant sap-feeding insects are also expected to respond positively to the effects of e\(\text{CO}_2\), or to remain unaffected (Docherty et al., 1997; Shi et al., 2014). Beyond this, physiological responses of individual species to e\(\text{CO}_2\) may alter interspecific competition and community structure, resulting in changed relative abundances of species (Stacey & Fellowes, 2002; Sun et al., 2009).

Psyllids have varying nutritional demands and feeding modes (Steinbauer, 2013; Steinbauer et al., 2014), thus their response to plant-mediated e\(\text{CO}_2\) effects may also vary. Australia has a highly diverse endemic psyllid fauna, including many species associated with Eucalyptus (Hollis, 2004). Several of these psyllid species are pests with considerable outbreak potential that can lead to area-wide defoliation and dieback of Eucalyptus over vast areas (Hall et al., 2015). These include species of the genera Cardiaspina (Clark, 1962; White, 1969) and Glycaspis (Moore, 1961). Some species are also significant invasive pest insects outside Australia (Paine et al., 2011), for example, Cardiaspina fiscella in New Zealand (Withers, 2001) and on Norfolk Island (Australian Government Department of Agriculture, 2014). In Australia, outbreaks of a single psyllid species on Eucalyptus have also been linked with observations of increased abundance in other psyllid species (Clark & Dallwitz, 1974).

About 50 % of Australian psyllid species are lerp builders (Hollis, 2004; Sharma et al., 2013). Lerps are solidified faecal excretions produced by individual psyllid nymphs, and function as protective shelters that are hinged to the leaves (Supplementary Fig. S1a-c) (Taylor, 1962; Sharma et al., 2013). The sessile psyllid nymphs feed underneath their lerps throughout their development until adult emergence (Hollis, 2004). The structure of the lerps is characteristic for psyllid genera and, in some instances, also for species (Taylor, 1962). Lerps can fall from the canopy, in particular after having
been vacated by the adult psyllids. Lerps collected from the woodland floor can therefore be used as a measure of lerp-forming psyllid abundance and diversity.

_Eucalyptus_ feeding psyllids have different leaf age preferences for oviposition which may constrain their responses to e[CO₂] even when feeding on the same host tree. Leaf chemistry can vary with leaf age (Ballhorn _et al._, 2011) and this may impact insect herbivores of _Eucalyptus_ at e[CO₂] based on their feeding preferences (Gherlenda _et al._, 2015). _Glycaspis_ generally prefers flush foliage while _Cardiaspina_ is a mature leaf specialist (White, 2015). Furthermore, in contrast to _Glycaspis_, _Cardiaspina_ psyllids can nutritionally enhance fully expanded mature leaves by disrupting the chloroplast thylakoid membrane, thereby effectively inducing leaf necrosis (Supplementary Fig. S1) and early leaf senescence that can result in progressive defoliation (Supplementary Fig. S1d,e) (Steinbauer _et al._, 2014). This enhancement of their food source may also alleviate any negative plant-mediated effects of e[CO₂], and potentially provide a competitive advantage over other co-occurring psyllids.

Here, we quantified population responses of three naturally occurring lerp-forming psyllid species within the _Eucalyptus_ free-air CO₂ enrichment (EucFACE) experiment, both at species and community scales, within the first two years of CO₂ fumigation. Throughout this period we also quantified the change in leaf area index (LAI), a key parameter for canopy processes (Bréda, 2003). The dynamics of LAI are driven by growth of new leaves and turnover of old leaves. For evergreen _Eucalyptus_ woodlands, the timing of new leaf production is highly dependent on rainfall (Pook _et al._, 1997), and this was also demonstrated at the EucFACE site by the detailed assessment of changes in LAI and leaf litter over time (Duursma _et al._, 2016). Evergreen _Eucalyptus_ trees do not constantly produce leaves throughout the year, but rather produce leaves under favourable conditions at any time of the year, and this is largely determined by rainfall patterns (Pook _et al._, 1997; Duursma _et al._, 2016). We expected that increases in LAI stimulated by rainfall of _Eucalyptus_ would drive population dynamics of psyllids, in particular of flush feeders (such as _Glycaspis_), and conversely, outbreaks of
senescence-inducing leaf feeders (such as *Cardiaspina*) would result in eventual but not instant canopy defoliation due to the progressive leaf senescence and necrosis. Therefore we predicted LAI feedback cycles driven by rainfall, leaf production, psyllid outbreak dynamics and eventual defoliation. Furthermore, we hypothesised that overall psyllid abundance would be impacted by e\([\text{CO}_2]\) and that flush-feeders would display different responses than senescence-feeders due to their different feeding strategies.

