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REGULAR ARTICLE

Precipitation, not CO_2 enrichment, drives insect herbivore frass deposition and subsequent nutrient dynamics in a mature *Eucalyptus* woodland

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Abstract

Background and aims Herbivorous insects are important nutrient cyclers that produce nutrient-rich frass. The impact of elevated atmospheric $[CO_2]$ on insect-mediated nutrient cycling, and its potential interaction with precipitation and temperature, is poorly understood and rarely quantified. We tested these climatic effects on frass deposition in a nutrient-limited mature woodland.
Methods Frass deposition by leaf-chewing insects and its chemical composition was quantified monthly over the first 2 years at the *Eucalyptus* free-air CO_2 enrichment experiment and contrasted with leaf nitrogen concentration, rainfall and temperature.
Results Leaf-chewing insects produced yearly between 160 and 270 kg ha^{-1} of frass depositing 2 to 4 kg ha^{-1} of nitrogen. Frass quantity and quality were influenced by rainfall and average maximum temperatures. In contrast, elevated CO_2 did not impact nitrogen concentrations in fully expanded leaves and frass deposition to the woodland floor.
Conclusions Two years of elevated CO_2 did not alter nutrient transfer by leaf-chewing insects. This may be due to the low nutrient status of this ecosystem, duration of CO_2 fumigation or climatic conditions. However, rainfall co-occurring with seasonally higher temperatures exerted strong effects on nutrient cycling, potentially through shifts in leaf phenology with consequences for insect population dynamics and insect-mediated nutrient transfer.

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Introduction

Insect herbivores living within forest canopies play major roles in terrestrial ecosystems; they can shape community structure and link above- and belowground processes via their roles in forest nutrient dynamics (Schowalter 2000; Bardgett and Wardle 2003; Frost and Hunter 2004). Insect herbivores can directly affect soil nitrogen (N) availability by the deposition of frass (insect excrement), transferring N from leaf tissue to the forest floor within the same season and prior to leaf senescence (Frost and Hunter 2007). Herbivores also indirectly impact nutrient transfer through alterations in plant community composition that can change leaf decomposition rates and nutrient release (Schweitzer et al. 2005; Chapman et al. 2006).

It is well established that elevated atmospheric CO_2 concentrations, hereafter e[CO_2], alter leaf chemistry and physical properties of plants (Ainsworth and Rogers 2007). e[CO_2] often leads to a decrease in foliar [N] (Ellsworth et al. 2012) and increases in carbohydrate concentrations resulting in an increased C:N ratio (Stiling and Cornelissen 2007; Duval et al. 2012; Sardans et al. 2012; Sistla and Schimel 2012). Some secondary compounds, such as phenolics, are also expected to increase under e[CO_2] (Ryan et al. 2010). Alterations to foliar chemistry at e[CO_2] generally have negative impacts on insect herbivore performance, such as increased developmental time and reduced pupal weights (Robinson et al. 2012; Murray et al. 2013a; Gherlenda et al. 2015). Leaf consumption is also expected to increase, potentially via compensatory feeding in response to reduced foliar nutrient concentrations (Stiling and Cornelissen 2007). Changing patterns of leaf consumption or varied insect herbivore abundance as a result of plant-mediated e[CO_2] effects may therefore have implications for forest nutrient transfer mediated by frass deposition.

Leaf consumption by insects results in the production of frass which contains nutrients such as N and phosphorus (P) in higher concentrations and in a more labile form than in leaf litter (Lovett et al. 2002; Madritch et al. 2007). Nutrients contained within frass, particularly N, can quickly be re-assimilated by forest plants (Frost and Hunter 2007), transported from the forest via water runoff (Townsend et al. 2004; Frost and Hunter 2007) or immobilised by the microbial community (Hillstrom et al. 2010; Kaukonen et al. 2013). It has been suggested that the role of frass deposition within forest nutrient cycling is most pronounced during insect outbreaks (Hollinger 1986; Reynolds et al. 2000). Recent studies, however, have shown that insect herbivores at normal population levels also significantly impact nutrient cycling and soil processes (Hunter et al. 2003; Meehan et al. 2014).

Changes in leaf chemistry mediated by environmental factors such as e[CO_2], precipitation and temperature may result in altered frass chemistry, including the amount of phenolic compounds and C:N ratio. This may have implications on forest processes utilising frass as a nutrient source. For example altered frass C:N ratios may influence plant associations with microbial symbionts (Katayama et al. 2010; Kagata and Ohgushi 2012). Incomplete understanding of the intricate relationship between insect-mediated nutrient cycling and environmental factors such as rainfall and seasonal temperature variations, adds further uncertainty to how forest nutrient cycling will be impacted by climate change.