**Materials and methods**

The large EucFACE experimental site (35 ha) is located within a native remnant of the critically endangered Cumberland Plain Woodland (CPW) in Richmond, NSW, Australia. *Eucalyptus tereticornis*, a species characteristic of the CPW (Tozer, 2003) is the dominant tree species within the site where it forms an open canopy. Prior to the establishment of EucFACE, the woodland site was undisturbed for 75 years, and therefore retains old growth with some re-growth. The site includes six rings (25 m diameter), surrounded by a 28 m high scaffold that extends above the tree canopy and supports the CO\(_2\) supply system. Three rings received CO\(_2\) fumigation via a proportional-integral-derivative type of control algorithm (Lewin et al., 2009) while the remaining three rings received air with no CO\(_2\). This set up does not impact mobility of insects other than through plant-mediated effects of e\([\text{CO}_2]\) (Hamilton et al., 2012). Beginning from September 2012 until February 2013 (CO\(_2\) ramp-up phase), [CO\(_2\)] was increased at a rate of 30 \(\mu\)mol mol\(^{-1}\) every month. After this ramp-up phase, the set point of diurnal [CO\(_2\)] was at 150 \(\mu\)mol mol\(^{-1}\) above ambient levels.

Over two years, psyllid lerps were collected after they had fallen from trees together with insect frass of chewing insects and leaf litter fallen from the canopy (Gherlenda et al., 2016). Per ring, eight collection baskets (0.1979 m\(^2\) ellipsoid surface area, 0.56x0.45 m axes, 0.40 m high) were staked to the ground. The baskets were lined with a fine nylon mesh (200 \(\mu\)m aperture) which allowed precipitation to pass through freely. During the CO\(_2\) ramp-up psyllid lerps were collected monthly.
from mid-November 2012 to mid-January 2013. After the ramp-up, psyllid lerps were collected for the months of March, June, September and December from mid-March 2013 until mid-December 2014.

Psyllid lerps were visually sorted from frass and plant material that had fallen into the baskets. Lerps were grouped into three types that were characteristic of the genera *Cardiaspina*, *Glycaspis* and *Spondyliaspis* (Supplementary Fig. S1) (Hollis, 2004). Adults of *Cardiaspina* were further identified as *Cardiaspina fiscella* Taylor (Hemiptera: Aphalaridae) by following the identification key by Taylor (1962) that lists *E. tereticornis* as host tree species, and comparison with the holotype kept at the Australian National Insect Collection (CSIRO). *Cardiaspina fiscella* can have five generations each year in the Sydney region (Taylor, 1962), while *Glycaspis* may have up to six, depending on the region (Moore, 1961; Sharma et al., 2013). Generations of *Cardiaspina* sp. on *E. moluccana* are not overlapping (Hall et al., 2015) and this was also observed for *C. fiscella* (J.L.E. and A.A.G.H., personal observation). Therefore up to ten consecutive generations of *C. fiscella*, *Glycaspis* sp. and *Spondyliaspis* sp. were sampled over the two-year study period. Identification to species level for *Glycaspis* and *Spondyliaspis* specimens, both also of the Aphalaridae family within Psylloidea, was not obtained; therefore they are referred to as *Glycaspis* sp. and *Spondyliaspis* sp.. Furthermore, *E. tereticornis* leaves with nymphs and lerps of each lerp type were placed into separate zip-lock bags. Three adult specimens emerging from underneath each lerp type were DNA barcoded (primers CI-J-2441 and TL2-N-3014). Sequence reads were aligned and divergence was calculated using pairwise distance analysis (Hall et al., 2015). All three *Spondyliaspis* sp. individuals had identical mitochondrial haplotypes, while individuals of *C. fiscella* and *Glycaspis* sp. both had two haplotypes with intraspecific nucleotide divergences of 0.4 and 11.2 %, respectively (Supplementary Table S1). Individuals of *C. fiscella* of EucFACE were further genetically characterised on nuclear, mitochondrial and endosymbiont loci (Hall et al., 2016).
The lerps of psyllid species were counted from individual collection baskets, averaged by ring and converted to fallen lerps per square metre soil surface for each month. Dry weights of all lerps (oven dried at 40 °C for 72 hours) were averaged by ring for the samples from December 2012 (CO₂ ramp-up), December 2013 (when all species were low in abundance) and at the peak of each species’ abundance (March 2014 for Glycaspis sp., June 2014 for Spondyliaspis sp., and December 2014 for C. fiscella).

Canopy LAI was estimated from canopy transmittance, using three photosynthetically active radiation (PAR) sensors (LI-190, LI-COR, Lincoln, NE, U.S.A.) located 1 m aboveground, and one PAR sensor extending above the canopy at 23.5 m height, with all four sensors installed in each ring. Only highly diffuse light conditions were used to estimate LAI. This restriction to cloudy days resulted in an irregular time-series, and, therefore, data were interpolated with a semi-parametric smoother (see Duursma et al., 2016). From the smoothed data, the net change in LAI for every month was calculated (dLAI) for every FACE ring separately. The variable dLAI can thus be positive (indicating net leaf growth) or negative (indicating net leaf loss). In general, rainfall events which resulted in soil moisture greater than 0.2 m³ m⁻³ caused an increase in leaf production, as measured by positive dLAI and increases in leaf litter fall as old leaves were replaced with flush leaves (Duursma et al., 2016; Supplementary Fig. S2 and S3).

A linear mixed effect model fitted by restricted maximum likelihood was constructed in R (v3.2.2 R Development Core Team, 2015) using the nlme package. The fixed effects were [CO₂] (as a categorical variable), psyllid species, collection date and all interactions. FACE ring was included as a random effect to account for repeated measures. An autocorrelation function was used in order to test for temporal autocorrelation, and an autoregressive moving average (ARMA) correlation structure was employed to model dependence among observations using a first-order autoregressive structure (AR1) (Bader et al., 2013). Psyllid abundance data was log + 1 transformed to meet the assumptions of normality. Relationships between psyllid abundance and dLAI (dLAI

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values from the current month or one month prior) were modelled and $R^2$ values were calculated according to Nakagawa and Schielzeth (2013) as implemented in the MuMIn R package. To assess the community relationship between the three psyllid species at e[CO$_2$] and across time at EucFACE, Principal Components Analysis (PCA) and Permutational Multivariate Analysis of Variance (PerMANOVA) with Hellinger transformation were performed using the vegan R package.

**Results**

In this study, 28,972 lerps of three psyllid species were counted. During the CO$_2$ ramp-up phase no significant differences were observed in the abundance of lerps collected between CO$_2$ treatments (Fig. 1a; Table 1). After the CO$_2$ ramp-up, the abundance of collected lerps was reduced at e[CO$_2$] when compared with a[CO$_2$] (Fig. 1b; Table 1). A significant species by month interaction was detected both during and after the CO$_2$ ramp-up (Table 1). This was the result of sequential abundance peaks for different psyllid species. From the abundance data collected in this study; the abundance of *Glycaspis* sp. lerps was highest in March 2014 with approximately 5,800 m$^{-2}$ versus 2,400 m$^{-2}$ woodland surface area at a[CO$_2$] and e[CO$_2$], respectively (Supplementary Fig. S4); the abundance of *C. fiscella* lerps was highest in December 2014 with approximately 140 m$^{-2}$ versus 80 m$^{-2}$ woodland surface area at a[CO$_2$] and e[CO$_2$], respectively (Supplementary Fig. S5); and *Spondyliaspis* sp. lerps had an overall lower abundance and displayed a bimodal abundance pattern (Supplementary Fig. S6).