This study assessed frass deposition by leaf-chewing insect herbivores from the canopy to the woodland floor of the *Eucalyptus* free-air CO_2 enrichment (EucFACE) experiment. We aimed to assess the impact of e[CO_2] on aspects of nutrient cycling driven by leaf-chewing insects within this nutrient-limited woodland ecosystem described by Crous et al. (2015) and Nielsen et al. (2015). It was hypothesised that frass deposition in this mature *Eucalyptus* woodland would increase at e[CO_2] due to compensatory feeding by leaf-chewing insects in response to plant-mediated e[CO_2] effects (if any were detected). Such compensatory feeding was observed in most laboratory studies (Stiling and Cornelissen 2007; Robinson et al. 2012), including two key *Eucalyptus* herbivore species found at EucFACE, *Paropsis atomaria* (Coleoptera: Chrysomelidae) and *Doratifera quadriguttata* (Lepidoptera: Limacodidae) (Murray et al. 2013a; Murray et al. 2013b; Gherlenda et al. 2015). However, potential compensatory feeding responses have so far only been tested in few field studies (Meehan et al. 2014; Couture et al. 2015).

As a secondary aim, we investigated the effect of environmental variation in rainfall and temperature on seasonal and annual variability in insect herbivore frass production in this mature and evergreen *Eucalyptus* woodland over a 2 year period. Previous studies have assessed frass and associated nutrient deposition in *Eucalyptus* woodlands mostly as individual time snapshots, resulting in considerable variation in the frass production reported for these ecosystems (Ohmart et al. 1983; Ohmart 1984; Lowman and Heatwole 1992). However frass deposition may depend on rainfall that drives production of new foliage in evergreen *Eucalyptus*, and on temperature, a key factor for insect development and activity (Gherlenda et al. 2015). Previous research demonstrated that the production of new foliage (flush growth) in *Eucalyptus* due to rainfall (Pook 1984) can stimulate herbivory while insect activity is highest in the summer (Ohmart et al. 1983; Heatwole et al. 1997). Therefore, we hypothesised that precipitation during periods with higher average maximum temperatures would also increase frass deposition as a function of new leaf production and increased insect activity.

Methods

This study was conducted at the EucFACE experiment which is located in a remnant of native Cumberland Plain Woodland in Richmond, NSW, Australia. The EucFACE experiment comprises six 25 m diameter FACE rings which extend above the tree canopy. Three rings were supplied with ambient CO_2 and the other three rings received $e[CO_2]$; CO_2 was supplied to all rings via a proportional-integral-derivative control algorithm (Lewin et al. 2009), and without changing airflow in rings so that it would not impact insect mobility (Hamilton et al. 2012). CO_2 enrichment began in September 2012 and was gradually increased at a rate of 30 $\mu mol\ mol^{-1}$ per month until the maximum concentration of 150 $\mu mol\ mol^{-1}$ above ambient $[CO_2]$ was reached in February 2013; it was then kept at this diurnal $[CO_2]$ target within the elevated rings. Except for the fumigation and experimental set up, the study site has remained undisturbed for at least 75 years and is dominated by mature *Eucalyptus tereticornis* trees forming an open tree canopy with a grassy understory characteristic for the Shale Plains Woodland community, the most widely distributed vegetation community of Cumberland Plain Woodland (Tozer 2003; see Crous et al. 2015 for detailed site description).

Eight plastic baskets with a collection surface area of 0.1979 m^2 (an ellipsoid with lengths of 0.56×0.45 m) and with a depth of 0.40 m were randomly allocated within each treatment and control ring, and staked approximately 20 cm above the woodland floor. Each basket was lined with a fine nylon mesh (200 μm aperture) weighted down in the centre. This allowed collection of material deposited from the *E. tereticornis* canopy while allowing precipitation to freely pass through. Five baskets were initially installed in each ring in mid September 2012 and additional three baskets were added to each ring in mid October 2012. Frass was collected monthly for 2 years from all eight traps per ring starting from mid November 2012 until mid October 2014. Therefore, year 1 was defined as from November 2012 to October 2013; year 2 as from November 2013 to October 2014. Frass was oven-dried at 40 °C for 72 h. Frass of leaf-chewing herbivores was identified by shape, texture and colour, and sorted from other material collected (e.g., litterfall, bark) under a dissecting microscope (Couture et al. 2015). For the purpose of this study we have excluded lerps, starchy excretions by plant-sucking psyllids commonly found on *Eucalyptus* (Hall et al. 2015). This is consistent with other frass studies that have quantified frass exclusively from leaf-chewing insects (Ohmart et al. 1983; Couture et al. 2015). After sorting, frass samples were weighed and then pooled per ring before being ground to a fine powder for chemical analysis.

For each month, [C] and [N] of frass samples were measured with a CHN elemental analyser after combustion (TruSpec micro, LECO Corp., St. Joseph, MI, USA; and FLASH EA 1112 Series CHN analyser, Thermo-Finnigan, Waltham, MA USA). Monthly [P] were determined by placing 50 mg of each sample in a muffle furnace (550 °C) for 8 h. The resulting ash was dissolved in 5 mL of 1 % perchloric acid and total [P] quantified by ascorbic acid-molybdate reaction (Murphy and Riley 1962) that was run in technical triplicates. Monthly total phenolics (TP) content was determined as described in Salminen and Karonen (2011), in technical triplicates, using a Folin-Ciocalteu assay with gallic acid monohydrate (Sigma-Aldrich, St. Louis, MO, USA) as the quantification standard.

Two fully expanded and mature leaves from each of three trees per ring were collected in October 2012 (during ramp-up), and February (after ramp-up), May and September 2013 (constituting year 1 of frass collection) and in November 2013, February, May and November 2014 (constituting year 2 of frass collection). A single collection of flush leaves was also conducted in February 2014 for comparison with mature leaves collected at the same time. Throughout the study, leaf flushing periods were observed starting in late January 2013 (minor flushing in year 1) and December 2013 (major flushing in year 2; D.S. Ellsworth, personal communication). Leaves from each ring were freeze-dried and ground to a fine powder with a ball mill (MM400 Retsch, Haan, Germany). Foliar N was determined with a CHN elemental analyser after combustion (TruSpec micro, LECO Corp., St. Joseph, MI, USA; and FLASH EA 1112 Series CHN analyser, Thermo-Finnigan, Waltham, MA, USA). Data of leaf [N] were analysed for elevated CO_2 effects in JMP (JMP v. 11, SAS Institute, Cary, NC, USA) with a repeated measurements ANOVA.

For each monthly frass sample, the dry weight was divided by the duration in days between monthly collections and multiplied by 30 to obtain the monthly frass deposition. Annual amounts of C, N, P and TP transferred to the woodland floor by herbivorous insects were calculated as the mass of frass in a given month multiplied by the concentration as determined by chemical

analysis for each respective month. Linear mixed effects models fitted by restricted maximum likelihood were used to determine the effect of e[CO_2], time, rainfall and average maximum temperature on frass deposition and chemistry in R (v3.2.0, R Development Core Team 2015) using the *nlme* package (v3.1-118, Pinheiro et al. 2015). Ring identity and month were included as random factors in order to account for repeated measures. An autocorrelation function was used in order to test for temporal autocorrelation and model residuals. Monthly rainfall values (starting with the 15th day of each month and ending with the 14th day of the following month, in order to match the mid-monthly periodicity of the frass collection) were obtained from automated tipping buckets (Tipping Bucket Rain gauge TB4, Hydrological Services Pty Ltd, Liverpool, NSW, Australia) located 23.5 m high on the central tower in three rings, while average monthly maximum temperatures were obtained from a HUMICAP sensor (HMP 155 Vaisala, Vantaa, Finland) located 23.5 m high on the central tower in all six rings. Data obtained from both sensors were logged every 15 min using CR3000 data loggers (Campbell Scientific, Townsville, Australia). Average monthly and minimum temperatures did not significantly affect frass deposition, thus only maximum temperatures were modelled. Rainfall occurring 30 days prior to the frass collection period and average maximum temperature was modelled, and R^2 values were obtained using the *r.squaredGLMM* function in the *MuMIn* R package (Johnson 2014; Barton 2015).

Results

The quantity of insect herbivore frass falling to the woodland floor was not influenced by the e[CO_2] treatment ($P=0.698$; Fig. 1a). In year 1, frass deposition peaked in November and from March to May, with a smaller third peak in September, while in year 2 frass deposition peaked in January and March (Fig. 1a). No significant difference in total yearly rainfall was observed between the two years ($P=0.873$) but the distribution of rainfall differed between years (Fig. 1b). In year 1, rainfall peaked in February and March, while in year 2, it peaked in December with a smaller peak in April. Monthly frass deposition was positively correlated with rainfall 30 days prior to the collection period ($P<0.001$, $R^2=0.080$; Fig. 2a). Higher monthly average maximum temperatures also significantly stimulated
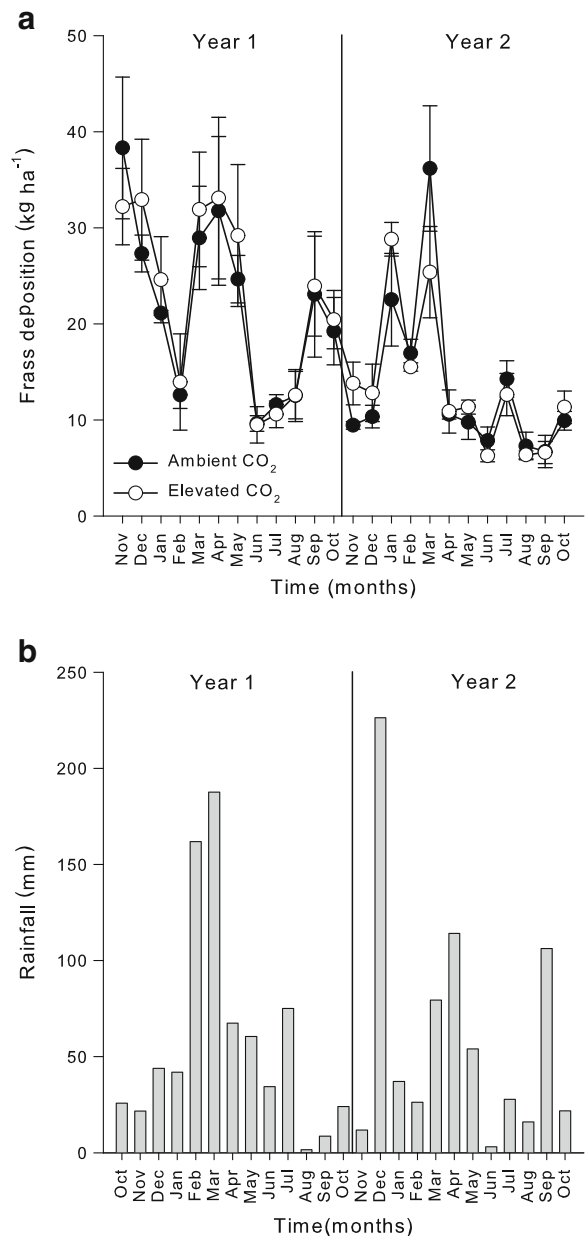
Fig. 1 Mean (± S.E.) monthly frass deposition by leaf-chewing canopy insects (**a**) and monthly rainfall (**b**) in the first and second year of the *Eucalyptus* free-air CO_2 enrichment experiment at ambient or elevated [CO_2]