During the first two years of EucFACE, monthly net change in LAI (dLAI; m$^2$ m$^{-2}$ month$^{-1}$) was unaffected by e[CO$_2$] (Duursma et al., 2016), indicating that the difference in psyllid abundance between CO$_2$ treatments was not due to different resource availability between treatments. However, the abundance of *Glycaspis* sp. lerps increased with a preceding increase of dLAI as measured in the month prior to the lerp collection, indicating that new leaf growth stimulated
Glycaspis sp. lerp abundance one month later (Fig. 2a; Table 2). In contrast, no significant correlation was observed between Glycaspis sp. abundance and dLAI within the same month ($P = 0.613$). For C. fiscella and Spondyliaspis sp., however, an increase in the abundance of lerps resulted in a concurrently reduced dLAI (Fig. 2; Table 2). Exclusion of outlier values in these models did not impact the relationship and significance found between dLAI and psyllid abundance (Supplementary Table S2).

The community composition of psyllids both during and after the CO$_2$ ramp-up phase rapidly shifted over time (PerMANOVA $R^2 = 0.737$, $P < 0.001$; $R^2 = 0.771$, $P < 0.001$, respectively; Fig. 3). The significant effect of time on community composition was due to the sequential outbreak of two psyllids species altering the abundance of the other co-occurring species. Cardiaspina fiscella and Spondyliaspis sp. had a stronger association with each other and co-occurred more frequently than with Glycaspis sp., while Glycaspis sp. appeared to not strongly associate with either of the other two species (Fig. 3). These species associations were maintained during and after the CO$_2$ ramp-up phase. However, at very high abundances of C. fiscella (December 2014) or Glycaspis sp. (March 2014) the abundances of the other two co-occurring species were greatly reduced (Fig. 3).

During the CO$_2$ ramp-up phase, lerp weight was not different between the CO$_2$ treatments (Fig. 4a; Table 3), while a significant CO$_2$ effect was observed after one year of CO$_2$ fumigation (Fig 4b; Table 3). Individual lerp weight of Glycaspis sp. was higher under e[CO$_2$] than a[CO$_2$], while lerp weights for C. fiscella or Spondyliaspis sp. were similar between CO$_2$ treatments (Fig. 4b). The higher lerp weight for Glycaspis sp. but not for C. fiscella or Spondyliaspis sp. after the CO$_2$ ramp-up was also maintained during the abundance peak of each respective psyllid species (Supplementary Fig. S8).

The monthly deposition of Glycaspis sp. lerps to the woodland floor during its highest abundance was 69.6 kg ha$^{-1}$ month$^{-1}$ at a[CO$_2$] and 33.6 kg ha$^{-1}$ month$^{-1}$ at e[CO$_2$], while the inputs from Spondyliaspis sp. and C. fiscella were considerably lower with values between 0.1 and 1 kg ha$^{-1}$ month$^{-1}$ during their abundance peaks (Supplementary Table S3).

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Discussion

We studied the population dynamics of three psyllid species in combination with changes in LAI of a mature *Eucalyptus* woodland within the first two years of free-air CO\(_2\) enrichment. Based on this, we report, for the first time, an outbreak of a pest insect at a FACE site and link it with changes in canopy leaf area index. During the two-year study period psyllid populations were at low abundance in the first year, and at high abundance in the second year, with two consecutive and significant population peaks of two species; first by flush-feeding *Glycaspis* sp. and then by senescence-inducing *C. fiscella*, with the latter causing significant canopy loss.

While e[CO\(_2\)] did neither initiate the psyllid outbreaks nor drive the significant defoliation in the second year, it reduced the abundance of psyllid lerp at EucFACE. This negative response contradicted our expectation that plant sap feeders generally respond positively to e[CO\(_2\)]. In contrast, psyllid lerp abundances were not different between CO\(_2\) treatments during the CO\(_2\) ramp-up. The population response of psyllids to e[CO\(_2\)] was consistent across three species from different genera that naturally occurred at EucFACE on the same host tree species, *E. tereticornis*, yet with a different preference for either flush or mature foliage. This CO\(_2\) effect on abundance after the ramp-up was also maintained after a significant change in the psyllid community composition due to the rapid transition (within one year) from high *Glycaspis* sp. abundance to high *C. fiscella* abundance.