frass deposition ($P<0.001$, $R^2=0.155$, Fig. 2b) up to about 30 °C. Above 30 °C frass production dropped .

For both years, monthly frass deposition to the woodland floor ranged between 10 and 40 kg ha^{-1} (Fig. 1a). In year 1, average monthly frass deposition was 22.3± 2.6 kg ha^{-1} and this significantly decreased to 13.5± 2.2 kg ha^{-1} in year 2, regardless of CO_2 treatment
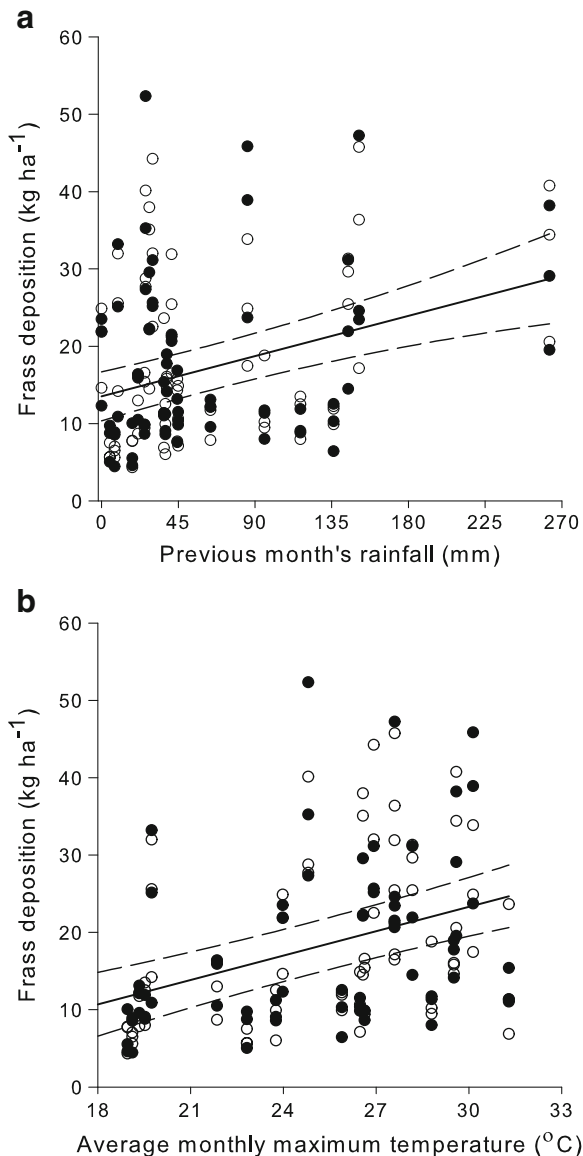
Fig. 2 Relationship between frass deposition by leaf-chewing canopy insects and rainfall occurring 30 days prior to the frass collection period (**a**) and average monthly maximum temperatures (**b**) over a 2 year period. *Solid line* indicates regression curve with *dashed lines* indicating predicted 95 % confidence intervals from the statistical model (mean ring monthly frass deposition values displayed). *Filled circles* represent ambient [CO_2], and open circles represent elevated [CO_2]

(Fig. 3a; Table 1). Similarly, frass [N] and [P] were 44 and 45 % lower in year 2 ($P=0.002$; $P=0.009$, respectively; Fig. 4) while the C:N ratio increased by 16 % (Table 2); no other frass chemical characteristic was altered between the years (Table 2). Frass [N] and [P] were also not significantly altered by e[CO_2] ($P=0.809$;

$P=0.709$, respectively; Fig. 4, Table 2), however, they fluctuated throughout both years. Frass [N] was highest in February of year 1 while in year 2, [N] was highest in December (Fig. 4a); this seasonal pattern was also consistent for frass [P] (Fig. 4b). Based on these concentration values and total frass deposition, annual deposition of frass components (C, P, N and TP) were significantly reduced in year 2 (Fig. 3b-e, Table 1), while e[CO_2] did not significantly alter the total quantity of N, P, C and TP transferred from the canopy to the soil via frass deposition (Fig. 3b-e; Table 1).

The average [N] in leaves of *E. tereticornis* was 16.6 ±0.4 mg g^{-1} at ambient and 15.9±0.4 mg g^{-1} at e[CO_2] in year 1, while in year 2 average foliar [N] was 16.6± 0.9 mg g^{-1} at ambient and 16.4±0.9 mg g^{-1} at e[CO_2] (Fig. 3f). Overall, foliar [N] was not impacted by e[CO_2] ($P=0.681$), and no significant difference was observed between years ($P=0.808$; Fig. 3f); however, a significant monthly effect was observed ($P<0.001$; Fig. 5). The single collection of flush foliage in February 2014 revealed higher foliar [N] when compared to mature leaves collected at the same time point ($P=0.003$; Fig. 5), and after a significant amount of rainfall in December 2013 (Fig. 1b) that had stimulated the production of new flush foliage (D.S. Ellsworth, personal communication).

Discussion

Over a 2 year period, and at the start of the EucFACE experiment, significant temporal variations were observed both in the total amount of frass deposited and its nutrient composition while CO_2 treatment itself did not impact frass quantity or composition in this mature *Eucalyptus* woodland. Within each year, at least two peaks in frass deposition and therefore nutrient transfer to the woodland floor were apparent between September to May (spring to autumn) with lowest frass production in winter. Peaks in frass deposition were a function of rainfall in the month prior to the frass peak and average monthly maximum temperature; the frass deposition peaks were also linked with the observed production of new foliage with higher [N] than mature leaves measured at the same time point. Furthermore, frass deposition and frass nutrient content was lower in the second year, possibly due to a shift of rainfall events in the second year. Overall our results are in strong support of our second hypothesis regarding rainfall and
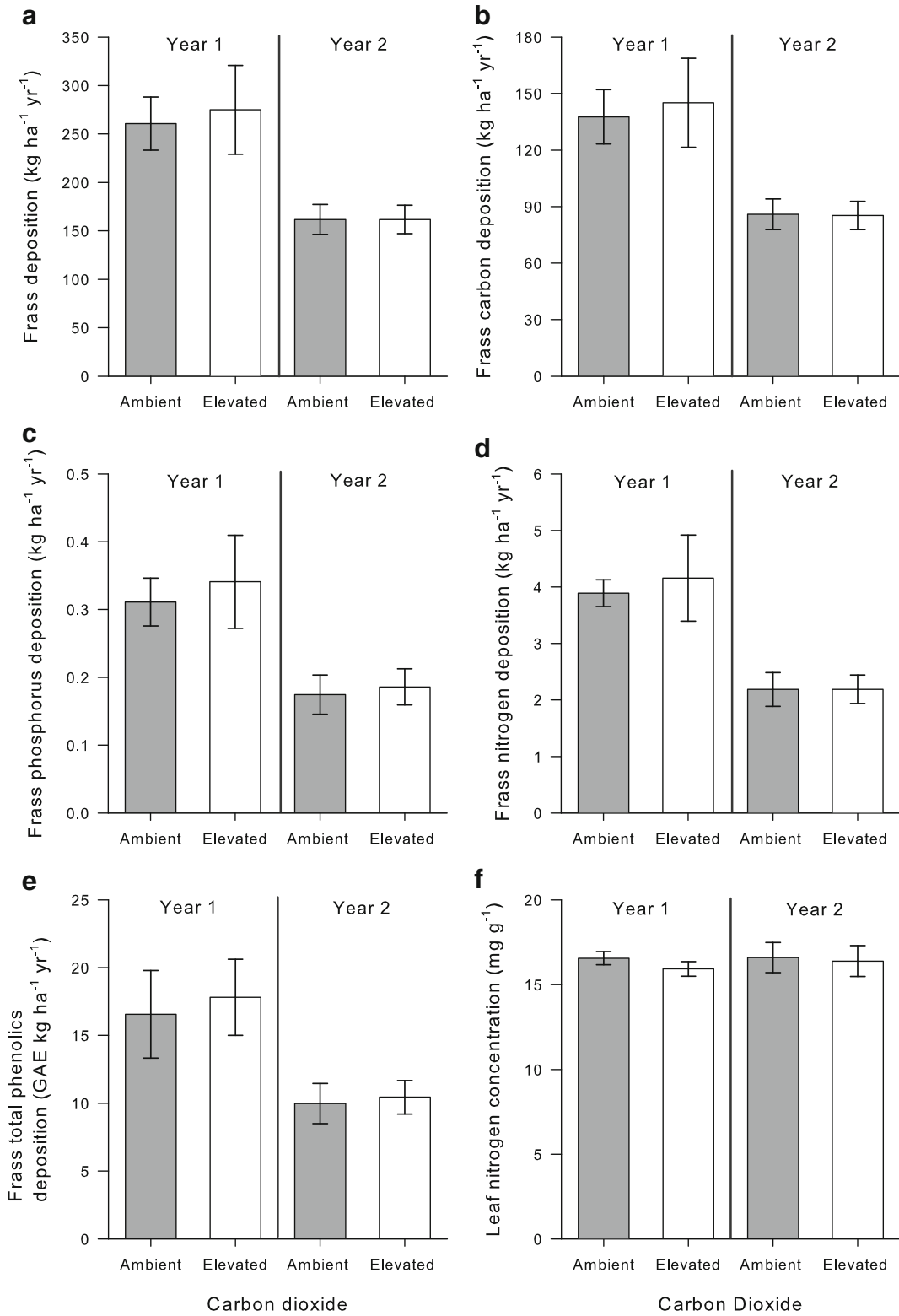
temperature as key drivers of frass deposition in the mature and evergreen *Eucalyptus* woodland of the EucFACE site, while we did not find support for our first hypothesis of e[CO₂] effects on leaf [N], frass quantity and composition 2 years into the EucFACE experiment.