The rapid sequence of population peaks by different psyllid species and the stark psyllid community turn-over may have occurred due to intra- or interspecific competition in combination with tree responses as it was observed in pecan aphids (Petersen & Sandström, 2001; Cottrell *et al.*, 2009) while progressing leaf phenology may have favoured the different feeding strategies of first flush-feeding and then senescence-inducing psyllid species.
The large population size of *Glycaspis* sp. did not impact canopy leaf area index but this species thrived after an increase in dLAI in March 2014. This dLAI increase was driven by the production of new flush foliage after rainfall (Duursma *et al.*, 2016). As this new cohort of *Glycaspis* sp. infested leaves matured, the *Glycaspis* sp. abundance dropped, while *C. fiscella* abundance increased and peaked. At high abundance, *C. fiscella* caused significant defoliation, demonstrated by a dramatic decrease in dLAI in September 2014 (the largest rapid loss in canopy leaf area index throughout the study period). This decrease in dLAI was not accompanied by an instant increase in leaf litter, as *C. fiscella* cause leaf senescence and necrosis resulting in a delayed leaf drop and defoliation. This progressive psyllid-caused defoliation on *Eucalyptus* is typical for this system and results in the brown and burnt appearance of the canopy that still carries wilted leaves before they are dropped (Steinbauer *et al.*, 2014; Hall *et al.*, 2015).

Many chewing insect herbivores increase their food intake at eCO$_2$ (Robinson *et al.*, 2012) and some evidence of compensatory feeding exists for aphids feeding on nutrient altered diets (Simpson *et al.*, 1995). The dry weight of individual *Glycaspis* sp. lerps increased at eCO$_2$ suggesting that consumption by this species may have increased as a behavioural feeding compensation to altered nutrient composition of leaves. Gherlenda *et al.* (2016) reported that flush foliage at EucFACE had a higher nitrogen concentration than mature foliage during the same period of the *Glycaspis* sp. outbreak. However, the nitrogen concentration of flush and mature foliage between CO$_2$ treatments was not different. Similarly, there were no differences in nitrogen and phenolic content of frass produced by chewing insects between CO$_2$ treatments during this period (Gherlenda *et al.*, 2016). Therefore, the increase in lerp weight at eCO$_2$ may be an indication of another, yet unknown change in leaf composition. An increase in lerp weight was not observed for the two defoliating species that were correlated with negative dLAI, *C. fiscella* or *Spondyliaspis* sp., and this may be due to their feeding strategy as leaf senescence inducers. *Cardiaspina* species secrete salivary enzymes that break down the chloroplast thylakoid membranes and mobilise nutrients in leaf tissue.
(Steinbauer et al., 2014), perhaps limiting the need for compensatory feeding while inducing early leaf senescence that causes leaf necrosis and ultimately defoliation.

In aphids, plant-mediated effects of e[CO$_2$] may also include the alteration of amino acid metabolism by their primary endosymbiont Buchnera (Guo et al., 2013). Similarly, psyllids carry a primary endosymbiont, ‘Candidatus Carsonella ruddii’ and complementing secondary endosymbionts that may be responsible for nutrient provisioning (Sloan & Moran, 2012). However, it appears that these bacterial endosymbionts did not mitigate the negative effects of e[CO$_2$] on psyllid abundance. Furthermore, compensatory feeding may increase the incidence of plant pathogen transmission, as some psyllid species vector plant-pathogenic bacteria (Pelz-Stelinski et al., 2010; Casteel et al., 2012). However, psyllid species associated with Eucalyptus have not yet been found to carry any plant pathogens.

Reduced psyllid abundance at e[CO$_2$] may also impact other species which rely on psyllids or their excretions as a food source. Psyllids can be an important food source for a number of insectivorous birds (Clarke & Schedvin, 1999; Lockwood & Gilroy, 2004), parasitoids and predatory insects (Hall et al., 2015) which may be negatively impacted by lower psyllid abundance at e[CO$_2$]. Psyllid lerps may also be a rich source of carbon (White, 1972) for soil biota and alterations in lerp deposition to the soil may alter abundance and composition of soil biota, and thus impact decomposition of organic matter and nutrient cycling within forests.

Rapid and large compositional shifts in the psyllid community occurred as a result of the population peaks of Glycaspis sp. and C. fiscella, and this may have concealed any small effects that e[CO$_2$] may have had on community structure. The Glycaspis sp. outbreak did not impact dLAI but benefited from its increase, and its outbreak could have potentially gone unnoticed by LAI-based or remote sensing approaches often applied in tree health surveys (Trumbore et al., 2015). Conversely, increases in C. fiscella and Spondylaspis sp. abundance were associated with reduced dLAI. Rainfall has previously been reported to increase dLAI at EucFACE when a soil moisture threshold is reached,

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while long periods without rain can reduce dLAI (Duursma et al., 2016). Only one such dry event was recorded in our study in from August to October 2013 causing an increase in leaf litter fall (Fig. S2 and S3) and an associated decrease in LAI (and hence a negative dLAI). This dry period occurred in the year prior to the Glycaspis and C. fiscella population peaks. It needs to be noted that it is difficult to tightly link the timing of psyllid population peaks with leaf litter fall. This is because damage caused by Cardiaspina psyllids does not result in immediate defoliation such as with leaf chewers; infested leaves become increasingly necrotic before they die, and can then still be held in the canopy for considerable time before being dropped. Furthermore, Cardiaspina defoliation does not result in instant stunted growth, because psyllids preferentially infest mature leaves. However a consequence of chronic Cardiaspina defoliation can be dieback and epicormic growth of Eucalyptus (Hall et al., 2015). Given that our study documented a first Cardiaspina defoliation event at EucFACE without any Cardiaspina defoliation directly prior to it, we did not observe stunted growth during the period of our study.

Cardiaspina outbreaks often result in extensive canopy damage and this can lead to significant tree dieback and mortality during prolonged or repeated outbreaks (Clark, 1962; Hall et al., 2015). Because of feedback mechanisms in combination with other biotic and abiotic factors such as resource availability, temperature and rainfall patterns (Hall et al., 2015), outbreaking psyllid populations in Australian woodlands often experience boom-and-bust cycles. A previously reported collapse of an outbreak by a different Cardiaspina species and area-wide defoliation of several thousands of hectares of Eucalyptus moluccana in Western Sydney’s CPW surrounding EucFACE has been linked to a combination of resource depletion and summer heat waves (Hall et al., 2015). Furthermore, the outbreak of C. fiscella was not restricted to the EucFACE site, as during our study, and prior to September 2014, E. tereticornis was severely impacted by C. fiscella in a larger area of the Hawkesbury region, about 10 km to the west of the EucFACE site.
In contrast to the reduced lerp deposition at e[CO$_2$], frass deposition by chewing insect herbivores, as demonstrated in another study at the EucFACE site, was not influenced by CO$_2$ treatment (Gherlenda et al., 2016). However, frass deposition by chewing insects dropped significantly in the second year while lerp abundance increased. It appears that this clear shift from chewing to sap-feeding insects was independent of CO$_2$.

Psyllids are so far an under-represented group in climate change research, yet an important herbivore in native and plantation forests of *Eucalyptus* (Paine et al., 2011) and many crop plants (Hollis, 2004). Consistently, all three psyllid species had reduced abundances at e[CO$_2$] despite their plant sap-feeding strategy, and this is contrary to the general trend of improved performance of sap-feeders at e[CO$_2$] (Robinson et al., 2012), including at other forested FACE sites with trees (Hillstrom et al., 2014). e[CO$_2$] did not alter the outbreak potential and community compositional shifts of psyllid species at EucFACE but it reduced abundances of psyllids by approximately 10-22 % and therefore also modulated the population peaks. To our knowledge, herbivorous insect outbreaks with severe impacts on canopy cover, such as high levels of defoliation, have previously not been observed in other FACE studies with trees that have assessed pest species at low abundance levels (Percy et al., 2002; Hamilton et al., 2012; Couture et al., 2015). The study of insect outbreaks, including psyllid outbreaks, is often opportunistic. This presents many challenges in isolating the key factors leading to an outbreak and its cessation, and the overall impact of insect outbreaks. The study of fortuitous insect pest outbreaks at FACE sites will therefore provide important information for modelling and predicting insect outbreak dynamics, their ecological impacts, tree recovery after disturbance and therefore woodland ecosystem resilience at e[CO$_2$]. Our study also highlights functional links of canopy leaf processes, driven by rainfall, and psyllid population dynamics resulting into a rapid sequence of psyllid outbreaks and significant defoliation caused by outbreaks of some but not all psyllid species.
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Data deposition: The mtDNA sequences reported in this paper have been deposited in the GenBank database (accession numbers KT388055-KT388063).