Many *Eucalyptus*-feeding insects are known to preferentially feed on young expanding leaves because of their higher [N] (Lowman 1985; Steinbauer et al. 1998; Nahrung et al. 2008; Gherlenda et al. 2015). The production of *Eucalyptus* flush foliage can occur anytime throughout the year and is mostly driven by water availability and temperature (Davison and Tay 1989; Pook et al. 1997; Myers et al. 1998). Therefore, rainfall and temperature stimulating flush production may indirectly impact herbivorous insect population dynamics (and with it, frass deposition and nutrient cycling) while insect activity will also be directly stimulated by higher temperatures. Shifts in the timing of leaf production may thus impact insect populations and community composition (Heatwole et al. 1997), and with it frass production.

Frass deposition at the EucFACE experiment decreased in the second year, and this may have been due to several factors acting independently or interactively on insect abundance or composition; (1) the functional relationship between plant growth and herbivorous insect development may have been disrupted because of a shift in rainfall and leaf phenology; (2) less frass may have been produced because of a shift to a different insect community composition. For example, the herbivore community composition may have changed towards senescence leaf-chewers (White

2015), or alternatively there may have been a shift from leaf-chewing (therefore frass-producing) to plant-sap feeding herbivores; or a population increase at higher trophic levels may have suppressed insect herbivores via increased predation or parasitism (Hamilton et al. 2012). However, detailed characterisation of the insect herbivore community is not yet available for the EucFACE site to address this question.

Frass nutrient concentrations, including frass [N], significantly decreased in the second year, while foliar [N] was not different between both years. While in principle an interesting disparity, any functional interpretation of this difference needs to be made with extreme caution. The frass collection effort was far more comprehensive than leaf collection throughout our study. Furthermore, leaf samples represent a single plant species response while frass samples (albeit from the same single plant species) are derived from an insect species community response. For example, the ability of insects to extract foliar nutrients from the same plant species may differ and vary in time depending on insect species, and this may result in diverging chemistry of frass from leaves (Knepp et al. 2007; Couture and Lindroth 2014; Meehan et al. 2014). Similar to the reduction of frass mass deposited, the reduction in frass [N] may be due to nutrient shifts away from leaf-chewing insects towards other functional herbivore groups (i.e., plant sap feeding insects) for which our frass collection approach was not appropriate.

Research of another forest FACE site, Aspen FACE, has demonstrated that e[CO₂] can enhance nutrient transfer by leaf-chewing herbivorous insects (Hillstrom et al. 2010; Meehan et al. 2014; Couture et al. 2015). Frass deposition analyses in Aspen FACE were started 10 years after CO₂ fumigation began, and were undertaken over 2 years between late May to early September each year (Couture et al. 2015) because of their deciduous nature. In contrast, frass by leaf-chewing insects at EucFACE was collected from evergreen *Eucalyptus* trees during the first 2 years of elevated CO₂ fumigation.

Table 1 Linear mixed effect model results (*P* values) on total frass, carbon, nitrogen, phosphorus and total phenolics deposition at the EucFACE site. d.f. represents degrees of freedom and bold values indicate significance at *P*<0.05

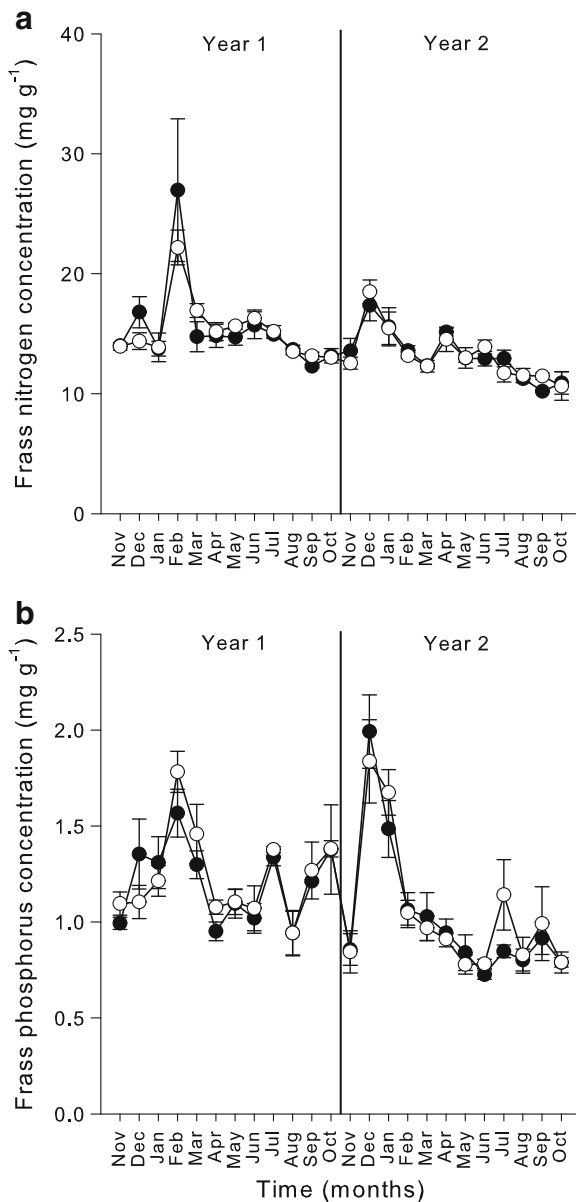|	*d.f.*	Frass	Carbon	Nitrogen	Phosphorus	Total phenolics
CO₂	1,4	0.750	0.742	0.694	0.651	0.723
Year	1,4	**0.017**	**0.016**	**0.012**	**0.011**	**0.019**
CO₂ x year	1,4	0.708	0.682	0.659	0.691	0.762

Fig. 4 Mean (± S.E.) monthly [N] (**a**) and [P] (**b**) contained within frass deposited by leaf-chewing canopy insects at ambient (*filled circles*) or elevated (*open circles*) [CO$_2$] at the *Eucalyptus* free-air CO$_2$ enrichment experiment

There is evidence to suggest that the age of trees and the total length of exposure influences tree responses to e[CO$_2$] (Gunderson and Wullschleger 1994; Curtis 1996). Moreover, trees at Aspen FACE have been exposed to e[CO$_2$] from the seedling stage throughout the establishment phase (Dickson et al. 2000) while in EucFACE fumigation occurred on already established and mature trees. Furthermore, and in contrast to Aspen

Face, the EucFACE site is P-limited (Crous et al. 2015) and this may limit the e[CO$_2$] response of *E. tereticornis* in growth, photosynthetic rates or leaf chemistry, with consequences for the insect community and insect-mediated nutrient transfer. P limitations at the EucFACE site has also been observed to define soil biota (Nielsen et al. 2015). The overall difference in tree ontogeny and duration of fumigation between both FACE experiments may be responsible for the contrasting responses; *E. tereticornis* trees at EucFACE may have had the ability to offset short-term e[CO$_2$] exposure by accessing nutrient reserves. Given that there was no effect of e[CO$_2$] on leaf [N] and therefore no basis for an expectation of compensatory feeding, our hypothesis of increased frass production at e[CO$_2$] within the first 2 years of EucFACE was rejected. Future analyses of leaf [N] and frass deposition after prolonged CO$_2$ exposure may show significant differences between ambient and elevated CO$_2$ treatments, in particular as there was a trend towards lower leaf [N] at e[CO$_2$] for some months. Previous studies at other FACE sites such as Duke FACE have also reported delayed onset of reduced leaf [N] at e[CO$_2$] (Ellsworth et al. 2012). In the future it will also be important to consider the effects of e[CO$_2$] on different developmental stages of leaves because herbivore species have different preferences for either flush, mature or senescent foliage (Gherlenda et al. 2015; White 2015).

There are several limitations which constrain the interpretation of results when reporting frass inputs from herbivorous insects to the forest floor: FACE rings and CO$_2$ fumigation do not prevent insect movement allowing insects to freely move between treatments (Hamilton et al. 2012). However, insects may respond behaviourally to plant-meditated effects of CO$_2$ treatments such as changes in the emitted volatile carbon profile. This could impact adult insects in their choice of diet, or adult female insects in their choice of oviposition sites, but is unlikely to impact larval stages that are restricted in their movement. Frass collected within our study was more likely from the larval stages that probably completed their development within a single treatment ring. Excretions from phloem-feeding insects were not measured yet are known to also impact nutrient transfer (Stadler et al. 2001) and this may be significant if at high abundance such as observed for psyllids on *Eucalyptus* (Steinbauer et al. 2014; Hall et al. 2015). High numbers of psyllid lerps were observed in frass traps throughout our study, and psyllid populations