References


Ballhorn DJ, Schmitt I, Fankhauser JD, Katagiri F, Pfanz H (2011) CO₂-mediated changes of plant traits and their effects on herbivores are determined by leaf age. Ecological Entomology, 36, 1-13


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Gherlenda AN, Haigh AM, Moore BD, Johnson SN, Riegler M (2015) Responses of leaf beetle larvae to elevated [CO₂] and temperature depend on *Eucalyptus* species *Oecologia, 177*, 607-617

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Hillstrom ML, Couture JJ, Lindroth RL (2014) Elevated carbon dioxide and ozone have weak, idiosyncratic effects on herbivorous forest insect abundance, species richness, and community composition. Insect Conservation and Diversity, 7, 553-562


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Stacey DA, Fellowes MDE (2002) Influence of elevated CO$_2$ on interspecific interactions at higher trophic levels. *Global Change Biology, 8*, 668-678


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Supporting information captions

**Figure S1.** Lerps excreted by three psyllids species and leaf damage by *Cardiaspina fiscella* on *Eucalyptus tereticornis* at the *Eucalyptus* free-air CO$_2$ enrichment (EucFACE) site.

**Figure S2.** Mean monthly leaf litter fall at *Eucalyptus* free-air CO$_2$ enrichment (EucFACE) site.

**Figure S3.** Total monthly rainfall occurring at the *Eucalyptus* free-air CO$_2$ enrichment (EucFACE) site.

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Figure S4. Mean monthly number of *Glycaspis* sp. lerps collected from the woodland floor and dLAI values from the previous month at the *Eucalyptus* free-air CO$_2$ enrichment (EucFACE) site.

Figure S5. Mean monthly number of *Cardiaspina fiscella* lerps collected from the woodland floor and dLAI values at the *Eucalyptus* free-air CO$_2$ enrichment (EucFACE) site.

Figure S6. Mean monthly number of *Spondyliaspis* sp. lerps collected from the woodland floor and dLAI values at the *Eucalyptus* free-air CO$_2$ enrichment (EucFACE) site.

Figure S7. Psyllid lerp abundance against dLAI values.

Figure S8. Mean dry weight of individual lerps from three psyllid species collected at the EucFACE experimental site under ambient and elevated CO$_2$ treatments during the peak abundance for each respective species.

Table S1. Reference specimens for the three psyllid species for which lerp abundance was assessed at the EucFACE site.

Table S2. Model output of the relationship between psyllid species and dLAI reanalysed with outliers removed.

Table S3. Weight of dry psyllid lerps (kg ha$^{-1}$) falling to the woodland floor under ambient and elevated CO$_2$ treatments during the month in which their abundance peaked at the EucFACE site.
Table 1. Results of mixed-effects models of psyllid lerp abundance with CO$_2$, psyllid species and month during the CO$_2$ ramp-up and after CO$_2$ concentrations were at 150 µmol mol$^{-1}$ above ambient levels.

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**Table 2.** Results of mixed-effects models of psyllid lerp abundance with dLAI at the EucFACE site.

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<th>Psyllid species</th>
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<td><em>Glycaspis</em> sp.</td>
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<td><em>Cardiaspina fiscella</em></td>
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<td><em>Spondyliaspis</em> sp.</td>
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<td>&lt;0.001</td>
<td>0.406</td>
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**Table 3.** Results of mixed-effects models of psyllid lerp weight with CO$_2$ and psyllid species during the CO$_2$ ramp-up and after CO$_2$ concentrations were at 150 µmol mol$^{-1}$ above ambient levels.

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