Table 2 Mean (± S.E.) yearly concentrations (% dry weight) of carbon, nitrogen, phosphorus, and total phenolics as well as C:N and N:P ratios of frass falling from the canopy under ambient ($a[CO_2]$) or elevated ($e[CO_2]$) concentrations, with analysis results of the linear mixed effects model

		Carbon	Nitrogen	Phosphorus	Total phenolics	C:N	N:P
Year 1							
$a[CO_2]$		52.71 (0.14)	1.55 (0.08)	0.12 (0.001)	6.01 (0.55)	35.66 (0.94)	13.05 (0.65)
$e[CO_2]$		52.79 (0.21)	1.53 (0.04)	0.12 (0.007)	6.15 (0.21)	35.41 (1.03)	12.6 (0.45)
Year 2							
$a[CO_2]$		52.99 (0.29)	1.32 (0.04)	0.10 (0.006)	5.84 (0.20)	41.21 (1.22)	13.76 (0.70)
$e[CO_2]$		52.96 (0.24)	1.32 (0.06)	0.10 (0.007)	5.86 (0.22)	41.30 (1.85)	13.50 (1.04)
	d.f.	P-value					
CO_2	1,4	0.832	0.824	0.691	0.781	0.899	0.690
Year	1,4	0.176	**0.050**	**0.026**	0.626	**0.038**	0.413
$CO_2 \times$ year	1,4	0.663	0.873	0.906	0.824	0.903	0.870

culminated into large numbers causing considerable canopy defoliation after frass collections ceased (A.N.G. and M.R., personal observation), and this will require further investigation in the future. Finally, precipitation may have leached out collected frass mass and its nutrient content. Estimates of reductions in the mass of frass as a result of precipitation can be as high as 34 % depending on the producer of frass and the intensity of precipitation events (Mizutani and Hijii 2001). Therefore, the values reported in our study should be considered as an under-estimate of frass deposition and nutrient content.

Our study demonstrated in monthly accumulative sampling over 2 years that leaf-chewing herbivorous insects, even at non-outbreak levels, can transfer significant amounts of frass mass (160–270 kg ha^{-1} per year) and nutrients (e.g., 2–4 kg N ha^{-1} per year) from the canopy to the floor of a *Eucalyptus* woodland. This comprehensive measurement allowed correlation of frass deposition by leaf-chewing insects with the temporal and seasonal variation of precipitation and temperature. The most likely link between rainfall, temperature and frass deposition is the production of new canopy foliage, with higher [N] content that then drives

Fig. 5 Average monthly foliar [N] of *Eucalyptus tereticornis* from mature leaves (*circles*) and a single collection event of flushing leaves (*triangles*) under ambient (*filled symbols*) or elevated (*open symbols*) [CO_2] at the EucFACE site between October 2012 and November 2014. Increased leaf [N] over the seasons represents a new leaf cohort (i.e., May 2013 and Feb 2014)
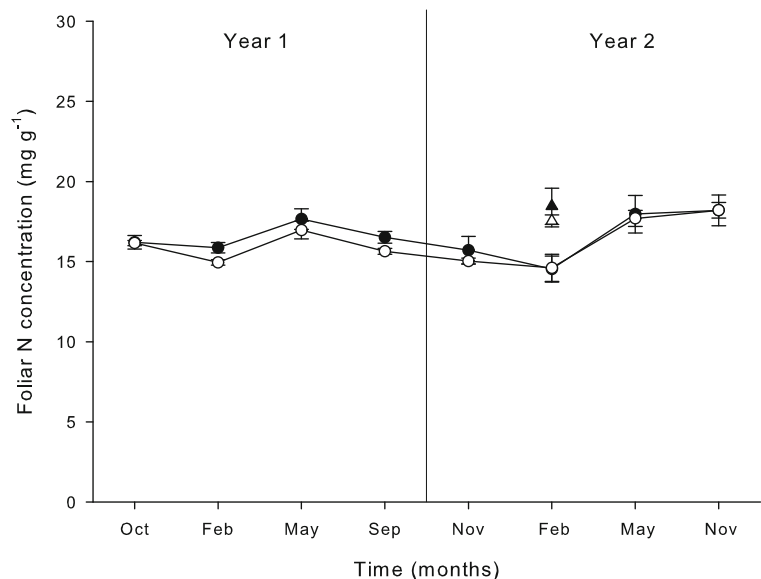
insect herbivore abundance and population dynamics when they are most active during the warmer parts of the year. The interactions of environmental variables such as rainfall and temperature, with e[CO_2] is still rarely addressed (Murray et al. 2013a; Murray et al. 2013b; Gherlenda et al. 2015) yet may be an important aspect in modelling climate change responses of insect herbivores specifically, but also of forest ecosystems more widely. In nutrient-poor ecosystems, the effects of e[CO_2] on frass deposition and composition may be masked by abiotic factors such as rainfall and temperature which drive leaf phenology and insect population dynamics.

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